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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, and poster sessions of the 46th Annual Meeting of the Society of Toxicology, held at the Charlotte Convention Center, Charlotte, March 25–29, 2007.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 449.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 480.

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1

REACH – A NEW FRAMEWORK FOR THE REGULATION OF CHEMICALS.

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The European Commission (EC) is increasing its regulation of chemicals through a comprehensive new program known as REACH (Registration, Evaluation, and Authorization of Chemicals). The legislation, which is based on the precautionary principle, shifts the burden from government to industry to prove that chemicals can be used safely before they are marketed. As a part of this new system, manufacturers and importers of chemicals will be required to submit hazard, use, and risk data for many substances manufactured in or imported into the EU. Chemicals which exist in preparations or as part of articles sold in the marketplace will also be subject to these regulations. Thus, while REACH is a European regulation, it will impact manufacturers worldwide. A key aspect of the program is the requirement to submit data concerning the physicochemical properties, toxicity, and environmental effects of those chemicals. Registration will be mandatory before a new chemical can be marketed, as well as to keep an existing chemical on the market, and chemicals of greatest concern will be subject to formal authorization procedures much like pharmaceuticals. The amount of information required for registration will be proportional to the chemical's health risks and production volumes. Chemicals that are classified as carcinogenic, mutagenic, toxic to reproduction, persistent, or bioaccumulative may be banned entirely if the manufacturer cannot demonstrate that risks associated with the chemical can be controlled. REACH will impact the global chemicals industry and their downstream users. The estimated costs to industry for complying with REACH have been reported to be as high as \$64 billion ([1] 150 billion). Chemicals produced in quantities greater than 1000 tons, as well as the most toxic chemicals, will have to be registered within 3 years of the regulation entering into force, and all chemicals over 1 ton must be registered within 11 years. Compliance with this regulation will require significant toxicology and laboratory resources.



2

CONCEPTS, METHODS AND APPLICATIONS OF DISCOVERY AND INVESTIGATIVE TOXICOLOGY.

J. Davis¹, D. Badger², J. McKim³, J. Steidl-Nicholls⁴, B. Car⁵ and K. Kolaja⁶. ¹DSRD, PGRD, Chesterfield, MO, ²Drug Safety Evaluation, Allergan, Irvine, CA, ³Cee-Toxicology, Kalamazoo, MI, ⁴DSRD, PGRD, La Jolla, CA, ⁵Discovery Toxicology, Bristol-Myers Squibb, Princeton, NJ and ⁶Investigative Toxicology, Roche, Palo Alto, CA.

Drug development is a long, complex and expensive process. Typical development timelines are between 10 and 15 years with attrition rates that are often too high for companies to sustain productive pipelines. Investigational and discovery toxicology are extensions of the field of general toxicology, created to fulfill the growing need for generating higher throughput, integrative, and predictive toxicological information, in an effort to reduce attrition at later stages of drug development. These novel ideas have begun to be employed more frequently and its widely anticipated that this will pave the way for future drug testing paradigms. This course will provide first an overview on the role of toxicology in discovery units including a description of how the discovery toxicologist can support non-clinical drug development. The next lecture will cover in vitro methods and interpretation of cytotoxicity. Following this, an iterative approach to de-risking strategies of QT prolongation will be discussed. The forth speaker will discuss strategies to partner with pharmacology colleagues to probe and understand toxicology. The course will conclude with a lecture discussing emerging technologies that hold near-term potential for improving drug safety evaluation. By the conclusion of this course it is anticipated that the attendees will be able to better assess which approaches would be most appropriate for their organization.



3

GENOMICS: FROM NOVICE TO EXPERT, FROM CHALLENGES TO PROMISES.

B. Sen¹, J. Tucker², S. Hester¹, H. Auer³ and H. Hamadeh⁴. ¹U.S. Environmental Protection Agency, Research Triangle Park, NC, ²National Institute of Environmental Health Sciences, Research Triangle Park, NC, ³Columbus Children's Research Institute, Columbus, OH and ⁴Amgen, Inc., Thousand Oaks, CA.

Microarrays, when used properly, can be a powerful tool for genomics research. The complexity of the technology provides numerous challenges and potential pitfalls to the uninitiated. The FDA has developed guidelines for submission of genomics data and the EPA has guidelines for submission under development. It is becoming increasingly necessary for toxicologists in many fields, including regulatory review, to have some level of understanding of the design and interpretation of microarray based studies. The ability to utilize this technology and generate reproducible re-

sults requires a detailed understanding of the process. The first presentation will focus on the design considerations of a successful microarray experiment. In a typical microarray experiment, lists of significant differentially expressed genes are created. The common task faced by any researcher is to translate these gene lists into a better understanding of the biological phenomenon involved. The second presentation will discuss the use of various bioinformatic tools for data mining. From drug discovery, to a better understanding of the disease process, to unraveling the toxicity profile of unknown chemicals, genomics offers many promises. The second half of the course will highlight the applications and limitations of this emerging technology in disease and toxicity profiling. The third presentation will focus on the use of microarray in disease outcome prediction. The final presentation will examine the potential of genomics in evaluating toxicity and identifying biomarkers. This course is aimed at providing investigators contemplating or pursuing studies in differential gene expression, and practicing toxicologists involved in data interpretation for safety assessment and regulation, valuable knowledge of experimental design, sample processing, data analysis, and an understanding of the scope and limitations of this powerful tool.



4

ALLERGY AND ALLERGIC DISEASE: A PRIMER FOR TOXICOLOGISTS.

I. Kimber¹ and G. Gerberick². ¹Central Toxicology Laboratory, Syngenta, Cheshire, United Kingdom and ²Miami Valley Innovation Center, Procter & Gamble, Cincinnati, OH.

Allergy to chemicals and proteins is an important health issue, and some forms of allergy are increasingly prevalent diseases. Moreover, there is no doubt that skin sensitization represents the most common manifestation of immunotoxicity in humans. Allergy may take a variety of forms, those of greatest relevance to toxicology being allergic contact dermatitis resulting from skin sensitization, respiratory allergy and occupational asthma associated with both proteins and chemicals, gastrointestinal and systemic allergic responses caused by food proteins, and a variety of local and systemic adverse reactions to drugs where allergic reactions are frequently implicated. The purpose of this Continuing Education Course is to explore, in the context of toxicological sciences, and following a general introduction to the principles of allergy, each of the four main types of allergic disease mentioned above. In each case clinical manifestations of disease, biological mechanisms, and approaches available for toxicological evaluation will be described. This Basic Level course will be of value to immunotoxicologists, and also to those with interests in respiratory, skin and gastrointestinal toxicology, occupational medicine, the development of approaches to hazard characterization, and risk assessment.



5

FUNDAMENTALS OF HUMAN HEALTH RISK ASSESSMENT WITH A CASE STUDY APPROACH.

T. Leavens¹, J. Orme-Zavaleta², G. Foureman², E. Cohen-Hubal², H. Clewell¹ and M. Gargas³. ¹CIIT Centers for Health Research, Research Triangle Park, NC, ²U.S. EPA, Research Triangle Park, NC and ³The Sapphire Group, Inc., Beaver Creek, OH.

This course will provide a general overview of the process of human health risk assessment to educate students and post doctorates interested in careers in risk assessment and researchers interested in how their work can impact and strengthen the science upon which the assessments are based. Risk assessment is used by toxicologists to estimate the likelihood of an adverse event from exposure to a hazardous agent. In 1983 the National Academy of Sciences-National Research Council published "Risk Assessment in the Federal Government: Managing the Process" in which the process of risk assessment was clearly outlined into 4 components including hazard identification, dose-response assessment, exposure assessment, and risk characterization. This course will focus on each of these 4 components and will use vinyl chloride, especially the U.S. EPA IRIS assessment, as a case study to illustrate the development and application of the 4 components. The presentations will cover historical studies incorporated into hazard identification including acute, subchronic, and chronic animal assays as well as epidemiology studies, in vitro assays, predictive models, and genomics research. Information will also be presented on determination of the mode-of-action from non tumor data, and how it may guide and direct the assessment process to reduce uncertainty in extrapolating effects. Various methods of dose-response assessment will be presented including statistical and mechanistic models used to extrapolate effects among doses and species. Available exposure factor data, exposure modeling tools, biomonitoring data, and potential sources of variability and uncertainty in exposure assessment will be highlighted. The final component, risk characterization, will address statistical and biological issues, such as appropriate models and target populations, as well as connect the process of risk assessment with risk management.



6

TOXICOLOGY AND MOLECULAR BIOLOGY OF TISSUE REPAIR.

H. M. Mehendele² and C. Corton¹. ¹NHEERL, U.S.-EPA, Research Triangle Park, NC and ²Department of Toxicology, University of Louisiana, Monroe, LA.

After chemical exposure, injury may progress unabated leading to tissue and organ dysfunction and failure, or regress leading to recovery. When recovery occurs, tissue injury resolves as a result of cell division, restored tissue architecture, structure and function. The molecular biology of these events, and their dynamic relationships to progression or regression of tissue injury are of significant mechanistic interest, because risk of adverse effects from chemical exposure should consider not only the level of tissue damage but the dynamics of recovery from that damage. In addition, interventional therapeutic opportunities may be developed through this understanding. These dynamic events apply to acute injuries such as ischemia-reperfusion, drug or toxicant-induced necrosis, or chronic tissue injuries. The first lecture will cover the sequential events that occur during tissue regeneration using recent genomic analyses of the partial hepatectomy model and other models of tissue regeneration. The second lecture will describe various models of liver injury, dose-response relationships and modulations of injury and progression leading to hepatic failure, and regression of injury leading to recovery from injury. The third lecture will cover the cellular and biochemical changes associated with renal injuries, and how injury initiated by drugs, toxicants, and ischemia-reperfusion may progress leading to renal failure. Both renal as well as hepatic injury and tissue repair models will be discussed in the context of modulation by disease, dose, duration of ischemia before reperfusion, and nutritional factors. The last lecture will cover the role of the dynamic events of tissue destruction and restoration focusing on hydrolysis of extracellular matrix components by activated matrix metalloproteinases in preparation for restoration of tissue architecture. The role of angiogenesis in restoring extracellular matrix, cell mobility and placement are dynamic and critical events needed in re-establishing tissue homeostasis.



7

THE BIOLOGY AND TOXICOLOGY OF THE PERI- AND POST-NATAL DEVELOPMENT.

D. J. Auyeung-Kim¹, R. Chapin², R. Tyl³, L. W. Jackson⁴, G. Cappon² and S. Makris⁵. ¹Toxicology, Charles River Laboratories, Preclinical Services, Sparks, NV, ²Pfizer Global Research & Development, Groton, CT, ³Center for Life Sciences & Toxicology, RTI International, Research Triangle Park, NC, ⁴Epidemiology & Biostatistics, Case Western Reserve University School of Medicine, Cleveland, OH and ⁵ORD NCEA, U.S. Environmental Protection Agency, Washington, DC.

The period of post-natal development has always been of societal, scientific, and regulatory concern. This concern is heightened by the growing literature which shows that exposures and health status in early (prenatal and post-natal) life can have significant health consequences much later in life; in humans, this may mean 50-60 years later. This basic course will begin with a review of the recent data showing the connection between early-life events and later-life health, and move on to a discussion of the comparative developmental biology of rodents and humans. The third talk will describe regulatory and tailored study designs which address perinatal exposures and adult outcomes and discuss endpoint selection. The last speaker will address the issues and challenges of data interpretation and regulatory action. Attendees will take home an appreciation of the complex biology, the length of the health trajectory that is set up by very early exposures, and the challenges of dealing with these issues from a regulatory perspective.



8

TOXICOLOGICAL EVALUATION OF CHEMICAL AND DRUG MIXTURES.

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Drugs, consumer and personal care products, pesticides, environmental chemicals, and foods are often assessed for safety and risk without extensive consideration of numerous potential pharmacological and toxicological interactions that might occur as these agents are encountered as mixtures by patients, consumers, and through environmental exposures (e.g. mixtures present in air, water, soil). Such assessments are complex due to the potential pharmacokinetic and pharmacodynamic mechanisms that underlie interactions in mixtures, necessitating study designs, methods of analysis, and limits on interpretation not required for single chemicals. This course will cover the fundamentals of study design and data analysis for mixtures that apply to all uses of chemicals, regardless of market application. The first presentation will cover the basic principles underlying component-based and whole mixture techniques as well as study designs available for evaluating the toxicity of mixtures. The second presentation will review the concepts for evaluat-

ing data on mixtures, including data on binary and higher order interactions, and will apply those concepts to various examples from the published scientific literature. The third talk will focus on the pharmacokinetic and pharmacodynamic mechanisms of interactions among drugs and chemicals. The final talk will present the current approaches and guidance for conducting risk assessment of chemical mixtures and safety assessment of drug combinations. The course will be of interest to experimentalists who wish to conduct studies on mixtures that are more probative and relevant for safety and risk assessment, as well as for safety and risk assessors who must evaluate and apply data on mixtures and interactions in assessments.



9

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING FOR RISK ASSESSMENT APPLICATIONS.

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Pharmacokinetics is a key step in mechanistically based risk assessments, linking chemicals exposures with the mode of action leading to toxicity. Physiologically based pharmacokinetic (PBPK) modeling is one approach for incorporating pharmacokinetics into risk assessment, particularly quantitative dose-response analyses. This course will guide the student through the PBPK modeling process, from model development through the use of the modeling results in risk assessment. The basic principals of PBPK model development will be described, focusing on the range of different model structures that have been developed to address compounds with a variety of physicochemical characteristics, toxicological properties, and exposure pathways. Models for endocrine active compounds will be described to illustrate issues of serum protein and receptor binding and modeling in vitro and in vivo systems. Characterizing variability of the human population can be incorporated in PBPK models using distributions of physiological and chemical-specific parameters (e.g., metabolism) and Monte Carlo methods. Bayesian statistical approaches implemented with Markov Chain Monte Carlo analyses and maximum likelihood methods will be described as these have been the major approaches to characterizing uncertainty in model estimated dose metrics. Qualitative and quantitative evaluations of PBPK models are a critical step in their application in risk assessments. A variety of risk assessment applications (e.g., route-to-route extrapolation) and examples of using PBPK models to develop reference concentrations/doses and cancer unit risks will be presented.



10

PROTEIN THERAPEUTICS: ASSESSMENT AND IMPACT OF IMMUNOGENICITY ON STUDY DESIGN AND INTERPRETATION.

Y. Ouyang¹, L. LeSauter², D. Wierda³, D. Finco-Kent⁴, R. House⁵ and S. Kirshner⁶. ¹CDER/OND/OODP/DMIHP, U.S. FDA, Silver Spring, MD, ²Laboratory Sciences, Preclinical Services, Charles River Laboratories, Senneville, QC, Canada, ³Biopharmaceutical Immunotoxicology, Eli Lilly & Company, Greenfield, IN, ⁴World Wide Safety Sciences, Pfizer Inc., Groton, CT, ⁵DynPort Vaccine Company LLC, Frederick, MD and ⁶CDER/OPS/OBP/DTP, U.S. FDA, Bethesda, MD.

Immunogenicity is a unique and significant safety and efficacy concern in developing protein therapeutics and could have significant impact on safety, pharmacokinetics, and efficacy. Anti-erythropoietin (EPO) antibodies-elicited pure red cell aplasia has highlighted the significant impact of immunogenicity on human safety of protein therapeutics. Immunogenicity is also a critical issue in designing and interpreting animal toxicity studies. The purpose of this basic CE course is to highlight important aspects of immunogenicity including an overview of current FDA regulation with regards to nonclinical and clinical immunogenicity assessment. Specifically, the course will address what makes protein therapeutics potentially immunogenic, and how an antibody response is produced. The development and validation of common methods used to detect an antibody response will be presented by describing development/optimization parameters and validation criteria for immunogenicity assays, followed by case studies describing antibody screening approaches, and total and neutralizing antibodies determination. This course will also review the current practice in immunogenicity evaluation in animal models and recent development of new animal models. The consequences of immunogenicity will be discussed using specific case studies highlighting safety evaluation, risk assessment, potential clinical consequence and the impact of immunogenicity on designing and interpreting toxicity studies. With the current drug development approach on targeted therapeutics, protein therapeutics are becoming increasingly important. Immunogenicity is a challenge for anyone involved in immunotoxicity research and in the research and development of protein therapeutics.

**11** RODENT IMAGING OVERVIEW FOR THE TOXICOLOGIST.

K. Gabrielson¹, R. Maronpot², B. Tsui³ and P. Contag⁴. ¹Molecular and Comparative Pathobiology, Johns Hopkins University, Baltimore, MD, ²Laboratory of Experimental Pathology, National Institute of Environmental Health Sciences, Research Triangle Park, NC, ³Radiology, Johns Hopkins University, Baltimore, MD and ⁴Imaging, Xenogen Corporation, Alameda, CA.

In the last decade, in vivo imaging methods have become established tools in basic science research. This course is designed to provide a general overview of imaging modalities utilized in basic science with applications to drug discovery and toxicological research. This review will focus on five imaging modalities including: MR, PET, SPECT, optical and ultrasound. Each speaker will give a basic overview of the modality and present specific examples relevant to drug discovery and toxicological research. In this review, several applications of MRI, Magnetic Resonance Spectroscopy and Electron Paramagnetic Resonance in oncology, cardiovascular disease and morphological phenotyping during toxicological studies will be described. PET and SPECT imaging of radioactively labeled compounds will be reviewed in the setting of drug discovery and toxicological research. The application of the multi-modality imaging scanners will also be discussed. These multi-modalities studies provide comprehensive evaluation that enables the toxicologist to study structure, function, in tandem with distribution and metabolism of radioactively labeled drugs. PET imaging of gene expression during pharmacology studies will be reviewed. Optical imaging will be reviewed in the context of imaging gene expression in transgenic mice engineered with gene promoters from enzymes (CYP1A2 or CYP3A4) driving luciferase expression. Signal transduction pathway discovery in toxicological research will be discussed using optical imaging in transgenic mice with a Smad-responsive luciferase reporter. Ultrasound imaging will be overviewed using applications of toxicity and drug discovery in cardiovascular and cancer mouse models. Following completion of this workshop, attendees should have basic understanding of the various imaging modalities and applications to drug discovery and toxicological research.

**12** APPLICATION OF HUMANIZED MOUSE LINES TO TOXICOLOGICAL AND PHARMACOLOGICAL STUDIES.

R. S. Pollenz. *Biology, University of South Florida, Tampa, FL.*

A major challenge to human risk assessment is the translation of toxicological and pharmacological results produced in rodents to human populations. This is further complicated by the fact that many toxicologically relevant enzymes and receptors exhibit differences in response to activating compounds that are species specific. In addition, for any chemicals that have carcinogenic properties, it is often impossible to obtain results in human populations for comparisons to rodents. In an effort to address some of these issues, "humanized" mouse lines are being generated in which human genes have been used to replace orthologous mouse genes. Such models are termed "knock-in" mouse lines. Alternatively, mouse lines can be generated in which human hepatocytes have been transplanted into mice. In these studies it often possible to obtain mice that show 70-98% replacement ratios of the mouse hepatocytes. The advantage to such humanized mouse lines is that they are likely to show phenotypes closer to that of humans and be applicable to a wide variety of in vivo studies. Such lines make it feasible to carry out risk assessment studies in rodents that may be directly applicable to human populations. This symposium session is aimed at exploring the generation and analysis of mouse models harboring hCYP1A1, hCYP1A2, pregnane X receptor (hPXR), and the peroxisome proliferator-activated receptor (hPPAR). These studies will be contrasted to those in mice harboring human hepatocytes. These novel animal models have relevance to pharmacology, toxicology, drug development and human risk assessment.

**13** MOUSE METABOLOMICS IN THE SEARCH FOR ENDOGENOUS P450 METABOLITES AND METABOLIC PROFILING.

F. Gonzalez¹, C. Chen¹, K. Krausz¹ and J. Idle². ¹NCI, Bethesda, MD and ²Charles Univeristy, Prague, Czech Republic. Sponsor: D. Nebert.

Metabolomics is the study of changes in small molecules in cells, tissues and biological fluids such as serum and urine. Metabolomics, using 1H-NMR, has gained prominence and been embraced by the pharmaceutical industry as a means to determine the potential toxic effects of drugs. For example, a chemical is administered to rodents and urine examined for the presence of metabolites that reflect organ-specific toxicity. In an attempt to find biomarkers for cytochromes P450 expression, P450-null and humanized mice were compared. By use of metabolic cages, urine was collected and subjected to ultra performance liquid chromatography coupled time-of-flight mass spectrometry (UPLC-TOFMS). The UPLC-TOFMS data

were deconvoluted by principle component analysis software in order to find compounds that correlate with the presence or absence of a specific P450 form. To study drug metabolism, drugs were administered to mice and serum and urine examined for the production of metabolites that were not found in untreated mice. In this manner, metabolites derived from the drug and endogenous metabolites that reflect the drugs biological activity or toxicity can be resolved. This high resolution of MS-based metabolomics offers great promise in the fields of drug metabolism and toxicology.

**14** PREGNANE X RECEPTOR (PXR) HUMANIZED MICE IN TOXICOLOGY STUDIES.

W. Xie. *University of Pittsburgh, Pittsburgh, PA.* Sponsor: D. Nebert.

The regulation of hepatic CYP enzymes is important in drug metabolism and toxicological studies. The CYP genes are up-regulated by numerous foreign chemicals (xenobiotics), yet the inducibility shows distinct species specificity. The orphan nuclear receptor PXR (NR1I2) in the mouse and its human ortholog, have been established as species-specific xeno-sensors that regulate CYP3A enzymes. By knocking-out the mouse Nr1i2 gene (encoding mPXR) and replacing it with the human NR1I2 gene (encoding hPXR), a "humanized" mouse model has been established. In addition to the original liver-specific hPXR mouse line, we have recently also created a mouse line with hPXR expressed in both the liver and intestine. We have found that the humanized mice readily responded to human P450 inducer drug rifampicin in the equivalent range of the standard oral dosing regimen in humans (300-600 mg per 70-kg man). These humanized mouse lines, which display human drug response profiles, represent unique tools for dissecting responses to drugs and foreign chemicals and should aid in the development of safer drugs. —Supported in part by NIH grants ES012479 and CA107011.

**15** REPOPULATION OF MOUSE LIVER WITH HUMAN HEPATOCYTES: CHARACTERIZATION OF THIS MOUSE LINE AND USE IN DRUG METABOLISM STUDIES.

K. Yoshizato. *Biological Science, Hiroshima University, Higashihiroshima, Hiroshima, Japan.* Sponsor: D. Nebert.

Chimeric mice were produced by transplantation of human hepatocytes into immuno-deficient and liver-diseased mice (urokinase-type plasminogen activator-transgenic severe combined immune deficiency mice). These animals were normal and survived for more 100 days post-transplantation. Some animals were given bromodeoxy uridine (BrdU). Liver sections were stained with antibodies against human cytokeratin 8/18 (hCK8/18) and BrdU. The hCK8/18-positive-hepatocyte colonies gradually increased their size. Some chimeric mice showed an almost-complete replacement with human hepatocytes after 9 weeks post-transplantation. Approximately a half of the chimeric mice had a replacement index (RI, the number of hCK8/18-positive human hepatocytes divided by total number of hepatocytes) of more than 70%. BrdU-labeling index was about 9% at 1 week and gradually decreased to a background level at 11 weeks post-transplantation. The chimeric mice showed normal liver lobules and sinusoid structures: the sinusoids were lined up with mouse endothelial cells; the space of Disse was occupied with mouse stellate cells; mouse Kupffer cells were distributed on the luminal aspect of the endothelium. The human hepatocytes in the chimeric liver expressed major human cytochrome P450 (hCYP) subtypes in a manner nearly equivalent to those of the original donor hepatocytes. hCYP3A4 and hCYP1A1/1A2 were specifically induced in the liver by rifampicin and 3-methylcholanthrene, respectively. The profile of bile acid composition of taurocholic and glycocholic acids was closer to human than to mouse. Efficient infection and propagation of both hepatitis B and C viruses were observed in the chimeric mice: known anti-hepatitis virus agents dramatically decreased the level of viremia in the infected mice. This animal model will have wide usefulness for human liver-related studies, especially for predicting the metabolism of drugs in the human liver and to screen an anti-human hepatitis virus drug. —Supported in part by CLUSTER Project.

**16** REPLACEMENT OF THE MOUSE *CYP1A1* AND *CYP1A2* GENES WITH THE HUMAN *HCYP1A1-CYP1A2* LOCUS.

D. W. Nebert, S. Derkenne, T. Doetschman and T. P. Dalton. *Department of Environmental Health, University Cincinnati Medical Center, Cincinnati, OH.*

The human *CYP1A1* and *CYP1A2* genes are oriented head-to-head, sharing a common 5'-flanking region of 23.3 kb. The mouse *Cyp1a1* and *Cyp1a2* genes are also oriented head-to-head and share a common 5'-flanking region of only 13.3 kb; this region is so small that one would need to generate thousands of offspring from a

Cyp1a1(-/-) x Cyp1a2(-/-) cross to obtain a double-knockout. Using Cre-mediated interchromosomal recombination between *loxP* sites inserted at the 3' ends of both the *Cyp1a1* and *Cyp1a2* genes, we have succeeded in generating a *Cyp1a1/1a2(-/-)* double-knockout mouse line. This lab has also created a bacterial artificial chromosome (BAC)-transgenic mouse line, carrying both human *CYP1A1* and *CYP1A2* genes, the 23.3-kb spacer region, >55 kb of *CYP1A1* 3' flank, and >85 kb of *CYP1A2* 3' flank. Both lines have been backcrossed into C57BL/6J (B6) so that their genomes comprise >99.8% B6 genes. The humanized *hCYP1A1_1A2* line—in the absence of the mouse *Cyp1a1* and *Cyp1a2* orthologous genes—expresses human *CYP1A1* and *CYP1A2* mRNA, protein and enzyme activities in all tissues in a pattern very similar to that of the mouse: basal *CYP1A1* is nil, whereas TCDD-inducible *CYP1A1* is robust and occurs in all tissues examined; basal *CYP1A2* is prominent in liver, whereas TCDD-inducible *CYP1A2* occurs in liver, duodenum, stomach and lung. Theophylline was chosen to prove human P450 participation in metabolism; the drug is predominantly metabolized by *CYP1A2* in humans and rodents. Replacement of mouse *Cyp1a2* with the functional human *CYP1A2* gene restored the capacity to metabolize theophylline, and the metabolism changed from the mouse to the human profile (*i.e.* 3-methylxanthine formation was now seen, which does not occur in wild-type mice). This humanized *hCYP1A1_1A2* BAC-transgenic line will be helpful in estimates of human risk assessment, following exposure to a variety of drugs, carcinogens, and other environmental pollutants that are *CYP1A1* and/or *CYP1A2* substrates or inducers. —Supported in part by NIH grants R01 ES08147 and P30 ES06096.



17 GENOMICS AND DOSE-RESPONSE MODELING.

M. Andersen. *CIIT Centers for Health Research, Research Triangle Park, NC.*

In recent years, with the completion of the Human Genome project and evolving capabilities and technologies in the area of genomic research, there has been a significant increase in information about consequences of chemical exposure on gene expression. While these types of information are currently being used qualitatively to make decisions, the challenge remains to be able to incorporate genomic information quantitatively into the risk/safety assessment process. The main challenge facing scientists in the regulatory arena is not only attempting to identify a genomic signature or threshold associated with chemical exposures, but to also correlate this information with a phenotypic threshold, whether that is at the level of a protein, cell, tissue or behavior. The purpose of this symposium is to present examples of the gathering of dose-dependent genomic information on toxic compounds and to discuss how this information might be considered in dose-response modeling for risk assessment application. Several case studies will be presented, as well as reviews of the types of information or studies needed to facilitate the consideration of genomic data in dose-response modeling.



18 THE CURRENT STATE OF THE USE OF GENOMIC INFORMATION IN RISK ASSESSMENT.

R. J. Preston. *U.S. Environmental Protection Agency, Research Triangle Park, NC.*
Sponsor: R. Gentry.

The recently released U.S. EPA Guidelines for Carcinogen Risk Assessment provide a framework for the consideration of mechanistic data in the cancer risk assessment process. A tumor is induced by a set of key events that are required for the conversion of a normal cell into a transformed one and ultimately to produce a malignant phenotype. The key events for a particular chemical are described by the mode-of-action (MoA) for that chemical; for example, DNA-reactivity, cytotoxicity and regenerative cell proliferation, and receptor interactions. It is likely that multiple modes of actions are operating for any particular chemical. Given the complexity of tumor formation and that multiple key events are involved, it is of considerable value, if not essential, to assess effects of potential or known carcinogens at the whole-genome level, and at the tissue/organ level. It is this systems (cell or tissue) approach that is likely to be the most fruitful for assessing cancer risks at low, environmental exposure levels. In this way, it is feasible to enhance the ability to conduct the various extrapolations that have to be a part of the risk assessment process. These extrapolations include high to low dose, animal to human and tumor site to site. Genomics data at the DNA, mRNA and protein levels have already been informative in the identification of specific genetic and phenotypic alterations associated with tumor formation. This approach can lead to the development of informative biomarkers of tumor response and their use as surrogates for tumors for the types of extrapolation noted above. The use of genomics approaches is just entering the realm of risk assessments; there remains considerable groundwork before a clearly defined use can be established. (This abstract does not necessarily reflect U.S. EPA policy).



19 GENOMIC CONSIDERATIONS IN THE DOSE-RESPONSE MODELING FOR FORMALDEHYDE.

M. Andersen. *CIIT Centers for Health Research, Research Triangle Park, NC.*

This presentation will provide a discussion of the development of a mechanistic dose-response model with genomic data for formaldehyde. Inhalation exposures in rats were conducted at concentrations ranging from 0.7 to 15 ppm, 6 hours per day, 5 days per week, for three weeks. These concentrations were previously examined in long term inhalation studies in rats. Instillation exposure to 400 mM formaldehyde served as a positive control. Gene expression was evaluated with a whole genome rat array. Dose-response analyses of genomic responses at several time-points showed dose-dependent transition for the mode of action of formaldehyde nasal carcinogenicity, in which increasing exposure concentration activates different gene pathways. Qualitatively large scale changes in gene expression occurred with increasing concentration from 6 to 15 ppm. The 15 ppm more resembled the instillation results. The differential activation of these pathways correlates with different cellular responses noted by histological observations and measurements of cell proliferation rates.



20 USING GENOMIC DOSE-RESPONSE INFORMATION TO INFORM A POTENTIAL MODE OF ACTION FOR ARSENIC.

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A comprehensive literature search was conducted that identified more than 400 studies containing information on gene expression changes following exposures to inorganic arsenic compounds. This information was organized by compound, exposure, dose/concentration, species, tissue, and cell type. Initial dose-response analysis focused on in vitro data obtained with primary human cells exposed to arsenite. A concentration-related hierarchy of responses was observed, beginning with changes gene expression associated with adaptive responses (e.g., pre-inflammatory responses, delay of apoptosis). Between 0.1 and 10 μ M, additional gene expression changes related to oxidative stress, proteotoxicity, inflammation, and proliferative signaling occur along with those related to DNA repair, cell cycle G2/M checkpoint control, and induction of apoptosis. At higher concentrations (10 - 100 μ M), changes in apoptotic genes dominate. Comparisons of primary cell results with those obtained from immortalized or tumor-derived cell lines were also evaluated to determine the extent to which cell lines predict the responses of primary cells. While immortalized cells appear to respond similarly to primary cells, caution must be exercised in using gene expression data from tumor-derived cell lines, where inactivation or overexpression of key genes (e.g., p53, Bcl-2) may lead to altered genomic responses. Data from acute in vivo exposures is of limited value for evaluating the dose-response for gene expression, due to the transient, variable, and uncertain nature of tissue exposure in these studies. The available in vitro gene expression data, together with information on the metabolism and protein binding of arsenic compounds, supports a mode of action for inorganic arsenic carcinogenicity involving the superposition of highly-specific direct interactions with critical proteins such as those involved in DNA repair, overlaid against a background of chemical stress, including proteotoxicity and depletion of non-protein sulfhydryls, resulting in increased oxidative damage to DNA.



21 GENE EXPRESSION DOSE-RESPONSE IN THE MOUSE BLADDER FOLLOWING EXPOSURE TO ARSENATE IN DRINKING WATER.

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The association between drinking water exposures to inorganic arsenic and life-threatening tumors in the human is strongest for bladder cancer. Moreover, a working model for the pathogenesis of human bladder cancer has been developed. To investigate the mode of action for inorganic arsenic carcinogenicity in the bladder, a study was conducted to explore the dose-response for genomic expression in bladder epithelial cells resulting from exposure of C53Bl/6J mice to arsenate in drinking water for up to 12 weeks at concentrations ranging from 0.05 to 50 mg As/L. Bladder and urine concentrations of all arsenic species, including arsenite and the methylated metabolites, were also determined in order to support the design of in vitro analyses on primary bladder epithelial cells at concentrations of arsenite equivalent to those achieved in the in vivo study. Animals exposed at 0.05 mg/L arsenate showed no significant differences from controls in urinary arsenic species, tissue concentrations, or bladder gene expression. However, tissue concentrations were significantly increased at the higher exposures, with MMA as the dominant species in kidney tissue and DMA in bladder and urine. In addition, a dose-dependent up-

regulation in a variety of genes related to DNA repair, cell cycle control, proteotoxicity, and apoptosis was observed. This presentation will discuss the implications of these genomic data for understanding the mode of action of arsenic bladder carcinogenicity and will also describe how similar data obtained in primary human bladder epithelial cells will be used to inform the quantitative dose-response assessment for the bladder carcinogenicity of inorganic arsenic.

22 THE FUTURE OF GENOMICS IN DOSE RESPONSE MODELING: USEFUL LESSONS FROM SYSTEMS BASED APPROACHES.

E. Faustman, X. Yu and B. Griffith. *Environmental and Occupational Health Sciences, University of Washington, Seattle, WA.*

The increasing availability of "Omic" information requires a reassessment of quantitative risk assessment interpretative tools. Although microarray technology has emerged as a powerful tool to explore expression levels of thousands of genes or even complete genomes after exposure to toxicants, the functional and quantitative interpretation of microarray datasets still represents a time-consuming and challenging task. Gene ontology (GO) and cell signaling pathway mapping have both been shown to be powerful approaches to generate an overall global view of biological processes and cellular responses impacted by toxicants. However, current methods do not allow for adequate comparisons across dose and time points and have limited capabilities for translation across levels of biological complexity. Frequently results are presented in extensive gene lists with minimal or limited quantitative information, data that is crucial in the application of toxicogenomic data for risk assessment. To facilitate quantitative interpretation of dose or time dependent genomic data, we propose several approaches. First, we have developed an extended program (GO-Quant) to extract quantitative gene expression values and to calculate the average intensity or ratio for those significantly altered by functional gene category based on MAPPFinder results. An application of this approach will be given. Secondly, computational approaches will be discussed that allow for integration of GO-Quant across cellular, organ, and organism level responses. To evaluate these responses across biological systems necessitates the use of toxicokinetic and dynamic models that can incorporate biological information. These studies were supported by USEPA and NIEHS ES08601, ES07033, ES 11387, ES 10613 and R 826882.

23 ENVIRONMENTAL RISKS FACTORS FOR AUTISM.

E. Silbergeld. *Environmental Health Sciences, Johns Hopkins School of Public Health, Baltimore, MD.*

Autism spectrum disorders (ASD) are a prevalent and severe health issue for children and their families, with estimated prevalence rates between 1:200 and 1:600 live births. It has been generally recognized that ASD involves gene:environment interactions, but very little information has been developed on environmental risk factors that may contribute to these serious diseases. There are many new initiatives in research on ASD with both private and public support. The opportunity to discuss current research on the cellular and molecular biology of brain development, new information on the molecular events in ASD as well as on candidate susceptibility genes makes a workshop on environmental risks timely and appropriate. This workshop will bring together experts on ASD, neuroscientists, and neurotoxicologists to discuss the state of knowledge, promising experimental models, and methods for translating research into clinical practice and public health policy.

24 TRENDS IN THE INCIDENCE AND PREVALENCE OF AUTISM SPECTRUM DISORDERS: CLUES FOR GENERATING HYPOTHESES ON ENVIRONMENTAL RISK FACTORS.

C. J. Newschaffer. *Epidemiology, Johns Hopkins University, Baltimore, MD.*
Sponsor: E. Silbergeld.

Data on secular trends in autism prevalence have fueled the current debate over the potential role of environmental risk factors in autism etiology. This presentation will summarize existing data on secular and cross-national trends in autism prevalence. Challenges to the meaningful etiologic interpretation of these ecologic data will be highlighted. Results from a simulation analysis will also be presented that will underscore the complexities of interpreting incidence, prevalence, and cumulative incidence trend data for a developmental condition, like autism, whose diagnosis is behaviorally based.

25 CELL AND MOLECULAR MECHANISMS OF NEURODEVELOPMENT: CLUES FOR IDENTIFYING ENVIRONMENTAL RISK FACTORS FOR AUTISM?

P. Lein. *Center for Research on Occupational and Environmental Toxicology, Oregon Health & Science University, Portland, OR.*

Autism spectrum disorder (ASD) is a behaviorally defined syndrome with diverse clinical presentations. Although altered neurodevelopment is widely recognized as the underlying neuropathological cause of ASD, the etiology and pathogenesis of ASD is poorly defined at the cellular and molecular levels. Genetic factors exert a strong influence in aggregate; however, it is clear that ASD susceptibility is determined by not only complex interactions between genes but also gene-environment interactions. There is, therefore, significant interest in identifying and characterizing epigenetic and environmental risk factors for ASD. One approach towards realizing this goal is to identify and characterize the cell and molecular mechanisms that control normal neurodevelopment in order to identify molecular targets whose disruption contributes to ASD-related traits. This presentation will provide an overview of recent advances in our understanding of the cellular and molecular mechanisms of neurodevelopment in the context of the neurobiology of autism. The major goals are to familiarize attendees with developmental mechanisms that control (1) cell migration; (2) the patterning of the balance between excitatory and inhibitory signaling pathways; and (3) synaptogenesis and synaptic plasticity. In addition, recent research delineating the influence of immune mediators on neurodevelopment will be discussed in light of the emerging theme that immunological challenge represents a point of convergence between genetic and environmental causal factors in ASD.

26 IDENTIFYING ENVIRONMENTAL CONTRIBUTORS TO AUTISM RISK: INSIGHTS FROM NEUROBIOLOGY AND IMMUNOLOGY.

I. N. Pessah^{1,2}. ¹VM: *Molecular Biosciences, University of California, Davis, Davis, CA* and ²*Center for Children's Environmental Health, Davis, CA.*

Autism is among the most complex neurodevelopmental disorders with potential susceptibility genes (current estimates range from 3 up to 100 genes) spread across the entire genome. Concordance between monozygotic twins for autism is significantly less than unity and phenotypic presentation can vary broadly between sibs. Autism therefore presents unique opportunities and challenges to study interactions among multiple susceptibility genes, and how epigenetic factors and exposure to environmental modifiers may contribute to variable expression of autism, and autism-related traits. The major goals of this presentation are to familiarize attendees with (1) genetic and epigenetic mechanisms implicated in altering the ratio of excitation/inhibition within central processing circuits of the autistic brain; including nicotinic, glutamatergic, gabaergic, and calcium signaling systems; (2) early immunologic differences in humoral and cellular immunity identified in autistic children that may impact brain development and behavior; and (3) approaches that toxicologist can develop in mouse models to better understanding how specific genetic or epigenetic defects alter sensitivity to chemical exposure(s) of current concern to children's health and autism. The development of mouse models to study gene-environment interaction relevant to autism present special challenges because the impact of altered immunity on the development of complex behaviors, key brain structures and neurotransmitter receptors relevant to autism must be studied in concert. How these challenges can be met will be discussed by presenting specific approaches used by the UC Davis Center for Children's Environmental Health and Disease Prevention. A better understanding of the mechanisms by which environmental, immunologic, and molecular factors interact to influence the risk and severity of autism is essential for developing relevant strategies for intervening and/or mitigating autism's devastating impact.

27 TERATOGENS AND AUTISM.

P. M. Rodier. *Departments of Obstetrics and Gynecology and Environmental Health Sciences, University of Rochester School of Medicine, Rochester, NY.*

Five teratogens are known to increase the risk of autism: rubella, thalidomide, valproic acid, ethanol and misoprostol. The critical period of exposure is early in the first trimester for each of these. The discovery of autism cases among the thalidomide-exposed population in Sweden has led to greater understanding of autism's origins by pinpointing the time of neural tube closure as important in the development of the disorder. In these cases, dysfunctions of the cranial motor nerves indicated brain stem injury and hearing impairments occurred with ear malformations. A newly discovered genetic syndrome (BSA) includes similar anomalies of the motor innervation of the eye muscles and facial muscles, deafness, and autism. It is

caused by homozygous inheritance of a large mutation of the gene, HOXA1. An animal model based on valproic acid exposure during neural tube closure has been studied anatomically and behaviorally. It resembles the histology of human cases of autism in having a reduction of the volume of the cerebellar cortex, reduced numbers of Purkinje cells, reduced volume and neuron number in the inferior olive and reduced volume and neuron number in the deep nuclei, especially the nucleus interpositus. Because these injuries involve several parts of the neural circuits responsible for eyeblink conditioning, one might expect that behavior to be disturbed in both human cases of autism and the animal model. The result is the opposite: eyeblink conditioning is enhanced in both. Further, the animal and human data exhibit identical abnormalities in the timing of the blink response. These results are noteworthy because many neurological and psychiatric disorders have been tested for eyeblink conditioning and autism is the only one in which enhancement has been observed. Human cases exposed to valproate in utero are now being studied for eyeblink conditioning. We are comparing exposed children who developed autism to exposed children who did not, and comparing both to typically-developing children.

28 AUTISM: WHAT DOES MERCURY HAVE TO DO WITH IT?

E. Silbergeld. *Environmental Health Sciences, Johns Hopkins School of Public Health, Baltimore, MD.*

Research on complex neurological disorders has utilized animal models exposed to agents that do not contribute to the burden of human disease, such as the use of 6-hydroxydopamine to emulate pathophysiological mechanisms in Parkinsonism or specific gene knockouts in mice to study dementias. In this sense, research on mechanisms of mercury-induced developmental neurotoxicity may be valuable in understanding pathophysiological mechanisms involved in some aspects of autism spectrum disorders. Exposures to inorganic and organic mercury (Hg) compounds induces developmental neurotoxicity, involving glial activation and altered neuronal migration. The neuroimmunological effects of Hg are highly dependent upon genotype in rodent models. In addition to developmental neurotoxicity, early exposure to Hg also affects the development of the immune system, and increases the likelihood and severity of autoimmunity consequent to infection. Because these events are also reported in many cases of ASD, these similarities suggest that further mechanistic research on Hg, the role of genotype, and interactions with infection may be valuable in increasing understanding of early and potentially reversible or compensable events in ASD. Research supported by Cure Autism Now Foundation.

29 THE FUTURE OF RISK ASSESSMENT IN ENVIRONMENTAL HEALTH – CAN CURRENT APPROACHES FULFIL PUBLIC HEALTH NEEDS? INTRODUCTORY THOUGHTS.

A. Tritscher¹ and **C. Portier²**. ¹ *World Health Organization, Geneva, Switzerland* and ² *National Institute of Environmental Health Sciences, Research Triangle Park, NC.*

Risk assessment needs to use all available information to address the relevance of environmental exposures to human health. In practice, current risk assessment approaches tend to consider single hazards, most sensitive toxic endpoints, and often simple exposure scenarios. Most available data are from basic toxicological tests, which have been standardized and accepted for regulatory purposes. Also, most risk assessments are based on deterministic approaches and variability in the population and during life-stages may not be taken sufficiently into account. Real life situations are much more complex and human exposures occur to mixtures of agents and also never in isolation from other factors such as life-style and environmental issues. New scientific developments may provide tools to address more complex questions in the evaluation of environmental hazards, quantification of exposure, calculation of risks and quantification of associated uncertainties. However, they are often not sufficiently integrated into the regulatory process. Application of new scientific knowledge and tools to medicine and public health could change the nature of risk assessments in the future. Questions to be addressed are e.g.: can new 'omics' technologies be used to quantify interindividual variation in susceptibility? Can these tools be used to identify and quantify consequences of exposures to multiple environmental agents? Can dose-response assessment methods address exposures to mixtures of compounds? How can new scientific developments and new mechanistic knowledge be integrated in the regulatory risk assessment process? This session tries to address if currently applied risk assessment procedures, following the defined steps of hazard identification, hazard characterization with dose-response assessment, exposure assessment and ultimately risk characterization, are appropriate to address complex real life situations, and how new scientific knowledge and tools can be integrated to improve risk assessment.

30 THE FUTURE OF HAZARD IDENTIFICATION.

S. M. Roberts. *Center for Environmental & Human Toxicology, University of Florida, Gainesville, FL.*

Currently, hazard identification is based primarily on the results of toxicity studies conducted in laboratory animals. Hazard identification that is well conducted using the current approach is both costly and time consuming. New tools made available by advances in biotechnology, such as the "omics" and high-throughput screening, have the potential to provide a more comprehensive assessment of hazard, more rapidly, and perhaps at reduced cost. "Omics" tests can provide a much more complete picture of the biological responses to an agent than traditional studies, offering alerts for possible adverse effects as well as aiding in the identification of modes of action. With high throughput screening, information on specific effects at the cellular and sub-cellular level can be obtained for tens of thousands of chemicals in a short period of time. There is general agreement regarding the value of new technologies such as these as aids in hazard identification and as screening tools to help prioritize chemicals for conventional toxicity studies. What it would take for these technologies to supplant conventional in vivo studies in hazard identification is more controversial, especially for use in risk assessments to support regulatory action. If hazard identification is to move from a foundation in observational studies to one in predictive studies (i.e., from in vivo to in vitro and in silico), several obstacles must be overcome. Perhaps one of the more difficult challenges will be to resist the temptation to oversimplify toxic responses, trying to reduce them to one or a few key biological events. Lessons learned from decades of trying to predict carcinogenesis, for example, indicate that the complexity of pathophysiology needs to be acknowledged and embraced. Nevertheless, a future can be imagined in which hazard identification is shaped primarily by predictive modelling of biological responses, with in vivo studies reserved for validation and confirmation.

31 EXPOSURE ASSESSMENT.

L. W. Reiter and **C. Trent.** *National Exposure Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC.*

Environmental exposures are a known etiology of preventable disease. The quantitative assessment of these exposures is a critical component of the risk assessment paradigm. Yet, despite its obvious importance in the risk assessment continuum, exposure assessment often is the weak link, resulting in large uncertainties that can perpetuate throughout the risk assessment process. Major sources of these uncertainties arise from either a lack of adequate data to characterize exposure or a poor understanding of the relationships between environmental concentrations, exposure and health outcomes. This presentation will discuss how recent scientific/technical advances will likely improve our ability to collect data needed to reduce these uncertainties. Advances in sensor technology for example, will provide more sensitive, real time and affordable methods to measure both ambient concentrations and human exposures. The availability of biosensors when applied in tandem will allow us to better define and understand the relationships between external exposure and internal dose. Novel applications of emerging electronics and information technologies (e.g. GIS) provide new possibilities to accurately record human activities and describe their relationship to exposure. Improvements in computational methods are providing new opportunities to link exposure models with pharmacokinetic and dose-response models and hence our ability to conduct more integrated quantitative risk assessments. In the specific case of air pollution, data fusion techniques are also being developed to add air quality models to these linkages. Finally, the rapid advances in genomics are providing new and better biomarkers of exposure which are mechanistically linked to health outcomes. Consequently, they serve to closely link exposure assessments to health outcome including how gene environment interactions affect sensitivity/susceptibility. Potential applications as well as challenges for using these biomarkers as part of the exposure assessment process will be discussed.

32 DOSE-RESPONSE ANALYSIS – THE QUANTITATIVE CHALLENGE.

C. J. Portier. *Office of Risk Assessment Research, NIEHS, Research Triangle Park, NC.*

Dose-response analysis is that part of risk assessment tasked with developing a quantitative description of the relationship between a given level of exposure and the risk of disease morbidity or mortality in human populations. The major challenges in quantifying these risks are presenting an accurate picture of the variability of the estimated risks for known components that contribute to variation in response and providing a clear discussion of the uncertainty in the risk estimates due to untestable assumptions that were needed for the analysis. This talk will focus on the future of dose-response analysis in its broadest context (modelling at all levels, sensitivity to assumptions, statistical objectivity, uncertainty analysis, etc.) in light

of our emerging understanding of interactions between organisms/organs/tissues/cells/biomolecules with exposures that alter these systems. The talk will be from the perspective of the analyst attempting to use the broadest array of current and emerging tools and approaches to evaluate dose-response relationships with a focus on the “the good, the bad and the ugly” regarding the strengths of new tools and approaches for risk assessments, their weaknesses and the really difficult aspects of using tools that are at the cutting edge of risk assessment research. Additionally, there will be discussion of the linkage between the usage of these data for risk assessment and feedback into the laboratory setting to develop appropriate designs to improve their utility. Finally, the discussion will switch to emerging data sources and some of the advantages and challenges seen for their use in the next 5 years. Examples of dose-response evaluations that have used emerging tools historically and currently will be included in the talk to illustrate the points being made.

33 RISK CHARACTERIZATION - THE BOTTOM LINE.

S. Olin. *Research Foundation, ILSI, Washington, DC.*

The true product of a risk assessment is not simply a number (e.g., a tolerable daily intake or a cancer slope factor). Rather, it is an integrative analysis and discussion of the risk assessment findings, including their scope and applicability, assumptions and uncertainties, and interpretation(s) for human health or the environment. This integrative analysis and discussion has been called the risk characterization. It is the foundation, the basis for communication between the risk assessor and those who need to understand and use the risk assessment findings. Because a risk characterization reflects the identified hazard(s), whatever is known about the relationship between dose and effect(s), the available information on exposure, and the approach(es) chosen to describe and sometimes quantify the risks, it must adapt to developments in all of these areas as they are incorporated into the risk assessment. Some specific examples include the focus on problem formulation as a first step in a risk assessment and its impact on the risk characterization; the availability of new tools and approaches for characterizing the human relevance of modes of action occurring in test species; the increasing use of Margin of Exposure in risk characterization; the incorporation of life-stage-related differences; and the call for improved characterization of uncertainties. The complex problem of characterizing combined risks from multiple stressors or at multiple endpoints has received some attention, and sophisticated models for estimating cumulative risks (from exposure to multiple chemicals operating by the same mode of action) have been developed and applied in a few cases; issues and prospects for further progress will be noted. Overlaying all of these is the challenge of finding better ways to reach consensus on the adoption of this new science in risk assessments at national and international levels. Strengths and weaknesses of current approaches will be discussed, and opportunities to improve “the bottom line” will be considered.

34 RISK ASSESSMENT AND REGULATION.

L. L. Smith. *CTL, Syngenta, Cheshire, United Kingdom.*

Risk assessment should be a scientific process with the outcome based on a holistic evaluation of good quality data on hazard characterisation and exposure. Regulation of risk requires an appreciation of the process of risk assessment and the incorporation of precautionary safety factors. Also, regulation must necessarily take account of societal mores, often resulting from non-scientific drivers particular to individual countries. However, it is vital for the good governance of regulation that the process is transparent, and the rationale for the decision clearly stated. This is important so that the scientific community, NGO's and other regulatory authorities can critique the outcome.

Originally hazard characterisation was essentially an observational science that relied on an understanding of the physiology and biochemical function of individual organs and of various cell types. Progressively, hazard characterisation has improved with the advent of mechanistic toxicology which increases the reliability of the extrapolation of animal data to humans. However, this extrapolation requires expert interpretation and greater openness on the limitations of the utility of animal data. The availability of new technology platforms provides insights into the individual genes and biochemical pathways that are altered as a consequence of exposure to particular chemicals. While our ability to improve hazard characterisation continues, the competence and speed of some regulatory authorities to incorporate these new concepts is mixed. However, perhaps the real challenge to better risk assessment, and hence regulation, is our ability to determine realistic exposure measurements.

Hence, scientifically robust models of exposure need to be developed.

The future of risk assessment should be driven by a more mechanistic approach to hazard characterisation combined with improved measures of exposure. Only by the thoughtful application of these capabilities will the predictability of human

health hazards improve now. This approach, together with the exquisite balance of judgement, by regulators should lead to a more certain protection of humans without the damaging consequences of exaggerated concerns.

35 HEALTH RISKS OF OF CARBON NANOTUBES: WHAT CAN WE LEARN FROM MINERAL FIBERS OR ULTRAFINE PARTICULATES?

J. S. Tsuji¹ and F. S. Mowat². ¹*Exponent, Bellevue, WA* and ²*Exponent, Menlo Park, CA.*

As major building blocks in nanotechnology, carbon nanotubes (CNTs) are among the most studied nanomaterials, although understanding of their health risk is far from complete. Factors affecting toxicity include chemistry, shape and structure, persistence, and surface characteristics. Exposure characterization is also critical given the complexity of particle size and shape distributions. Similar issues have been extensively investigated for mineral fibers and ultrafine particulates (UFPs). For example, increasing toxicity with aspect ratio has been established for asbestos fibers, and biopersistence of synthetic vitreous fibers (SVFs) influences disease potential. Although CNTs may have similar properties, they differ in their chemistry and in some other aspects (e.g., electrical conductivity, photochemistry, tensile strength). In addition, differences among types of CNTs are apparent. Single-walled CNTs (SWCNTs) agglomerate and have different morphology from SVFs, as they are typically very flexible. By contrast, multi-walled CNTs (MWCNTs) do not agglomerate, are more rigid, and will therefore react differently than SWCNTs. Besides producing lung lesions, CNTs may potentially cause cardiovascular toxicity by either translocation of CNTs from the lung or by cytokine release (as seen with UFPs). Recent identification of MWCNTs in fine particulate matter ($\leq \text{PM}_{2.5}$) from natural gas combustion indicates that environmental exposures to MWCNTs may be common and could play a role in air pollutant-induced diseases, although a direct link has yet to be established. Information from other well-studied aerosols may help guide research and assessments of the health effects of CNTs, although the complexity of these structures may also inform understanding of exposure and health effects of different types of aerosols in general.

36 CHALLENGES OF MONITORING EXPOSURE TO AIRBORNE CARBON NANOTUBES.

A. Maynard. *Project on Emerging Nanotechnologies, Woodrow Wilson International Center for Scholars, Washington, DC.* Sponsor: J. Tsuji.

Single and multi-walled carbon nanotubes form complex aerosol particles that are not easily characterized using conventional exposure monitoring methods. For instance, recent research on single walled carbon nanotubes has demonstrated that the chemistry and morphology of aggregates released from as-produced material may differ over different size ranges. At the same time, research is indicating that physical and chemical structure is critical to determining the behavior of these particles in the lungs.

The characteristics of carbon nanotube aerosols will depend on the source material—whether it is as-produced or processed in some manner—and the mechanisms of aerosolization. Likewise, the characteristics of carbon nanotube aggregates used in toxicity studies will depend on how they are prepared and introduced to the test system. Sophisticated methods are needed to characterize and monitor the material appropriately if potential impact is to be evaluated. Advanced aerosol analysis techniques such as differential mobility analysis, aerosol particle mass analysis, and high resolution electron microscopy can be used to provide insight into the nature of airborne carbon nanotube aggregates.

Simpler approaches are needed for routine exposure monitoring. However, representing such a complex material with a single index of exposure presents many challenges. The diverse morphologies of carbon nanotube aggregates makes analogy with other airborne materials difficult. Nevertheless, there may be sufficient similarities to inform future approaches. For instance, some aggregates of carbon nanotubes will be typified by high surface-to-area ratios, as are many aerosols of aggregated nanometer-diameter particles. Others may share similar morphologies to mineral fibers—both natural and synthetic—that would suggest microscopy-based monitoring would be applicable. But while we will be able to learn from previous experience when monitoring exposure to carbon nanotubes, future methods must be responsive to biologically relevant characteristics and unique carbon nanotube attributes.

37 BIO-PERSISTENCE OF SYNTHETIC VITREOUS FIBERS AND TUMORIGENIC POTENTIAL.

T. W. Hesterberg. *International Truck and Engine Corp., Warrenville, IL.*

Because inhalation of asbestos, a naturally occurring, inorganic fibrous material, is associated with lung fibrosis and thoracic cancers, concerns have been raised about the possible health effects of synthetic vitreous fibers (SVFs). SVFs include a very

broad variety of inorganic fibrous materials: fiber glass, mineral wool, and refractory ceramic fiber. Ongoing epidemiology studies of SVF manufacturing workers have provided very little evidence of harmful effects in humans. Research using rodents exposed by inhalation have confirmed that SVF pulmonary effects are determined by fiber dose (to the lung), dimension, and durability. Fiber dose over time is determined by fiber deposition and biopersistence in the lung. Deposition is inversely related to fiber diameter. Biopersistence is directly related to fiber length and inversely related to fiber dissolution and fragmentation rates. Inhaled short fibers ($< 5\mu\text{m}$) are cleared from the lung relatively quickly by mobile phagocytic cells, but long fibers ($> 20\mu\text{m}$) persist until they dissolve or fragment. In contrast to asbestos, most of the SVFs tested in rodent inhalation studies are cleared rapidly from the lung and were innocuous. However, several biopersistent SVFs induced chronic inflammation, lung scarring (fibrosis), and thoracic neoplasms. Thus, biopersistence of fibers is now generally recognized as a key determinant of the toxicologic potential of SVFs. In vitro dissolution of fibers in simulated extracellular fluid correlates fairly well with fiber biopersistence in the lung and pulmonary toxicity, but several exceptions suggest that biopersistence involves more than dissolution rate. In vitro cell culture studies indicate that the biological effects of fibers are coordinated by a complex system of inter-cellular messengers and cytotoxic cell products; phagocytic lung cells appear to be central players. Research demonstrating the relationship between biopersistence and SVF toxicity has provided a scientific basis for hazard classification and regulation of SVFs. It is unclear whether the mechanisms of SVF lung clearance and toxicity apply to carbon nanotubes.



38 HOW THE PROGRESSION OF PULMONARY TOXICITY RESEARCH IS INFORMING COMPARISONS OF CNT WITH MINERAL FIBERS.

D. B. Warheit, DuPont Haskell Laboratory, Newark, DE.

Previously we reported that intratracheal instillation exposures to laser ablation-type single wall carbon nanotubes (SWCNT) in rats produced a non-dose-dependent series of multifocal granulomas; evidence of a foreign tissue body reaction. In addition, instillation of high-dose SWCNT produced some mortality, resulting from mechanical blockage of the upper airways by the agglomerated instillate. Other investigators have demonstrated similar granulomatous lesions in mice exposed to a variety of SWCNT-types. In our study in rats, we have noted that the development of these granulomatous lesions is not consistent with a normal dust-related paradigm, including adverse pulmonary reactions caused by cytotoxic dusts such as quartz particles or asbestos fibers. These lesions generally develop following lung inflammatory responses at the anatomical sites of bronchoalveolar junctions. In contrast, we observed the presence of SWCNT-nanorope-induced multifocal granulomas located primarily in the distal airways. As a consequence, we have questioned the physiological relevance of our findings following instillation exposures. Moreover, it is conceivable that due to the electrostatic nature and tendency of SWCNT to agglomerate into nanorope structures, instillation of SWCNT may produce very different pathological results when compared to exposures via the inhalation route. In contrast to SWCNT, chrysotile asbestos fibers are highly respirable, tend to fibrillate, and produce similar lung pathological responses following either inhalation or instillation exposures. The main criteria for the pathogenicity of chrysotile asbestos fibers is biopersistence concomitant with the length dimension, with chrysotile fibrils greater than 10 microns producing lung inflammation, fibrosis and tumors in rats. Indeed, the pathological sequelae of these pulmonary lesions are very different from those reported thusfar with exposures to SWCNT. This issue of similarities or differences in responses to SWCNT vs. asbestos can be reconciled following the implementation of inhalation toxicity studies in rats with aerosolized SWCNT samples.



39 COMPARISON OF PULMONARY RESPONSES TO SINGLE-WALLED VS. MULTI-WALLED CARBON NANOTUBES.

V. Castranova, PPRB, NIOSH, Morgantown, WV.

Exposure of mice to purified single-walled carbon nanotubes ($<0.25\%$ iron) by pharyngeal aspiration resulted in a rapid but transient inflammatory response as well as an early onset and progressive fibrotic reaction. Labeling of single-walled carbon nanotubes with gold nanoparticles indicated that granulomas occurred at deposition sites of large agglomerates, while interstitial fibrosis was associated with deposition of more dispersed nanotube structures. Techniques were developed to improve the dispersion of single-walled nanotubes. Aspiration of this dispersed single-walled carbon nanotube sample resulted in greater interstitial fibrosis in the absence of granulomas. Aspiration of a dispersed preparation of gold-labeled single-walled carbon nanotubes resulted in rapid movement of nanotube structures into the alveolar interstitium and measurable translocation to systemic organs. Mice are currently being exposed by pharyngeal aspiration to a dispersed preparation of pu-

rified multi-walled carbon nanotubes. Results of these studies will be compared to those of single-walled carbon nanotubes to evaluate the effect of nanotube dimension (diameter) on pulmonary response. Single-walled carbon nanotubes were also evaluated in vitro to determine their ability to generate reactive species and stimulate oxidant production from macrophages. In vitro assays were not very good predictors of the in vivo fibrogenic potential of single-walled carbon nanotubes. In conclusion, results indicate that it would be prudent to implement strategies to limit exposure to carbon nanotubes in the workplace.



40 TOXICITY OF CARBON NANOTUBES AND ITS IMPLICATIONS FOR OCCUPATIONAL AND ENVIRONMENTAL HEALTH.

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Carbon nanotubes (CNTs), which possess desirable electrical and mechanical properties, potentially have wide industrial applications. CNTs exist in two forms, single-wall (SW) and multi-wall (MW). There has been great concern that if CNTs enter the work environment as suspended respirable particulate matter (PM), they could pose an inhalation hazard. The results of recent rodent studies have collectively shown that CNTs can produce inflammation, epithelioid granulomas, fibrosis, and biochemical changes in the lungs. Studies in mice given equal amounts of test dusts showed that CNTs were more toxic than quartz and produced lesions that became progressively more pronounced. These results have led us to recommend that respirable CNT dust be considered a serious occupational health hazard, and that exposure limits be established in the expectation of expanded industrial applications. CNTs, which are totally insoluble and fibrous, would be expected to be more biopersistent than mineral fibers. Biopersistence is the key factor determining the long-term toxicity of mineral fibers and certainly of CNTs too. We have postulated that the electrical and fibrous properties of CNTs also play important roles in the toxicity of CNTs in the lungs. Recently, MWCNTs have been found in ultra-fine PM aggregates in combustion streams of methane, propane, and natural-gas flames of typical stoves; indoor and outdoor fine (< 2.5 micron) PM samples were reported to contain significant fractions of MWCNTs. Environmental fine PM is mainly formed from combustion of fuels, and fine PM has been reported to be a major contributor to the induction of cardiopulmonary diseases by pollutants. Given that manufactured SWCNTs and/or MWCNTs have elicited pathological changes in the lungs and heart, we have postulated that exposure to combustion-generated MWCNTs in fine PM in the air may play a significant role in air pollution-related cardiopulmonary diseases. Therefore, CNTs from manufacturing and combustion sources in the environment could have adverse effects on human health.



41 HEALTH RISKS OF CARBON NANOTUBES (CNTS): WHERE DO WE GO FROM HERE?

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Health concerns of CNTs are related to their 1) nano-size and hence high surface area and increased reactivity relative to mass, as has been shown for ultrafine particulates (UFPs); 2) fiber-like shape and potential for respiratory effects; 3) reported fibrosis, inflammation, granulomas, and other effects, 4) challenges for quantifying exposure; and 5) high biopersistence, like asbestos and synthetic vitreous fibers (SVFs). While research to date indicates similarities of CNTs with SVFs and UFPs, differences are also apparent and must be considered in drawing analogies between CNTs and more well-studied substances to assess health risks. Comparisons to UFPs, SVFs, and asbestos also raise interesting issues. For example, single-walled CNTs that agglomerate to larger bundles may have similar size and shape as SVFs that are retained in the lung. Mixtures of single and multi-walled CNTs may not agglomerate and could be cleared from the lung; however, although health risk is often discounted for SVFs that are not retained because of smaller size and aspect ratio, translocation to the heart (and other organs) or effects on cytokines affecting the heart is a concern for UFP with some similarities indicated for CNTs. Moving forward, testing and evaluation of CNT health effects should examine issues such as: particle size/number distribution; shape and surface area; type of administration/exposure; dose delivered to the target site; chemical composition, including metal content (intended and as impurities); structure, whether multi-walled or single-walled, and distribution of types; use of standard reference dusts (carbon black, quartz, and TiO₂) to benchmark CNT toxicity; CNT properties, including surface treatments/charges, agglomeration, and characteristics of larger structures; biopersistence; and translocation and fate within the body and in the environment. As has been shown for asbestos, SVFs, and UFPs, characterization of each type of CNT will be imperative, as not all nanotubes are similar, and differences in the above properties can greatly affect exposure, target organ effects, and resulting health risk.

**42 METHODS FOR INVESTIGATING SKIN IRRITATION.**

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Occupational skin diseases are believed to be more prevalent than reported with annual costs (including lost workdays and loss of productivity) reaching over \$1 billion. Skin irritation can be defined as a non-specific inflammatory response of the skin to direct chemical damage which is characterized by erythema, mild edema and scaling (Irritant contact dermatitis). All the chemical agents (e.g. soaps, cleansers, solvents) damage the skin by causing structural change in lipids leading to a release of inflammatory mediators predominantly from cells in epidermis. Absorption of the chemical into skin reflects the potential for irritation. Differences in blood flow, vascular permeability, skin thickness and leukocyte infiltration may vary depending on the irritant. Understanding the signaling mechanisms involved in chemical induced irritation has been recognized to be a very important topic and this is being currently studied at several different levels. Most mechanistic studies have used cultured cells and also human stratified epidermis. Also, various publications do exist from whole animal studies but there are very few studies which have a) correlated in vitro and in vivo data and b) used a model capable of investigating the molecular effects of chemicals in the epidermis and dermis based on measured skin concentrations in an effort to demonstrate cause and effect. This workshop will discuss the basic issues with skin irritation (irritant and contact dermatitis), update the audience about the current status of in vivo methods to assess skin irritation, discuss some novel in vitro culture methods being employed in skin irritation (3-D cultures), enumerate the use of skin microdialysis in assessing skin irritation by monitoring various neuropeptides (Substance P, alpha-MSH etc.) and finally discuss the regulatory issues important in this process. This symposium is timely because of the international interest in protecting individuals from skin irritation from chemicals.

**43 OVERVIEW OF SKIN IRRITATION: INCIDENCE, MECHANISMS AND MANAGEMENT.**

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A major task of the epidermis is to defend the organism against outside harm, which can be of biological, physical or chemical nature. Today's lifestyle involves frequent exposure to chemicals with irritating properties, making irritant contact dermatitis a common dermatologic problem, with a prevalence of up to 10% of the population. In addition, occupational contact dermatitis resulting from exposure to chemicals in the workplace, depending on the country, comprise from 20 to 70 per cent of all occupational diseases.

Skin irritation is a local inflammatory reaction induced by primary contact with chemicals, which is characterized by erythema, edema and scaling. Irritant contact dermatitis is a multifactorial disease, the onset of which depends on both intrinsic (i.e. age, genetic background, race, gender) and extrinsic factors (i.e. chemical properties of the molecule itself, concentration applied, duration of exposure, etc.). Substances that are keratin solvents, dehydrating agents, oxidizing or reducing agents, and others may be irritants.

Skin inflammatory reactions involve resident epidermal cells and fibroblasts of dermis, as well as invading leukocytes, each interacting under the control of a network of cytokines and lipid mediators. Generally, the inflammatory reaction is initiated by external damage to the stratum corneum, resulting in disruption of the epidermal barrier and release of inflammatory mediators by keratinocytes, which in turn rapidly puts the innate immune system on alert in order to prevent invasion of pathogens through the weakened barrier.

Considering the increasing number of occupational skin diseases, it is essential that the mechanism of protection and efficacy be assessed together with a proper therapeutic approach. The prevention of skin diseases is accepted as an important part of occupational dermatology. Correctly identifying the inciting irritants permits appropriate personal avoidance. Barrier creams, mild topical cortisones may represent valuable treatment options, while pramoxine, phenol, camphor, and menthol may be valuable in allaying symptoms of pruritus.

**44 UNDERSTANDING THE CASCADE OF EVENTS IN IRRITATION AT A MOLECULAR LEVEL - IN VIVO METHODS.**

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Tests for chemical-induced skin irritation have identified a variety of chemicals that have the potential to initiate inflammatory processes in the skin. The Draize rabbit skin test has been used for over 50 years to identify chemicals that may irritate the skin. Patch testing for chemical irritation in humans has been used as an alternative

and perhaps more reliable test. Once a chemical irritant is identified, rational therapeutic or prophylactic interventions require an understanding of the irritant cascade at the molecular level. The irritant response could be blocked or lessened with appropriate pharmacological intervention. *In vivo* experiments in laboratory animals have been used to aid in the understanding of the molecular events that occur during skin irritation. Many of the mediators of inflammation involved in the acute inflammatory response and vascular permeability have been identified, but the "triggers" or initiating events in chemical-induced irritation are not completely understood. These first responses in the skin would be expected to be the most likely points for intervention if they can be recognized. Several types of molecular studies have the potential to help in our understanding of the sequence of events in skin irritation. The sequence of changes in signaling protein levels, including cytokines and chemokines, can be used to understand the crosstalk between the epidermis and the dermis. Gene expression studies may be useful to identify activation of molecular pathways involved. Immunohistochemistry studies can be used to localize the molecular responses to specific skin layers or appendages. A systems biology approach may be useful to integrate a variety of responses and provide a coherent framework for the inflammatory response. The potential for understanding chemical-induced skin irritation is tremendously improving as molecular methods rapidly advance.

**45 IN VITRO MODELS FOR ASSESSING SKIN IRRITATION POTENTIAL.**

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In vitro models for assessing skin irritation include submerged monolayer cell cultures (e.g. mouse 3T3 cells or human keratinocytes), as well as differentiated 3-D human epidermal (partial-thickness) or epidermal/dermal (full-thickness) skin equivalents cultured at the air-liquid interface. Submerged monolayer cultures may be useful for irritation screening in some instances, and have been validated for applications such as phototoxicity. However, submerged cultures do not adequately model the physiologic and biochemical properties of differentiated epithelial tissues, and do not allow for topical application of water insoluble materials. Endpoints for submerged monolayer culture assays include cell viability (e.g. neutral red assay) or cytokine release (e.g. IL-1 α , IL-8, TNF α). 3-D human skin equivalents cultured at the air-liquid interface offer differentiated properties including barrier function (i.e. stratum corneum) and Phase I/II drug metabolizing enzyme activity, and allow for more realistic in vivo-like treatment options, including topical application of creams, lotions and other water insoluble treatments. Endpoints may be culture viability, cytokine release or histological evaluation as well as more recent genomic and proteomic technologies. Ongoing validation programs for skin irritation with 3-D skin equivalents have shown promising correlation with in vivo results.

**46 IN SITU METHODS FOR IRRITATION- SKIN MICRODIALYSIS.**

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Microdialysis is an in vivo sampling technique for measuring endogenous and exogenous solutes in the extracellular space of tissues. It is of great value in pharmacokinetic & pharmacodynamic investigations and in monitoring disease status and progression. Applications of Dermal microdialysis can be classified as (a) measurement of inflammatory mediators in the dermis (b) studies of skin metabolism (c) determination of the absorption of drugs or other agents in the skin and (d) utilization as an alternate route of drug administration. Skin irritation is an observable physiological reaction to a stimulus and is visualized as erythema and edema as a result of local inflammatory processes. Neurogenic inflammation refers to the ability of the neurological system to evoke local inflammatory responses through the release of neuropeptides such as Substance P (SP), calcitonin gene-related peptide (CGRP) and alpha-melanocyte stimulating hormone (alpha-MSH). There is accumulating evidence to suggest that these mediators contribute to inflammation and skin irritation. Conventional methods to assess irritation include measurements of skin condition [transepidermal water loss (TEWL), skin capacitance & erythema], release of inflammatory cytokines (IL-1 α & TNF α) and chemokines. We hypothesize that the relationship between skin irritation and exposure period can be quantified well enough by measurement of neuropeptides in the skin by dermal microdialysis in response to topical exposures with irritant chemicals. Jet fuel (JP-8) and its component chemicals were used as model skin irritants. In order to validate dermal microdialysis, release of neurochemicals after topical exposure with irritant chemicals was correlated with TEWL and cytokine (IL-1 α & TNF α) expression in skin & blood from experimental animals exposed under similar conditions. The results from these studies will be discussed.



47 REGULATORY ISSUES WITH SKIN IRRITATION: AN FDA PERSPECTIVE.

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Guidelines for evaluation of pharmaceuticals have been developed by the International Conference on Harmonization (ICH). However, these guidelines do not address skin irritation, other than under the umbrella of local effects. The Organization for Economic Cooperation and Development (OECD), which the United States is a party to, develops guidelines that describe protocols, including those for skin irritation and skin corrosion. Recently, laws in the EU have been enacted that either ban the use of animals in testing for certain products or mandate the development and validation of alternative methods to animal testing. Research has resulted in much activity in the development of in vitro methods intended for use as screens, adjuncts, and replacements for current in vivo standards. In the United States, the Interagency Committee for the Validation of Alternatives Methods coordinates validation of alternative methods and the FDA is a very active participant. Because it is desirable to use fewer animals for evaluation of toxicity and to cause less pain and suffering and because of the impetus of legislation in the EU regarding cosmetics evaluation, several in vitro skin corrosion assays have been developed and validated and several in vitro skin irritation methods are being worked on. Although knowledge of dermal irritation of clinical formulations rather than of neat materials is desirable and despite limitations of the various in vitro assays, such assays may be useful in evaluation of a number of pharmaceutical products in a number of contexts.

48 ROLE OF RELB IN AH-RECEPTOR (AHR) SIGNALING.

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The AhR is a member of basic helix-loop-helix (bHLH-PAS) transcription factors including Period (Per), ARNT, and single minded (SIM) regulating hypoxia, circadian rhythm, and cellular processes like differentiation and apoptosis. We show that the NF- κ B subunit RelB is functionally associated with the AhR and mediates the transcriptional regulation of e.g. IL-8 via AhR and protein kinase A (PKA) activation. Thus, RelB/AhR complexes bind to an unrecognized response element (RelBAhRE) linking two signaling pathways to activate IL-8. Furthermore, RelB/AhR complexes are found to bind on NF- κ B consensus elements and xenobiotic regulatory elements (XRE) without addition of any stimuli implicating the true endogenous function of the AhR. The interaction of RelB with AhR-regulated genes and AhR with NF- κ B signaling represents a new mechanism of cross talk between the two receptor paradigms.

49 LACK OF A ROLE OF CALPAIN PROTEASES IN THE LIGAND-INDUCED DEGRADATION OF THE AH RECEPTOR.

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The Ah receptor (AHR) is rapidly degraded both in vivo and in vitro following binding to ligands. The exact enzymes involved in the ligand-induced degradation have not been identified, but there is compelling evidence that the degradation is mediated via the ubiquitin proteasome pathway (UPP). To firmly establish the role of the UPP in AHR degradation, mouse Hepa-1, rat retinal epithelial and human retinal epithelial cell lines were exposed to several novel inhibitors specific to the UPP, calpain proteases or both pathways and then exposed to TCDD. The level of AHR as well as CYP1A1 protein was then measured by quantitative Western blotting. Exposure of cells to MG-132 (an inhibitor of both UPP and calpains) blocked TCDD-induced degradation in all lines at 5-10 μ M and did not impact the induction of CYP1A1. In contrast, treatment of cells with the calpain inhibitors: calpeptin, calpain inhibitor III, or PD150606 either individually or in combinations up to 75 μ M did not affect TCDD-induced degradation of the AHR or the induction of endogenous CYP1A1. Since the results with the calpain inhibitors were negative, a reduction in calpain activity was verified in Hepa-1 and RPE cells using an in vivo calpain assay. Importantly, exposure of cells to the specific proteasome inhibitors: epoxomicin (5 μ M), proteasome inhibitor I (10 μ M) or lacitactine (15 μ M) completely blocked the TCDD-induced degradation of the AHR. Inhibition of AHR degradation with these compounds did not reduce the induction of endogenous CYP1A1, and in some treatments actually enhanced the level of CYP1A1 protein or mRNA that was detected. Finally, Western blot analysis of the DNA bound AHR showed that the molecular mass was unchanged in comparison to unliganded AHR. Thus, these studies conclusively implicate the UPP and not calpain proteases in the ligand-induced degradation of the mouse, rat and human AHR and suggest that the pharmacological use of UPP inhibitors may impact the time course and magnitude of gene regulatory events mediated through the AHR. Supported by ES10991.

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ROLE OF CYCLIN-DEPENDENT KINASE INHIBITORS P21CIP1 AND P27KIP1 IN AH RECEPTOR-MEDIATED SUPPRESSION OF LIVER REGENERATION.

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Numerous studies indicate that aryl hydrocarbon receptor (AhR) activity regulates cell cycle progression. Exposure to the persistent agonist 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) elicits a G1 arrest in hepatocyte-derived cell lines as well as the regenerating liver following 70% partial hepatectomy (PH). Several cell culture studies link this G1 arrest with the AhR-mediated upregulation of p27Kip1 or p21Cip1, which are G1 phase cyclin-dependent kinase inhibitors that negatively regulate cell cycle progression. However, little is known regarding the role of these regulatory proteins in the AhR-mediated suppression of in vivo cell cycle progression. In the present study, p21Cip1^{-/-} mice were used to evaluate how the Cip/Kip inhibitors regulate the AhR-mediated G1 arrest observed during liver regeneration. Mice were gavaged with TCDD (20 μ g/kg) or peanut oil, immediately subjected to 70% PH, and sacrificed 12-96 hours later. Liver tissue was fixed for histological analysis, homogenized for western blotting, or used to prepare RNA for quantitative real-time PCR. In contrast to results obtained in wild-type mice, treatment of p21Cip1^{-/-} mice with TCDD failed to suppress liver regeneration as measured by BrdU incorporation. Accordingly, cyclin-dependent kinase-2 (CDK2) activity was largely unaffected by TCDD treatment. Lack of TCDD-induced G1 arrest in these mice was not due to impaired AhR function, based on increased transcript levels of several AhR-regulated target genes including CYP1A1, PAI-1, and p27Kip1. However, despite increased levels of p27Kip1 transcript, there was no evidence of a compensatory role of p27Kip1 in the absence of p21Cip1. These findings suggest that p21Cip1 protein expression is critical for the AhR-mediated G1 arrest observed in cycling hepatocytes during liver regeneration.

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ANALYSIS OF ARNT2 FUNCTION IN AH RECEPTOR-MEDIATED SIGNAL TRANSDUCTION.

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The aryl hydrocarbon nuclear translocator (ARNT) is a member of the basic-helix-loop-helix PER/ARNT/SIM (bHLH-PAS) family of proteins and functions as a dimerization partner for a variety of bHLH-PAS proteins such as the aryl hydrocarbon nuclear receptor (AHR), hypoxia-inducible factor 1 and the SIM protein. Importantly, there are several ARNT isoforms and ARNT and ARNT2 exhibit high levels of amino acid identity and appear to dimerize with the same partners. Although knock-out of ARNT or ARNT2 is lethal, the mice show different phenotypes. This could be due to differential tissue expression or the specificity of each ARNT to a given pathway. Analysis of ARNT and ARNT2 protein levels in total tissue lysates shows that both ARNT and ARNT2 are detected in the brain, eye, and kidney and to a lesser extent in the heart, spleen, and thymus. Thus, it was of interest to determine how ARNT and ARNT2 functioned in various signaling pathways when the proteins were expressed individually or in combination. Studies show that stable or transient expression of ARNT in the ARNT defective LA-I cell line can restore TCDD-mediated induction of CYP1A1. In contrast, LA-I cell lines stably expressing ARNT2 by means of retroviral transfection exhibit some complementation of TCDD-mediated induction of CYP1A1 but to a much lower magnitude than cells expressing ARNT. To validate these findings under endogenous conditions, the human retinal pigmented epithelial cell line (hRPE) was evaluated. hRPE cells co-express both ARNT and ARNT2 protein. siRNA reduction of ARNT results in complete ablation of TCDD-mediated induction of CYP1A1 even though ARNT2 levels are unaffected. However, while siRNA reduction of ARNT2 does not ablate TCDD-mediated induction of CYP1A1, reduction of ARNT2 does result in a decrease in the time course and magnitude of TCDD-mediated induction of CYP1A1. Thus, under endogenous stoichiometry, both ARNT and ARNT2 may participate in the AHR-mediated induction CYP1A1 to produce a maximal response. Supported by ES10991 (RSP).

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CHARACTERIZATION OF LIGAND-INDUCED CONFORMATIONAL CHANGES IN THE ARYL HYDROCARBON RECEPTOR BY PROTEOLYSIS.

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The Ah receptor (AhR) is a ligand-dependent transcription factor, which regulates the biochemical and toxic effects of structurally diverse chemicals. Ligand binding to the AhR is thought to result in a conformational change leading to its activation. The activated AhR dimerizes with a homologous protein Arnt to form a transcriptional factor capable of stimulating transcription from promoters with adjacent dioxin responsive elements (DRE). Utilizing limited proteolysis to detect AhR con-

formational changes, we detected formation of the AhR/Arnt dimer, and the results were equivalent to those obtained with the DRE-based gel-retardation assay. The ability of a representative group of structurally diverse AhR ligands was subsequently analyzed by gel retardation analysis and limited proteolysis. Strong agonists of the Ah receptor such as beta-naphthoflavone were found to proportionally induce AhR:Arnt dimerization and DRE-binding, and to stimulate the formation of an approximately 40 kDa protected fragment by limited proteolysis. Common antagonists of AhR function neither stimulated AhR transformation or DNA binding, nor induced the proteolysis resistant pattern. Modifications of the limited proteolysis assay are necessary to allow detection of ligand-specific differences in AhR protein conformation. (NIH ES07685, ES012498)

53 ARYL HYDROCARBON RECEPTOR GENOTYPE IN BIRDS: A POTENTIAL BIOMARKER FOR SPECIES SENSITIVITY TO DIOXIN-LIKE COMPOUNDS.

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Risk assessment for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and dioxin-like compounds in avian wildlife is challenged by a scarcity of species sensitivity data and dramatic differences in sensitivity among species. Because standard toxicity tests are difficult to carry out in wildlife, we are interested in determining if the aryl hydrocarbon receptor (AHR) might be useful for predicting sensitivity. Using chimeric AHR proteins and site-directed mutagenesis we discovered that amino acid substitutions in the ligand binding domain (LBD) of chicken and common tern AHR (Ile324 and Ser380 in chicken) contribute to important differences in TCDD binding affinity and TCDD-dependent transactivation in these species. We then determined the sequences of the AHR LBD in over twenty species from diverse avian orders and compared previously published LD50 data to AHR LBD genotypes. Chickens, the only species classified as very sensitive to dioxins (LD50: < 1 ppb TCDD equivalents (TEQ)), were unique in having a Ser residue at position 380. Two species of galliforms classified as moderately sensitive (LD50: 1 – 10 ppb TEQ) had a chicken-like Ile-324, and a tern-like Ala-380. Common terns, as well as other fish-eating birds from several different orders were classified as insensitive to dioxin-like compounds (LD50: > 10 ppb TEQ) and all had Val-324 and Ala-380 substitutions. Interestingly, Japanese quail, the only galliform with tern-like residues at both positions, was also classified as insensitive from LD50 data, suggesting that AHR LBD genotype is predictive of dioxin sensitivity both within and between avian orders. These findings further our understanding of species differences in sensitivity to dioxin-like compounds in avian wildlife and suggest the possibility of a simple assay that would use either RNA or DNA sequence of the AHR to classify avian species as sensitive, moderately sensitive or relatively insensitive to dioxin-like compounds.

54 EPIGALLOCATECHIN-3-GALLATE, A MAJOR COMPONENT IN GREEN TEA, ACTS AS AN ARYL HYDROCARBON RECEPTOR INHIBITOR BY INTERACTING WITH HSP90.

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Numerous animal studies have shown that epigallocatechin-3-gallate (EGCG), a major component of green tea, protects against certain types of cancers, although the mechanism has not yet been determined. Our laboratory demonstrated that EGCG blocks the Aryl hydrocarbon Receptor (AhR) -mediated transcriptional ability induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Unlike other AhR antagonists that directly bind to the AhR, EGCG blocks AhR-mediated transcription by binding to hsp90. Since hsp90 inhibitors, such as 17-allylaminogeldanamycin (17-AAG), are being intensively studied for cancer therapy in clinical trials, it is attractive to hypothesize that EGCG exerts anti-AhR AND anti-cancer effects by acting as an hsp90 inhibitor. The use of a proteolytic footprint assay implicates that EGCG directly modulates the conformation of hsp90 and binds to the C-terminus of hsp90. Hsp90 dimerization, which occurs at the C-terminal end of hsp90, is also inhibited by EGCG treatment. These data suggest that EGCG acts as an hsp90 inhibitor. Coimmunoprecipitation studies show that EGCG decreases the association of aryl hydrocarbon nuclear translocator (Arnt) to ligand-activated AhR. This is consistent with electrophoretic mobility shift assays showing that EGCG inhibits TCDD-induced dioxin response elements (DRE) binding. Thus, EGCG through its ability to bind to AhR-associated hsp90, alters the AhR conformation, resulting in decreased association with Arnt and loss in DRE recognition. These studies indicate a novel mechanism whereby EGCG blocks ligand-induced DRE binding and inhibits the AhR-mediated transcription activity. Future studies will investigate how EGCG binds to hsp90 and affects its functions such as the ability to interact with other client proteins in addition to the AhR. These studies will provide directions for future research to investigate the possibility that the anti-cancer effects of green tea could be attributed to EGCG's interference with hsp90 functions. Supported by NIH grants ES014364 and ES01247.

55 ANTI-TUMOUR BENZOTHAZOLE DRUGS: POTENT ARYL HYDROCARBON RECEPTOR LIGANDS.

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The Aryl Hydrocarbon Receptor (AhR) is a ligand-dependent transcription factor that mediates the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). It has been suggested that the AhR may mediate the action of anticancer test agents; the 2-(4-aminophenyl)benzothiazole analogues (series 1) (Trapani et al 2003) and the dimethoxyphenylbenzothiazoles derivatives (series 2). This study quantifies the interactions of these agents with the AhR and investigates the structure activity relationships for binding to the AhR and biological activities.

Interactions of chemicals with AhR were studied in rat hepatic cytosols, using a competitive binding assay. The apparent kd and Bmax for [3H]-TCDD were 0.37 nM and 40fmole/mg, respectively. Among the 24 agents tested, 18 competitively inhibited TCDD binding to the Ah receptor with subnanomolar IC50 values. These molecules are co-planar, and less hydrophobic than typical Ah receptor ligands. AhR binding was enhanced by hydrophobic substituents and weakened by polar groups. The 4-amino group in the phenyl moiety is required for binding in series 1 and the 3,4-dimethoxy group in series 2. The most potent inhibitors were the halogenated-derivatives 5F203 and GW610 with IC50 of 12nM and 25nM respectively. Fluorination at position 5 enhances potency in series 1 and is important for activity in series 2 (Hutchinson et al 2001) but not affinity in both series. A good correlation appears between AhR binding affinity and growth inhibitory potency in all the compounds in series 1 but this is not true in series 2. These data identify benzothiazoles as potent ligands for the AhR, and provide insight into the mechanism of action of these agents and the structure activity relationships for ligand-binding to the AhR.

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56 ROLE OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR-ALPHA (PPAR α) IN MEDIATING THE DEVELOPMENTAL TOXICITY OF PERFLUOROOCTANOIC ACID (PFOA) IN THE MOUSE.

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PFOA is a member of a family of fluorinated chemicals that have a variety of commercial/industrial applications. PFOA persists in the environment and is found in wildlife and humans. PFOA is developmentally toxic, reducing *in utero* and neonatal survival, development and growth. PFOA binds to PPAR α and activation of this receptor is considered causal for induction of hepatic tumors in rodents. This study used PPAR α knockout (KO) and 129S1 wild type (WT) mice (Jackson Laboratories, Bar Harbor, ME) to determine if the PPAR α pathway is required for induction of developmental toxicity by PFOA. After mating overnight, the next day was designated gestation day (GD) 0. Pregnant females were dosed orally from GD1-17 with water (vehicle), 0.1, 0.3, 0.6, 1, 3, 5, 10 or 20 mg PFOA/kg/day. PFOA did not affect the number of embryos implanted (not significant across all dams and groups) or the weight gain in pregnant females with surviving litters. However, PFOA significantly increased the rate of full litter resorption in both WT and KO mice at doses of 5 mg/kg or higher. These embryos died early in gestation (dams did not gain weight but had uterine embryonic implantation sites) and, of the 33 pregnant mice exposed to 5 mg PFOA/kg or higher, only 2 had litters that survived to term (1 KO litter each at 5 and 10 mg/kg). Neonatal survival of WT pups was significantly reduced by PFOA at 0.6 and 1 mg/kg, but was not adversely affected in KO litters at any dose. In WT pups, PFOA exposure at 1 mg/kg reduced weight gain compared to WT controls and eye opening was delayed as dose increased with significant delays in pups exposed to 1 mg vs 0.1 or 0.3 mg/kg. However, weight gain and eye opening in KO pups were not significantly affected at 1 or 3 mg/kg. It is concluded that PFOA-induced early gestational lethality is not mediated by PPAR α . However, neonatal lethality, developmental delay, and growth deficits are dependent on activation of PPAR α . This abstract does not necessarily reflect US EPA policy.

57 LONG TERM EFFECTS OF ADVERSE DEVELOPMENTAL ENVIRONMENTS: EFFECTS IN ADULT RATS EXPOSED TO TOXICANTS OR UNDERNUTRITION *IN UTERO*.

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Studies have shown correlations between in utero and early life environments and adult diseases including hypertension, coronary heart disease, diabetes, obesity, schizophrenia, early onset chronic renal failure, cancer and compromised reproduction. Current study designs for developmental toxicity do not assess long-term

health in offspring. This study was undertaken to determine if toxicant exposure during pregnancy in rodents would have long-term health effects on offspring. Pregnant rats were exposed to perfluorooctane sulfonate, atrazine or dexamethasone in regimens designed to result in reduced birth weight. Another group was subjected to undernutrition (50% of control intake). At birth, offspring were cross-fostered to naive dams with each group subdivided into large (n = 12) or small (n = 6) litters. Body weight, blood pressure, fasting serum glucose, insulin response, and overall health of offspring were monitored through adulthood. At 18 months, these rats were killed for tissue analysis. Maternal exposure to toxicants or undernutrition resulted in lower birth weight. While body weight lagged in adulthood, initial recovery of body weight was observed, with those in small litters preceding the large. Male offspring in all groups had higher blood pressure than controls at seven weeks of age, while female offspring did not exhibit elevated blood pressure until 37 weeks. Rats reared in small litters had slightly higher blood pressure than those reared in large. Fasting serum glucose levels were not affected, but insulin response to glucose challenge was altered in experimental groups. These studies indicate that prenatal toxicant exposure may result in elevated blood pressure as well as other adverse adult health effects. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

58 LONG-TERM HEALTH EFFECTS OF PFOA AFTER PRENATAL AND LACTATIONAL EXPOSURE IN MICE.

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Perfluorooctanoic acid (PFOA), an environmentally persistent chemical detected in humans and wildlife, is a surfactant with wide consumer and industrial applications. It has been shown to induce tumors in lifetime fed adult rats and to delay mammary gland development in mouse pups exposed to the compound prenatally. To evaluate the long-term health effects of PFOA, pregnant CD-1 mice were given 1, 3, 5 or 10 mg/kg PFOA by oral gavage daily from gestation days (GD) 2-18 (n=15-20/dose group) and their female offspring were evaluated until 18 months of age. Exposure to PFOA at 10 mg/kg caused neonatal mortality shortly after birth. Postnatal survival of the offspring improved at lower doses and was not compromised at 1 or 3 mg/kg, but significant growth retardation was noted in the 3 mg/kg group. Body weights recovered to the control level by 6 weeks of age and, these mice continued to gain more weight compared to controls as they aged. This led to a dose-related increase in body weight by 16 months (p<0.05, 4-17%), at a time when little to no residual serum PFOA was detectable. At 18 months, gross evaluations revealed a significant dose-related increase in >1mm ovarian cysts visible in 25, 47, 60, and 80% of the animals in the 0, 1, 3, 5 mg/kg PFOA groups, respectively (p<0.05). In whole mount preparations of the mammary glands, macroscopic examination revealed abnormal nodules in the glands and the number of nodules per affected gland increased with dose. In addition, a marked reduction in spleen weight and an increase in brown adipose tissue were noted in the PFOA exposed mice. There was no significant change in liver:body weight ratio at 18 mo, but significant centrilobular hepatocellular hypertrophy was evident in all PFOA groups upon pathological examination. These results suggest that in utero and lactational exposure to PFOA may lead to obesity in adulthood as well as abnormalities in a number of tissues. (This abstract does not necessarily reflect EPA policy).

59 NADH BALANCE DURING ROTENONE TOXICITY: A NONGENOMIC, MITOCHONDRION-BASED EFFECT OF TUMOR SUPPRESSOR P53.

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Tumor suppressor p53 is an environmental-responsive transcription factor that can influence NADH metabolism in mouse embryos. Rotenone, a general use pesticide, alters NADH metabolism by inhibiting respiratory complex I. To test for interaction between rotenone and p53 we exposed NIH 3T3 and p53-null mutant mouse embryonic fibroblast cell lines to rotenone (20 – 1000 nM) in DMSO for 3–48h. Mitochondria were labeled with mitotracker; p53 was visualized as recombinant protein fused to EGFP and transfected in forms competent for nuclear translocation (p53-EGFP) or sequestered in the mitochondrion (m53-EGFP); microtubules were visualized by anti-tubulin; and nuclear counterstain was visualized with Hoechst. Cell growth was monitored by MTT assay, flow cytometry, and anti-BrdU. Effects of exposure became evident at 500 nM rotenone with respect to cell rounding (3h), polyploidy (24h), and growth arrest (48h). These effects correlated with disruption of the microtubular network in 3T3 and p53-deficient cells and were insensitive to taxol. Recombinant p53-EGFP or m53-EGFP protein distribution was not altered by rotenone. On the other hand, HPLC revealed differential

effects on NADH content. Whereas NADH declined in 3T3 cells at 3h it markedly increased in p53-null cells. Transfection of p53-null cells with m53-EGFP (mitochondrial wild-type p53), m53DD (mitochondrial dominant negative mutant p53), or p53-EGFP (nucleo-cytosolic wild-type p53) suppressed rotenone-induced accumulation of NADH in the rank order m53 > m53DD > p53. We conclude that microtubule disruption and NADH imbalance are early effects in rotenone toxicity. Although rotenone toxicity does not appear to mobilize p53 the modulation of NADH metabolism may reflect a non-genomic effect of p53 on the inner mitochondrial membrane. (Supported by NIH grants RO1 ES09120 and T32 ES014559 and a pilot grant from the UoFL CGEMM).

60 THE EFFECTS OF 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN ON MOLARS DIFFER AMONG NON-RESISTANT INBRED STRAINS OF MICE.

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is an environmental toxicant and teratogen that causes tooth abnormalities and molar agenesis. Variations in susceptibility to TCDD's effects have been attributed primarily to differences at the Aryl hydrocarbon receptor (*Ahr*) locus. Differences in endpoint-specific sensitivity to TCDD, however, suggest that other genes influence TCDD's effects on a given character. We therefore hypothesized that the effect of TCDD on molar development would differ even among inbred mouse strains possessing similar *Ahr* alleles. To test this, we evaluated the effects of several different levels of TCDD (control, 0.01, 0.1, and 1.0 µg/kg) on molar development in mice of several different inbred strains bearing TCDD-sensitive *Ahr* alleles. We evaluated the amount of molar agenesis, the prevalence of an observed morphological variant, and differences in molar size, shape and asymmetry in the offspring of dosed females from five different inbred strains of mice possessing TCDD-sensitive *Ahr* alleles. We found that a maternal dose of 1 µg TCDD/kg body weight on gestation day 13 altered the shape (but not the size or asymmetry) of the first two molars and increased the amount of third molar agenesis in mice from the CBA/J and C3H/HeJ strains of mice. We concluded that the CBA/J and C3H/HeJ strains were most sensitive to disrupted molar development due to TCDD exposure and that the C57BL/6J strain was the least sensitive. Therefore, it would be appropriate to cross the C57BL/6J strain with either the CBA/J or the C3H/HeJ strain in order to conduct a QTL study designed to locate the genes responsible for this sensitivity. Support for this research was provided by grant # 1 R21 DE 015597-01A1 from the National Institute of Dental and Craniofacial Research to L.J. Leamy.

61 DEVELOPMENTAL METHYLMERCURY EXPOSURE IN ZEBRAFISH AND THE POTENTIAL INFLUENCES OF MATERNAL DIET ON EARLY LIFESTAGE TOXICITY.

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Methylmercury (MeHg) affects the developing nervous system, and the children of women that consume high quantities of wild-caught fish during pregnancy may be at risk for neurological impairment. Data suggest that diets containing significant amounts of selenium (Se) may reduce the toxic effects of MeHg. The goal of this project is to identify neural and molecular biomarkers as predictors of MeHg exposure and deficits in specific neurobehavioral endpoints, and to explore the effect of Se on toxicity. Specifically, we are investigating the effects of sublethal MeHg exposure on developing zebrafish by analyzing early life stage (ELS) toxicity and changes in global gene expression by microarray analysis. We exposed zebrafish embryos using direct water-borne exposure (as methylmercuric chloride) with or without the addition of selenium (as selenomethionine). Uptake of MeHg in direct exposures was nearly linear through all exposure concentrations (0.01-0.3 µM). Selenium uptake was non-linear but still concentration dependent in the range of 0.03-0.3 µM. Interestingly, selenium co-treatment reduced the uptake of methylmercury and ameliorated the resultant ELS toxicity. We also exposed zebrafish embryos to varying concentrations of MeHg via maternal diet using freeze-dried wild-caught wall-eye fillets grouped as low, medium and high Hg content representing 1.0, 2.0 and 3.5 ppm dry weight, respectively. Maternal walleye diet resulted in an increase in embryo Hg content and an increase in ELS toxicity. Gene expression analysis in exposed embryos was performed using an 8,000 element zebrafish cDNA microarray. Microarray analysis revealed changes in the expression of a number of genes primarily associated with neurological structure and function, mitochondrial function, and immune system function. This is consistent with our phenotypic analyses which revealed a number of learning and neurobehavioral deficits.

62 ETHANOL ATTENUATES ALDH mRNA EXPRESSION IN JAPANESE MEDAKA (ORYZIAS LATIPES) EMBRYOGENESIS.

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The mechanisms of teratogenic effects of ethanol in Japanese medaka embryogenesis were investigated by testing the hypothesis that ethanol or its metabolite ameliorates the expression of ethanol metabolizing enzymes. Like human, ethanol metabolism in medaka is catabolized by the enzyme alcohol dehydrogenase (ADH) and Aldehyde dehydrogenase (ALDH). We have previously demonstrated that medaka embryos express two subtypes of ADH mRNAs during development (Adh5 and Adh8), and ethanol is unable to alter the expression pattern of these two mRNAs. We, therefore, extend our investigations to ALDH system, the next enzyme of alcohol metabolic pathway. Initially, we have cloned two Aldh cDNAs from the liver tissue of adult Japanese medaka (*Oryzias latipes*). The coding region spanned 1557 and 1515 nucleotides, and the deduced amino acid sequence data and phylogenetic analysis identified them as an ortholog of human Aldh1A2 and Aldh9A genes, respectively. Reverse transcriptase polymerase chain reaction (RT-PCR) analysis further showed that both Aldh mRNAs are constitutively expressed in all organs tested (brain, eye, gill, GI, heart, liver, kidney, muscle, skin, testis and ovary). Using quantitative real time RT-PCR, we have measured Aldh1A2 and Aldh9A mRNA expression during development. We have observed that Aldh9A which encodes the enzyme betaine aldehyde dehydrogenase was expressed at all time points analyzed and was lowest between 1-8 h post fertilization (hpf), however, ALDH1A2, which encodes for retinaldehyde dehydrogenase 2, expressed low copies in early stages (1-48 hpf) and increased several folds at hatching. Treatment of the embryos with ethanol for 48 hpf attenuates the expression of both Aldh mRNAs. This modulation may cause abnormality in embryonic ethanol metabolism and induce teratogenesis in medaka embryo. [Support: National Center for Natural Product Research and the Department of Pharmacology, University of Mississippi].

63 URINE CORTICOSTERONE CORRELATES WITH IMMUNOLOGICAL CHANGES CAUSED BY EXOGENOUS CORTICOSTERONE AND CHEMICAL STRESSORS: IMPLICATIONS FOR SAFETY TESTING OF DRUGS.

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Drugs and chemicals at the high dosages used in safety testing often induce non-specific stress responses. The ICH S8 Guidance Document for Immunotoxicity testing indicates that changes in immunological end points typically affected by stress responses may not be sufficient to demonstrate that adverse immunological effects are mediated by stress. Therefore, the present study was performed to add a direct measure of stress to the usual immunological end points. Male Sprague-Dawley rats were treated for 5-10 days with chemical stressor or with exogenous corticosterone to produce a similar increase in serum corticosterone as noted in chemically treated rats. Urine samples were collected 0-6 and 6-24 hr after the last administration of chemical stressors and were analyzed for corticosterone metabolites using a commercial ELISA kit (Assay Designs), and immunological parameters were quantified 24 hr after the last dose. Urine samples collected over a period of time should provide a good representation of the cumulative increase in corticosterone expression in a similar manner as the area under the corticosterone concentration vs. time curve does for serum corticosterone data. Results were analyzed by linear and non-linear modeling, and r-squared values greater than 0.7 were obtained for the correlation between urine corticosterone (normalized to express the control group as 100%) and several immunological parameters including lymphocyte and neutrophil differential, thymus weight to body weight ratio, and NK cell percentage in the blood. The data collected thus far suggest a quantitative relationship between corticosterone and immunological parameters. This relationship may be used in the future to help determine if changes in immunological parameters are mediated by stress induced corticosterone release in rats. This work was funded by a grant from Pfizer Inc.

64 DELTA-9-TETRAHYDROCANNABINOL-INDUCED DRAMATIC INFILTRATION OF NEUTROPHILS IN MICE IS MAST-CELL DEPENDENT.

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Delta-9-tetrahydrocannabinol (THC), the major psychoactive component of marijuana, possesses significant immunomodulatory properties. We observed that i.p. administration of THC in C57BL/6 (WT) mice resulted in dramatic dose-depen-

dant accumulation of cells (THC 9±3.5, vehicle 1.8±0.6 million) in the peritoneal cavity, which peaked at 12-24 hrs. FACS analysis for CD3, F4/80 (macrophage) and Gr-1 showed infiltrating cells were predominantly granulocytes. Also, >90% of these granulocytes were found to be neutrophils when stained with mouse neutrophil-specific mAb (NIMP-R14). A significant increase in Gr-1⁺ cells was also observed in liver, but not in blood. Similar results obtained in C3H mice ruled out any effects due to possible contaminating LPS. Chronic nasal challenge of WT mice also resulted in significant increase in granulocyte number in lungs. Cannabinoids mediate their effect through G-protein-coupled cannabinoid (CB) receptors. CB1 receptors are expressed in brain and immune cells, whereas, CB2 receptors are found primarily on immune cells. Both CB1 and CB2 receptor selective agonists (JWH 133 & ACEA, respectively) independently induced intraperitoneal granulocyte infiltration comparable to THC. Moreover, pretreatment of animals with CB1 or CB2 select antagonists resulted in partial blocking, whereas, a combination completely blocked THC-induced granulocyte infiltration. Mast cells release potent inflammatory mediators, including chemotactic factors upon activation. To test if THC-induced granulocyte infiltration is caused by degranulation of tissue mast cells, we used mast cell-deficient (W/W^v) mice. Unlike WT mice, W/W^v mice did not show significant granulocyte accumulation upon THC injection. We found murine bone marrow derived mast cells expressed both CB1 and CB2 mRNA. In conclusion, THC can activate tissue mast cells *in vivo* leading to dramatic neutrophil-specific chemotaxis. (Grant support: NIH R01AI053703, R01ES09098, R01 AI058300, R01DA016545, R01HL058641).

65 DOSE-RESPONSE OF PERFLUOROOCTANOIC ACID-INDUCED IMMUNOMODULATION IN ADULT C57BL/6 MICE.

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Perfluorooctanoic acid (PFOA), used in fluoropolymer production, is environmentally persistent, present in human and wildlife populations worldwide, and associated with myriad health effects in laboratory animals. A preliminary risk assessment by the US EPA identified immunosuppression as an endpoint of concern, citing reports of splenic and thymic atrophy and reduced antibody synthesis in adult mice fed 30 mg PFOA/kg body weight (BW) for 15d (Yang et al., 2001, 2002). The level of Agency concern and lack of corroborating studies warranted a more thorough dose-response assessment. C57BL/6 female mice were given 0, 3.75, 7.5, 15, or 30 mg PFOA/kg BW in drinking water for 15d. Mice were immunized with SRBC on d11 of dosing and serum for evaluation of IgM titers was collected 1d post-dosing (PD). Booster immunizations with SRBC were given 10d PD and serum for evaluation of IgG titers was collected 15d PD. Mice were sensitized to BSA on d11 of dosing, challenged with BSA 3d PD, and footpad swelling measured 4d PD. IgM titers were suppressed at all PFOA doses; IgG titers were suppressed by 30 mg PFOA/kg but were elevated at lower doses, suggesting recovery and rebound. DTH responses were suppressed by 7.5 and 15 mg PFOA/kg. At 1d PD, 15 and 30 mg PFOA/kg decreased body, spleen and thymus weights; liver weights increased at all doses. By 15d PD, body, spleen and thymus weights were normalizing but liver weights remained elevated. These data indicate that 15d of PFOA exposure suppresses IgM titers but that humoral function recovers within 15d PD at doses below 30 mg PFOA/kg. Similar recovery was observed in spleen and thymus weights 15d PD. DTH responses were suppressed by PFOA exposure but appear less sensitive than humoral responses. Although we failed to identify a NOAEL for the immune functions evaluated, our LOAEL for IgM titers (3.75 mg PFOA/kg) was nearly 10-fold lower than the 30 mg PFOA/kg reported by Yang et al. (2002). [This abstract does not reflect EPA policy and was supported by UNC/EPA Cooperative Training Agreement CT829472.]

66 TISSUE DISTRIBUTION AND PRO-INFLAMMATORY CYTOKINE INDUCTION BY THE TRICHOHECENE DEOXYNIVALENOL IN THE MOUSE: COMPARISON OF NASAL VS. ORAL EXPOSURE.

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Ingestion of the trichothecene mycotoxin deoxynivalenol (DON), a common contaminant of cereal grain, causes adverse effects on growth and immune function in experimental animals and thus poses a health risk to consumers. However, relatively little is known about the risks posed to farmers and grain handlers who are exposed to DON via inhalation of contaminated grain dust. The purpose of this research was to test the hypothesis that DON will distribute in tissues and induce proinflammatory cytokines to a greater extent following nasal exposure than by oral challenge. B6C3F1 mice were treated with a single dose of DON (5 mg/kg) either by

nasal instillation or oral gavage. After 0, 15, 30, 60, 120 minutes, mice were euthanized and serum, spleen, liver, lung and kidney samples were collected. An ELISA was devised for the sensitive measurement of DON burden in serum and tissue. In both oral and nasal treatment groups, DON serum and tissue concentrations peaked after 15 minutes and declined by 75 to 90% after 120 minutes. However, DON concentrations were 1.5 to 3.5 times higher in serum and other tissues of mice exposed by the nasal route at all time points assayed as compared to orally exposed mice. The functional significance of different DON tissue concentrations was assessed by measuring the expression of the pro-inflammatory cytokines IL-6, TNF α , and IL-1 β in spleen. As expected, oral exposure to DON induced cytokine mRNA expression after 60 and 120 minutes. However, mRNAs for these cytokines were 2 to 3 times higher in spleens of mice exposed to DON by the nasal route. Taken together, these toxicokinetic and functional data indicate that DON is potentially more toxic when inhaled than when ingested. Furthermore, they suggest that adverse human health effects could potentially result from inhalation of DON-contaminated grain dust. (This study was supported by USDA U.S. Wheat and Barley Scab Initiative)

67 LOW CONCENTRATIONS OF VANADIUM MODULATE EXPRESSION OF INNATE IMMUNE GENES IN AIRWAY EPITHELIUM.

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Airway epithelial cells utilize the innate immune system to eliminate bacteria, yet the mechanisms of many of these processes are still being investigated. Vanadium, a transition element, is a major component of residual oil fly ash (ROFA), which can exacerbate respiratory infection and cardiovascular disease. These airway pollutants can inhibit innate immunity against microorganisms but the mechanism of how such pollutants do so is not well studied. We previously published that low doses of vanadium, either as V₂O₅ or as VOSO₄, inhibit lipopolysaccharide (LPS) induction of the antimicrobial peptide, β -defensin-2, in both human A549 alveolar type II and bovine tracheal epithelial cells (BTE). We hypothesized that other key mediators that participate in innate immune mechanisms to eliminate infection are inhibited as well. BTE were stimulated with 0, 0.145, 0.29, 0.59, 1.5, or 2.5 μ g/cm² VOSO₄ for 6 hours, washed with PBS, and then stimulated with 100ng/mL LPS in fresh medium for 18 hours. RNA was harvested, purified and gene expression of interleukin-8 (IL-8), matrilysin, and inducible nitric oxide synthetase (iNOS) was quantified using quantitative RT-PCR. Vanadium inhibited LPS-mediated IL-8 gene expression in a similar manner to β -defensin 2 gene expression. At concentrations of 0.145 to 2.5 μ g/cm², LPS-mediated induction of IL-8 mRNA was inhibited, which would lessen the ability of the airway to recruit neutrophils in the event of bacterial infection or an inhalation exposure to LPS. LPS did not induce iNOS in BTE in the absence of vanadium. However, when cells were pretreated with vanadium, LPS induced a 10-fold increase in iNOS mRNA levels. Matrilysin mRNA levels were not affected by vanadium, LPS or the combination of the two. These results suggest that lower concentrations of vanadium could impact innate immune responses in the airway independent of the inflammatory response observed with higher concentrations of particulate matter and their metal components.

68 PRE-TREATMENT WITH LOW DOSE INORGANIC MERCURY MODULATES THE INNATE IMMUNE RESPONSE TO COXSACKIE VIRUS-INDUCED MYOCARDITIS IN MICE.

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Mercury is a widespread environmental contaminant with neurologic and immunotoxic impacts observed even at low dose exposures. Inorganic mercury (iHg) has a range of immunotoxic effects, including induction of autoimmunity in some rodent strains. Epidemiological studies have demonstrated an association between Hg exposure and risks of autoimmune disease or development of biomarkers of autoimmune disease. We have previously treated mice with low doses of iHg and induced autoimmune cardiac disease using coxsackievirus B3 (CVB3) in BALB/c mice. CVB3 induces a biphasic cardiac disease which is characterized by a primary acute phase, peaking at day 12 post-infection (pi), and a chronic phase, peaking at day 35 pi. The animals were pre-treated with iHg (200 μ g/kg, every other day for 15 days) before infection with CVB3. In these preliminary studies, animals were euthanized 6 hours pi and peritoneal lavage and spleen cells were harvested and analyzed for cell phenotype and markers of activation by flow cytometry (FACS). Additionally, cytokine levels were measured in spleen samples by ELISA. In the peritoneum, we found that expression of co-stimulatory molecules (CD80 and

CD86) was decreased on macrophages while the regulatory molecule, tim-3, was increased; however, the opposite was true of expression on mast cells. Further, we found that IL-4 and IL-10 levels were increased in the spleen of iHg-treated animals. These results suggest that Hg alters the innate immune response following CVB3 infection and sets the stage for exacerbated autoimmune disease later in the immune response to infection.

This work was supported by NIH training grants (MMI and EHS) and donations from the Heinz Family Foundation.

69 POTENTIAL ROLE OF CYCLOXYGENASE-2 (COX-2) IN NI-DEPENDENT ALTERATIONS IN RELEASE OF IMMUNE-MODULATORS FROM HUMAN LUNG FIBROBLASTS (HLF) BY TLR-2 AGONIST, MALP-2.

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We have previously shown using HLF that NiSO₄ can synergistically enhance IL-6 release in response to Mycoplasma fermentans-derived lipopeptide (MALP-2). Here we evaluate the ability of Ni and MALP-2 to interact and modulate other cytokine and chemokine targets, as well as document changes in expression of various toll-like receptor signaling components that may account for Ni-dependent alterations in MALP-2 responsiveness. A RT2-PCR array that screens for 84 genes involved in TLR signaling pathways revealed that pre-exposure to Ni synergistically enhanced MALP-2-induced IL-8/CXCL-8, as well as IL-6, mRNA compared to cells treated with Ni or MALP-2 alone. The effect of Ni on MALP-2-induced monocyte-chemoattractant protein 1 (MCP-1) was minimal and Ni-pretreatment inhibited MALP-2-induced CXCL10 (interferon- γ -inducible protein, IP-10) expression. ELISA assay of cytokine proteins in media from Ni and MALP-2-exposed cells paralleled the changes in the level of mRNA. Ni or MALP-2 did not alter the mRNA level for any of the known toll-like receptors (1-10) or downstream signaling mediators (MyD88, TRAF6, TBK1, TICAM1 & 2, among other). Exposure to Ni alone, however, produced a 3.5-fold increase in COX-2 mRNA, which was synergistically enhanced by subsequent exposure to MALP-2 (19-fold increase). The specific COX-2 inhibitor, NS-398, attenuated the synergistic release of IL-6 and IL-8/CXCL8 following Ni + MALP-2 exposure. Basal MCP-1 release, as well as that following MALP-2 and Ni + MALP-2 was also attenuated by NS-398. In contrast, the COX-2 inhibitor reversed the Ni-dependent inhibition of MALP-2-induced CXCL10. These data indicate that Ni qualitatively alters the pattern of inflammatory mediator release from HLF in response TLR-2 dependent stimuli. Reversal of these changes via inhibition of COX-2 suggests that eicosanoid-like mediators may, in part, selectively amplify or retard cytokine production during mixed exposures to Ni and microbial stimuli.

70 DIESEL EXHAUST PARTICLE COMPOSITION AND THE METHOD OF SONICATION INFLUENCE THE ADJUVANCY EFFECT AND TARC PRODUCTION.

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Numerous reports have shown diesel exhaust particles (DEP) can act as an immunological adjuvant in asthma. Recent interest has focused on thymus and activation-regulated chemokine (TARC) as an important modulator of this effect. This study evaluated the adjuvancy effects of three chemically different DEP prepared by two different sonication methods and measured TARC production for all conditions. The samples were NIST SRM 2975 (NDEP) generated from a heavy forklift, an automobile generated DEP (ADEP), and DEP (CDEP) generated from a diesel engine used to power a compressor. These samples differed in the percentage of extractable organic material; NDEP, ADEP, and CDEP contained 1.5%, 67%, and 18.9%, respectively. Samples were sonicated in a waterbath for 40 min or with a point sonicator for 10 min. BALB/C mice were instilled intranasally with saline or 150 μ g of NDEP, ADEP, or CDEP with 20 μ g of ovalbumin on days 0 and 13, and then challenged with ovalbumin on days 23, 26, and 29. Immune and inflammatory endpoints in the lung were assessed 18 and 48 hrs after the last challenge. With the waterbath sonicated samples, mice exposed to NDEP had significantly higher neutrophil infiltration and IL4, IL5, and IL13 cytokine production in the BAL fluid after 18 hrs relative to ovalbumin alone. By 48 hrs, these mice had the highest levels of eosinophils and lung injury compared to all groups. However, exposure to point sonicated ADEP or CDEP caused greater adjuvancy than NDEP for these same indicators of allergic lung disease. In addition, airway hyperactivity was measured and found to be highest in the ADEP exposed mice. Analysis of TARC showed that this chemokine was associated with the strongest adjuvant effect depending on the preparation technique. These results show that source and composition as well as methods of sonication are important in regulating the extent of adjuvancy, and that these effects are associated with the presence of the Th2 chemokine, TARC. (This abstract does not reflect EPA policy)

71 CHRONIC ARSENIC EXPOSURE ALTERS GLUCOSE METABOLISM IN MICE.

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Millions worldwide suffer from the effects of chronic arsenic exposure, which typically occurs via ingestion of contaminated drinking water or agricultural products. Epidemiological studies link arsenic exposure to increased risks of cancers of the skin, kidney, lung, bladder and liver, as well as a variety of non-cancerous conditions including diabetes mellitus. However, although many of these studies have demonstrated the diabetogenic effects of arsenic, the biological and molecular mechanisms by which it exerts its effects remain elusive. In our studies with adult male C57BLK/6 mice we have found alterations in glucose metabolism in response to chronic, low-level arsenic exposure. Mice were exposed to 10 ppb or 50 ppb arsenite in their drinking water for times varying between 3 and 21 days, after which the mice were euthanized and their tissues and fluids collected for analysis. Increased blood glucose levels were observed in the arsenic treated mice as early as 2 days after exposure began, and excreted glucose was observed in their urine within 5 days. Additionally, insulin levels in the blood of the exposed mice were significantly higher than in the control mice. Harvested tissues were also analyzed for enzymatic and transporter expression. Increased hexokinase II (HKII) expression was observed in the renal cortex of the arsenic exposed mice. The discovery of high levels of cortical HKII has significant biological implications. HKII is a key enzyme in cellular metabolism, growth and survival, with its primary function being the phosphorylation of glucose to yield glucose 6-phosphate. Overall, our data suggests that chronic, low-level exposure to arsenic induces diabetogenic effects in mouse model systems. In addition, the dysregulation of glucose metabolism and alteration of renal HKII expression may be involved in arsenic-induced pathological conditions involving the kidney.

72 CHRONIC LOW DOSE ARSENIC EXPOSURE IN MICE CAUSES GENE EXPRESSION CHANGES ASSOCIATED WITH CHOLESTEROL BIOSYNTHESIS.

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Chronic arsenic (As) ingestion has been associated with increased risks of cancer, cardiovascular disease, diabetes, developmental problems and other diseases. Gene arrays represent a powerful tool to examine patterns of gene expression changes that can reveal underlying mechanisms. However, previous studies of As effects on gene expression in mice have used high, often cytotoxic doses. In this study, we exposed C57Bl/6 mice (n=5 per group) for 5 weeks to purified drinking water or with As (10 or 100 ppb) and a purified AIN-76A mouse chow diet with minimal As (5 ppb total) or chow with added As (10 ppb). At 5 weeks, groups of control mice (As-free water and chow) were injected either with saline, dexamethasone (Dex, 1mg/kg), As (1mg/kg), or Dex+As 8 hr prior to sacrifice. Tissues were obtained and gene expression changes measured with Affymetrix Mouse Whole Genome 430 2.0 oligonucleotide arrays. We observed significant alterations in liver expression of cholesterol biosynthesis genes with Dex, and these genes were further altered in the Dex+As group. Cholesterol biosynthesis genes were also altered in the mice exposed for 5 weeks via both drinking water and chow, though not as extensively as with the acute injections. These results suggest that dysregulation of cholesterol homeostasis may be an early contributor to As-related diseases. Epidemiology studies have suggested cholesterol dysregulation as a contributing pathway in many As-related diseases including type 2 diabetes and vascular disease, though the precise mechanism is poorly understood. This current work provides new insight into early changes in gene expression associated with As exposure at doses typically encountered in contaminated water and food in the US and throughout the world. (NIH-NIEHS P42 ES07373)

73 TOXICOGENOMIC STUDIES WITH NORMAL HUMAN LUNG FIBROBLASTS REVEAL GLOBAL EFFECTS OF LOW-DOSE ARSENIC ON GLUCOCORTICOID RECEPTOR SIGNALING.

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Chronic arsenic (As) intake has been associated with increased risk of various cancers, developmental and reproductive problems, cardiovascular disease, and other diseases. This disease burden may be caused in part by the potent endocrine disruption

activity of As. Low dose As has been shown to disrupt transcription mediated by all five steroid receptors as well as by other nuclear hormone receptors. Here we have examined the acute global gene expression changes in normal, non-transformed, telomerase-immortalized human lung fibroblasts (WHTBF-6) treated for 8 hr with As alone (25 ppb, ~0.3 uM, the 24hr-LC01) or in combination with dexamethasone (Dex) to stimulate glucocorticoid receptor (GR)-mediated gene expression. We determined that WHTBF-6 cells have ~20,000 GR per cell and exhibit a robust GR-mediated gene response due to 50 nM Dex. Background levels of total As in the complete cell media were determined to be < 0.6 ppb. We used Affymetrix GeneChip U133 Plus 2.0 human whole genome arrays (n=4 per treatment group) to analyze gene expression using robust statistical analyses. As alone altered many genes including those involved with chromatin modification/assembly/disassembly, immune response, and protein ubiquitination and phosphorylation. Exposure of cells to As+Dex resulted in a gene profile largely different from that of cells exposed to Dex alone. Effects caused by As+Dex vs. Dex exposure that were not caused by As alone include genes associated with nucleosomes, translation regulation, histone modification, and various signal transduction processes. These differences provide important clues for elucidating the basis for endocrine disruption by As, as well as other As effects on normal cells. (NIH-NIEHS: P42 ES07373, R01 ES011819)

74 ARSENIC DISRUPTS THYROID HORMONE-INDUCED GENE TRANSCRIPTION IN GH3 CELLS AND AMPHIBIAN TAIL METAMORPHOSIS.

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Arsenic (As) is a significant human health threat and has been associated with increased risks of multiple cancers, diabetes, heart disease, and reproductive and developmental problems. We previously demonstrated that arsenic acts as a potent endocrine disruptor at very low levels, altering steroid signaling at the level of receptor-mediated gene regulation for all five steroid receptors plus the type II retinoic acid receptor (RAR). We investigated whether thyroid hormone signaling through the thyroid hormone receptor (TR), a type II receptor like RAR, is also disrupted by As. Rat GH3 cells were treated with 0.01-5 uM sodium arsenite for 6 or 24 hr, with or without thyroid hormone (T3). Arsenic significantly altered expression of a transiently transfected TRE-luciferase construct as well as the endogenous TR-regulated Type I Deiodinase (D1) gene, and in a manner very similar to that observed previously for the other nuclear receptors suggesting a common mechanism. We then used an amphibian tail metamorphosis assay to determine whether such disruption would have patho-physiological consequences. Tail metamorphosis is principally regulated by thyroid hormone via TR and is tightly controlled by the sequential activation of specific genes at specific stages and in specific tissues. Xenopus tails were removed, cultured ex vivo and treated with As with or without thyroid hormone. Ex vivo tail metamorphosis closely mimics natural tail metamorphosis. TR-dependent tail metamorphosis was inhibited in a dose-dependent manner by As from as low as 7.5 ppb up to 300 ppb. Thyroid hormone is also critical to human development as well as adult function. Disruption of amphibian development by arsenic is therefore not only direct evidence of a patho-physiological effect of As-induced endocrine disruption but is also directly relevant to human health. (NIH-NIEHS P42 ES07373)

75 TRANSPLACENTAL EXPOSURE TO INORGANIC ARSENIC AT A HEPATOCARCINOGENIC DOSE INDUCES FETAL GENE EXPRESSION CHANGES INDICATIVE OF ABERRANT ESTROGEN SIGNALING AND DISRUPTED STEROID METABOLISM.

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Exposure to inorganic arsenic *in utero* produces hepatocellular carcinoma in male offspring when they reach adulthood. To help define the molecular events associated with the fetal onset of arsenic hepatocarcinogenesis, pregnant C3H mice received drinking water with 0 (control) or 85 ppm arsenic from day 8 to 18 of gestation. At the end of the arsenic exposure period, male fetal livers were removed and RNA isolated for microarray analysis using the Agilent mouse 22K oligo chips. Arsenic exposure *in utero* produced significant (p < 0.001) alterations in expression of 187 genes, and approximately 25% of aberrantly expressed genes were related to either estrogen signaling or steroid metabolism. Real-time RT-PCR confirmed these changes on selected genes. Various genes controlled by estrogen, including X-inactive-specific transcript, anterior gradient 2, trefoil factor 1, CRP-ductin, ghrelin, and small proline-rich protein 2A, were dramatically over-expressed. Estrogen-regulated genes including cytokeratin 1-19 and Cyp2a4 were over-expressed,

although Cyp3a25 was suppressed. Several genes involved with steroid metabolism also showed remarkable expression changes, including increased expression of 17 β -hydroxysteroid dehydrogenase 7 (HSD17 β 7; involved in production of estradiol) and decreased expression of HSD17 β 5 (involved in production of testosterone). The expression of key genes important in methionine metabolism, such as methionine adenosyltransferase 1a, betaine-homocysteine methyltransferase and thioether S-methyltransferase, were decreased. Thus, exposure of mouse fetus to inorganic arsenic during a critical period in development significantly alters the expression of various genes encoding estrogen signaling and steroid or methionine metabolism, which could disrupt genetic programming at very early life-stage leading to tumor formation much later in adulthood. Funded in part by DHHS #NO1-CO-12400.

76 ARSENIC EXPOSURE EXACERBATES ATHEROSCLEROSIS IN APOE-KNOCKOUT MICE.

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Chronic arsenic (As) ingestion causes inflammation and peripheral vascular disease in humans. In the present study, we examined the effect of As-feeding on atherogenesis in apoE-knockout mice. Three week old mice were maintained either on tap water or water containing 49 ppm As. The mice were sacrificed at age 10 or 16 wk. Plasma lipoprotein profiles, indices of oxidative stress and inflammation, and atherosclerotic lesion formation were examined. Plasma cholesterol and phospholipids were unchanged in 10 or 16 wk old mice. Plasma triglycerides were decreased 15-20% in 10 wk old As-fed mice. Fatty streak formation in aortic roots and arch in 10 wk old As-fed mice (n=9) were increased 3-4 fold (P<0.01) relative to controls (n=12). Enhanced lipid accumulation (P<0.01) occurred at lesion prone sites in 16 wk old As-fed mice (n=14) relative to controls (n=20). Immunohistochemical analysis at 16 wk showed a >2-fold increase in macrophage content in aortic valves of As-fed mice, whereas very little if any T-cells or smooth muscle cells were detected in these lesions. As-fed mice also showed increased (P<0.01) staining for markers of inflammation (monocyte chemotactic protein-1, MCP-1) and oxidative stress [protein-4, hydroxynonenal (HNE) and protein-malondialdehyde (MDA)]. Significant increases (P<0.05) in levels of free HNE, MDA and inflammatory cytokines and chemokines (TNF- α , IL-1 β , MCP-1, VEGF) were observed in plasma of As-fed mice. Vasoreactivity studies, performed on the distal aorta from 10 wk old mice, indicated that the ability of phenylephrine (10-5M) pre-contracted aortic rings to relax in response to acetylcholine (10-6-10-5 M) was compromised by 15-20% in As-fed mice. Collectively, our data suggest that early life-exposure to As causes endothelial dysfunction, enhances oxidative stress and inflammation and accelerates atherosclerosis in apo E-knockout mice. (Supported in part by PHS grants R01HL65618, R01ES011314, and a grant from UoFL Center for Genetics and Molecular Medicine)

77 IN UTERO ARSENIC EXPOSURE INDUCES EARLY ONSET OF ATHEROSCLEROSIS IN APOE-KNOCKOUT MICE.

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Consumption of arsenic contaminated drinking water has been linked to higher rates of coronary disease, stroke and peripheral arterial disease. Exposures to toxicants during development may play a significant role in the onset of chronic adult diseases. The potential for *in utero* arsenic exposure to accelerate the onset of cardiovascular disease was investigated by exposing pregnant ApoE-knockout mice to 49 ppm arsenic as sodium arsenite in their drinking water and examining the aortic trees of their male offspring for evidence of early disease 10 weeks after birth. Offspring mice were maintained on normal chow after weaning. Mice exposed to arsenic in utero showed a >2-fold increase in lesion formation in the aortic roots as well as the aortic arch compared to control mice. Total plasma cholesterol, phospholipids and total abundance of VLDL or HDL particles were unchanged in arsenic exposed mice. However, arsenic exposed mice had a >40% decrease in total plasma triglycerides. A decrease in large VLDL particles was apparent upon sub-fractionation of VLDL particles. Disturbance of endothelial cell signaling was suggested by a vasorelaxation defect in response to acetylcholine in phenylephrine pre-contracted aortic rings from arsenic exposed mice. These results indicate that in utero arsenic exposure induces an early onset of atherosclerosis in ApoE-knockout mice without a hyperlipidemic diet and support the hypothesis that in utero arsenic exposure may be atherogenic in humans. (Supported in part by PHS grants R01HL65618, R01ES011314, and a grant from UoFL Center for Genetics and Molecular Medicine)

78 CARCINOGENICITY OF AN ACTIVE METHYLATED ARSENIC (MMAIII) AND IT'S EFFECTS ON GENE REGULATION IN THE MOUSE.

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Although arsenic is a proven human carcinogen, lack of a proper animal model until recently makes it difficult to understand the mechanism of its carcinogenicity. MMAIII (monomethylarsonous acid) is formed as an intermediate metabolite during arsenic methylation. Arsenic metabolism was considered to be a detoxification mechanism. However, recent in-vitro studies have shown that MMAIII is more potent cytotoxic and genotoxic than inorganic arsenic. Our aim is to study whether MMAIII is carcinogenic to mice and the differences between spontaneous tumours and arsenic induced tumours at the molecular level. Groups of female C57Bl/6J mice were exposed to 0, 100, 250 or 500 μ g As/L as MMAIII in drinking water for up to 2 years. 24h pooled urine specimens were collected at time intervals of 0, 1, 2, 4, 8 weeks and then every 8 weeks over 2 years for arsenic speciation by HPLC-ICP MS. The major metabolite was found to be DMAV and neither MMAV nor MMAIII was detected. The apparent absence of these metabolites justifies future metabolic profile study in the available tissue bank. MMAIII treated mice showed statistically significant increases in the incidences of keratoacanthoma, lymphoma, plasmacytoma, histiocytic sarcoma and multiple tumors compared to the control group. Over 10,900 genes were significantly upregulated in tumors in mice from the treatment groups compared to those of the control group. Up regulated genes included those involved in cell cycle, cell proliferation, oncogenes, stat genes and DNA damage genes. The genes which are involved in NF κ B signaling pathway such as Traf, NF κ B, IKK β , Tank and Bcl2 are all present in the treated tumors but not in the control tumors. Our result shows MMAIII alone can cause tumors in vivo without any promoter, and supports the contention that arsenic metabolism is not only a detoxification pathway but rather it could also be a toxic process.

79 ALTERED GAIT IN RATS SUB-CHRONICALLY EXPOSED TO ARSENITE PROTECTION OF ALPHA TOCOPHEROL SUPPLEMENTATION.

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Exposure to inorganic arsenic (iAs) results impairment of superior neurological functions and demyelination and morphological alterations in axons of the peripheral nerve, evoking changes in the generation and propagation of action potentials. Recent studies have shown that iAs exposure causes oxidative damage in rat brain, spinal cord and peripheral nerves. Thus, the nervous injuries induced by iAs exposure can be defended by alpha tocopherol (α -TOC) administration, due to their antioxidant role. To better understand the functional consequences of iAs exposure, we compared the quantitative assessment of the two-dimensional angular kinematics based on flexion, extension and balance during walking in adult male Wistar rats according the follow experimental groups **A) Control**; **B) α -TOC**. Rats supplemented with α -TOC, 230 IU/kg bw/d, by gavage, daily for 20 days. **C) Arsenite**. Rats supplemented with a daily dose of sodium arsenite included in water (10 mg/kg of bw/d, by gavage, during 30 d). **D) Arsenite + α -TOC**. Rats arsenite-treated (10 mg/kg bw/d, by gavage, during 30 d) and supplemented with α -TOC (230 IU/kg bw/d, by gavage), initiated 10 d after administration of the first dose of arsenite and continued for 20 d.

The results no showed alterations in the phases of flexion, extension and balance of locomotion in all groups. Nevertheless, the rats showed an increment in the support phase (24%) and the duration of the elevation phase of the leg (25%), a decrease in the length of step (10%), in velocity of step (20%), with displacement slow and eventual only in the group exposed to arsenite. The group co-exposed with arsenite- α -TOC no showed altered locomotion. Our results suggest that the gait alteration is related with the oxidative damage induced by iAs exposure in several nervous regions, affecting the normal nervous transmission from periphery to the central nervous system.

80 CD EXPOSURE INCREASES CHOLINE TRANSPORT IN CHOROID PLEXUS OF IMMATURE RATS.

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The potential for heavy metal accumulation to alter choline transport in choroid plexus was examined in Sprague-Dawley rats administered 0, 0.25 or 0.50 mg Cd per kg by gavage from PND 8-28 (n=12 per dose). Among dose groups, there were

no differences in growth rate or development and no evidence of renal or hepatic dysfunction. In Cd-treated rats, final Cd concentrations in choroid plexus exceeded that in blood or brain by 30- and 40-fold. Discrete changes in the epithelium's ultrastructure were also observed. There was no detachment from the basement membrane or attenuation of the microvilli, and tight junctions remained intact. However, Cd increased the incidence of enlarged cytosolic vacuoles and dilation of intercellular spaces. Moreover, as compared to that in control tissues, 30-min uptake of 10 micromolar 3H-choline in choroid plexus isolated from rats treated with 0.25 and 0.5 mg Cd/kg increased by 30% and 45%, respectively. This suggested increased removal of choline from CSF. Central neural deficits associated with choline-deficiency in other studies were observed in Cd-treated rats. Specifically, in rats treated with 0.5 mg Cd/kg, brain weight (normalized to bod. wt.) was decreased, and operant learning was impaired. Thus, in the absence of pronounced toxicity, low dose exposure to Cd may stimulate choline transport in choroid plexus; this may limit availability of the nutrient in the developing brain. NIEHS-P30-ES01247

81 THE EFFECT OF MANGANESE ACCUMULATION ON NEUROTRANSMITTER UPTAKE.

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Manganese (Mn) is a trace element important in many cellular processes, but over-exposure is characterized by accumulation in dopamine-rich brain regions leading to Parkinson's like symptoms in extreme cases. Previous studies have shown Mn accumulation is exacerbated by dietary iron deficiency (ID) and disturbances in other neurotransmitters have been reported. Because behaviors associated with Mn neurotoxicity are complex, the goal of this study was to examine the effects of Mn-exposure on dopamine (DA), serotonin (5-HT) and norepinephrine (NE) uptake in synaptosomes derived from three rat brain regions. Sprague-Dawley rats were randomly assigned to four dietary groups (n=12 per group): control (CN), ID, CN with Mn supplementation (CNMn), and ID with Mn (IDMn). Synaptosomes from striatum (STR), locus ceruleus (LC), and cerebellum (CB) were isolated from treated rats at four, five, and six weeks and 3H-DA, 3H-5HT and 3H-NE uptake was measured. We found that 3H-NE uptake decreased significantly ($R=-0.753$, $p=0.001$) with increased Mn concentration in the LC at six weeks. ID was associated with decreased uptake of DA, 5-HT and NE in the STR at six weeks ($p<0.05$). A comprehensive look at the data collected indicates that after six weeks of dietary treatment, ID decreases uptake of DA, NE, and 5HT in the STR and exacerbates the effect of Mn supplementation in the LC. Supported by NIEHS #ES013791-01.

82 DOSE-DEPENDENT DEPOSITION OF MANGANESE IN OLFACTORY BULB AND DOPAMINERGIC STRUCTURES AFTER NOSE-ONLY INHALATION.

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Parkinson's disease has been linked to environmental exposures. This research examines whether inhalation of environmental concentrations of Mn, which produces a Parkinson's-like syndrome occupationally, results in olfactory uptake and subsequent deposition to the substantia nigra. The role of Fe status on uptake and deposition is also examined. Adult mice were nose-only inhalation exposed to air, low (2 mg/m³) or high dose MnCl₂ aerosol (7-10 mg/m³) for 10 or 30 days (5 d/wk, 6h/d). Additional mice were fed Fe-deficient diets and exposed to air or low MnCl₂ for 29-30 days. Mn and Fe were measured in olfactory glomeruli (OG) and tuberculum, globus pallidus (GP), caudate putamen (CPu) and substantia nigra pars compacta (SN) at 7 DPE using proton-induced X-ray emission. A dose-dependent Mn uptake was seen in all brain regions except CPu where increased Mn was only seen after high-dose inhalation. Mn deposition was greatest in the OG. These results are in line with our previous Mn brain deposition time-course studies. Although Fe concentration in brain regions was not affected by Mn inhalation alone, Fe-deficient diet reduced Fe levels in all brain regions except GP. The effect was most pronounced in OG and SN. There was, however, no clear impact of iron-deficiency on inhaled Mn uptake. Dose-dependent deposition of Mn in the SN, the region vulnerable in Parkinson's disease, identifies a mechanism for direct toxicity to the dopaminergic system, and time course and localization patterns implicate neuronal pathways rather than systemic or bulk-flow mechanisms in deposition of inhaled Mn. Evaluations of neuronal degeneration, astrogliosis and inflammation in these brains at 7 or 180 DPE is in progress and will contribute to our understanding of the CNS toxicity of environmental concentrations of inhaled Mn.

83 IRON (FE) TRANSPORT ACROSS THE BLOOD-CSF BARRIER *IN VITRO* AS AFFECTED BY MANGANESE (MN) EXPOSURE.

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The blood-cerebrospinal fluid (CSF) barrier (BCB) resides within the choroid plexus, with the apical side facing the CSF and the basolateral side towards the blood. Previous studies from this laboratory demonstrate that Mn exposure in rats decreases Fe concentration in blood, but increases Fe in the CSF, indicating a compartmental shift of Fe possibly by crossing the BCB. In the present study, a primary culture of rat choroidal epithelial cells growing on the transwell chamber system was used to investigate the transepithelial transport of Fe. Mn treatment (100 μ M for 24 hr) significantly reduced the transepithelial electrical resistance, an indicator of the tightness of the barrier. Transferrin-bound 59Fe (Tf-59Fe) or free 59Fe was added to the outer chamber and the radioactivity in the inner chamber counted to determine the influx of Fe; the efflux was determined vice versa. Under the normal condition, the influx rate of Tf-59Fe was significantly higher than the efflux rate ($p<0.05$). Following Mn exposure, the efflux rate of Tf-59Fe was increased by 135% as compared to controls ($p<0.05$); this effect was blocked by a transferrin receptor inhibitor, bafilomycin A1. At 24 hrs, the storage of Tf-59Fe in primary choroidal epithelia was 60.6% higher in influx than efflux studies. Unlike Tf-59Fe transport, the influx rate of free 59Fe was much lower than that of the efflux ($p<0.01$). Mn exposure significantly increased the efflux rate of 59Fe ($p<0.01$). Pre-treatment of the *in vitro* BCB model with the antibody against divalent metal transporter-1 (DMT1) blocked 59Fe transport. Taken together, these data suggest that Tf-bound Fe appears to be favorably transported from the blood toward CSF, whereas free Fe is favored for the opposite direction from the CSF to blood. Mn exposure facilitates Fe transport across the BCB by increasing cell permeability and by facilitating the fluxes of Tf-59Fe and 59Fe in their natural directions. (Supported in part by NIEHS ES08146 and ES 013118)

84 MANGANESE DISTRIBUTION IN BRAINS OF CYNOMOLGUS MONKEYS SHOWING HIGH MRI T1 SIGNAL INTENSITIES AFTER 8 MONTHS OF WELDING-FUME EXPOSURE.

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Several pharmacokinetic studies on inhalation exposure to Mn have already demonstrated that Mn readily accumulates in the olfactory and brain regions. However, the absorption and transportation of Mn from welding fumes may be due to the influence of various other metals contained in the welding fumes. Furthermore, the idea of transporters competing for binding sites makes more complicated. Accordingly, to clarify the Mn distribution in the brain and blood concentration of Mn after welding-fume exposure, non-human primate cynomolgus monkeys were assigned to 3 dose groups: unexposed, low (31 mg/m³ TSP, 0.9 mg/m³ of Mn), and high (62 mg/m³ TSP, 1.95 mg/m³ of Mn). The primates were exposed to manual metal-arc stainless steel welding fumes for 2 hrs per day in an inhalation chamber system equipped with an automatic fume generator for 8 months, their blood concentrations of Mn monitored, and the Mn distribution examined using MRI technology. Subsequently, the tissue Mn were measured after reaching the maximum MRI T1 relaxation time. Dose-dependent increase in the Mn was found in the lungs, and significant increases in the Mn were found in the various tissues such as liver, kidneys, epididymus, testes and spleen. Slight increases in the Mn were found in the caudate, putamen, and substantia nigra, a dose-dependent significant increase was found only in the globus pallidus. Our results indicated that a high MRI T1 signal intensity is well correspond with Mn concentration and the presence of a certain amount of iron or other metals, such as Cr and Ni, in the inhaled welding fumes may not have a significant effect on the uptake of Mn into the brain.

85 RECOVERY OF WELDING-FUME EXPOSURE INDUCED MRI T1 SIGNAL INTENSITIES AFTER CESSATION OF WELDING-FUME EXPOSURE IN BRAINS OF CYNOMOLGUS MONKEYS.

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The shortening of the MRI T1 relaxation time has been known to be an effective biomarker for Mn exposure after short-term welding-fume exposure. Our previous study indicated that the T1 relaxation times decreased time-dependently after exposure, and a visually detectable high signal intensity appeared after 150 days of exposure. The plateau for the shortening of the T1 relaxation time corresponded well with the blood Mn, as previously shown in welders, suggesting a correlation between a prolonged high Mn concentration in the blood and a shortening of the T1 relaxation time. Accordingly, to clarify the Mn clearance in the brain concentration of Mn after cessation welding-fume exposure, non-human primate cynomolgus monkeys were acclimated for a month and assigned to 3 dose groups: unexposed, low (31 mg/m³ TSP, 0.9 mg/m³ of Mn), and high dose (62 mg/m³ TSP, 1.95 mg/m³ of Mn). The primates were exposed to manual metal-arc stainless steel welding fumes for 2 hrs per day in an inhalation chamber system equipped with an automatic fume generator for 8 months, their blood concentrations of Mn monitored, and the Mn distribution examined using MRI. After reaching the maximum T1 relaxation time, the primates were allowed to recover from the high MRI T1 signal intensities by ceasing the welding-fume exposure. The primates showed recovery of MRI T1 signal intensities nearly same level as the unexposed within 2 months. Our results indicated that a high MRI T1 signal intensity induced by welding fume exposure can be reversible even after prolong exposure but tissue concentrations of brains and recovery of other parameters remain to be studied further.

86 NEUROBEHAVIORAL CHANGES AFTER PROLONG WELDING-FUME EXPOSURE IN CYNOMOLGUS MONKEYS.

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Chronic human exposure to high concentrations of metal fumes, as observed among welders, has been shown to induce adverse health effects. A neurological and neurophysiological study of workers occupationally exposed to Mn exhibited an increased emotional irritability, dysmnnesia, concentration difficulties, and sleepiness, and limb paresthesia. Our previous study indicated that a high MRI T1 signal intensity is well correspond with Mn concentration in the globus pallidus. Accordingly, to clarify the relationship between brain concentration of Mn and neurological signs after prolong welding-fume exposure, non-human primate cynomolgus monkeys were acclimated for a month and assigned to 3 dose groups: unexposed, low dose (31 mg/m³ TSP, 0.9 mg/m³ of Mn), and high dose (62 mg/m³ TSP, 1.95 mg/m³ of Mn). The primates were exposed to manual metal-arc stainless steel welding fumes for 2 hrs per day in an inhalation chamber system equipped with an automatic fume generator for 8 months. The profiles of home cage locomotor activity and its rhythmicity were investigated using a camera system for consecutive days in cynomolgus monkeys. Our results indicated that the home cage locomotor activity increased 5-6 times at 25 and 32 week in the high dose exposed comparing with the unexposed, and the increased locomotor activity was maintained 7 weeks after cessation of welding-fume exposure, but changed to 3 times at 13 weeks after the cessation. Taken together, our results indicated that prolong welding-fume exposure inducing MRI T1 high signal in globus pallidus and increased blood Mn concentration can cause neurobehavioral change in non-human primates.

87 MORPHOLOGICAL AND BEHAVIORAL EVIDENCES THAT INHALED MANGANESE INDUCES A PARKINSON'S DISEASE MODEL.

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Manganese is known to produce irreversible damage to the central nervous system in humans and other primates. The mechanism by which this metal injures neurons of the basal ganglia remains unclear. Some reports assume that Mn induces alterations mainly in globus pallidus and substantia nigra pars reticulata, but no experimental information is available about its inhalation, for that reason our goal was to determine if the structures directly involve in Parkinson's disease are affected by Mn inhalation through immunocytochemical and ultrastructural analysis and to correlate those changes with motor performance. CD-1 male mice 35g inhaled 0.04 M MnCl₂ and 0.02 M AcMn₃, one hour twice a week for 38 weeks. Animals were trained on the reaching task and on the beam walking test before the inhalation and evaluated each week after the Mn exposure, then were sacrificed, perfused by intracardiac puncture with saline and fixed with glutaraldehyde and paraformaldehyde. Corpus striatum and substantia nigra pars compacta were processed for electron microscopy and for tyroxine hydroxylase (TH) immunocytochemistry. The animals showed poor performance in both motor tests after Mn inhalation, decreased number of TH-positive cells, necrosis and apoptosis in both substantia nigra and striatum and evident neuropile alterations. Our data provides evidence that Mn inhalation produces similar morphological and behavioral alterations to those observed in Parkinson disease providing a useful experimental model for the study of this neurodegenerative disease.

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88 CERULOPLASMIN MEDIATES MANGANESE DISPOSITION AND ALTERS OXIDATIVE STRESS LEVELS IN MANGANESE-TREATED MICE.

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The protein ceruloplasmin (Cp) is a copper containing ferroxidase that is expressed as a soluble form circulating in plasma and as a GPI-anchored form on the outer surface of membranes in the CNS. Cp is thought to oxidize manganese (Mn) in humans and promote the loading of Mn onto plasma transferrin. Studies have suggested that GPI-anchored Cp in the brain may facilitate cellular iron efflux and modulate extracellular oxidative stress. Transgenic mice deficient in Cp have been shown to accumulate iron in the CNS and exhibit neurodegeneration, consistent with animal and human studies showing that elevated CNS accumulation of transition metals promotes cytotoxicity and neurodegeneration. Here we tested the hypotheses that the Cp mediates the distribution of Mn in peripheral and CNS tissues, alters the levels of oxidative stress associated with Mn toxicity, and mediates motor deficits of Mn toxicity. Wild type and Cp null (Cp -/-) mice were injected with a cumulative dose of 0, 90, or 180 mg/kg of Mn over 4 weeks. Total Mn content of blood and liver was not significantly different between Cp null and wild type mice at any treatment level, but Mn accumulation in brain tissue was greater in the Cp null mice compared to wild type. The 90 mg/kg Mn treatment produced higher levels of lipid oxidation than controls, based on cerebellum TBARS levels, and the Mn-treated Cp nulls had lower TBARS than treated wild type mice. Consistent with this, GSH levels in cerebellum were higher in Mn-treated Cp null versus treated wild type mice. Finally, while Mn treatment caused a reduction in motor activity, this effect was not significantly different between Cp null and wild type mice. The greater oxidative stress apparent in brain tissue of wild type mice than knockouts is consistent with Cp acting as a pro-oxidant, despite the greater Mn amounts present in the brains of Cp null mice. Collectively, these data suggest that Mn-induced oxidative stress in the brain is facilitated by Cp.

89 ANTIOXIDANTS SUPPRESS MANGANESE-INDUCED OXIDATIVE INJURY, ALTERATIONS IN MEMBRANE PERMEABILITY AND ENERGY METABOLISM.

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Chronic exposure to manganese (Mn) leads to neurotoxicity (manganism) and is characterized by neuropsychiatric symptomatology that often resembles Parkinson's disease. It is well-established that astrocytes sequester Mn and therefore, protect neurons from Mn-induced neurotoxicity. Although the mechanisms by which Mn induces neuronal cell death are not well defined, its neurotoxicity is regulated by a number of factors, including mitochondrial dysfunction and oxidative injury. We

investigated the mechanisms underlying Mn neurotoxicity and showed that biomarkers of oxidative damage, F2-isoprostanes (F2-IsoPs) were significantly ($p < 0.001$) increased (140%) in rat primary astrocytes exposed to 500 μM MnCl_2 for 30 min. Additionally, primary astrocytes exposed to Mn showed significant ($p < 0.001$) decrease in high energy phosphate (ATP) and mitochondrial membrane potential to 37% and 85% of control, respectively. Finally, Mn-induced alterations in F2-IsoPs, ATP and mitochondrial membrane potential were suppressed when primary astrocytes were pretreated with spin trapping agent, PBN (alpha phenyl-N-tert butyl nitron) and antioxidants tempol (4-hydroxy-2,2,6,6-tetramethylpiperidinyl-n-oxy) or a hydrophilic vitamin E analog, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The present study established that Mn-induced oxidative stress in astrocytes may indirectly lead to neuronal death and that the effects of Mn toxicity in astrocytes are fully reversed by antioxidants (Supported by NIEHS 007331 and DoD W81XWH-05-0239).

90 GENE EXPRESSION CHANGES IN THE FRONTAL CORTEX OF MANGANESE EXPOSED NON-HUMAN PRIMATES.

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Environmental exposure to high levels of manganese has been associated with a complex neurological syndrome including psychiatric, cognitive, and motor abnormalities. Research has focused on the characterization of manganese-induced deficits in the basal ganglia, which modulates motor activity, but less is known about the effects of manganese in other parts of the brain that could lead to the cognitive and psychiatric sequelae of manganese exposure. In the present study, we have examined gene expression in the frontal cortex in a non-human primate model of manganese exposure. These animals have been shown to express subtle deficits in cognitive and motor function (Schneider et al., Brain Res. 2006) as well as deficits of in vivo striatal dopamine release (Guilarte et al., Exp. Neurol. 2006) and cortical deficits in brain metabolites reflective of neuronal dysfunction (Guilarte et al., Tox. Sci. 2006). Cynomolgous macaques (5-6 years of age) were given i.v. injections of 10-15mg/kg MnSO_4 (n=4) once a week for approximately nine months. Microarray analysis revealed that 61 genes were consistently up-regulated and 4 genes were down-regulated in the frontal cortex of animals exposed to manganese compared to naive control (n=3). We identified clusters of up-regulated genes involved in apoptosis, cholesterol homeostasis, ubiquitin-proteasome function, cell cycle regulation, and inflammation. Interestingly, the most highly up-regulated gene was amyloid beta (A4) precursor-like protein 1, a member of the amyloid precursor protein family that has been associated with the neuropathology of Alzheimer's disease. Our analysis reveals that manganese exposure causes changes in the expression of a network of interconnected genes in the frontal cortex that are involved in neural injury. These results suggest that some of the early gene expression changes in the frontal cortex may be involved in the neurotoxicity of manganese. [supported by ES10975]

91 A POSSIBLE ROLE FOR MITOGEN ACTIVATED PROTEIN KINASE-3/6 IN THE PHOSPHORYLATION OF P38 KINASE IN MICROGLIAL CELLS EXPOSED TO MANGANESE AND LPS.

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Manganese (Mn) neurotoxicity resembles many of the features of Parkinson's disease (PD), and it has been suggested that activated microglia are involved in both pathologies. In previous work, we have demonstrated that Mn enhances the production of inflammogen (lipopolysaccharide; LPS)-induced proinflammatory cytokines such as IL-1 β , IL-6, and TNF- α by microglial cells and that the Mn-caused enhancement of LPS-induced cytokine production occurs via a p38-alpha mitogen activated protein kinase alpha (p38 α) and nuclear factor kappa B (NF- κ B)-dependent mechanism. Although exposure to Mn+LPS increased and prolonged p38 α activation, the mechanism of this activation is not understood. In our current study we are examining the mitogen activated protein kinases (MAPK) upstream of p38 α , including the MAPK kinase 3 and 6 (MKK-3/6). N9 microglial cells were exposed to 250 μM Mn, 100 ng/ml LPS, or Mn+LPS for various time periods. At the end of exposure, cell lysates were isolated and examined using western blot analysis with antibodies specific for MKK-3/6. Compared to controls, exposure to Mn, LPS, and Mn+LPS for 1h increased MKK-3/6 phosphorylation, with the greatest effect being observed in the cells exposed to Mn+LPS. Additionally, preliminary data suggest that exposure to Mn+LPS may increase MKK-3/6 phosphorylation as early as 15min. Besides examining the effect of Mn on MKK-3/6 activation in greater detail, future experiments will also focus on other kinases that may

be involved in the phosphorylation of p38 α . As enhanced production of proinflammatory cytokines by microglial cells may cause damage to surrounding cells (i.e. neurons), determining the mechanism of Mn-enhanced proinflammatory cytokine production will increase our understanding of Mn neurotoxicity and similar neuropathologies. (Supported by NIEHS ES11654).

92 MANGANESE-INDUCED ASTROCYTE APOPTOSIS VIA MITOCHONDRIAL-DEPENDENT PATHWAYS.

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Manganese (Mn) is essential for maintaining the proper function and regulation of multiple biological processes, including various antioxidant enzymes, such as superoxide dismutase (Mn-SOD) and glutamine synthetase (GS). Chronic exposure to excessive Mn results in a movement disorder termed manganism, which resembles Parkinson's disease. The pathogenic mechanisms underlying this disorder are not fully understood. Several lines of evidence implicate astrocytes as an early target of Mn neurotoxicity. In the present study, we investigated the hypothesis that cytotoxicity of Mn in astrocytes is mediated via mitochondrial control of apoptosis. Primary astrocyte cultures were prepared from cerebral cortices of one day old Sprague-Dawley rats. Cultures were grown to confluency in minimal essential media (MEM) with Earle's salts supplemented with 10% heat-inactivated horse serum. Cells were treated for 30 min and 1, 2, or 6h with Mn at 0, 100, 500, and 1000 μM . Astrocyte metabolic activity was assessed by lactate dehydrogenase (LDH) leakage into the culture medium. Mn cytotoxicity was determined by measuring the conversion of a tetrazolium salt (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, MTT) to a formazan product. The potentiometric dye tetramethylrhodamine ethyl ester (TMRE) was used to assess the effect of Mn on astrocytic mitochondrial membrane potential ($\Delta\Psi\text{m}$). Our results show that Mn-induced a concentration-dependent ($p < 0.05$ by ANOVA) release of LDH into the culture media and MTT reduction. Mn treatment (1 and 6 hrs) significantly ($p < 0.01$) dissipated the $\Delta\Psi\text{m}$ in astrocytes as evidenced by a decrease in mitochondrial TMRE fluorescence. These results suggest that Mn plays a pivotal role in oxidative responses in astrocytes and that mitochondrial-dependent pathway is involved in this response to Mn neurotoxicity.

93 DOPAMINE NEURON DEGENERATION IN C. ELEGANS: IDENTIFICATION OF GENETIC AND CHEMICAL MODULATORS IN A NOVEL MODEL OF PARKINSON'S DISEASE AND MANGANISM.

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Parkinson's disease (PD) and manganism are characterized by motor deficits and dopamine neuron dysfunction, and dopamine or its metabolites are believed to contribute to the disorders. Furthermore, expression of the pre-synaptic protein α -synuclein, and the oxidative stress-induced protein parkin have been proposed to contribute to the pathogenesis of both disorders, and occupational exposure to Mn^{2+} has been invoked to predispose individuals to PD. Despite the initial characterization of these disorders well over a century ago, and intensive research within the past several decades, the origin of the pathogenesis and the molecular determinants involved in PD and manganism have yet to be fully elucidated. A significant hindrance in dissecting the molecular components is the high complexity of the vertebrate brain and lack of facile *in vivo* genetic models to determine and explore the mechanisms involved in the cell death. We have developed a novel pharmacogenetic model using the genetically tractable nematode *C. elegans* to dissect and characterize the molecular components involved in DA neuron degeneration. We show that the DA neurons are sensitive to the PD-associated neurotoxin 6-OHDA and Mn, and cell death likely occurs through a novel pathway. We also show that a number of PD-associated genes contribute to the cell death. We have now instituted genetic and chemical screens for regulators of toxin-mediated cell death, and we have isolated several mutants and compounds that suppress the neurodegeneration. This system will allow us a facile test to examine the role xenobiotics and Mn^{2+} play in the degeneration of DA neurons.

94 BHT PROTECTION OF RAT HIPPOCAMPAL ASTROCYTES FROM MANCOZEB-INDUCED CYTOSKELETAL ALTERATIONS.

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Mancozeb belongs to the subclass of dithiocarbamate pesticides having a metal atom coordinated with ethylene-bis-dithiocarbamate (EBDC). Specifically, it is a polymeric complex of Mn-EBDC with zinc salts. The extreme reactivity of dithiocarbamates have been shown to involve their metal-chelating properties and high

affinity for -SH groups. The effects of manganism are associated with considerable neurological damage. Cecconi et al. (2006) demonstrated that Mancozeb can adversely affect reproduction by perturbing microtubular organization during meiotic maturation. In vitro studies have shown that Mancozeb is considered a prooxidant and has the ability to produce oxidative stress in cells by increasing reactive oxygen species and 8-hydroxy-2'-deoxyguanosine (8-OHdG). Astrocytes play an essential role in protecting neurons since they are equipped with a full range of antioxidant machinery. Previous studies from this lab examining the effects of antioxidants and chelators on Mancozeb-treated astrocytes revealed BHT as a sole protectant against Mancozeb induced cytotoxicity. This investigation further studied the protective role of BHT in Mancozeb-induced cytoskeleton destruction. Rat hippocampal astrocytes were exposed to 7.5 μ M of Mn-Zn dithiocarbamate for an hour at 37 degrees C. The cells were re-fed complete media or media containing 50 μ M BHT. Immunofluorescence and scanning electron microscopy were performed to study cytoskeletal structures. Results showed that BHT partially restored Mancozeb-induced destruction as evidence by immunofluorescent staining with α -tubulin antibody at 4, 8, 12 and 24 hr post-treatment. Scanning electron microscopy of cytoskeletal preparations at 8 hr post-treatment also revealed the protective effects of BHT on actin sheets, intermediate filaments and microtubules. Antioxidant data indicated that BHT significantly increased ($p < 0.05$) antioxidant activity within the cells.

95 **ROLE OF GLYCOLYSIS IN DIFFERENTIAL RESPONSE OF NON-DIFFERENTIATED (ND) AND DIFFERENTIATED (D) PC12 CELLS EXPOSURE TO MN, MPP+ AND ROTENONE.**

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Exposure to Mn, MPP+ and rotenone (Rot) cause Parkinson symptoms in humans and other primates by affecting dopaminergic neurons. The PC12 cell system is a neoplastic dopaminergic cell line with a high rate of glycolysis accompanied by a high lactate production and low use of glucose carbon through the Krebs cycle. MPP+ and Rot target complex I in mitochondrial respiration and elicit less cytotoxicity in cells using a high rate of glycolysis as their primary energy pathway. Previous studies have shown that MPP+ has greater toxicity in DPC12 cells compared to NDPC12, and that a high rate of glycolysis reduces the cytotoxicity of MPP+ on NDPC12 cells. This study examines the role of glycolysis on the toxicity of Mn, MPP+ and Rot in DPC12 and NDPC12 cells. Glycolysis was inhibited by using glucose free media and iodoacetic acid (IAA) exposure prior to treatment with neurotoxins. Cytotoxicity studies revealed Mn was toxic in both PC12 cell types following 24, 48 and 72 hrs exposure compared to MPP+ and Rot which were both toxic only in DPC12 cells and only at 48 and 72 hrs. The EC50s for Mn in NDPC12 cells were 6.1 mM, 1.8 mM and 1.2 mM at 24, 48 and 72 hrs, respectively, and in DPC12 cells 25.0 mM and 6.4 mM at 48 and 72 hours. The EC50 for MPP+ in DPC12 cells was 120 μ M at both 48 and 72 hrs and for Rot 500 nM at 24 hr and 30 nM at 48 and 72 hrs. Glycolysis inhibition by IAA revealed NDPC12 and DPC12 cells are equally sensitive, but NDPC12 cells show significantly greater ($p < 0.05$) sensitivity to glucose free media. In both NDPC12 and DPC12 cells, cytotoxicity of Mn, MPP+ and Rot was significantly increased ($p < 0.05$) during glycolysis inhibition. The results suggest that cell differentiation status and glycolysis use are involved in PC12 cell susceptibility to neurotoxins.

96 **CONTROL OF THE LATERAL CILIATED GILL EPITHELIUM OF *CRASSOSTREA VIRGINICA* AND *MYTILUS EDULIS* (BIVALVIA) AND THE NEUROTOXIC EFFECTS OF MANGANESE.**

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Lateral cilia of gill of *Mytilus edulis* is known to be controlled by a reciprocal serotonergic-dopaminergic innervation from their ganglia. Other bivalves have been studied to lesser degrees, and lateral cilia of most respond to serotonin and dopamine when applied directly to gill, indicating a possible neuro or endocrine mechanism. Lateral cilia in *Crassostrea virginica* are affected by serotonin and dopamine, but little work has been done with respect to ganglionic control of cilia. Presently we examined the role of the cerebral and visceral ganglia in innervating lateral gill cilia of *C. virginica* and *M. edulis*. Ciliary beating rates were measured by stroboscopic microscopy of gill preparations which had ipsilateral cerebral and visceral ganglia attached. Superfusion of gill with serotonin increased ciliary beating rates. This response was antagonized by pretreating with methysergide. Superfusion with dopamine decreased beating rates and this was antagonized by ergonovine. Furthermore, superfusion of the cerebral ganglion or visceral ganglion with serotonin increased ciliary beating rates which was antagonized by pretreating with

methysergide. Superfusion with dopamine decreased beating rates and this was antagonized by ergonovine. Acute treatment with 1 mM manganese, a neurotoxin known to induce Manganism, a Parkinson's-like Disease in humans, reduced the cilio-inhibition caused by dopamine, which is in agreement with the method of action of manganese in humans. This study demonstrates there is a reciprocal serotonergic-dopaminergic innervation of the lateral gill ciliated cells of *C. virginica* which originates in the cerebral ganglion of the animal, similar to that of *M. edulis*, and this preparation is useful as a model to study manganese neurotoxicity and the pharmacology of drugs affecting it and biogenic amines.

97 **NEUROTOXIC EFFECTS OF MANGANESE ON BIOGENIC AMINES OF THE NERVOUS SYSTEM AND INNERVATED ORGANS OF *CRASSOSTREA VIRGINICA*.**

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Manganese is a neurotoxic substance which induces Manganism, in individuals exposed to high airborne levels or ingesting high concentrations. Manganism occurs because too much manganese injures dopaminergic neurons in the brain. *Crassostrea virginica* is identified by the EPA as a test animal for studying marine pollutants. It possesses a simple nervous system with monoaminergic neurons. We studied if acute exposure of *C. virginica* to manganese results in increases in tissue levels of manganese and decreases in biogenic amine levels. Oysters were exposed to 1 mM and 10 mM manganese by maintaining them in containers of artificial sea water at 18°C for 3 days, and metal levels were determined by Atomic Absorption Spectrophotometry using electrothermal vaporization with deuterium lamp background correction in a Perkin Elmer AA800 Atomic Absorption spectrophotometer with a THGA graphite furnace. Biogenic amines were measured by HPLC with fluorescence detection using a Beckman HPLC system with a Phenomenex Gemini 5 μ C18 column and a Jasco FP 2020 Spectrofluorometer. Manganese was distributed in the tissues of untreated animals in μ g/g dw amounts, with palps and gill having the highest. Treated animals accumulated about a 10 fold increase in manganese in the tissues. The 1mM manganese treatment caused statistically significant reductions in dopamine levels in both the cerebral and visceral ganglia (77% and 74% respectively) and both gill and palps (79% and 63% respectively), but no changes in the levels of norepinephrine, octopamine or serotonin. The results are consistent with reported mechanisms of action of manganese in human and mammalian systems and shows marine invertebrates can be damaged by high environmental levels of manganese and *C. virginica* is a suitable test animal to further study neurotoxic actions of manganese and related agents.

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98 **PROTECTIVE EFFECTS OF VERAPAMIL ON MERCURY TOXICITY IN *C. ELEGANS*.**

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The free living soil nematode *C. elegans* has become an important model as an indicator of environmental contamination in soil and water. Specific cells and their lineage can be traced in the whole animal throughout development and into adulthood permitting analysis of effects of toxicant on individual cells. We are attempting to optimize the *C. elegans* model for studies on neurotoxicity of mercurials in attempts to facilitate studies of chronic and developmental effects of these neurotoxins on specific neurons in the whole organism. Mercurials, especially methylmercury (MeHg) impair neurodevelopment. Some of their effects are thought to be due to Ca^{2+} channels and perturbation of $[Ca^{2+}]_i$. We established a lethality index for MeHg and $(HgCl_2)$ and tested for protection by the Ca^{2+} channel blocker verapamil on cytotoxicity. Worms feeding on saturating liquid cultures of *E. coli* were exposed to varying concentrations of MeHg and $HgCl_2$ for 48 hrs. At that time motile (live) vs immotile (dead) worms were counted using motility as an index of viability. Over the concentration range of 0.25 - 10 μ M and 10-70 μ M for MeHg and Hg^{2+} , respectively there was a concentration-dependent increase in lethality. Treatment with 20 μ M MeHg and 70 μ M $HgCl_2$ caused > 90% lethality in wildtype (N2) *C. elegans* and a strain KC136 expressing green fluorescent protein under control of a heat shock gene promoter. Worms were then exposed to 70 μ M $HgCl_2$ in the presence of varying concentrations of the L-type Ca^{2+} channel blocker verapamil for 48 hrs. Simultaneous treatment with 50 μ M verapamil resulted in reduction of lethality by $HgCl_2$, to 20%. This suggests that Hg^{2+} induced mortality in *C. elegans* is mediated by pathways associated with membrane Ca^{2+} channels. The work was supported in part by NIH grants R01ES116622 and R21ES01457. L. Koselke was supported by an ASPET Summer Research Fellowship.

99 DIFFERENTIAL EFFECTS OF Ca^{2+} CHANNEL α_1 SUBUNITS ON METHYLMERCURY-INDUCED CHANGES IN $[\text{Ca}^{2+}]_i$ IN HEK293 CELLS.

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Methylmercury (MeHg) disrupts Ca^{2+} homeostasis in a biphasic manner in rat cerebellar granule cells. The 1st phase is due to release of intracellular Ca^{2+} , and the 2nd to Ca^{2+} influx through nifedipine and ω -conotoxin-MV1IC-sensitive pathways, suggesting a role for the L-, N- and P/Q-type Ca^{2+} channels (VGCC). To investigate the role of these individual VGCC subtypes directly, HEK293 cells were cotransfected with a constant VGCC $\alpha_{2\delta}$ and β subunit, and either α_{1A} , α_{1B} , or α_{1C} subunits, corresponding to P/Q-, N-, and L-type VGCCs, respectively. The Ca^{2+} -sensitive fluorophore fura-2 and cell imaging was used to examine the effect of in vitro exposure to MeHg on Ca^{2+} homeostasis. In WT cells, which express no VGCCs, 1.0 μM MeHg caused a transient increase in $[\text{Ca}^{2+}]_i$ after 68 ± 17 sec (mean $\pm$ SEM). In cells transfected with VGCC, MeHg induced a biphasic increase in $[\text{Ca}^{2+}]_i$, consistent with data in rat cerebellar granule cells. At 1 μM MeHg, the 1st and 2nd phases occurred at 114 ± 22 and 686 ± 107 sec (α_{1B}) and 204 ± 42 and 863 ± 160 sec (α_{1C}). At 2.5 μM MeHg, 1st and 2nd phases occurred at 170 ± 30 and 689 ± 49 (α_{1A}), 28 ± 1.6 , and 270 ± 57 (α_{1B}) and 100 ± 30 and 560 ± 110 sec (α_{1C}), respectively. Thus cells with α_{1B} respond to MeHg more quickly, and MeHg may prefer to enter the cell through N-types VGCCs. When Ca^{2+} was removed, the 2nd increase in $[\text{Ca}^{2+}]_i$ was prevented. Additionally, cells expressing α_{1C} channels were exposed to 10 μM nifedipine for 5 min before and during exposure to 2.5 μM MeHg with 2 μM Ca^{2+} . Nifedipine did not affect the time-to-onset of the 1st phase but abolished the 2nd. These support further that the 2nd phase is due to Ca^{2+} influx through VGCC, and that MeHg disrupts Ca^{2+} homeostasis by inducing Ca^{2+} release from intracellular stores and extracellular influx. Ca^{2+} can reach cytotoxic levels within the cell and activate signal transduction cascades that lead to cell damage and death. Supported by NIH grant ES03299.

100 BOTH RYANODINE RECEPTOR-SENSITIVE AND INSENSITIVE INTRACELLULAR Ca^{2+} POOLS PLAY A ROLE IN METHYLMERCURY (MeHg)-INDUCED INITIAL STIMULATION OF SPONTANEOUS INHIBITORY SYNAPTIC CURRENTS.

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We have previously shown that the time to onset of MeHg-induced change in $[\text{Ca}^{2+}]_i$ in the entire molecular layer and granule cells in cerebellar slices appears to be correlated to the time to onset of initial increases in frequency of spontaneous inhibitory synaptic currents (sIPSCs) recorded from Purkinje cells. In addition, pretreatment of slices with caffeine, a ryanodine receptor opener, or cyclopiazonic acid (CPA), an inhibitor of smooth endoplasmic reticulum (SER) Ca^{2+} -ATPase, markedly reduced MeHg-induced increases in $[\text{Ca}^{2+}]_i$ in both molecular and granule layers in cerebellar slices. To determine further if these treatments are also effective at reducing or preventing MeHg-induced initial stimulation of spontaneous release of inhibitory neurotransmitter, effects of caffeine and CPA on sIPSC frequency of Purkinje cells in cerebellar slices were examined using whole cell current recording techniques. Consistent with previous findings of confocal Ca^{2+} imaging, pretreatment of slices with 5 - 10 mM caffeine or 10 - 25 μM CPA apparently reduced MeHg-induced increase in sIPSC frequency in Purkinje cells. However, both treatments failed to alter the general pattern of effects of MeHg on sIPSC frequency i.e. MeHg initially stimulates and subsequently suppresses sIPSC frequency. Neither caffeine nor CPA pretreatment significantly changed the time courses of effects of MeHg on sIPSC frequency and amplitudes. These results suggest that Ca^{2+} release from both ryanodine receptor-sensitive and SER intracellular Ca^{2+} pools play a role in MeHg-induced increase in $[\text{Ca}^{2+}]_i$ and the frequency of sIPSCs, although other Ca^{2+} sources or mechanisms may also contribute to the early stimulatory effects of MeHg on spontaneous transmitter release. Supported by NIH grants R01ES03299 and R01ES11662.

101 DISRUPTION OF INTRACELLULAR CALCIUM REGULATION IN A MOTORNEURON CELL LINE-NSC34 BY METHYLMERCURY.

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Environmental effects have been postulated to contribute to the onset of the sporadic form of amyotrophic lateral sclerosis (ALS). Among the environmental factors proposed as potentially contributory, are neurotoxic metals such as Pb and Hg. The prospective role of these metals in ALS has never been tested directly. We are attempting to determine whether chronic exposure to Hg can enhance excitotoxicity

in spinal motoneurons. As a 1st step in this, we tested the effect of acute exposure of a neuroblastoma motoneuron cell line -NSC34- to methylmercury (MeHg), the most prominent environmental form of Hg exposure. The Ca^{2+} indicator fura-2 and single cell fluorescence imaging was used to examine the effect of *in vitro* exposure to MeHg on Ca^{2+} . Over the range of 0.5-2 μM , MeHg induced a biphasic increase in fura fluorescence. The two phases frequently were separated by a plateau phase. Times to onset of 1st and 2nd phases were MeHg-concentration-dependent - 80, 120 and 220 sec respectively at 0.5, 1 and 2 μM , for the 1st phase, and 590, 640 and 800 sec for 2nd phase. The total increase in fluorescence ratio and rate of rise of fluorescence during the initial phase of the increase were both also MeHg concentration-dependent. Concomitant treatment with the Na^+ channel activator veratridine (30 μM) and 1 μM MeHg was used to depolarize the cell and potentially increase the rate of entry of MeHg. Veratridine did not affect the time of onset of either phase of increased $[\text{Ca}^{2+}]_i$, but did speed up the rate of rise of fluorescence at 1 μM MeHg. Thus in a spinal motoneuron cell line, exposure to MeHg induces changes in $[\text{Ca}^{2+}]_i$ consistent with those seen in native neurons, so the NSC-34 cell line should be an acceptable model system for further studies of effects of chronic exposure to MeHg. Supported by NIH grant R21ES01457. C. Dupré was supported by a Summer Research Fellowship from ASPET.

102 METHYLMERCURY-INDUCED APOPTOSIS BY MITOCHONDRIA-DEPENDENT PATHWAY IN RAT PRIMARY ASTROCYTE CULTURES.

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Methylmercury (MeHg) preferentially accumulates in astrocytes. Mitochondrial dysfunction is a prominent feature of MeHg-induced cell death, leading us to test the hypothesis that MeHg affects critical pathways in these organelles. Primary astrocyte cultures were prepared from cerebral cortices of one day old Sprague-Dawley rats. Cultures were grown to confluency in minimal essential media (MEM) with Earle's salts supplemented with 10% heat-inactivated horse serum. Cells were treated for 0.5, 1, 2, 6, 12, or 24 h with MeHg at 0, 1, 5, and 10 μM . Cytotoxicity was evaluated by lactate dehydrogenase (LDH) leakage into the culture medium and proteins were collected for immuno-blotting analysis. The potentiometric dye tetramethylrhodamine ethyl ester (TMRE) was used to probe mitochondrial membrane potential ($\Delta\psi_m$). Our studies show that MeHg induced significant LDH release into the culture media in a concentration-dependent manner ($p < 0.05$ by ANOVA); MeHg treatment also resulted in significant activation of astrocyte phosphorylated extracellular signal-regulated kinase (p-ERK) ($p < 0.05$), peaking at 30 min and sustained through 6 hrs of exposure. MeHg also markedly increased apoptosis-inducing factor (AIF) expression ($p < 0.05$) and the effect was maintained for 24 hrs. MeHg treatment (1 and 6 hrs) significantly ($p < 0.01$) dissipated the $\Delta\psi_m$ in astrocytes as evidenced by a decrease in mitochondrial TMRE fluorescence. These studies are consistent with the hypothesis that MeHg exerts its effect via oxidative stress, and that its toxicity is associated, at least in part, with the dissipation of astrocytic mitochondrial membrane potential and activation of apoptotic cascades. These results suggest that early activation of ERK and AIF is involved in MeHg-induced neurotoxicity via mitochondrial-dependent pathways in astrocytes.

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103 IN UTERO EXPOSURE TO METHYLMERCURY CAUSES DELAYED CHANGE IN MITOCHONDRIAL MEMBRANE POTENTIAL OF EARLY POSTNATAL MOUSE CEREBELLAR GRANULE CELLS.

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Methylmercury (MeHg) is a neurotoxicant that affects both adult and developing brains. Our lab has previously shown that chronic low doses of MeHg cause a decrease in mitochondrial membrane potential (MMP) in adult mouse cerebellar granule cells (CGC). In addition, MMP was also decreased in human SH-SY5Y cells used as a neuronal developmental model system, when exposed to chronic low doses of MeHg. Therefore, in the current study, we exposed pregnant C57BL/6J mice to a total MeHg dose of 0.3 $\mu\text{g}/\text{kg}$ or 1.0 mg/kg body weight over a 10-day period from gestational day 8 to 18. CGC from newborn pups at postnatal (P) day 0 and P10 were analyzed for MMP using tetramethyl rhodamine methylester (TMRM) dye and fluorescence microscopy. No difference in MMP was observed in non-treated CGC between P0 and P10. MMP was different among treatments at P10; MMP at 0.3 μg was less than that for controls, and MMP at 1.0 mg was greater than that for controls. In addition, MMP at 0.3 μg was decreased at P10 compared to P0, and MMP at 1.0 mg was increased at P10 compared to P0. These data indicate that *in utero* MeHg exposure has a delayed affect on MMP of CGC,

that can be observed by P10. The increase in MMP in P10 cells at 1.0 mg may be a result of the need for increased cell activity to compensate for the effects of MeHg neurotoxicity. This research was funded in part by CERH NIEHS Grant #P30ES09106 to LCA.

104 METHYLMERCURY (MeHg) CAUSES A BIPHASIC EFFECT ON SPONTANEOUS SYNAPTIC CURRENTS IN LAYERS II/III AND IV NEURONS IN VISUAL CORTEX SLICES OF RAT.

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One of the most conspicuous and consistent findings in MeHg-induced neurological disorders in humans and animals following acute and chronic MeHg exposure is disturbance of visual function. Pathological evidence suggests that lesions in the visual cortex are primarily responsible for MeHg-induced visual functional deficits. To characterize the effects of MeHg on visual synaptic transmission, we examined *in vitro* acute effects of MeHg on spontaneous synaptic responses in layers II/III and IV neurons in visual cortex slices of rat using whole cell voltage-clamp recording technique. At 10 -100 μ M, MeHg initially stimulated and subsequent suppressed both the frequency and amplitudes of glutamate receptor-mediated spontaneous excitatory postsynaptic currents (sEPSCs) in layer II/III pyramidal neurons and layer IV nonpyramidal neurons. Times to onset of stimulation and suppression of sEPSCs were MeHg concentration-dependent. Similar pattern of effects of MeHg on GABAergic spontaneous inhibitory postsynaptic currents (sIPSCs) was also observed in these neurons. Thus, consistent with its effects on synaptic function in other brain slice preparations such as cerebellar slices, MeHg may also act at both pre- and postsynaptic sites to affect synaptic transmission in the visual cortex slices. These results again suggest that mechanisms underlying the effects of MeHg *in vitro* on spontaneous synaptic responses are generally similar. Supported by NIH grant 5R21ES013767-02 and MSU funding 06-HBRI-II-616.

105 TROLOX, A VITAMIN E DERIVATIVE, PROTECTS AGAINST PERSISTENT NEUROBEHAVIORAL DISRUPTION INDUCED BY NEONATAL METHYLMERCURY EXPOSURE.

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Methylmercury (MeHg) is a neurotoxicant that poses a potential health risk to humans, particularly when exposure occurs during neural development. Antioxidants protect against MeHg-induced toxicity *in vivo* and *in cell culture*. However, the protective effect of antioxidants on long-term behavioral function following early MeHg exposure has not been well studied. We examined whether Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water soluble vitamin E derivative, would ameliorate MeHg-induced disruptions of a number of behavioral measures in mice. Mice received 4 mg/kg MeHg or vehicle on alternate days from postnatal days 3-15. Some mice were pretreated with 2.5 mg/kg or 1 mg/kg Trolox sc 1 hr prior to each MeHg administration. Body weight, reflex development, spatial learning, and social interaction/aggression were examined beginning in early postnatal life through 3-4 months of age. MeHg treatment resulted in decreased weight gain and a defective mid-air righting reflex. Treated mice showed poorer spatial learning performance in the water maze as adolescents. Increased aggressive behavior and reduced social investigation were also observed in MeHg treated mice in the resident-intruder paradigm at 3-4 months of age. Trolox pretreatment attenuated the MeHg-induced reduction in weight gain and the mid-air righting deficit in a dose-related fashion. Trolox also reduced learning deficits observed in the water maze, with the higher dose showing superior protection. Finally, 2.5 mg/kg Trolox completely prevented the increase in aggressive behavior and decrease in social investigation in adult mice elicited by early MeHg exposure. These data indicate that Trolox offers significant protection against long-term MeHg-induced neurobehavioral deficits.

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106 SELENIUM'S PROTECTION AGAINST METHYLMERCURY. CHRONIC EXPOSURE TO MEHG WHILE ON HIGH OR LOW SELENIUM DIET TO TEST HYPOTHESIS THAT SELENIUM BLOCKS MEHG NEUROTOXICITY.

J. C. Heath, E. Pesek and M. C. Newland, *Auburn University, Auburn, AL.*

Chronic exposure to at least 5 ppm of MeHg produces a constellation of neurological signs indicative of sensory and motor dysfunction. Selenium has been shown to protect against some of these signs in short-duration, high-dose experiments but lit-

tle is known about dose or time-related protection in long-term experiments. Female rats were exposed chronically to methylmercury via drinking water containing 0, 0.5 or 5 ppm of Hg as MeHg, producing an estimated 0, 40, or 400 μ g/kg/day of Hg, while consuming a purified diet containing 0.06 or 0.6 ppm of Se (as sodium selenite) for more than a year. The rats' were tested every two to three months for sensitivity to tail pinch, the appearance of hind-limb cross (clasp reflex) and limb flexion, grip strength, and overnight activity in a running wheel. This formed a 2 (lifespan diet) X 3 (chronic MeHg) X N (repeated testing) factorial design. Grip strength and sensory function diminished with age, this loss was accelerated by MeHg exposure and the loss was attenuated by dietary selenium. Selenium delayed but did not prevent the appearance of hind-limb cross and flexion. Selenium did not prevent MeHg's effects on running but did ameliorate changes seen with aging. Selenium confers protection against some overt neurological signs of chronic, moderate-to-high dose MeHg exposure [Supported by NIH ES 10865]

107 "LOW-RATE CHALLENGES" IN RATS EXPOSED DEVELOPMENTALLY TO METHYLMERCURY AND SELENIUM.

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Developmental exposure to methylmercury produces response perseveration and disrupted sensitivity to reinforcing consequences. High-rate behavior in adulthood may be especially sensitive to developmental MeHg exposure. We examined MeHg's effects on the acquisition and persistence of high-rate behavior. Female rats were exposed *in utero* to methylmercury via maternal exposure to drinking water containing 0, 0.5 or 5 ppm of Hg as MeHg, producing an estimated 0, 40, or 400 μ g/kg/day of Hg. The rats' mothers also consumed a purified diet containing 0.06 or 0.6 ppm of Se (as sodium selenite), and the offspring continued these diets throughout life. This formed a 2 (lifespan diet) X 3 (developmental MeHg) factorial design. As adults, the offspring acquired lever-pressing under two schedules of reinforcement designed to maintain high-rates of lever-pressing. A differential reinforcement of high-rate (DRH) schedule imposed a rigid, unchanging response requirement. A Percentile schedule also reinforced high-rate behavior but constantly adjusted the response requirement according to recent performance. Responding under each schedule could be described by bouts of high-rate (2-5 rs/sec) responses separated by 2-20 sec pauses. Acquisition of responding and the result of "low-rate challenges," in which low rates of lever pressing were reinforced, were examined. Developmental methylmercury exposure promoted the acquisition of high-rate responding and tended to produce higher response rates during steady-state performance. When rats were abruptly required to respond at low, rather than high, response rates, exposed rats showed considerable difficulty, compared to controls, in adjusting to the low-rate challenge. They required many sessions to achieve steady state and continued to respond at higher response rates than controls. Dietary selenium did not influence MeHg's effects. This extends to high-rate operant behavior the phenomenon of perseveration previously reported using choice procedures. [Supported by NIH ES 10865]

108 MODIFICATION OF BASAL AND DEXAMETHASONE-INDUCED SUPPRESSION OF CORTICOSTERONE LEVELS IN FEMALE OFFSPRING EXPOSED TO MATERNAL LEAD EXPOSURE, MATERNAL STRESS OR THE COMBINATION.

M. Virgolini, A. Rossi-George, R. Lisek, D. Weston and C. A. Deborah, *Environmental and Occupational Medicine, Environmental and Occupational Health Sciences Institute, University Med. Dent New Jersey, Piscataway, NJ.*

Prior experimental studies from our laboratory demonstrate that maternal Pb exposure permanently alters basal and stress-induced corticosterone levels in both male and female offspring. To evaluate the role of negative feedback inhibition of corticosterone as a mechanism of these effects, the ability of the synthetic glucocorticoid dexamethasone (DEX) to suppress corticosterone levels (DEX suppression test, 0, 10 or 15 mg/100g i.p.) over time was examined in adult female offspring subjected to either maternal Pb exposure (0, 50 or 150 ppm initiated 2 mos prior to breeding and continuing through lactation; corresponding mean blood Pb of <2, 12 and 30 μ g/dl, respectively), maternal restraint stress (45 min restraint, 3x per day on gestational days 16-17) or both. Maternal Pb alone notably delayed the post-injection reduction in corticosterone. Maternal stress interacted with maternal Pb exposure. Specifically, in controls, maternal stress delayed the post-injection reduction in corticosterone, but the blunting of the corticosterone response by DEX was equivalent in both normal and maternal stress offspring, with dose-related reductions peaking

4 hrs post DEX and returning to control levels at 8 hr. In contrast, DEX suppression showed a blunted pattern in response to combined maternal Pb and maternal stress at all 3 time points post-injection, particularly at 50 ppm. Thus, both maternal Pb and maternal stress alone reduce negative feedback inhibition in the HPA axis, and combined maternal Pb and maternal stress increases resistance to control of corticosterone feedback. Given that DEX appears to be mediated by its actions on the pituitary, these findings suggest a peripheral mode of action of Pb that can impact CNS functions. ES012712.

109 BEHAVIORAL CONSEQUENCES OF CONTINUOUS LEAD EXPOSURE COMBINED WITH MATERNAL AND OR OFFSPRING STRESS.

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Pb exposures are highest in low socioeconomic status populations that are also thought to experience the highest levels of environmental stress. Our prior studies confirm permanent alterations in baseline fixed interval (FI) performance and its alteration following stress challenges in response to maternal Pb, maternal stress and their combination. The current study extended these observations to an experimental model that would most closely approximate human parameters of these risk factors. Dams were exposed to 0, 50 or 150 ppm Pb in drinking water, maternal restraint stress, or both. Offspring were continued on the same Pb exposure as the dam, and a subset were also subjected to offspring stress challenges. Mean blood Pb values of offspring ranged from <2, 11-12 and 27-29 $\mu\text{g/dl}$, respectively with slightly elevated levels for males and for those subjected to offspring stress. Performance on an FI schedule of food reinforcement, a baseline with known sensitivity to Pb exposure, was examined over time, including sessions preceded by one of three different stress challenges (restraint, cold, novelty). Behavioral consequences were more pronounced in females than males, with significant increases in FI response rates across behavioral session in the groups exposed to Pb alone or Pb combined with either maternal or maternal +offspring stress, whereas only marginal increases occurred in the 0 ppm maternal or 0 ppm maternal + offspring stress groups. These changes were largely attributable to increased run rates and decreased inter-response times, with postreinforcement pause times not systematically affected. Similar trends were evident in males, but restricted to the 50 and 150 ppm + maternal stress groups. These findings confirm that stress can enhance the behavioral toxicity of Pb even at very low blood Pb levels and highlight a preferential vulnerability of females to these effects. ES012712

110 DEFICITS IN EXTINCTION OF CONDITIONED FEAR IN LEAD-EXPOSED YOUNG ADULT RATS.

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Pavlovian fear conditioning is a model of emotional learning in which a neutral stimulus such as a tone is paired with an aversive stimulus such as foot shock. Presentation of a tone with a foot shock in a context (test box) elicits a freezing response representative of stereotypic fear behavior. Daily presentation of the context (test box) in the absence of the conditioned (tone) and unconditioned stimulus (shock) causes extinction of the fear response. Rats chronically exposed to environmentally relevant levels of lead (Pb^{2+}) and controls were tested in a contextual fear (CF) paradigm at 50 days of age (PN50). Littermates to CF rats received an immediate shock (IS) when placed in the test box with no tone or were placed in the test box with no tone or shock (naïve). Blood Pb^{2+} levels in control and Pb^{2+} -exposed animals were (mean $\pm$ sem): 0.76 ± 0.11 ($n=15$) and 25.8 ± 1.28 $\mu\text{g/dL}$. Freezing behavior was recorded during acquisition or during 4 consecutive extinction days. Control and Pb^{2+} -exposed CF rats exhibited the same level of freezing time in the acquisition day. No freezing behavior was measured in IS or naïve rats regardless of treatment. Presentation of context 24 hrs later produced an enhanced freezing response on both control and Pb^{2+} -exposed-CF rats but not in IS or naïve. When tested in the extinction phase, Pb^{2+} -exposed-CF rats exhibited deficits in extinction compared to control-CF rats. That is, when presented with context on 4 consecutive days after acquisition of the fear response, Pb^{2+} -exposed-CF rats exhibited a greater freezing response than control-CF rats. These findings indicate that chronic Pb^{2+} exposure produces a deficit in extinction learning and the animals remain more fearful than controls. Since long-term retention of extinction involves a transfer from NMDA receptor-independent to NMDA receptor-dependent memory (Santini et al., J. Neurosci. 21, 9009, 2001), our present findings add additional evidence that Pb^{2+} -exposed rats have deficits in NMDA receptor-dependent memory [supported by grant ES06189 to TRG].

111 EFFECTS OF LEAD EXPOSURE ON SYNAPTIC TARGETING OF NMDA RECEPTOR SUBUNITS AND SIGNALING PATHWAYS IN RAT HIPPOCAMPAL NEURONS.

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Lead (Pb^{2+}) exposure in children and in animal models produces deficits in cognitive function and synaptic plasticity. In animals, Pb^{2+} -induced deficits in spatial learning and long-term potentiation in the hippocampus have been attributed to alterations in the expression of NMDA receptor subunits and NMDA receptor composition at the cellular level. However, information on the effects of Pb^{2+} exposure on the expression of NMDA receptor subunits at the synaptic level and their effects on downstream signaling pathways is lacking. In the present study, we used immunohistochemistry and integrated morphometric analysis to examine the effects of Pb^{2+} exposure on the number of dendritic spines expressing different NMDA receptor subunits and their synaptic targeting in primary cultures of rat hippocampal neurons. Neurons at 14 days *in vitro* were exposed for 5 days to 0.0, 0.01, 0.1 and 1.0 μM Pb^{2+} -acetate. The data indicates that the number of puncta per unit length of dendrite labeled with NR1 or NR2A subunits was significantly increased by the Pb^{2+} exposure with no change in NR2B labeled puncta. At the lowest concentration of Pb^{2+} tested, there was an increase in the co-localization of the NR2A subunit with the postsynaptic density protein PSD-95 but decreased co-localization of the NR2B subunit. No significant change was measured on the co-localization of NR1. We also examined the functional consequences of these changes by assessing the level of CREB and ERK phosphorylation (p). Under non-stimulated conditions, Pb^{2+} -exposure produced a significant decrease in both pCREB and pERK in hippocampal neurons. Further, using a protocol that increases synaptic activity, Pb^{2+} -exposure produced a dose-dependent decrease in pCREB and a general increase in pERK relative to the control condition. These data indicate that chronic exposure of hippocampal neurons to Pb^{2+} results in significant changes in NMDA receptor subunit composition and targeting to the synapse altering activity-dependent changes in nuclear pCREB and pERK [ES06189 to TRG]

112 LIFETIME EXPRESSION PROFILE OF THE AMYLOID PRECURSOR PROTEIN IN BOTH THE BRAIN AND BLOOD OF DEVELOPMENTALLY Pb-EXPOSED MICE.

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Alzheimer's disease is a neurodegenerative disease hallmarked by senile plaques composed of Amyloid-beta ($\text{A}\beta$) protein aggregates. Definitive diagnosis is achieved through post-mortem examination of brain tissue, therefore utilizing $\text{A}\beta$'s precursor, the Amyloid Precursor Protein (APP), as a potential biomarker allows one to follow disease progression and/or the effects of treatment. Our lab has previously shown that lead (Pb) exposure alters APP expression in the brain by inducing transcriptional up-regulation, initiating an increase in APP leading to a rise in $\text{A}\beta$. However, it is not known if changes in the brain are reflected in the blood. The objective of this study is to compare alterations in AD-related proteins in the brain with those in the blood so as to test the utility of blood screening as means to report on events occurring in the brain. Control and developmentally Pb-exposed animals were sacrificed at different time-points of their entire lifespan and their cortices were removed. Quantitative RT-PCR was performed to monitor the expression of APP over the mouse's lifetime (24 months). Data analysis showed that Pb-exposed mice unregulated their brain APP expression both early and late in life. APP expression was also altered in the blood following Pb exposure. These preliminary studies suggest that APP expression in blood cells may be used as an indicator for changes in APP expression in the brain.

113 INFANTILE EXPOSURE OF PRIMATES TO LEAD ELEVATES ALZHEIMER-RELATED PROTEINS LATE IN LIFE VIA ALTERATIONS IN EPIGENETIC PATHWAYS.

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Alzheimer's disease (AD) is a progressive neurodegenerative disease clinically manifested by deterioration of memory and dementia. The intracellular accumulation of beta-amyloid ($\text{A}\beta$), which is a cleavage product of the amyloid precursor protein (APP), has been implicated in the development of AD. Greater than 90% of AD cases are sporadic and have no genetic predisposition; thus environmental factors

may play a key role in the development of AD. Our lab has previously demonstrated that early life exposure of rodents to lead (Pb) enhances cerebral APP and A β production late in life suggesting that epigenetic mechanisms are involved in mediating this early exposure/late onset of AD pathology. Recently, we acquired aged monkey brains which were exposed to lead as infants. Again APP and A β levels were elevated in the monkeys with a past history of infant exposure to Pb and the brains of these aged monkeys exhibited more intense AD plaque pathology. The activity of DNA methyltransferase was also found to be low and was accompanied by disturbances in DNA methylation. These data suggest that environmental exposure to Pb is a risk factor for AD and that exposure during brain development pre-determines late life pathogenesis through an epigenetic pathway.

114 NEUROPATHOLOGICAL LESIONS AND ALTERATIONS IN BRAIN MORPHOMETRY FOLLOWING TREATMENT OF MALE RATS WITH TRIETHYLtin BROMIDE.

D. P. Myers, I. Taylor, S. Cooper and A. Broadmeadow. *Huntingdon Life Sciences, Eye, Suffolk, United Kingdom.* Sponsor: D. Mitchell.

As part of a positive control validation study, triethyltin bromide was administered by oral gavage to groups of 70 day old male Crl:CD (SD) rats at doses of 1 or 2 mg/kg on Days 1, 4, 7, 10, 14 and 17 of study. A concurrent Control group was dosed daily with water for formulation. All animals were killed on Day 29 by an overdose of barbiturate and perfused with glutaraldehyde:paraformaldehyde fixative followed by immersion in this fixative. A range of nervous system tissues were processed and examined microscopically and the thickness of the neocortex, hippocampus, corpus callosum and folia of the cerebellum were estimated in standard sections of the brain. Treatment-related neuropathological lesions included widespread vacuolation of the white matter in the brain and spinal cord, myelin vacuolation in the optic nerves and degenerate fibres in the peripheral nervous system at both doses of triethyltin bromide. The incidence and severity of these changes showed a clear dose-response. In addition, morphometric analyses on standard sections of the brain indicated that treatment at 2 mg/kg was associated with statistically significant reductions in the mean thickness of the neocortex and hippocampus and an increase in the mean thickness of the folia of the cerebellum. Treatment at 1 mg/kg was associated with a reduction in the mean thickness of the hippocampus. The results from the study demonstrated that morphometric measurements of various regions of the brain were sensitive enough to detect changes in brain architecture following administration of a known neurotoxicant.

115 CHRONIC EXPOSURE TO DEPLETED URANIUM (DU) INCREASES HIPPOCAMPAL GLUTAMATE (GLU) RELEASE AND RECEPTOR DENSITY IN RATS.

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Studies utilizing acute exposure to uranyl ion in vitro have demonstrated an inhibition of hippocampal GLU release with an IC₅₀ ~ 2 μ M. This study was performed to determine if chronic DU exposure in vivo disrupts K⁺-stimulated GLU release and activation of the NMDA receptor channel. Male rats received 0, 300, or 600 mg of DU implanted i.m. at 70-90 days of age. Animals were sacrificed after 15-17 months exposure, 400 μ m thick hippocampal slices prepared, and superfused with 10 mM HEPES-sucrose buffer, pH 7.4. Endogenous release was stimulated with 50 mM K⁺, and collected fractions quantified by liquid chromatography. For receptor studies a crude membrane fraction was prepared by homogenization of hippocampal tissue in 50 mM Tris acetate (pH 7.4) followed by multiple washing steps. Membrane aliquots were incubated with 25 nM tritiated MK-801 and 8 concentrations of unlabeled MK-801 in the range of 1-1000 nM. The incubation occurred in the presence of GLU and glycine at 20 μ M. Nonspecific binding was defined by 100 μ M MK-801. In contrast to the effects of acute uranyl ion exposure, chronic DU produced an increase in the response of GLU release compared to slices from control animals; the effect was most apparent at the 600 mg DU dose, but also could be seen in the 300 mg group. The response under Ca²⁺-free perfusion conditions exhibited a smaller increase in GLU after high K⁺ relative to controls at the high DU exposure level. In addition chronic DU exposure resulted in an up-regulation of high affinity hippocampal NMDA receptors by more than doubling receptor density in both the 300 and 600 mg dose groups compared to control values. The enhancement in stimulated GLU release may be due to uptake of DU into astrocytes, disruption of mitochondrial function, and consequent inhibition of GLU uptake, similar to actions reported for MeHg. Further studies are required to determine whether the NMDA up-regulation reflects a direct effect on the receptor or a compensatory response to presynaptic changes. (Supported by USAMRMC DAMD17-02-1-0212)

116 ENVIRONMENTAL ESTROGENIC COMPOUND BISPHENOL A INDUCED ROS SIGNALING MOLECULES AND THEIR ADVERSE ROLE IN BRAIN DEVELOPMENT.

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Bisphenol A (BPA) is an estrogenic chemical, and humans are usually exposed through food and beverage containers, dental composites and various work place and domestic products. Bisphenol A perturbs the development of the neocortex and possesses the capacity to alter important events during critical periods of brain development. However, the mechanism by which BPA alters brain development and function is thus far unclear.

In this study, we have used normal human brain cells (HCN-1A) as a cell model for studying the effects of environmental estrogen on the brain. Low doses (environmental exposure levels) of BPA and 17- β -Estradiol (E2) were used for this study. The level of ROS was measured using 2',7'-Dichlorodihydrofluorescein diacetate (DCFH) assay while DNA synthesis was measured by means of Bromodeoxyuridine (BrdU) assay. Environmental concentrations of bisphenol A (1 pg - 100 ng/ml) triggered a dose-dependent and rapid production of intracellular ROS ranging from a one- to several-fold increase in HCN-1A cells. The formation of ROS continued beyond 2hrs. E2 also increased ROS formation dose dependently. Low doses of BPA induced ROS formation while high doses inhibited it. BPA at 100pg/ml increased DNA synthesis by 40 percent. Recently, we showed that estrogen-induced ROS are signal transduction messengers. Our data therefore allude to the role of environmental estrogen mediated ROS signaling in the perturbation of brain development. The biological significance of our findings from this study provide the basis for the development of novel antioxidant-based drugs or gene therapy targeted for the prevention and treatment of defects in brain development.

117 THE PENALIZED OPTIMAL EXPERIMENTAL DESIGN: THE PRECISE ESTIMATION OF AN INTERACTION THRESHOLD IN A MIXTURE OF EIGHTEEN POLYHALOGENATED AROMATIC HYDROCARBONS.

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Crofton et al. (EHP, 2005) conducted a study of 18 polyhalogenated aromatic hydrocarbons (PHAHs) on serum total thyroxine (T₄). Young female Long-Evans rats were dosed with the 18 single agents or a fixed-ratio mixture, and serum total T₄ was measured via radioimmunoassay. The initial analysis found significant interaction among the chemicals, with evidence of synergy at high doses (Crofton et al., 2005). To address the subsequent question of dose-dependency, Gennings et al. (JABES, 2006) fit an interaction threshold model to the data. The resulting estimate of the interaction threshold was positive and within the observed dose region, however, the corresponding confidence interval was wide and included zero. In order to more precisely estimate the location of the interaction threshold, a Ds-optimal second stage design was determined, with the objective of minimizing the variance of the hypothesized interaction threshold. Substantial concerns regarding the practicality of this design led to the use of the penalized optimality criterion (Parker and Gennings, JABES, under review), which uses desirability functions to penalize impractical experimental designs. Results demonstrate that, while the Ds-optimal second stage design can be used to more precisely define the interaction threshold, the penalized Ds-optimal second stage design has the characteristics deemed important in practice, while allowing only a minimal increase in the variance of the interaction threshold. (*This abstract does not necessarily represent EPA policies.*)

118 EFFECTS OF 2, 3, 7, 8-TETRACHLORDIBENZO-P-DIOXIN (TCDD) ON GENE EXPRESSION IN THE RAT PITUITARY GLAND AND PITUITARY-DERIVED GH3 CELLS.

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Exposure to the dioxin, TCDD, has been shown to cause reproductive dysfunctions. The effects of dioxin are mediated by the ligand-activate arylhydrocarbon receptor (AhR), a basic helix-loop-helix PAS transcription factor. The AhR regulates transcription of target genes and also exerts crosstalk with a number of other nuclear signaling pathways, particularly with estrogen receptor signaling. TCDD likely acts throughout the reproductive axis, but exposure to AhR ligands affects plasma levels gonadotropins and prolactin, suggesting that the anterior pituitary gland is a target. Previously we used cDNA microarray analysis of pituitary glands and found that the prolactin gene, as well as the glycoprotein hormone alpha subunit gene, are possible targets of both TCDD and estradiol (E2). In the present

study, we verified these findings with real-time PCR in vivo. We showed that TCDD had no effect on Prl gene expression, but it interfered with E2 upregulation of the gene. In contrast, TCDD upregulated expression of the alpha subunit gene, but E2 downregulated expression of this gene. Interestingly, E2 interfered with the ability of TCDD to upregulate the alpha subunit gene. We obtained similar findings when we examined the effects of E2 and TCDD on LH beta subunit gene expression. We also showed that E2 interfered with the ability of TCDD to induce the cyp1A1, but not cyp1B1. Subsequently, we used dual-luciferase assays and pituitary-derived GH3 cells to show that TCDD interfered with E2-induced upregulation of the prolactin promoter. Mutation of the putative DRE sequence did not affect the ability of TCDD to decrease E2-induced Prl promoter activity, except at low (0.1 nM) and high (100 nM) doses. These findings show that TCDD and E2 show crosstalk in the regulation of pituitary hormones important for reproductive functions, but the molecular mechanisms remain to be determined. Funded by NIH grants ES08774 and ES013885 to SLP

119 EFFECTS OF ATRAZINE (ATR) AND METABOLITE, DIAMINO-S-CHLOROTRIAZINE (DACT), ON THE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS IN RATS.

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Previously, we reported that a single dose of atrazine (ATR) caused an immediate release of adrenocorticotrophic hormone (ACTH) in male Wistar rats. These dose and time-dependant changes in ACTH were correlated with increased serum corticosterone (C) and progesterone (P), and indicated that ATR may have a direct effect on the brain and/or pituitary. Here, we continue to evaluate the effects of ATR and the primary metabolite, DACT, on the HPA axis. Sixty-day-old male rats were acclimated to dosing with vehicle (methylcellulose) for 7 days by gavage. On day 8, the rats were divided into groups of 10, given a single dose of DACT (0, 3.37, 33.7, 67.5, 135 mg/kg) by gavage at 0900 (12/12 light cycle, on @ 0700) and killed by decapitation 5, 15, 30, or 60 min later. Plasma ACTH and serum steroids were measured by radioimmunoassay. Doses of DACT were equimolar to those used in our previous study with ATR (0, 5, 50, 100, 200 mg/kg). Another group, dosed with ATR (200 mg/kg), served as a replicate of our previous study with ATR and enabled the direct comparison of ATR and DACT. ATR significantly increased plasma ACTH by 2.6, 3.9, 3.9-fold at 15, 30 and 60 min, respectively. Serum progesterone (P) and corticosterone (C) were also increased after ATR exposure. However, while DACT (33.7 and 135 mg/kg) increased ACTH at 30 and 60 min. by 1.3-fold, this increase was not entirely dose responsive and was moderate when compared with the response to an equimolar dose of ATR. Small increases in P and C were also observed for animals dosed with DACT (135 mg/kg) at 30 and 60 min. Thus, this study shows that while both ATR and DACT can elevate ACTH and adrenal steroids, the parent chlorotriazine herbicide, ATR, may be more active when compared to equimolar doses of DACT. Additional studies are ongoing to identify the primary target site for these effects, and to determine the role that adrenal steroids may play in mediating ATR-induced adverse effects on the reproductive system. This abstract does not necessarily reflect EPA policy.

120 ASSESSMENT OF THE GRAVIMETRIC, HISTOPATHOLOGICAL AND PROTEOMIC CHANGES INDUCED BY THE WEAK ANTI-ANDROGENS DDE AND LINURON, IN THE TESTOSTERONE-PROPIONATE STIMULATED WEANLING RAT.

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The rapid in vivo identification of anti-androgens generally relies on the conduct of the Hershberger assay, which monitors weight changes of androgen-sensitive tissues following compound exposure for 10 days. However, as the assay requires the use of castrated male rats, the OECD is currently investigating the testosterone-propionate (TP) stimulated weanling rat as a suitable alternative. As part of this validation programme, we investigated the activity of two weak anti-androgens, DDE and Linuron using a range of dose levels and included the potent anti-androgen, Flutamide (FLUT) as a positive control. In addition to gravimetric measurements, histopathological assessment of the testes, seminal vesicles and prostate of vehicle control animals, TP-stimulated animals and TP-stimulated animals co-exposed to 160 mg/kg/d DDE, 100mg/kg/d Linuron or 3mg/kg/d FLUT was conducted. The proteomic profile of the prostates of these same animals was also investigated. Our gravimetric data confirmed the weak anti-androgenic activity of DDE and Linuron previously recorded in the classic Hershberger assay. A strong correlation between the weight changes and the sexual maturity of the tissue, based on the histopatho-

logical findings, was recorded. Finally, a novel protein thought to be associated with apoptosis, L-amino acid oxidase (LAO), was up-regulated in the TP-stimulated weanling prostate by DDE and Linuron. As this protein was recently identified in adult rat prostate following exposure to the anti-androgens, FLUT and Finasteride, it is possible that LAO could be used as a biomarker for anti-androgenic effects. In conclusion, the TP-stimulated weanling rat appears to be a suitable animal model to use in the Hershberger assay. Our investigations also indicate that the assay has the potential to provide additional information on the MOA of a compound if required.

121 EFFECTS OF LOW CONCENTRATIONS OF ESTROGENS AND THEIR METABOLITES ON THE CACO-2 CELL LINE.

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Through our daily diet, humans are continuously exposed to low concentrations of estrogens, their metabolites and compounds that might act as estrogens. Exposure to these compounds has been associated with higher incidences of fertility problems and hormone-dependent cancers. A scientific controversy exists with respect to effects of estrogens on colonic cells in either stimulating cell proliferation or apoptosis. ER- β is predominant in human colon and mediates apoptosis, whereas ER- α , which mediates proliferation, is less present in colon cells. Based on ER- β occurrence in colonic cells, apoptosis would be expected upon exposure to estrogens. Cytochrome-P450-1A1/1B1 are present in colonic tissue and formation of estrogen metabolites is therefore likely. Presently, we study effects of estrogens and their metabolites on Caco-2 colon cells. The potent phytoestrogen 8-prenyl naringenin is also included in our experiments. Possible proliferating effects of 17 β -estradiol (E2) and its potentially genotoxic 4-OH metabolite were assessed with the MTT assay. E2 or 4-OH-E2 did not induce cell proliferation in the range of 0.01 nM-100nM.

Preliminary data from the Apo-ONE apoptosis assay (Promega) showed a minor induction of apoptosis by E2 and 4-OH-E2 at concentrations higher than 10 nM. Similar experiments are ongoing with other estrogenic compounds and estrogen hydroxy- and methoxy-metabolites. So far, these data suggest that low concentrations of dietary estrogens appear not to contribute to colon cancer risk.

122 EFFECTS OF MARINE ORGANISM-DERIVED INDIRUBINS ON PROSTATE-SPECIFIC ANTIGEN (PSA) EXPRESSION AND CELL PROLIFERATION IN LNCAP HUMAN PROSTATE CANCER CELLS.

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Certain indirubins and related compounds are potent cyclin-dependent kinase (CDK) inhibitors and may be effective in the treatment of various cancers. We tested the effects of olomoucine, roscovitine, indirubin-3'-oxime (I-3'-M), 6-Br-I-3'-M, aminopurvalanol and aloisin A on cell proliferation and PSA expression in androgen-dependent LNCaP human prostate cancer cells. The overall goal was to identify structural derivatives with antiandrogenic properties that may be suitable candidates for the treatment of hormone-dependent prostate cancer. LNCaP cells were exposed to the test compounds (1.0 and 10 μ M in steroid-free serum containing 0.1% DMSO) for 5 days in the presence or absence of DHT (0.3 nM) after which cell proliferation was assessed by MTT reduction assay. PSA expression (mRNA) was determined by semi-quantitative RT-PCR after a 24 h exposure. Olomoucine did not affect DHT-induced cell proliferation but it increased basal cell proliferation at 1.0 μ M. At 1.0 and 10.0 μ M, basal PSA expression was statistically significantly increased; DHT-induced PSA expression, on the other hand, was decreased to basal levels. Roscovitine had no effects on DHT-induced cell proliferation, but had a biphasic effect on basal cell proliferation with an increase at 1.0 and decrease at 10 μ M. Roscovitine (10 μ M) significantly reduced basal and DHT-stimulated PSA expression. Indirubin-3'-monoxime concentration-dependently stimulated basal and DHT-induced LNCaP cell growth. Its 6-Br-analog stimulated basal and DHT-induced cell proliferation at 1.0, but strongly reduced it at 10 μ M. Aminopurvalanol produced a similar biphasic response. Aloisin A had no effect on basal or DHT-stimulated cell growth at 1.0 μ M, but reduced DHT-induced proliferation to basal levels at 10.0 μ M. Our results indicate that certain CDK inhibitors exhibit pro- and/or antiandrogenic effects in LNCaP cells and are interesting candidates for more detailed studies concerning their effectiveness against hormone-dependent prostate cancer.

123 MASPIN EXPRESSION IN LNCAP CELLS AS MARKER FOR CHEMOPREVENTIVE ACTIONS BY PHYTOCHEMICALS.

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Curcumin, the active ingredient of the Indian curry spice turmeric, is often studied as anticancer agent for example against prostate cancer. In this study, the effects of curcumin on maspin expression were determined in human prostate cancer cell line LNCaP. Maspin expression is negatively correlated with tumor suppression in breast and prostate cancers. Although the mechanism is not clear, maspin is suggested to inhibit tumor-induced angiogenesis and sensitize tumor cells to undergo apoptosis.

Incubation of LNCaP cells with curcumin (10 microM) increased maspin mRNA expression, while DHT (dihydrotestosterone, 10 nM) decreased maspin expression. Since curcumin actions appear to be mostly mediated by interactions with PPAR-gamma (peroxisome proliferator-activated receptor gamma), effects on PPAR-gamma mRNA levels were also determined. Incubation with curcumin resulted in a slight increase of PPAR-gamma mRNA, while DHT decreased PPAR-gamma expression. These results concur with the hypothesis that curcumin acts as anticancer agent in prostate cancer cells and the clinical findings that androgen withdrawal increases maspin expression, which results in better prognosis for prostate cancer. Effects of other suggested chemopreventive compounds sulforaphane and lycopene on maspin and PPAR-gamma in prostate cancer cells are currently under investigation in our laboratory.

124 CIMICIFUGA EXTRACT FROM BLACK COHOSH IS NO PHYTOESTROGEN.

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Aromatase catalyses the six electron transfer to form the estrogens estrone and estradiol from androstenedione, testosterone and other endogenous precursors. We have developed a product based assay for aromatase activity allowing to separate and quantify individual aromatase products i.e. estrone and estradiol, as well as other possible microsomal metabolites like estradiol sulfate. Placental microsomes formed only estradiol and estrone with a dose dependent ratio of 3 : 1 to 5 : 1 in favor of estradiol. Extracts from plants like Cimicifuga, also known as black cohosh, have long been used to treat menopausal symptoms like hot flashes and postmenopausal osteoporosis. Since these symptoms can also be treated by estrogen supplementation cimicifuga extracts have been assumed to act as phytoestrogens, possibly by modulating estrogen metabolism. In preliminary experiments we found a decrease in estradiol but not in estrone formation at high concentrations. However, dose dependent addition of cimicifuga extract showed no effect on aromatase activity in lower concentrations comparable to those that may be achieved by therapeutic doses. The shift in the product ratio at high concentrations may result from an interaction of cimicifuga extracts with 3-hydroxysteroid dehydrogenase catalysing the product equilibrium. We found no evidence to support a proestrogenic activity of cimicifuga extracts by modulation of estrogen formation; in cell culture experiments cimicifuga extracts possessed antiestrogenic activity which may be explained by the observed shift to the hormonally inactive estrone. Therefore we conclude that cimicifuga extracts cannot be considered phytoestrogenic; its therapeutic effects on perimenopausal symptoms appear to be due to central nervous effects.

125 USE OF THE H295R CELL TO EVALUATE THE EFFECT OF ENVIRONMENTAL COMPOUNDS ON STEROID HORMONE PRODUCTION.

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H295R cells constitute a pluripotent cell line that has retained the enzymatic ability to produce steroids along the entire steroidogenic pathway, including C19 androgens and C18 estrogens. For this reason, they have been a valued research tool, and have been employed in an ever-increasing number of studies. The US EPA is interested in the utility of these cells as an *in vitro* screen for environmental compounds which may have direct effects on steroidogenesis. This study examined the effects of selected chemicals on the production of progesterone, androstenedione, testosterone, estradiol, estrone and corticosterone by these cells *in vitro*. After a 48h incubation period, the concentration of each hormone was readily detectable in the medium. Using a 24-well plate format with 200,000 cells per well, it was possible to measure by RIA concurrently up to 4 - 5 hormones per well in a single incubation.

Exposure of the cells to chemicals known to modify steroidogenesis affected concentrations of the appropriate hormones as expected. For example, forskolin (which increases cyclic AMP activity) induced a dose-dependent increase of each hormone examined. In contrast, incubation with aminoglutethimide (which inhibits multiple P450s) caused a dose-dependent decrease in the production of several hormones. As part of this work, cells were also examined for any potential effects of compound application on cell viability. These data support the usefulness of this approach as a screen for toxicant effects on steroid hormone production and can also provide valuable insight into sites of impact along the steroidogenic pathway. (This abstract does not necessarily reflect EPA policy.)

126 SOY ISOFLAVONE AND ORAL CONTRACEPTIVE EFFECTS ON ESTROGEN METABOLISM.

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Estrogen metabolism may play an important role in mammary carcinogenesis in postmenopausal women. We evaluated the long-term effects of prior oral contraceptive (OC) use and soy isoflavones (IF) on endogenous estrogen metabolism in a primate model. One hundred eighty-one female cynomolgus monkeys were randomized to receive OC or placebo for 28 months premenopausally, then ovariectomized and randomized to either an IF-depleted soy protein isolate (SPI-) diet or a diet containing soy protein isolate with a human equivalent of 129 mg IF/day (SPI+) for 36 months. At month 35 of the postmenopausal phase overnight urine samples were collected. Reversed-phase HPLC-ESI-MS/MS was used to measure the concentrations of 15 estrogens including estrone (E₁), estradiol (E₂), and 2-, 4-, and 16 α -OHE₁/E₂ catabolites. Overall prior OC treatment was associated with lower urinary 2-OHE₁, 16 α -OHE₁, 4-OHE₁, E₁, E₂, and total estrogens, although those differences were only statistically significant in the animals who were fed an IF free diet postmenopausally (p<0.05 for each). Over all animals isoflavone consumption resulted in no significant changes in the aforementioned metabolites. However, an IF+ diet in monkeys receiving premenopausal placebo was associated with a significant increase in the ratio of urinary 2-OH/4-OHE₁ (p<0.001), with 45% higher levels of 2-OHE₁ (p=0.2) and 48% lower 4-OHE₁ (p=0.1). IF consumption in OC-treated monkeys resulted in 121% higher 16 α -OHE₁ (p<0.001) compared to 22% higher levels in the placebo-treated IF fed group (p=0.4). Thus: (1) premenopausal OCs may have long term lowering effects on estrogen excretion postmenopausally; (2) a postmenopausal diet that is high in soy isoflavones may modulate the urinary estrogen metabolite profile towards a less carcinogenic profile; (3) prior OC use may alter postmenopausal estrogen metabolism pathways challenged by phytoestrogens. These findings suggest that OC use may modulate a woman's response to postmenopausal therapies.

127 THE COMPARATIVE SENSITIVITY OF THE CD-1 MOUSE, FISHER 344 RAT, AND THE CD SPRAGUE DAWLEY RAT TO THE PHYTOESTROGEN GENISTEIN.

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Dietary phytoestrogens can significantly alter the results of uterotrophic assays and vaginal opening (VO) endpoints in animals used to evaluate the estrogenic activity of endocrine disruptor compounds (EDC). Species and strains differ in their sensitivity to the phytoestrogen genistein (G). The objective of the study was to compare the sensitivity of the CD-1 mouse, Fisher 344 (F344) rat, and the CD Sprague Dawley (SD) rat to genistein. Dams with standardized litters of 10 female pups were housed in a polycarbonate microisolator cage containing autoclaved hardwood bedding. The dams were fed the Zeigler 5412-01 phytoestrogen reduced diet containing <10 μ g daidzein (D) and (G)/g diet and given RO/DI water ad libitum. Mouse pups were weaned at post-natal day (PND) 15 and rat pups were weaned at PND 19 and randomly assigned to cages. The weaned pups were fed the AIN-76A diet spiked with genistein at 0, 150, 300, or 450 μ g/g diet. Animals were observed daily and the time of VO was recorded daily. Body weights were determined at weaning, at weekly intervals, and at the time of VO. The CD-1 mice showed significant (p<0.05) differences in the mean time of VO between the control and all treatment groups. The F344 rat showed significant differences in the mean time of VO between the control and the 300 and 400 μ g/g diet groups. The SD rat showed significant differences in the mean time of VO only at the 450 μ g/g diet level. Significant differences in body weights at weaning and at all other time points were observed between the F344 and SD rat. It was concluded that: 1) the SD rat was less sensitive to (G) than the CD-1 mouse or the F344 rat, 2) the estrogenic effects were not due to total metabolizable energy, 3) species and strains significantly differ in their sensitivity to genistein, and 4) a phytoestrogen reduced diet (<10 μ g D&G/g diet) and the strain used should be stated in VO assays evaluating the hormonal activity of EDCs.

128 VARIATIONS IN PHYTOESTROGEN CONTENT BETWEEN DIFFERENT MILL DATES OF THE SAME DIET PRODUCES SIGNIFICANT DIFFERENCES IN THE TIME OF VAGINAL OPENING IN F344 RATS BUT NOT IN CD SPRAGUE DAWLEY RATS.

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The selection of the animal species, strain and optimum diet for conducting uterotrophic assays for the evaluation of the hormonal activity of Endocrine Disruptor Compounds (EDC) is a subject of current concern. This report describes the effects of a fourfold variation of the phytoestrogen content of three different mill dates of the PMI #5002 diet on the time of vaginal opening (VO) in F344 rats and Sprague Dawley (SD) rats. Dams with standardized litters of 10 female pups were fed the Zeigler phytoestrogen-reduced diet II containing < 10 µg daidzein and genistein (D&G)/g diet and given RO/DI water ad libitum. Rat pups were weaned at post-natal day (PND) 19 and were randomly assigned to the PMI #5002 test diets or control PMI #5K96 phytoestrogen-reduced diet (at least 16 pups/diet). The time of VO was recorded daily from PND 19 to time of VO. Body weights were determined at weaning, at time of VO and at weekly intervals until PND 46. Different mill dates of the same diet produced significant ($p < 0.05$) differences in the time of VO in F344 rats. In contrast, no significant differences were observed in the time of VO in SD rats fed different mill dates of the same diet even though the concentration of the D&G in the plasma of SD rats was twice as high as the plasma concentration in the F344 rat. Significant differences were observed in the body weight at PND 19, at time of VO, and at all time points between the two species. It was concluded that 1) the F344 rat was more sensitive to D&G than the SD rat; 2) the variation in the D&G content in different mill dates of the same diet produced significant ($P < 0.05$) differences in the time of VO in F344 rats, but not in SD rats; 3) a standardized open formula phytoestrogen reduced diet (<10µg of D&G/g diet) containing ~ 3.1 Kcal/g diet should be considered for studies evaluating the hormonal activity of EDCs.

129 BATCH TO BATCH VARIABILITY IN ESTROGENIC ACTIVITY IN COMMERCIAL ANIMAL FEED: IMPORTANCE AND IMPLICATIONS FOR LABORATORY ANIMAL RESEARCH.

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Significant undefined batch-to-batch variability in estrogenic components of commercial rodent feeds can influence phenotype. To address this issue, the NIEHS and the NIH Office of Dietary Supplements convened two workshops. The first determined the extent of the problem from the scientist's perspective and the second addressed solutions with feed manufacturers. Workshop discussions documented that batch to batch variability in estrogenicity of diets is responsible for failures to replicate results both within and between laboratories. In addition, variability in estrogenicity of animal diets will require a larger number of laboratory animals for adequate bio-statistical power. This will increase the cost of scientific research and significantly influence estrogenic effects of diets on positive controls, species, strain and life stage differences. Investigators will benefit from the availability of commercial soy- and casein-based animal diets for which manufacturers have identified the components responsible for the variability in estrogenic activity and applied appropriate manufacturing quality control procedures to assure control of the variability between batches of the feeds. Documentation of the estrogenic activity in these diets by chemical analysis coupled with bioassays of batch to batch estrogenic composition and activity can effectively meet this need. In summary, scientists should be aware of the implications of undefined phytoestrogens and other estrogenic components in the rodent diets used, to document the estrogenic content of the diets used by reporting the identity and details of diet composition and mill dates in publications, by using positive controls in every experiment, and by keeping historical samples of diets for future analyses.

130 INTERACTION OF RETINOID X RECEPTOR WITH ENVIRONMENTAL CHEMICALS.

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The retinoid X receptor (RXR) is a pleiotropic transcription factor that functions both independently and in concert with other nuclear receptors. RXR is highly conserved among the metazoa and regulates a variety of activities critical to normal physiology. Despite its important roles in regulating aspects of development, growth, and reproduction, little is known of the susceptibility of this receptor to

xeno-agonists or antagonists. We have undertaken the cloning, expression, and characterization of RXR isolated from the water flea (*Daphnia magna*) in an effort to provide a definitive in vitro assessment of the interaction of this receptor with environmental chemicals and establish in vivo consequences of such chemical-receptor interactions. The full length cDNA of the daphnid RXR was cloned by RACE yielding a 1888 bp product that coded for a 400 amino acid protein. The RXR protein shared significant identity with RXR from other species. Expression profiling revealed that daphnid RXR mRNA levels were greatest in embryos that were early in development and progressively declined through the five stages of embryo development. Adult females expressed higher levels of RXR mRNA than did males and exposure of females to the terpenoid hormone mimic pyriproxyfen reduced RXR mRNA in females to levels approaching males. Presently, recombinant RXR protein is being expressed for the assessment of xenobiotics-binding interactions with the receptor. Candidate xeno-ligands then will be evaluated for effects on development, growth, and reproduction in daphnids. Preliminary data suggests that some insect growth regulating insecticides activate RXR with consequences on sex determination of offspring.

131 EFFECTS OF BISPHENOL A AND CADMIUM ON GENOMIC AND NON-GENOMIC ACTIONS OF THE ESTROGEN RECEPTOR.

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Various hormonal and environmental estrogens activate the estrogen receptor (ER) and stimulate cell proliferation. The ER is a member of the nuclear steroid receptor family, and mediates the regulation of numerous estrogen-responsive genes such as the progesterone receptor (PgR). Recent evidence supports the existence of a plasma membrane ER, which, upon ligand binding activates various second messenger and protein kinase signaling pathways such as mitogen-activated protein kinase (MAPK) pathways. In this study, we investigated two environmental compounds, bisphenol A and cadmium, both of which had been shown to bind ER, on their abilities to activate the genomic (through nuclear ER) and non-genomic (through membrane ER) actions, as well as their abilities to stimulate breast cancer cell proliferation. ER positive T47-D breast cancer cells were cultured in stripped serum for 5 days before treatment with different concentrations of bisphenol A or cadmium chloride. Cells were harvested after 6 days for a proliferation assay, or at different time points for determination of a) PgR mRNA levels by real time RT-PCR, b) ERK1/2-MAPK phosphorylation by western blot. Our results showed that Bisphenol A increased PgR mRNA level by 18 hours, and 100 nM bisphenol A induced maximal transcriptional activation. No significant ($p > 0.05$) PgR up-regulation was induced by cadmium, even at 10 µM. Different from their effects on genomic action, 1 nM concentrations of bisphenol A and cadmium activated ERK1/2-MAPK phosphorylation as early as 5 minutes. Bisphenol A and cadmium displayed different capability to modulate T47-D cell proliferation. The former increased cell proliferation at 10 nM, while the later did not, even at a concentration as high as 1 µM. These findings suggest that different ER ligands show different patterns of ER activation, and that genomic and non-genomic pathways of ER signaling may play different roles in breast cancer cell proliferation. Supported by Grants CA77550 and P30 ES 03819.

132 EVALUATION OF AN IN VITRO PRE-SCREENING STRATEGY TO PRIORITIZE ENVIRONMENTAL CHEMICALS FOR FURTHER TESTING: ANDROGEN AND ESTROGEN RECEPTOR MEDIATED ACTIVITY.

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This project evaluated an in vitro pre-screening strategy to identify chemicals that may act as androgen or estrogen receptor agonists or antagonists and prioritize them for further testing as potential endocrine disrupting compounds. Specifically, the approach was to examine the interaction of 117 chemicals with the androgen (AR) and estrogen receptor (ER) with in vitro cell-based transcriptional activation (TA) assays using the MDA-kb2 and T47D-KBluc cell lines, respectively. The chemicals identified as positive in TA assays were then examined for competitive receptor binding to confirm their ability to act via that receptor. Solubility and cytotoxicity assessments were also performed on all chemicals. Initially, seventeen compounds with varying, but well-established, affinities for either AR or ER were evaluated in cell-based TA and receptor binding assays to establish the methods and to demonstrate satisfactory performance of those methods. Then fifty chemicals were chosen that were primarily from the ICCVAM list of Proposed Substances and Selection Criteria for Assay Validation. Finally, fifty unknowns were evaluated in the final phase of the work. Taken together, the results of this project demonstrated the feasibility of this approach for chemical prioritization and identified several important issues that must be considered in evaluating in vitro data. In addition, these methods have the potential to be used in high through-put screening.

Disclaimer: This abstract does not necessarily reflect US EPA policy.

133 COMPOUNDS OF DIFFERENT CHEMICAL CLASSES ELICIT SUPRAMAXIMAL RESPONSES IN IN-VITRO ANDROGEN AND ESTROGEN RECEPTOR MEDIATED ACTIVITY ASSAYS.

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In vitro assays designed to identify estrogen and androgen activity were evaluated as an efficient high throughput method for screening a wide range of environmentally significant chemicals for endocrine activity. In the present study more than 120 chemicals were tested for androgen (AR) and estrogen receptor (ER) mediated activity using a cell-based transcriptional activation (TA) model consisting of MDA-kb2 and T47D-KBluc cell lines, respectively combined with competitive receptor binding assays. Receptor binding assays used a human recombinant receptor in combination with polarographic fluorescence detection. Chemicals that were positive in the TA assay were tested in the receptor binding assay as a confirmation of the chemical's ability to elicit response via ER or AR. Solubility and cytotoxicity assessments were also performed. Several of these compounds, which included cadmium chloride, genistein, benzoic acid, 2-hydroxy-, 4-(1,1-dimethylethyl)phenyl ester, benzyl salicylate, 1,3-benzenedicarboxylic acid, and diphenyl ester produced a supramaximal response in both assays with response values ranging from 0.5 to 3-fold greater than that obtained from endogenous high affinity ligands, such as estradiol. This supramaximal response was most pronounced when the compound was co-incubated with the reference agonist (Estradiol or DHT). One exception to this occurred with genistein which caused a 2- to 3- fold increase both with and without agonist co-incubation. Supramaximal responses have been observed by others in reporter gene assays. Possible explanations for this phenomenon, depending on the chemical class, could be ligand-dependent differences in the ability of receptor to bind co-activators or interaction of ions with transcription factors. Further study in light of these observations could help elucidate the mechanism of this phenomenon in transactivation models.

134 ESTROGEN RECEPTOR ACTION: GLOBAL GENE EXPRESSION PROFILING IN HUMAN ISHIKAWA CELLS WITH DISTINCT ESTROGEN RECEPTOR EXPRESSION REVEAL DISTINCT ESTROGEN PATHWAYS.

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Estrogens regulate diverse physiological processes in various tissues including the mammary gland and reproductive organs. Estrogenic effects are mediated by the estrogen receptors belonging to the superfamily of ligand-inducible transcription factors. We analyzed cellular responses to estrogens at the gene expression level in a time and dose-dependent manner. For that, global gene expression profiling (Illumina® Sentrix® BeadArray) was done in Ishikawa cells expressing endogenous estrogen receptor (ER) alpha (Ishikawa-plus) or lacking ER (Ishikawa-minus). Cells were treated with 17β-estradiol (E2) and a combination of E2 and the anti-estrogen ICI 182,780 (ICI) and harvested after 2, 24 and 72 hours. Principle component analysis (PCA) showed that the two cell lines accumulate in two well-defined, separate clusters, confirming their different properties in gene expression. We were able to identify more than 1300 significantly deregulated genes in response to E2 in Ishikawa-plus with a clear time-dependent pattern. The deregulation of the majority of these genes was suppressed by ICI, indicating their ER-dependent modulation. In contrast, only 374 genes were deregulated in Ishikawa-minus. Interestingly, eight genes were deregulated in both Ishikawa-plus and -minus and showed diametric directions of deregulation. These eight genes are therefore putative marker genes to discriminate ER-dependent from ER-independent signals. Overall, these results show that Ishikawa cell lines with distinct ER expression provide a suitable model system to screen for and to analyze estrogenic effects with respect to ER-dependent or ER-independent signalling pathways.

135 FOOD ASSOCIATED ESTROGENIC COMPOUNDS INDUCE ER-MEDIATED LUCIFERASE GENE EXPRESSION IN TRANSGENIC ER-LUC MALE MICE.

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The current research project reveals the in vivo estrogenic potency of seven in vitro estrogenic food associated compounds in ER-luc male reporter gene mice.

Estradiol-propionate (EP, positive control) was dosed at 0.3 and 1 mg/kg body-weight (bw), bisphenol A (BPA) or nonylphenol (NP) at 10 and 50 mg/kg bw, p,p'-DDE at 5 and 25 mg/kg bw, quercetin at 1.66 and 16.6 mg/kg bw, and diisooheptyl phthalate (DIHP), di (2-ethylhexyl) phthalate (DEHP) or di (2-ethylhexyl) adipate (DEHA) all at 30 and 100 mg/kg bw. DMSO was used as the solvent control. Generally, IP dosing caused higher inductions after 8 hours compared to oral dosing after 14 hours, except in the liver where the responses were comparable. Maximum induction was reached up to 20,000 times in the liver at 1 mg EP/kg bw. DDE, NP, DEHA and DIHP did not induce luciferase in any tissue. BPA induced luciferase in the liver via both exposure routes and after IP exposure in the testis, kidneys and tibia at 50 mg/kg bw. Orally dosed 100 mg DEHP/kg bw, induced luciferase in the liver 3-fold but reduced luciferase in the testis. Orally dosed 16.6 mg quercetin/kg bw induced luciferase 1.4 fold in the liver.

The results of this study show that food associated compounds can be estrogenic in vivo. Oral exposure generates lower estrogenic activity compared to IP exposure, thereby lowering the risks for orally ingested food associated estrogenic compounds. Luciferase levels were induced in livers of ER-luc male mice after exposure to BPA at levels 2300 times the TDI in humans. Based on these results no effects are expected upon average human exposure to estrogenic food associated compounds.

136 LONG-TERM ESTROGEN EXPOSURE (LTEE) UP-REGULATES ARYL HYDROCARBON (AH) RESPONSIVENESS IN MCF-7 BREAST CANCER CELLS.

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The long-term presence of estrogen is known to imprint gene expression patterns. In this study, the effects of LTEE on Ah responsiveness to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in MCF-7 cells were investigated. MCF-7 cells were continuously cultured in Dulbecco's-based medium containing 5% bovine calf serum (DC5 medium), which contains minimal estrogen, or DC5 medium supplemented with 0.01, 0.1 or 1.0 nM 17β-estradiol (E2). At passages 4 and 12, basal and TCDD-induced ethoxyresorufin O-deethylase activities (EROD) were determined, and samples from control and TCDD-induced (10 nM TCDD, 72 h) cultures were taken for isolation of total RNA, whole-cell extracts were prepared for Western immunoblot analysis of soluble proteins, and microsomal fractions were prepared for analysis of cytochrome P450 (CYP) expression. The results showed significant (P<0.01) elevations of basal and TCDD-induced EROD activities at the 0.01, 0.1 and 1.0 nM E2 levels. Western immunoblots showed up-regulation of Ah receptor protein expression and enhanced induction of CYP1A1 and CYP1B1 proteins by TCDD in LTEE compared with the long-term estrogen-deprived (LTED) cells maintained in DC5. Total RNA isolated from these cultures was subjected to reverse transcription and real-time polymerase chain reactions (PCR). These PCR results showed up-regulation of Ah receptor mRNA and enhancement of the induction of CYP1A1 and CYP1B1 mRNAs consistent with the Western blot and EROD activity measurements. These RNA samples were subjected to gene expression analysis on Affymetrix HG U133A 2.0 chips, and the data were analyzed for global changes in gene expression. The results revealed that LTEE enhanced TCDD-induced expression of a number of members of the Ah gene battery in MCF-7 cells. These studies suggest that LTEE, through the enhancement of Ah responsiveness, may ultimately affect the toxicity and carcinogenicity of environmental Ah receptor ligands and CYP1 substrates.

137 TIER-1 SCREENING CLASSIFICATION SYSTEM FOR ESTROGENIC EDCS USING THE LUMI-CELL® ER BIOASSAY.

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There is a growing need for a reliable tier-1 screening classification system for estrogenic endocrine disruptor chemicals (EDCs). The concern for EDCs arises from the detrimental effects on human and wildlife populations resulting from its bioaccumulation in the food chain. A classification system would help researchers and regulators determine which compounds pose the greatest potential threat to human and wildlife populations. Here we report a possible method for classification of xenoestrogenic EDCs based on dose response criteria using the LUMI-CELL® ER bioassay. Fifty compounds, which were on ICCVAM's priority list to validate an estrogen receptor transcriptional activation (ER TA) assay, were analyzed using the LUMI-CELL® ER bioassay. We evaluated gene induction data for all compounds to establish a classification scheme based on dose dependent response and induction efficacy. The data allowed chemicals to be divided into 4 classes depending on their EC50: Class IV (greater than 10⁻³M); Class III (10⁻³M to 10⁻⁷M); Class II (10⁻⁷M to 10⁻¹⁰M); and Class I (10⁻¹⁰M to 10⁻¹³M).

⁷M to 10⁻⁹M); and Class I (less than 10⁻⁹M). Compounds were further classified by the efficacy of their agonist ER TA response. Group D includes compounds with a maximal response of less than or equal to background. Group C includes compounds with a maximal response of greater than background, but less than 50% of the relative light unit (RLU) response for 17 β -estradiol (E2). Group B includes compounds with a maximal response of greater than 50% of the E2 response but less than 100% of the E2 response. Group A compounds include responses greater than 100% of the response of E2. This system of in vitro classification should help prioritize compounds for subsequent analysis in animal and cell systems and will help determine initial doses reducing animal use. Supported by NIEHS SBIR ES10533-03 and Superfund Basic Research Grant ES04699.

138 COMPARISON OF VISUAL INSPECTION TO CELLTITER-GLO[®] IN EVALUATING CYTOTOXICITY IN THE LUMI-CELL[®] ER BIOASSAY.

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There is a need to determine if a decreased response observed (for agonist and in particular antagonist testing) in an endocrine disruptor screening assay is a result of lower activity due to a chemical-receptor response or whether the compound is cytotoxic, resulting in a lower observed response. A comparison study was conducted in cooperation with NICEATM to compare cytotoxicity measured using the Visual Inspection assay with that obtained using Promega's CellTiter-Glo[®] assay which is a method of estimating viable cell number based on quantitation of ATP. Visual Inspection viability score codes were developed with: 1 = normal cell morphology and cell density, 2 = altered cell morphology and small gaps between cells, 3 = altered cell morphology and large gaps between cells and, 4 = few (or no) visible cells. Comparison of the Visual Inspection and CellTiter-Glo[®] assays demonstrate that, a score of 1 corresponded to greater than 80% viability, 2 corresponded to 80 – 60% viability, 3 corresponded to 60 – 40% viability and 4 corresponded to less than 40% viability. Eight coded compounds were selected to test for estrogenic activities and eight coded substances to test antagonist activities in the LUMI-CELL[®] ER assay, a cell based assay in which estrogenic chemicals induce firefly luciferase. The CellTiter-Glo[®] and Visual Inspection assays both were able to detect cytotoxicity, with a significant correlation between the two techniques for determining cytotoxicity. Accordingly, either of these methods may be useful in determining cytotoxicity of chemicals and extracts tested in the LUMI-CELL[®] ER assay, thereby eliminating cytotoxicity-dependent false positives. Supported by NIEHS Contract N01-ES-85424, NIEHS SBIR ES10533-03 and Superfund Basic Research Grant ES04699.

139 MIXTURES OF THYROID DISRUPTING CHEMICALS: TESTING ADDITIVITY OF HEPATIC INDUCERS AND THYROID PEROXIDASE INHIBITORS.

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Humans are exposed to chemical mixtures via diet, occupation, and the environment. Previous data demonstrated that low doses of polycyclic halogenated aromatic hydrocarbons (PHAHs) acting through similar mechanisms result in an additive reduction of thyroxine (T4). If xenobiotics acting through the same mechanism are able to disrupt thyroid hormone homeostasis, it is reasonable to expect that chemical mixtures acting through different mechanisms but targeting the same endpoint (i.e. serum thyroid hormone levels) would exert additive effects on T4. Along with PHAHs, which induce T4-eliminating hepatic enzymes, certain pesticides are shown to decrease production of T4 by inhibiting thyroid peroxidase in the thyroid gland. Female Long-Evans rats, 28 days of age, were dosed with a mixture of 18 PHAHs (2 dioxins, 4 dibenzofurans, and 12 PCBs, including dioxin-like and non-dioxin like PCBs) and a mixture of 3 pesticides (thiram, pronamide, and mancozeb) for 4 consecutive days. Stock solutions were prepared with concentrations of the PHAH mixture and each individual pesticide that individually produce a 20% decrease in circulating T4 levels. Six serial dilutions (66, 33, 10, 6.6, 3.3, and 1%) and a control were administered in the 4-day bioassay. Tissues were collected 24 hours after the last dose and serum total T4 was quantified via radioimmunoassay. Animals exposed to the four highest doses resulted in serum T4 decreases between 3 and 45% compared to control animals; the two lowest doses

showed a slight increase in circulating T4. Dose and response addition models fit to the data suggest that the combination of UGT-inducers and TPO inhibitors are antagonistic. The results of this research suggest that environmentally complex chemical mixtures interact in a complicated fashion to disrupt thyroid hormone homeostasis. (Funded by the EPA NHEERL/NCSU Cooperative Training Program in Environmental Sciences Research, CT826512010. This abstract does not represent USEPA policy).

140 BRAIN, LIVER, AND THYROID BIOMARKERS REFLECT ENHANCED SENSITIVITY OF THE DEVELOPING RAT TO THYROID HORMONE DEPLETION.

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Many developmental events are regulated at least in part by thyroid hormones. It was hypothesized that tissue biomarkers of thyroid status would be more accurate predictors of neurotoxicity than serum biomarkers in rats treated with the goitrogen propylthiouracil (PTU). Over several studies, timed pregnant rats were dosed 0, 0.3, 1, 3 or 10 ppm PTU solutions in drinking water from day 2 of gestation until weaning. Dams and pups were studied at 21-30 days after birth for thyroid status and hippocampal electrophysiology, focusing on synaptic potential and weak and strong long term potentiation (LTP). Brain tissue from animals dosed with 0, 1, 2, or 3 ppm PTU at the USEPA was studied. Cerebrocortical Type 2 5'-deiodinase (D2), which converts thyroxine(T4) into the metabolically active 3',3',5'-Triiodothyronine (T3), was found to be a very sensitive indicator of T4 depletion, and provided robust protection of brain T3 levels. Cortical T3 concentrations were reduced further in pups than in dams. Serum T3 concentrations were better protected than serum T4 concentrations. Serum T4 was more affected by doses >0.3ppm in PND4 and 14 pups than older animals. Serum T4 and cortical D2 activity correlated strongly, and results from two rat strains were nearly identical, strengthening the utility of D2 as a biomarker of thyroid disruption in the brain. In our first study, effects on synaptic potential in PND 21 pups showed a strong linear correlation to cerebrocortical D2(R=.75, p<.0001). In that study, synaptic potential decreased with increasing D2 and decreasing serum T4, and LTP was impaired in PND100 animals. Overall, exposure to PTU doses >0.3ppm had effects on at least serum T4 at PND4-21. This data will assist researchers developing a biologically based pharmacokinetic model of thyroid disruption in the developing rat. Funding was provided by USEPA STAR cooperative agreement #R832134. (Does not reflect USEPA policy)

141 EVALUATION OF THE REPRODUCTIVE TOXICITY OF A MIXTURE OF ANTI-ANDROGENIC COMPOUNDS IN FEMALE WISTAR RATS.

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Earlier studies have shown that anti-androgenic compounds elicit severe developmental effects on the male reproduction system and generate feminised male rats after perinatal exposure. However, the literature on effects of anti-androgenic compounds on female offspring is limited, and more research is needed in order to elucidate effects on endocrine endpoints in females. Furthermore, in vitro studies have revealed that several of these substances can have multiple effects on the endocrine system in addition to anti-androgenic effects, and only few studies in general have evaluated mixture effects of endocrine disruptors. The objective of this study was to evaluate whether prochloraz (PRO), vinclozolin (VIN), di-2-ethyl-hexyl-phthalate (DEHP) and finasteride (FIN) affected female offspring. Pregnant dams were given the test substances both singly and in a mixture at 'No Observed Adverse Effect Level' (PRO: 5 mg/kg, VIN: 5 mg/kg, DEHP: 3 mg/kg, FIN: 0.01 mg/kg) and 5 to 10 times higher doses. The dosages were given daily by gavage for a period of 31 days from gestation day 7 until postnatal day (PND) 16. Birth weights, anogenital distance (AGD), organ weights, ovarian steroidogenesis, uterine effects (histology, immunohistochemistry and gene expression) and hormone levels at PND6 and PND16 were evaluated. Furthermore onset of puberty (time of vaginal opening) and oestrous cyclicity were analyzed as end points for long-term effects. The results of this study showed only small effects on the female offspring. The highest dose of prochloraz (50 mg/kg) caused an increased AGD and so far no other significant short-term effects have been observed. With regard to long-term effects, no detectable effect on onset of puberty was observed. However, the highest mixture dose caused a disturbed oestrous cyclicity, which may be regarded as a sensitive endpoint for endocrine disruptors.

142 ARSENIC-INDUCED TOXICITY AND THE PROTECTIVE ROLE OF ASCORBIC ACID IN MOUSE TESTIS.

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Oxidative stress has been suggested to be a major cause of male reproductive failure. Here, we investigated whether arsenic, which impairs male reproductive functions in rodent models, acts by inducing oxidative stress. Male 8-week-old ICR mice were given drinking water containing 20 or 40 mg/l sodium arsenite with or without 0.75 or 1.5 g/l of the antioxidant ascorbic acid for 5 weeks. The arsenic-treated mice showed decreased epididymal sperm counts and testicular weights compared to untreated mice. These effects were reversed in mice that were co-treated with ascorbic acid. Similarly, arsenic treatment lowered the activities of testicular 3 β -hydroxysteroid dehydrogenase (HSD) and 17 β -HSD, which play important roles in steroidogenesis, and this was reversed by co-treatment with ascorbic acid. The testes of arsenic-treated mice had decreased glutathione (GSH) levels (which correlate inversely with the degree of cellular oxidative stress) and elevated levels of protein carbonyl (a marker of oxidative damage to tissue proteins). Ascorbic acid co-treatment reversed both of these effects. Thus, ascorbic acid blocks both the adverse effects of arsenic on male reproductive functions and the arsenic-induced testicular oxidative changes. These observations support the notion that arsenic impairs male reproductive function by inducing oxidative stress.

143 A FIVE GENERATION REPRODUCTIVE TOXICITY ASSESSMENT OF ETHINYL ESTRADIOL (EE) IN CD SPRAGUE-DAWLEY RATS.

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The toxicity of dietary EE (0, 2, 10, and 50 ppb), a potent estrogen, has been evaluated in a multigeneration design with NCTR CD rats. These concentrations led to ingested doses of approximately 0, 0.2, 1, and 5 μ g/kg/day in females and 0, 0.1, 0.7, and 4 μ g/kg/day in males. This study was one of a series examining estrogens of varying potencies, including the carryover and magnification of subtle effects across generations, reversibility of effects, and chronic effects. The parental generation was exposed to EE in a low phytoestrogen diet (Purina 5K96) from 28 days prior to mating until necropsy at PND 140, the age at which all generations were terminated. The F₁ and F₂ generations were exposed continuously from conception, F₃ from conception until wean, and F₄ was not exposed to EE. Body weight was lower than controls in the 50 ppb dose group for both sexes in the continuously exposed F₀ through F₂ generations, with terminal body weights 8-15% lower than controls. Body weights in all groups were similar after dosing was discontinued at weaning in the F₃ generation. Fertility and litter parameters did not show consistent dose-related effects. The primary effects observed in females were at 50 ppb: an acceleration of the time of vaginal opening by 4, 6, and 3 days in the F₁, F₂, and F₃ generations, respectively, and disrupted estrous cycles, which were approximately doubled in length, in the F₁ and F₂ generations. No clear adverse histopathological effects were observed in females at 140 days. In males, an increase in renal tubule mineralization was observed in the 50 ppb dose group of the continuously exposed F₁ and F₂ generations. Increases in alveolar/ductal hyperplasia of the male mammary gland were observed in the 50 ppb dose group of the F₀ through F₃ generations, with significant effects at 10 ppb in the F₁ and F₂ generations, and at 2 ppb in the F₁ generation. These effects overlap those observed previously at near 10,000-fold higher doses of the phytoestrogen genistein.

144 ALTERED UTERINE GENE EXPRESSION IN RATS IN RESPONSE TO P-TERT-OCTYLPHENOL EXPOSURE.

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P-tert-octylphenol (OP) is an environmental contaminant possessing estrogenic properties found to alter gene expression, interrupting the normal course of development and reproduction. OP, the most estrogenic among the alkylphenols, is an environmentally persistent industrial by-product, present in food, drinking water and household products. Multiple studies show that high doses of OP induce interruption of the estrous cycle, changes in uterine, prostate and epididymal weights and dose-dependent alterations in estrogen-responsive gene expression *in vitro* and *in vivo*. Exploiting uterine sensitivity to estrogens, we tested uterine response and gene expression as a biomarker of estrogenicity, using OP as a purported estrogenic chemical. Sprague-Dawley rats were given vehicle (propylene glycol) or OP (125mg/kg bw/day) by gavage either once or for 35 days. Uteri collected after 24hrs or 35 days were weighed and processed for microarray gene expression studies. OP did not affect uterine weights but altered uterine expression of certain genes re-

sponsive to estrogens, toxicants or stress. Acute exposures changed the expression of 21 genes ($p < 0.05$, >2-fold, 12 up- and 9 down-regulated), 3 of which have been related to estrogen changes (cytochrome P450 Cyp1a2, heterogeneous ribonucleoprotein Hnrpa1 and sulfotransferase Sult1c1). In the sub-chronic treatment, 8 genes had a >2-fold change in expression ($p < 0.05$, 3 down- and 5 up-regulated), one of which (cysteine rich protein Cyr 61) was responsive to estrogenic changes. Expression of expected estrogen-responsive genes in uterine tissues was not affected by OP exposures. Thus, results do not support that OP has estrogen-like activity in uterus at the dose tested here. Low oral bioavailability, rapid elimination, and route of exposure to OP may underlie these negative results, since previous studies required higher doses to observe estrogenic effects. However, altered expression of several other genes suggests that OP could alter uterine physiology through estrogen-independent mechanisms. (Funded by NSERC)

145 ACUTE DIMETHYLDITHIOCARBAMATE (DMDC) EXPOSURE ALTERS NEUROENDOCRINE CONTROL OF OVULATION (OV) BUT CONTINUED DOSING DOES NOT.

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Our laboratory has shown previously that a single exposure to dithiocarbamate pesticides on the morning of proestrus will block the OV surge of luteinizing hormone (LH), an effect attributed to a DMDC-induced decrease in hypothalamic norepinephrine (hNE) and decreased pulsatile release of gonadotropin releasing hormone (GnRH). Blocking the LH surge delays OV, alters oocyte viability, delays embryonic development and reduces litter size. We also reported that continued dosing (21 days) is without effect on pregnancy, indicating that any adverse reproductive effect observed following one treatment dissipates with repeated dosing. Here, we examined the underlying neuroendocrine changes associated with DMDC and pregnancy disruption to better define the events responsible for the initial adverse effects and characterize how these measures change with repeated dosing. Groups of ovariectomized (OVXed), estrogen-primed rats were dosed with DMDC at 1300 h (50 mg/kg, i.p.) for 1, 5 or 21 days. The induction of the surge was timed to occur on the last day of treatment. On day 1, DMDC decreased LH secretion and hNE. On day 5 and 21, LH secretion was not different from controls. hNE was still lower in treated rats on day 5 but not day 21. In a second experiment we examined the pulsatile release of LH in long-term OVXed females treated with DMDC for 1, 5 and 21 days using a cardiac catheter. Again, DMDC decreased the pulsatile LH release on 1 day, but not 5 and 21 days. These results demonstrate that the neuroendocrine mechanisms underlying the generation of the OV surge of LH are initially impaired by DMDC, but these adverse effects are ameliorated after only 5 days of exposure and appear to be completely compensated after 21 days later. This would explain our observation of pregnancy loss after acute but not subchronic DMDC treatment and emphasizes the importance of tolerance in the risk assessment process. This abstract does not necessarily reflect EPA policy.

146 PPAR GAMMA AND OVARIAN TOXICITY: AN ENDOCRINE STORY.

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Previous work in rats with PPAR (peroxisome proliferator-activated receptor) γ agonists has shown a clear association between treatment and the loss of corpora lutea. Recently, we also identified an increase in atresia of secondary follicles in rats treated with the gamma-weighted PPAR α, γ dual agonist, naveglitazar. The present study was designed to elucidate the interplay between PPAR γ activation and the endocrine system on ovarian function. Naveglitazar was administered daily by oral gavage to female F344 rats for 4 weeks. Serial blood samples were collected on the final day of dosing from unrestrained rats via an atrial cannula. Circulating levels of hormones including LH, FSH, Inhibin A, and progesterone (P4) were measured. The ovary, uterus, cervix, and vagina were examined for morphological changes. Treatment with naveglitazar resulted in normal levels of basal LH and elevated FSH. Decreased levels of P4 were associated with depletion of corpora lutea. Increased atresia was noted in follicles from the secondary follicle stage of development through the Graafian stage with associated decreases in Inhibin A levels. It is clear, given the corpora luteal depletion, that in spite of sufficient gonadotropin support, ovarian follicles failed to reach an ovulatory state. Further, the increase in circulating FSH levels did not result in an increased rate of follicular development. The increase in follicular atresia was observed in the same developmental stages at which PPAR γ expression in the follicle is increasing during normal follicular development. Additionally, previously reported evidence suggests that activation of PPAR γ is associated with factors favoring apoptosis such as induction of p53, inhibition of bcl-2, and interference with estradiol binding its receptor. Thus, the interpretation of the present study concludes that activation of PPAR γ in the ovary interrupts follicular development at early stages inducing apoptotic events and atresia resulting in a loss of follicles that are functionally able to undergo ovulation.

147 EFFECTS OF THE DITHIOCARBAMATE FUNGICIDE THIRAM ON THE REPRODUCTIVE AND THYROID AXIS FOLLOWING EXPOSURE IN THE JUVENILE AND ADULT MALE RAT.

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Thiram inhibits the synthesis of norepinephrine (NE) by inhibiting dopamine-beta-hydroxylase in the hypothalamus. NE is important for the regulation of gonadotropin releasing hormone and subsequent luteinizing hormone (LH) secretion. Thiram has been shown previously in our laboratory to inhibit the proestrous surge of LH in the female rat. Therefore, we hypothesized that thiram would disrupt testosterone secretion and affect pubertal onset in the juvenile rat. We exposed weanling males from postnatal day (PND) 23 to 53 by oral gavage with 0, 12.5, 25 and 50 mg/kg/d thiram. Preputial separation was delayed at 50 mg/kg. Lateral and ventral prostate and seminal vesicle weight at PND 53 was decreased by the same dose. There were no effects on testes or epididymal weights. Serum LH was decreased by 25 and 50 mg/kg, while testosterone was decreased at all doses on PND 53. These results demonstrate that thiram is able to alter pubertal progression in the male rat, which can be attributed to effects on LH. We also observed a dose-dependent decrease in serum thyroid stimulating hormone (TSH) at 25 and 50 mg/kg, while T4 was only decreased by 50 mg/kg. Since this pattern of effect on thyroid homeostasis indicated that thiram may be altering the pituitary or hypothalamic regulation of TSH, an additional study was conducted in the adult male rat to evaluate TSH release more closely. We dosed 60 day old male rats daily by gavage with 0, 25, 50, or 100 mg/kg thiram for 1, 7 or 21 days and measured TSH six hours after dosing. TSH was decreased 6 hours after either 1, 7 or 21 days of exposure to thiram or at doses of 50 and 100 mg/kg. These results demonstrate that effects are seen on TSH after only a single dose and that the brain or pituitary are likely target sites. Further studies are planned to evaluate this effect of thiram on the pituitary or hypothalamic regulation of TSH. This abstract does not necessarily reflect EPA policy.

148 EFFECT OF SIMAZINE (SIM) ON TESTICULAR FUNCTION IN THE JUVENILE WISTAR RAT.

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Chlorotriazine herbicides, such as SIM, are used extensively in the U.S. each year and both the parent compound and the metabolites are detected in ground water in areas of major usage. Previously we found that SIM exposure from postnatal day 23 to 53 increased serum testosterone (T) on postnatal day (PND) 53 in the rat. We hypothesized that SIM was either advancing the hypothalamic-pituitary-gonadal (HPG) activation of puberty by targeting the hypothalamic regulation of pituitary luteinizing hormone (LH) release or inducing the secretion of testosterone by altering testes function. The present study examines the effects of SIM on testes function more closely. We measured intra-testicular T concentrations and ex-vivo T production following a 20 day exposure to 0, 3.125, 6.25, 25 and 100 mg/kg of SIM by gavage (PND 23 to 43). Specifically, we measured interstitial fluid (IF) and seminiferous tubule fluid (SNF) in one testis of each rat and determined T content. There were no significant differences in testis weight or IF and SNF volume or T content. The other testis was incubated in supplemented Med 199 based upon the sliced testes assay and baseline and challenged collections (100 mIU/ml hCG) were obtained at seven 30 minute intervals. In the initial baseline collection, the concentration of T in the media was increased at simazine doses of 25 mg/kg or higher. All other incubation media T concentrations were not different from controls in the baseline or hCG stimulated collections. Lactate dehydrogenase activity from the last collection indicated no cytotoxicity. In conclusion, SIM did not alter testicular function and suggests that the previously observed effects of SIM on serum T concentrations are not due to any direct effects on the testes, but may be due to increased LH stimulation. Further studies are ongoing to determine the effect of simazine on the hypothalamic-pituitary regulation of LH secretion during the pubertal period. This abstract does not necessarily reflect EPA policy.

149 EFFECTS OF DI(N-BUTYL)PHTHALATE ON DEVELOPMENT OF REPRODUCTIVE ORGANS AND STEROIDOGENESIS IN SPRAGUE DAWLEY FEMALE RATS.

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The purpose of this study was to evaluate the effect of di(n-butyl)phthalate (DBP), a widely used plasticizer, on the reproductive organ development in F1 Sprague-Dawley (SD) female rats following in utero exposure. Development of female reproductive organs and vagina opening were dose-dependently delayed by DBP

treatment. High-dose DBP (700 mg/kg/day) increased the number of days in proestrus, whereas DES 50 ug/kg/day produced a persistent estrus state. In granulosa cells of ovary follicle, expressions of aromatase and ER α were decreased by DBP treatment, but were increased in the DES treatment group. In uterus epithelium cells, expression of ER α was increased in the DBP treatment group and was proliferated more than control group similar to DES treatment. The serum estradiol and IGF-I levels were significantly decreased by the DBP treatment but were significantly increased in the DES treatment groups. In the expression of peroxisome proliferator-activated receptors α was increased by the DBP treatment in ovary. These results showed that ER α , IGF-I and aromatase may be significantly involved in DBP-induced disruption of ovary development and estradiol synthesis. These results demonstrate that exposure to DBP during gestation days 10-19 causes several abnormal developments of female reproductive organs, and these effects may be due to disruption of the stage and tissue specific expression of genes and growth factors related to development of organs.

150 COMPARISON OF CYCLOSARIN (GF) AND SARIN (GB) TOXICOKINETICS AND TOXICODYNAMICS FOLLOWING A SINGLE INHALATION EXPOSURE IN THE GUINEA PIG.

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It is necessary to generate mathematical models of the adsorption, distribution, and elimination of nerve agents to improve toxicity estimates from sub-lethal exposures to chemical warfare nerve agents (CWNA). However, such models are based on representative data sets generated with different routes of exposure and in different species and are designed to interpolate between limited laboratory data sets to predict a wide range of possible human exposure scenarios. This study was performed to integrate CWNA sub-lethal toxicity data using different nerve agents in male Duncan Hartley guinea pigs (GP). Animals had a single whole body inhalation (IH) exposure of either GF or GB (0.1 or 0.4 x LC₅₀). Arterial catheterization allowed repeated blood sampling from the same animal at various time periods. Blood and tissue levels of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), as well as blood and tissue levels of regenerated GF (rGF) or GB (rGB) were determined at various time points during and following low level nerve agent exposure. The following pharmacokinetic parameters were calculated from the graph of plasma or RBC regenerated nerve agent concentrations versus time: time to reach the maximal concentration (t_{max}), maximal concentration (C_{max}), mean residence time (MRT), clearance (Cl), volume of distribution at steady state (V_{ss}), terminal elimination phase rate constant (k_{elim}), and the area under the plasma concentration time curve extrapolated to infinity (AUC) using WinNonlin[®] analysis program 5.0. The plasma and RBC t_{1/2} for rGF and rGB was also calculated. These data will be used to develop a mathematical model of the absorption of sub-lethal doses of GF and GB and its distribution into susceptible tissues. A specific goal was to compare uptake and clearance kinetics of similar sub-lethal doses of GF or GB in the blood and tissue of GP exposed to agent by acute inhalation exposure.

151 EVALUATION OF TOXICOKINETICS OF SUBCUTANEOUS EXPOSURE TO SUB-LETHAL DOSES OF CYCLOSARIN (GF) AND SARIN (GB) IN THE GUINEA PIG.

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Mathematical models of the absorption, distribution and elimination of chemical warfare nerve agents can be used to improve toxicity estimates, extrapolate to humans and predict response to a range of nerve agent exposures. The current study was performed to compare toxicity data of subcutaneous exposure to different nerve agents in male guinea pigs. Animals were given a single low-level subcutaneous (SC) dose of either GB or GF (0.1 & 0.4 x LD50). Blood levels of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), as well as of regenerated GB (rGB) or GF (rGF) were evaluated at multiple time points following exposure. In plasma, for each respective LD50 exposure of either GF or GB, toxicokinetic parameters such as the AUC, C_{max}, t_{max} and clearance were similar for each respective dose irrespective of the agent used; however, the t_{1/2} for rGF in plasma was

twice as long compared to rGB. In RBC, similar toxicokinetic results occurred except that clearance from RBCs was higher for rGF than rGB and the $t_{1/2}$ for the 0.1 x LD50 GB dose was higher than the other RBC $t_{1/2}$ values. Also the t_{max} was higher for RBCs than plasma. In summary, it appears that GF has greater inhibitory action upon guinea pig AChE activity that occurs earlier and persists longer than does GB. Although toxicokinetic parameters were similar for rGB and rGF, some differences did occur. These data will be utilized in a physiologically based pharmacokinetic model that was developed to simulate low level nerve agent exposures in guinea pigs.

152 THE EFFECTS OF MEDICAL COUNTERMEASURES ON SURVIVAL AND BEHAVIOR OF GUINEA PIGS EXPOSED TO REPEATED DOSES OF SARIN OR VX.

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This study assesses the therapeutic effectiveness of standard medical countermeasures against repeated exposures to the organophosphorous nerve agents sarin (GB) or VX. Male guinea pigs were subcutaneously injected once daily with 0.7 x LD50 GB or VX (LD50 for GB and VX are 42 and 9 µg/kg, respectively) for two weeks (5 days per week, Mondays–Fridays). During the exposure period, animals were pretreated with pyridostigmine bromide (PB) or treated with the countermeasures (CM) atropine sulfate/2-PAM. PB (25 mg/L) was provided in drinking water for the duration of GB or VX exposure to inhibit ~25% of basal blood cholinesterase activity. A single MARK I injector equivalent dose (by body weight) of atropine sulfate (0.03 mg/kg, im) and 2-PAM (8.5 mg/kg, im) was injected immediately following daily GB or VX. Toxic signs (TST) were collected at selected exposure and recovery days. The TST measures cholinergic toxicity in response to nerve agents. CM decreased mortality in GB- and VX-exposed guinea pigs from 55.6% (GB alone) to 9.1% (GB+CM) and 81.8% (VX alone) to 9.1% (VX+CM), respectively. PB pretreatment with GB and VX exposure reduced mortality rate from 55.6% (GB alone) to 0% (PB+GB) and from 81.8% (VX alone) to 40.0% (PB+VX). GB exposure alone produced higher TST scores ranging from mild to moderate only during the first week. Countermeasures failed to lower GB toxicity during the exposure period. VX exposure alone produced moderate levels of toxicity starting on the fifth day and continuing through the rest of the exposure period. With VX exposure, CM reduced TST scores, producing mild levels of toxicity below the TST scores of the VX alone and PB+VX groups. PB pretreatment of the GB-exposed group prevented the toxicity produced by either GB alone or GB+CM. Conversely, PB pretreatment with VX exposure did not reduce TST scores. In summary, pretreatment with PB or treatment with atropine sulfate/2-PAM had variable effects upon toxic signs. However, regardless of whether VX or GB was tested, PB or CM decreased mortality rates.

153 LOW LEVEL VX INCREASES HIPPOCAMPAL NEUROTROPHIN EXPRESSION INDEPENDENT OF BRAIN PATHOLOGY IN MOUSE HIPPOCAMPUS.

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The chemical warfare nerve agent VX is an irreversible inhibitor of the enzyme acetylcholinesterase and leads to neurotoxicity at nerve synapses and neuromuscular junctions through the accumulation of excess acetylcholine. Although high doses of VX and other nerve agents induce seizures, excitotoxicity and brain pathology, less is known about the effects of exposure to low doses of nerve agents. Brain derived neurotrophic factor (BDNF) is associated with neuronal development, synaptic plasticity, and cell survival, and is neuroprotective for certain types of hippocampal neurons following brain trauma. In the current study, we evaluated the effects of low-level VX exposure on protein levels of BDNF in a dose (0.1, 0.2, 0.4 LD50 VX (1/day x 5 days/wk x 2 wk)) and time course study (range 2 hr to 2 wk). In a parallel study, we assessed whether there was any hippocampal pathology three weeks following the same dose regimen of low-level VX exposure. A significant increase in the level of BDNF protein was observed in the hippocampal formation at 72 hr post-exposure compared with saline control mice. Pathology was not observed when evaluated 3 wk after the final VX exposure. The increase in BDNF protein expression in hippocampus is consistent with our previous observation of increased BDNF mRNA in the hippocampal formation following multiple VX exposures. This increase in BDNF may be an adaptive response to VX, since we do not observe brain pathology following exposure. Funding was provided by DTRA to LA Lumley.

154 ANALYSIS OF FREE AND BOUND VX FROM MINIPIG BLOOD FOLLOWING WHOLE BODY VAPOR EXPOSURE.

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Recent developments to improve nerve agent biomarker and dosemetric techniques include methods for measuring fluoride ion regenerated VX G-analog (VX-G) in blood and tissue. Protein bound V-series nerve agents regenerate a G-series analog upon addition of fluoride ion. In the case of VX, the regeneration product is O-ethyl methylphosphonofluoridate. Blood samples were taken serially before, during, and after whole body VX vapor exposures from Gottingen minipigs via venous catheter allowing agent exposure profiles to be generated. After processing the samples with fluoride ion and extracting with C-18 solid phase extraction cartridges the ethyl acetate extract was analyzed by GC/MS/MS. In addition to VX-G, significant levels of free VX were recovered from plasma using hexane solvent extraction and GC/MS/MS analysis. Plasma levels of VX-G maximized relatively soon after the end of the exposure. However, RBC levels rose for hours after the end of the exposure.

155 INVESTIGATION OF HAIR FOLLICLE AND PLASMA BIOMARKERS FOR LOW-LEVEL VX VAPOR EXPOSURE.

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Currently, there are no field-ready, expedient, and simple assays available that can determine whether someone has been exposed to very low levels of chemical warfare agent (CWA). Our laboratory has begun work to identify protein biomarkers in hair follicles and metabolite biomarkers in the plasma of rats exposed to low-level VX vapor. Both blood plasma and the active hair follicle carry a large number of easily accessible proteins that are responsive to toxicant exposure and are therefore excellent candidates from which to mine biomarkers. We identified more than 20 potential protein targets that are reportedly altered as a result of OP agent exposure and, using Western blotting, determined which of these potential targets are found in the rat whisker hair follicle. Using a novel whole mount immunohistochemistry (IHC) technique we investigated which proteins are responsive to VX exposure. Growth stage corrected expression of estrogen receptor (ER- α) was significantly lower in both male and female VX-exposed rats. Expression of androgen receptor (AR) was also reduced in VX-exposed male rats. Rats exhibiting physical symptoms of exposure (miosis) one hour post exposure had significantly lower expression of a cytochrome P450 enzyme (CYP1A1) in the whisker follicle bulb. The metabolomic profiles of the blood plasma of the same VX-exposed rats were determined using liquid chromatography coupled with mass spectrometry (LC/MS). The LC/MS data were analyzed using unsupervised hierarchical clustering to identify groups of perturbed metabolic components in the plasma. A panel of plasma metabolite biomarkers that can discriminate between VX exposure and control as well as level of VX exposure was identified. We have identified several potential biomarkers of low-level VX exposure that, once validated, may allow more rapid screening for exposure to a toxic agent.

156 ALTERATIONS IN BRAIN GENE AND PLASMA PROTEIN EXPRESSION IN SOMAN VAPOR-EXPOSED RATS.

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Since the end of the first Gulf War, there has been concern that there may be unknown consequences of low-level exposure to a number of military compounds, including nerve agents. In particular, it was not known whether exposures to chemical nerve agents, at peri- and sub-symptomatic levels, may have potential to induce measurable immediate and/or persistent neurological effects. It is widely accepted that small molecular level changes are often amplified by participation in larger biochemical networks. The aim of this study was to link gene and protein expression alterations with biochemical pathways that may have important operational or longer-term health implications. To begin to address this aim, transcriptomic and proteomic alterations in the brain and blood plasma of male and female Sprague-Dawley rats exposed to low-level doses of the aerosolized nerve agent soman (GD) were assessed. The transcriptomic changes in the brains were measured using DNA

microarray analysis and real-time RT-PCR. The proteomic analysis of the blood plasma was carried out using LC-MS-MS. To date, our results indicate that: 1) low-level inhalation exposure to Soman results the differential expression of a number of neuronal genes, including some that participate in neurological injury mediation and regeneration and 2) there are a number of key protein alterations in the blood plasma of soman-exposed animals, and 3) there are significant gender-associated differences in the degree and type of gene and protein expression alterations.

157 NEBULIZED SULFUR MUSTARD CAUSES ACUTE LUNG INJURY IN ANESTHETIZED RATS.

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Sulfur mustard (SM) is an alkylating chemical warfare agent used during World War I and in the Iran-Iraq (1984-88) conflict. SM poses a threat to the respiratory tract with short duration exposure resulting in acute lung injury. The detection of acute lung injury is important for therapeutic countermeasures to reduce toxicity in a timely manner. This study was designed to deliver nebulized dilute SM in a dose-response manner to investigate possible markers of lung injury. SM was delivered to male rats (240-270g) by a small animal ventilator set for a volume of 2.5 mL x 60-80 breaths/minute. SM was administered at doses of 0, 25, 50, 75, 125, 175, 225, 275, and 300 µg/min x 10 minutes. The effects from doses between 25-125 µg/min were not different from saline-exposed controls for any parameter measured. In euthanized animals, the trachea was excised, 5 mL of saline was instilled into the lungs which were washed five times, and bronchoalveolar lavage fluid (BALF) was taken at 0.5, 1, 3, 6, and 24 hours post-exposure. BALF was analyzed for protein concentrations, LDH, GSH, and GPx. Lung wet and dry ratios showed no change across dose and time. As a validation of exposure, blood levels of SM were analyzed; increasing plasma levels of SM were significantly higher across all time points and doses vs. saline-exposed controls. BALF results at doses 175-300 µg/min showed an increase in protein and LDH in a dose- and time-response manner. The protein data indicated a marked alteration in the air-blood barrier, with significance at 24 hours post-exposure for 225 and 300 µg/min ($p < 0.05$). The LDH data indicated an increase in cell lysis and tissue injury at 1, 6 and 24 hours post-exposure ($p < 0.05$). BALF GSH indicated signs of oxidative stress with significance at 6 and 24 hours post-exposure ($p < 0.05$). BALF GPx, another indicator of oxidative stress showed significance at 0.5, 6, and 24 hours post-exposure to SM ($p < 0.05$). These data indicate that nebulized SM exposure causes acute lung injury across dose and time.

158 NITROGEN MUSTARD-INDUCED ALTERATIONS OF MATRIX METALLOPROTEINASE-9 IN MOUSE KERATINOCYTES.

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Cutaneous exposure to sulfur mustard (SM) produces delayed vesication by targeting basal keratinocytes and causing blistering at the epidermal-dermal junction. MMPs, enzymes that degrade extracellular matrix proteins, play important roles in inflammatory and immune responses. We have previously observed that SM upregulates matrix metalloproteinase-9 (MMP-9, gelatinase B) in the mouse ear vesicant model (MEVM). To reduce animal testing and bridge the MEVM and in vitro human models, we have established primary murine epidermal keratinocyte (MEK) cultures. We tested whether nitrogen mustard (HN-2), a surrogate for SM, alters MMP expression in MEK as in the MEVM. Primary MEK were grown on collagen I, IV, or laminin 332 (formerly laminin-5) matrices. Cells were cultured in low and high calcium concentrations to induce differentiation. This was verified by monitoring biomarkers keratin 1 and profilaggrin. HN-2 produced a dose-dependent toxic effect between 1 and 100 µM. Undifferentiated cultures were more susceptible to HN-2 than differentiated cultures. At 100 µM, HN-2 increased the relative level of MMP-9 mRNA and the expression of the active protein form in differentiated, but not undifferentiated MEK, as evidenced by RT-PCR and gelatin zymography. The changes in MMP-9 were similar to our previous observations in the MEVM. MEK grew and differentiated equally well on each of the matrices tested. In conclusion, exposure of differentiated MEK to HN-2 produces effects similar to those of SM in the MEVM. This model may be useful for high-throughput testing of vesicant countermeasures such as anti-proteinases and for exploiting transgenic technology for mechanistic studies.

159 MECHLORETHAMINE TOXICITY IN SKIN CELLS IS INHIBITED BY BUTYLATED HYDROXYANISOLE.

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Mechlorethamine (HN2) is a nitrogen mustard alkylating agent used clinically to treat Hodgkin's lymphoma. HN2 produces toxic effects on the eyes, respiratory tract and skin. Presently, there is no effective treatment for HN2 poisoning. Butylated hydroxyanisole (BHA) is a fat-soluble phenolic compound commonly used as a food preservative. The present study evaluated the ability of BHA to reduce HN2 toxicity in the A431 human epidermoid carcinoma cell line. Cellular viability was measured by the reduction of 3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium (MTT). The cytotoxicity of HN2 after either 24 hr or 48 hr of treatment was found to be concentration-dependent (LC50 ~25 µM). At both experimental periods, a co-treatment with BHA (75 µM) resulted in significant protection against the cytotoxic effects of HN2 (LC50 > 50 µM). Scanning electron microscopy (SEM) confirmed viability results with cells co-treated with BHA and HN2 showing a reduction in morphological alterations when compared to cells treated with HN2 alone. Cells depleted of glutathione (GSH) with the γ-glutamyl-cysteine synthase inhibitor di-buthionine-S,R- sulfoximine (BSO) were found to be more sensitive to HN2 after 24 hr (LC50 < 12.5 µM). The effect of HN2 on cellular levels of GSH and its potential to induce lipid peroxidation were investigated. GSH levels were found to be elevated in a concentration-dependent manner 3 hr after a treatment with 50 µM HN2, an effect that was partially reversed by co-treatment with BHA (75 µM). Furthermore, thiobarbituric acid reactive-substances (TBARS) were observed in skin cells at 48 hr after treatment with 50 µM HN2 but not in control cells. A co-treatment with BHA (75 µM) reduced the formation of TBARS to a significant extent. Taken together, these results indicate that BHA is effective at protecting skin cells from the toxicity of nitrogen mustard *in vitro*, perhaps, by reducing lipid peroxidation.

160 INFLAMMATORY CYTOKINE RELEASE IN HUMAN ISOLATED CELL CULTURE AND TISSUE CONSTRUCTS FOLLOWING *IN VITRO* EXPOSURE TO SULFUR MUSTARD.

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Sulfur mustard (2,2'-dichlorodiethyl sulfide, SM) is a cytotoxic chemical warfare agent known for its vesicating properties. Prior to visible blister formation, observable edema and redness are prevalent within SM-exposed tissues. To elucidate these inflammatory effects, we examined cytokine production in normal human epidermal keratinocytes (NHEK) and normal human bronchial epithelial cells (NHBE). These cell types serve as principal target sites for *in vivo* toxicity in response to SM exposure. In addition we compared cytokine secretion by these cell cultures to commercially available skin and airway tissue constructs (EpiDermFT and EpiAirway, MatTek Corp.) Cytokines were measured by Luminex technology using Biosource Human Cytokine Inflammatory 10-plex kits. Media samples were collected at 0, 2, 4, 6, 8, and 24 hours following exposure to 0, 1, 10, 50, 100 and 300 µM concentrations of SM. Increases of IL-1β, IL-6, IL-8 and TNF-α were routinely detected in cell cultures at SM concentrations greater than 10 µM. Additionally, our results indicate that some tissue constructs appear to be releasing cytokines into the media prior to SM exposure. This high background level can mask SM-specific release of cytokines. The data obtained with isolated cell cultures confirms the ability of SM to initiate inflammatory cascades in skin and airway tissue. Further studies will determine if skin and airway tissue constructs can be routinely incorporated as working models for future SM research.

161 PROTECTIVE EFFECT OF A NOVEL ANTI-INFLAMMATORY PEPTIDE AGAINST SULFUR MUSTARD-INDUCED LUNG INJURY AND THERMAL BURNS.

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Exposure of airway to sulfur mustard (SM) causes bronchitis, chemical pneumonia and lung edema. The present study demonstrates the protective effect of a 9 amino acid fragment of histone H2A against SM-induced lung injury. Mouse trachea was instilled with 50 µl saline containing 6 µg SM. Bronchoalveolar lavage (BAL) was carried out 24 hours after SM exposure. The animals received a single iv injection of the peptide (1 mg/kg) 7 days prior to SM exposure. We observed a significant reduction in Interferon gamma and Tumor Necrosis Factor alpha levels in the BAL fluid of peptide-treated mice as compared to controls. The anti-inflammatory/counter-irritating effect of the peptide was also demonstrated in heat burns induced by exposure of guinea pig skin to hot water. Intradermal injection of a peptide analog 5 min prior to heat exposure resulted in a significant reduction in

skin irritation score as compared to control animals injected with the vehicle. The anti-inflammatory effects of the peptide may be useful in prophylactic treatment of chemical and thermal injuries (supported by the US Army Medical Research and Material Command under Cooperative Agreement DAMD17-03-2-0013).

162 MATHEMATICAL MODEL FOR REGIONAL BRAIN DISPOSITION OF CHEMICAL WARFARE AGENTS AND IMPACT ON CHOLINESTERASES.

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Inhibition of red blood cell acetylcholinesterase (AChE) activity has been previously used as a correlate of AChE inhibition in the brain. Recently, however it has been shown to be a poor predictor of AChE inhibition in nervous tissues (Anderson et al., 2005). Heterogeneity and complexity of cell types and function of the CNS are impediments in the prediction of neurotoxicity. Unfortunately, predictive physiologically-based pharmacokinetic (PBPK) models often represent the brain as a single well-mixed compartment, ignoring its regional variations. A better understanding of regional toxicant distribution could better illuminate the understanding of mechanisms behind differential vulnerability. Little research has been devoted to the inclusion of regional brain specificity in PBPK/PD modeling efforts. Here, a PBPK model for sarin, developed in our laboratory by Gearhart et al. (2006), is expanded to include regional brain specificity. Brain compartments included cerebrospinal fluid, frontal and parietal cortex, hippocampus, cerebellum, brain stem, and thalamus. Volumes and blood flows of brain regions for the rat and other species were obtained from the literature. Preliminary parameterization was based on limited published regional brain measurements of basal cholinesterase (ChE) synthesis and irreversible inhibition after sarin exposures. The potential for integrating a functional PBPK/pharmacodynamic (PD) model with an appropriate PD model to characterize excitotoxicity, namely the evolution of excessive intracellular calcium resulting from acetylcholine (ACh) overload, and a possible role for improving neuroprotecting therapeutics are discussed.

163 THE PROTECTIVE EFFECT OF CAPSAICIN ANALOGS ON NORMAL HUMAN EPIDERMAL KERATINOCYTES (NHEK) FOLLOWING *IN VITRO* EXPOSURE TO SULFUR MUSTARD.

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Sulfur mustard (2, 2'-dichlorodiethyl sulfide, SM) is a cytotoxic alkylating agent with mutagenic and vesicating properties. Our research focus is the mechanism of toxicity of SM for the purpose of developing medical countermeasures to this chemical threat agent. A major feature of SM tissue injury is inflammation. Capsaicin is known to block neurogenic inflammatory pathways. It has been reported that the capsaicin analogs, octyl homovanillamide and retro-ovaniol, have limited ability to prevent SM damage in the mouse ear model. We have evaluated the ability of capsaicin analogs to prevent SM-induced changes in cultured NHEK. Three capsaicin analogs were obtained from the Korea Research Institute of Chemical Technology (KRICT). These compounds are identified as KR-22010, KR-24095, and KR-25018. Retro-ovaniol and octyl homovanillamide were also included in this study. NHEK were pretreated with these compounds 15 minutes prior to SM exposure. Cellular viabilities were determined 24 hours post SM exposure via vital dye (propidium iodide, PI) uptake as measured by flow cytometry. Morphological changes were documented via light microscopy. SM at 300 μ M for 24 hrs caused significant up-take of PI. Retro-ovaniol, while preserving good cellular morphology, failed to prevent SM-induced vital dye uptake. KR-24095 and KR-22010 significantly reduced the uptake of vital dye following SM exposure. Further studies with KR-24095, a water soluble analog, will be done to determine the efficacy of this compound as a medical countermeasure against the sulfur mustard injury.

164 INTERACTION OF CYANIDE AND NITRIC OXIDE AT THE CYTOCHROME C OXIDASE BINUCLEAR CENTER BINDING SITE.

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Cyanide induces neurotoxicity by inhibiting mitochondrial respiration by binding to the cytochrome c oxidase (COX) binuclear center ($\text{Fe}_{3+}\text{-Cu}_{2+}$). Previous studies have shown that nitric oxide (NO), which also inhibits COX and is metabolized by the enzyme, antagonizes cyanide. The interaction of NO and cyanide at the binu-

clear center was studied by monitoring cellular oxygen uptake and COX enzymatic activity in the immortalized rat mesencephalic cell line N27. KCN (10-60 μ M) inhibited cellular respiration in a concentration-dependent manner, in competition with O_2 . The KCN IC_{50} for cellular respiration ($15.5 \pm 2.1 \mu$ M) was slightly greater than that for COX inhibition ($9.9 \pm 0.7 \mu$ M). A respiratory threshold analysis indicated that KCN had to inhibit a 45 % excess of COX before decreasing cellular respiration. The NO donor SNAP (100 μ M) enhanced the KCN inhibition of cellular respiration and COX at 2 to 15 μ M cyanide, but reversed it at higher cyanide concentrations (15-60 μ M). When endogenous NO generation was blocked by treatment with a NOS inhibitor (L-NMMA, 100 μ M), the KCN IC_{50} for cellular respiration and COX increased to $88 \pm 6.8 \mu$ M and $110 \pm 11 \mu$ M, respectively. This suggested that under normal cell conditions, endogenous NO contributes to KCN-mediated COX inhibition. To further evaluate the interaction of cyanide and endogenous NO, mitochondrial NO and nitrite levels were examined in the presence of KCN. KCN dose-dependently (15-60 μ M) decreased mitochondrial NO levels, while increasing mitochondrial nitrite (metabolite of NO) production. The results indicate that exogenous NO can antagonize cyanide-mediated inhibition of COX, whereas endogenous NO may contribute to the enzymatic inhibition. A triphasic model is proposed to characterize cyanide binding at the binuclear center in the presence of endogenous NO and O_2 . (This study was supported by NIH grant ES04140.)

165 RICIN IC_{50} AS DETERMINED BY A BALB/c MURINE FIBROBLAST NEUTRAL RED UPTAKE ASSAY.

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Ricin is well recognized as a potential bioterrorism agent and the identification of this protein toxin in a powder found in a U. S. Senate office (2004) has served to keep ricin in the public eye. Proteins may be denatured and inactivated by fairly mild factors (e.g. temperature or pH change), necessitating the ability to ascertain the activity as well as the identity of protein toxins in such unknown samples. The well-known Neutral Red Uptake (NRU) assay has been previously employed for ricin cytotoxicity using bovine pulmonary endothelial cells [JN Hughes *et al.*, *Human & Exp. Tox.* 1996, 15, 443]. However, because of ricin's potential impact as a bioterrorism agent, a protocol previously validated for use with other chemicals is desirable for standardization purposes. Here we report ricin toxicity as determined by the NRU assay using BALB/c murine fibroblasts (3T3). We chose to follow the NICEATM/ECVAM protocol involving 3T3 cells, which was part of a recently completed multi-laboratory validation study for the determination of IC_{50} values for 72 coded chemicals [MW Paris *et al.*, SOT06 poster V5]. The NICEATM/ECVAM protocol required minor modification for use with a protein toxin as opposed to other non-protein toxic chemicals to which it was applied in the multi-laboratory study. Triplicate assays showed that ricin (Vector Laboratories, product L-1090) has an IC_{50} of $1.75 \times 10^{-03} \mu\text{g/mL}$ as determined by NRU using 3T3 cells. In addition, preliminary experiments indicate quick-freezing on liquid nitrogen does not affect ricin's cytotoxicity. Support: Defense Threat Reduction Agency Project BA05TAS080. NICEATM/ECVAM: The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods/European Centre for the Validation of Alternative Methods.

166 DEVELOPMENT OF HUMANE END POINTS IN RICIN INHALATION TOXICOLOGY.

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Ricin is a potent protein toxin produced by the seeds of the castor oil plant, *Ricinus communis* and has been of concern for some time as a potential agent in connection with warfare or terrorist attacks, particularly in relation to inhalation. In animal models, lung damage leads to pulmonary oedema which can be fatal. Exposure to lethal quantities of ricin (10 – 20 $\mu\text{g/kg}$ bodyweight) by inhalation leads to the development of toxic signs and symptoms, typically around 24 h following challenge, depending upon the dose. Symptoms of intoxication typically occur around 24h post inhalation exposure of a supra-lethal quantity of ricin aerosol, and mice or rats become less active, show signs of piloerection, begin to show signs of respiratory distress by panting, which gradually worsens over the next 12 – 24h. Appetite appears to be lost, animals lose weight and body temperature falls, eventually accompanied by cyanosis of ear lobes and footpads. When an animal is moribund, it may have lost as much as 33% of its original bodyweight and, as well as being cyanotic,

feels cold to the touch. These symptoms follow the progressive lung damage and corresponding increase in pulmonary oedema, accompanied by reduction in respiratory gaseous exchange and congestive impairment of blood circulation. At Dstl, we have been interested in developing humane endpoints following inhalation of ricin, focusing on the decrease in body temperature. Initial studies using intraperitoneally-implanted transponders measuring deep body temperature have been used, in freely moving mice. Animals were group housed in cages, 6 to a group. Each cage was equipped with a perimetric antenna, enabling almost continuous temperature recording from each animal simultaneously, without human intervention. Results of a small preliminary study were most encouraging and suggested that temperature fall could be an early predictor of mortality. Confirmatory studies are required to consolidate preliminary data.
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167 CYCLOTRIMETHYLENETRINITRAMINE (RDX) CAN INCREASE CALCIUM FLUORESCENCE IN HUMAN NEUROBLASTOMA CELLS.

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Cyclotrimethylenetrinitramine (RDX) has use as an explosive. Past studies in animals have demonstrated spontaneous seizures as an adverse effect of oral administration (Burdette et al., TAAP 92, 436, 1988). Mechanisms for seizure production, however, have not been delineated. In the present study we examined the ability of RDX applied to human neuroblastoma SH-SY5Y cells to increase cellular calcium, as increased calcium levels are a possible contributing mechanism for excitotoxicity. For these experiments, cells were loaded with calcium-indicating fluorescent dye one hour before application of RDX in concentrations between 1.5 μ M and 7.5 mM. Fluorescence was immediately measured on a kinetic fluorescent microplate reader with autoinject, and recordings made for a total of 30 cycles of 11 seconds each. A concentration response was noted, with even the lowest concentration of RDX tested capable of raising calcium fluorescence above baseline. A return toward baseline was noted by the end of the observation period. The effect of 7.5 mM RDX was significantly greater than the effects of the other concentrations tested ($p < 0.05$; $n=4$). Glutamate could also increase calcium fluorescence, but incubations of neuroblastoma cells with RDX for 1 hr did not result in a significant effect on cellular glutamate levels as indicated by GC-MS. These experiments suggest the possibility that increased cellular calcium concentrations could be a contributing factor to RDX-induced neuronal excitability (Abstract does not reflect U.S. Army Policy).

168 GLOBAL GENE EXPRESSION IN RAT BRAIN FOLLOWING ACUTE ORAL HEXAHYDRO-1, 3, 5-TRINITRO-1, 3, 5-TRIAZINE (RDX) EXPOSURE.

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Hexahydro-1,3,5-trinitro-1,3,5-triazine is a synthetic, high-impact, relatively stable compound commonly known as Rapid Detonation Explosive (RDX) and has been used in munitions and formulations since World War II. Humans can be exposed to RDX in several ways: during the manufacturing process, at contaminated soils at or near military facilities, or in groundwater. The primary symptom of acute exposure to high doses of RDX in humans is transient and reversible seizure, often accompanied by nausea, muscle spasms, and disorientation, which are also reported in experimental animals. The occurrence of these preventable effects in humans is most often reported in occupational settings that lack standard industrial hygiene practices, resulting in high airborne exposures. For lower exposures in water, a chronic oral reference dose of 0.003 mg/kg/day has been derived by EPA-IRIS (Integrated Risk Information System), based on rat prostate inflammation. Though several important studies have correlated measurements of RDX in the brains and plasma of dosed animals with the occurrence of seizures, little work has been carried out on the effects of RDX at the molecular level. Previously, we examined temporal gene expression of rat brain at single doses of 3 and 18 mg/kg RDX and times 0, 4, 24, and 48 hours. Recently, two additional doses of 9 and 36 mg/kg were added to the dataset and the gene expression profiles reexamined. The combined results of both experiments are presented here and a tentative hypothesis for the mechanism of RDX induced neurotoxicity is proposed, based on in vitro work and gene expression analysis.

169 HEMATOTOXICITY OF MUNITIONS COMPOUND HEXAHYDRO-1, 3, 5-TRINITRO-1, 3, 5-TRIAZINE (RDX) AND ENVIRONMENTAL DEGRADATION PRODUCT MNX.

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RDX, a military explosive and contaminant of munitions manufacturing and artillery training sites, undergoes anaerobic metabolism by nitroreductases of environmental microbes to hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX). Previous acute toxicity studies determined comparable potency for RDX and MNX (LD50 187 mg/kg; Meyer et al., 2005; J. Appl. Toxicol. 25: 427-34). Survivors of the MNX study exhibited normochromic anemia accompanied by splenic hemosiderosis (NOAEL 47 mg/kg). Because anemia persisted for 14 days after a single exposure, we hypothesized that hematopoiesis was impaired due to bone marrow toxicity and that RDX would have comparable effects. The objective of the present investigation was to determine and compare effects of RDX and MNX on bone marrow cellularity and hematopoietic progenitor cells. Acute exposure effects on peripheral blood and bone marrow cells after 14 days for MNX and RDX at various doses (vehicle, 12, 24, 47 and 94 mg/kg) were studied. RDX was more potent than MNX in reducing bone marrow cellularity and peripheral blood leukocytes, in particular granulocytes. Further, RDX and MNX were found equipotent in decreasing Burst Forming Units-Erythroblasts, BFU-E (NOAEL 12 mg/kg), while RDX and MNX at all doses decreased Granulocyte/Macrophage-Colony Forming Units (CFU-GM). In conclusion, our studies support the hypothesis that RDX and MNX toxicity to bone marrow hematopoietic progenitors effects a persistent depletion of peripheral blood cells and further indicates that both erythroid and myeloid lineages are involved. (Support: DoD; W914Z-05-P-0145 and W81XWH-05-1-0537).

170 ACUTE RENAL FAILURE ALTERS THE KINETICS OF PRALIDOXIME IN RATS.

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Objective: Pralidoxime (PX), the antidote to organophosphate poisoning, is eliminated unchanged by the renal route. This study assessed the effect of acute renal failure (ARF) on the kinetics of PX.

Methods: In a male Sprague-Dawley rat model of ARF induced by potassium bichromate, PX (50 mg.Kg-1 as PX base) was administered intramuscularly. Control rats received isotonic sodium chloride solution instead of bichromate. Plasma PX concentrations were measured by HPLC, urine concentrations using capillary electrophoresis. Pharmacokinetics were determined using Apis® program. Results are expressed as mean $\pm$ SEM. Statistical analysis was performed using Mann-Whitney test.

Results: Maximal plasma creatinine levels occurred 48 hours following bichromate injection (22.4 $\pm$ 1.8 μ mol.L-1 control vs 169.6 $\pm$ 26.5 μ mol.L-1 ARF). No significant difference was observed regarding the Cmax (24.4 $\pm$ 1.1 vs 35.2 $\pm$ 4.9 mg/l). In contrast, the areas under the curve (AUC) in the treated group were about 3 times greater than in the control group 2661.0 $\pm$ 535.6 ARF vs 976.3 $\pm$ 29.7 mg.min.L-1 control ($p < 0.01$). The volumes of distribution were not modified 3.3 $\pm$ 0.3 control vs 3.6 $\pm$ 0.7 l/kg-1 ARF. The total clearances were significantly reduced in ARF (1.5 $\pm$ 0.2 vs 3.1 $\pm$ 0.1 l.kg-1.h-1; $p < 0.01$) resulting in a significant increase in the elimination half-life (102.8 $\pm$ 7.9 ARF vs 44.0 $\pm$ 1.1 min control; $p < 0.01$). A positive correlation ($r^2 = 0.7665$) existed between the AUCs and the plasma creatinine values.

Discussion - Conclusion: The kinetics are significantly different between control and ARF groups. Acute renal failure did not modify PX distribution but decreased significantly its plasma elimination. PX kinetics was correlated with creatinine clearance. From a clinical viewpoint, our data suggest that the dosage regimen of PX should be modified in patients with severe acute renal failure. Further investigations are required to determine whether the antidotal efficacy of PX is modified in ARF.

171 BIOANALYTICAL METHOD VALIDATION OF 2, 3-BUTANEDIONE MONOXIME (DAM) IN HUMAN SERUM AND WHOLE BLOOD.

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2, 3-Butanedione monoxime (diacetyl monoxime or DAM) may have therapeutic uses as a cardioprotective agent against ischemia and/or an antidote following exposure to organophosphorus compounds. A validated bioanalytical method was

needed for conducting non-clinical studies and clinical trials. A GC/MSD method was developed and validated for DAM in human serum with a limited validation in human whole blood. Standards, quality control samples (QCs), and blanks were treated with acetonitrile containing the internal standard (IS): 1, 4-dichlorobenzene (d4). A best fit curve was determined using a quadratic regression model weighted $1/x$. The coefficients of determination were greater than 0.99 over a concentration range of 50 to 10,000 ng/mL. There were no interfering peaks in the retention times of the analyte or IS (specificity). Serum standard accuracy (%RE) ranged from -1.9 to 2.3% and precision (%RSD) from 0.2 to 3.1%. The limit of quantitation was 50 ng/mL (sensitivity). Human serum or QC samples with DAM concentrations greater than 10,000 ng/mL diluted linearly. Storage stability was determined using spiked human serum/whole blood samples (150 and 5000 ng DAM/mL). DAM in serum stored frozen (-20 degrees C) was stable for up to 69 days and through three freeze/thaw cycles. DAM in whole blood stored refrigerated (2-8 degrees C) decreased at least 30 percent for the 150 ng/mL samples whether using acetonitrile or saline. The decrease was less pronounced at the higher concentration. These results indicated that a method for quantitation of DAM in human serum and whole blood was able to be validated. The method was used to quantitate serum and blood DAM concentrations in a human clinical trial.

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172 ADMINISTRATION OF GENISTEIN PROMOTES NEUTROPHIL AND PLATELET RECOVERY IN IRRADIATED MICE.

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Genistein is a non-toxic isoflavone from soybeans. We previously reported that a single subcutaneous injection or repeated daily oral administration of genistein, significantly increased survival of lethally irradiated mice. In this experiment, we investigated the mechanism for genistein-induced radioprotection by evaluating the recovery of bone marrow cells and peripheral blood hematology in lethally irradiated mice. CD2F1 male mice received a single non-toxic subcutaneous injection of genistein (200 mg/kg), 24 hr prior to a lethal total body irradiation dose (8.75 Gy) of cobalt-60 gamma radiation. Survival and hematopoietic reconstitution were evaluated over 9 weeks postirradiation. Hematopoietic progenitor colony forming cell assays and an endogenous splenic CFU assay were performed to assess the reconstitution of the bone marrow after radiation-induced myelosuppression. In this study, 97% of genistein treated mice survived after 30 days, while 31% of vehicle-treated mice survived. The improvement in survival was related to accelerated neutrophil and platelet recovery resulting from earlier and more pronounced multilineage hematopoietic progenitor cell reconstitution in the femoral marrow compartment. Hematopoietic recovery in the marrow compartment was observed primarily 9-28 days postirradiation. Myeloid and erythroid progenitor cell numbers at day 15 postirradiation were 6-20 fold higher and the number of endogenous pluripotent hematopoietic splenic stem cells on day 11 postirradiation were 14-fold higher in genistein-treated than in vehicle control animals. These results demonstrate that a single subcutaneous administration of genistein 24 hr before irradiation provides significant radioprotection to the hematopoietic progenitor and stem cell compartment. Pretreatment with genistein may be useful in mitigating radiation-induced hematopoietic injury associated with radiotherapy or exposure to radiation by first responders entering contaminated areas.

173 ENVIRONMENTAL AND INDUSTRIAL CHEMICAL HAZARD ANALYSIS SYSTEM.

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A prototype risk assessment system, Environmental and Industrial Chemical Hazard Analysis System (EICHAS) has been developed. In its final form, EICHAS will predict exposure concentrations associated with specific types of military operations and will estimate and display the threat to military personnel, including those impacts that threaten the success of a mission (e.g., disabling health effects). Information and predictions provided by EICHAS will inform military planners in their decisions about targeting industrial facilities as well as where and how to deploy troops in areas where contamination is likely, so that potential exposures and chemical casualties are minimized. The current prototype system is designed to estimate residual concentrations and human health risks associated with exposure to chemicals remaining at a chemical manufacturing or storage site, after its destruction. Human health threats are based on a risk assessment methodology specifically developed for this application that incorporates into Human Toxicity Doses (HTDs): 1) severity of adverse effects (minimally adverse, clinically adverse, life threatening; 2) dose route (ingestion, inhalation, dermal); 3) dose duration (acute, intermediate, chronic); 4) carcinogenicity; and 5) gender-specificity/sensitivity. An

HTD represents the most certain estimate possible of the lowest-observed-adverse-effect level for a given severity category. The method used to estimate residual chemical concentrations includes modeling and simulation approaches to incorporate into Exposure Point Concentrations (EPCs): 1) time-varying chemical concentrations in soil and air; 2) geographic extent of the contamination; 3) extent of any chemical reactions (e.g., hydrolysis); 4) degree of migration into the soil profile over time; and 5) extent of volatilization into the air from a spill. The latter two elements were developed over time frames most likely to be encountered during military operations.

174 UPREGULATION OF GCLM EXPRESSION RESTORES GLUTATHIONE AND PROTECTS AGAINST APAP-INDUCED LIVER INJURY IN GCLM NULL MICE.

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The tripeptide antioxidant, glutathione (GSH), has many functions including promoting cell growth, maintaining cellular redox status, protecting cells against oxidative damage from reactive oxygen species, and playing a crucial role in the detoxification of xenobiotics. The rate-limiting enzyme in the biosynthesis of GSH is glutamate-cysteine ligase (GCL), consisting of two subunits, a catalytic subunit (GCLC) and a modifying subunit (GCLM). We have previously demonstrated that by conditionally over-expressing GCL, acetaminophen (APAP) induced hepatic injury is attenuated. Our present hybrid model is a cross between the GCLM null mouse and conditionally restored GCLM utilizing the liver specific transactivator GLVP in the presence of mifepristone (RU486). Restoring GCLM expression with RU486 elevates GSH levels in these mice approximately 3-fold compared to control mice. APAP administration resulted in serum ALT levels averaging over 5000 U/ml in hybrid mice not receiving RU486. When given RU486 in addition to APAP, hybrid mice increased GSH levels 3.5 fold and ALT levels were down nearly 16-fold. We propose that restoring GSH in GCLM null mice by upregulating GCLM results in diminished APAP-induced hepatotoxicity. Furthermore, this model demonstrates the importance of varying GCLM levels with regard to toxicant-induced liver injury.

175 PPAR α -REGULATED MOLECULAR NETWORKS ARE RESPONSIBLE FOR THE DIFFERENTIAL EFFECTS OF DIETARY FATTY ACIDS ON OXIDATIVE STRESS AND DNA DAMAGE IN MOUSE LIVER.

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Epidemiological and animal model research shows that ω -3 and ω -6 polyunsaturated fatty acids (PUFA) can attenuate or enhance cancer risk, respectively. Mechanisms underlying the differential effects between these PUFA are not well understood but are thought to include the ability to generate bioactive lipids, modulate gene expression through nuclear receptors, and increase cellular oxidant injury. To identify potential mechanistic differences between the two PUFA, C57BL/6J mice were administered a high fat diet containing corn (ω -6) or fish oil (ω -3) for up to 3 weeks and gene expression profiling was performed on liver. Pathway mapping of significantly altered genes showed that fish oil induced biological processes related to fatty acid oxidation, glutathione metabolism and oxidative stress; in contrast, corn oil repressed these processes while inducing inflammatory response. These data suggested that the type of dietary fat may influence the cellular anti-oxidant capacity. Thus, oxidative stress biomarkers were evaluated. It was determined that a corn oil diet lead to significant increase in expression of DNA repair genes and the number of 8-OH-dG DNA adducts with concomitant decrease in reduced GSH content. Fish oil lead to significant reduction in 8-OH-dG DNA adducts while no induction of DNA repair genes was observed. Comparison of PUFA gene expression data with a potent PPAR α pan-agonist, WY-14,643, determined that over 50% of the significantly altered genes from fish oil treatment were driven by PPAR α . Moreover, the increases in gene expression for glutathione-S-transferases, heat shock and proteasome proteins by fish oil could also be attributed to PPAR α . In summary, dietary fatty acids can modulate the cellular redox state and the direction may be dependent on their ability to trans-activate PPAR α . The ability of nutrients to exacerbate or attenuate oxidative stress and DNA damage, processes linked to carcinogenesis, warrants further study as its implications for human health could be significant.

176 EFFECT OF FESCUE TOXICOSIS AND ACUTE HEAT STRESS ON HEPATIC OXIDATIVE ACTIVITIES.

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Much of tall fescue grass in the US is infected with an endophytic fungus. Intake produces fescue toxicosis, characterized by reduced productivity and hyperthermia during heat stress (HS). A rat model for this condition was used to evaluate the ef-

fect of endophyte toxins on hepatic gene expression under thermoneutral (TN) and HS conditions. Core temperature (T_c) was monitored continuously in rats (n=24) implanted with telemetric transmitters. Rats were fed ad libitum either endophyte-infected (E+) or uninfected (E-) diets and maintained under Tn conditions (21°C) for five days, followed by TN or HS conditions (31°C) for three days. Feed intake (FI) and body weight (BW) were measured daily; selected visceral organ weights and serum chemistry were evaluated at the end. Liver sections were either fixed in 10% formalin or frozen at -80°C. Protein expression of hepatic CYP3A4 was evaluated using immunohistochemistry. Hepatic enzyme activities of antioxidants, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were determined spectrophotometrically. Hepatic apoptosis rates were determined using TUNEL assay. Both E+ and HS induced alterations in liver-specific genes were evaluated using DNA microarrays. Intake of E+ reduced FI and BW under TN and HS conditions. Core temperature at TN decreased from pretreatment level, but increased during HS. Serum prolactin decreased with E+ treatment at TN, and increased during HS. Heat stress, E+, and the combination reduced liver weight relative to BW. Protein level of CYP3A4 was greater in E+ liver compared to E-. In addition, rats exposed to E+ and HS had less amounts of SOD, CAT and GPx; and greater apoptosis rates. Microarray analysis is being used to evaluate genes associated with antioxidant, detoxification and immune functions. Present findings suggest that rats respond to E+ and HS by inducing CYP3A4 expression and suppressing hepatic antioxidant enzymes, which could ultimately increase oxidative stress and damage.

177 EFFECTS OF MIXTURES OF THE CHLOROACETATES ON THE INDUCTION OF OXIDATIVE STRESS IN HEPATIC TISSUES OF MICE.

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Dichloroacetate (DCA) and trichloroacetate (TCA) are byproducts produced during the process of chlorination of the drinking water. The two compounds were found to be hepatotoxic and hepatocarcinogenic in rodents, with oxidative stress considered as one of the possible mechanisms of toxicity. Since the compounds always exist in the water as mixtures, rather than individual, it is very important to determine the interactivities between the two compounds. To achieve that, the compounds were administered to groups of male B6C3F1 mice, p.o., at doses of 150 and 300 mg/kg and in two mixtures, DCA+TCA 150, and DCA+TCA 300, that correspond, respectively to 75 and 150 mg/kg of each of the two compounds. The animals were sacrificed 8 h later, and changes in the biomarkers of oxidative stress, including production of superoxide anion (SA) and lipid peroxidation (LP), as well as activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) were determined in hepatic tissues. The compounds caused significant production of SA and LP, with Potentiation/ synergistic effects observed with the mixtures. Also, the compounds and the mixtures caused dose-dependent suppression of SOD and GSH-Px activities, and had slightly smaller effects on CAT activity. The results suggest that oxidative stress play an important role in the hepatotoxicity of the chloroacetates, with more effects produced when the compounds exist in mixtures.

178 ATP SYNTHASE AND UBIQUINONE ARE TARGETS FOR TCDD MITOCHONDRIAL TOXICITY.

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Mitochondria generate ATP and participate in signal transduction and cellular pathology and/or cell death. TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) decreases hepatic ATP levels and generates mitochondrial oxidative DNA damage, which is exacerbated by increasing mitochondrial glutathione redox state and by inner-membrane hyperpolarization. This study identifies mitochondrial targets of TCDD that initiate and sustain reactive oxygen production and decrease ATP levels. One week after treating mice with TCDD, liver ubiquinone (Q) levels were significantly decreased, while rates of succinoxidase and Q-cytochrome c oxidoreductase activities were increased. However, the expected increase in Q reduction state following TCDD treatment did not occur; instead, Q was more oxidized. These results could be explained by an ATP synthase defect, a premise supported by the unusual finding that TCDD lowers ATP/O ratios without concomitant changes in respiratory control ratios. Such results suggest either a futile cycle in ATP synthesis, or hydrolysis of newly-synthesized ATP prior to release. The TCDD-mediated decrease in Q, concomitant with an increase in respiration, increases complex 3 redox-cycling. This acts in concert with glutathione to increase membrane potential and reactive oxygen production. The proposed defect in ATP synthase explains both the greater respiratory rates and the lower tissue ATP levels.

179 VITAMIN E DEFICIENCY ENHANCES PULMONARY INFLAMMATORY RESPONSE AND OXIDATIVE STRESS INDUCED BY SINGLE WALLED CARBON NANOTUBES IN C57BL/6 MICE.

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Exposure of mice to single walled carbon nanotubes (SWCNT) induces an unusually robust pulmonary inflammatory response with a very early onset of fibrosis, which is accompanied by a significant oxidative stress and antioxidant depletion. The role of specific components of the antioxidant protective system, specifically vitamin E, the major lipid-soluble antioxidant of membranes and lipoproteins, in the SWCNT induced reactions required further investigation. We used C57BL/6 mice, maintained on a vitamin E-sufficient diet as well as on a vitamin E-deficient diet, to explore and compare the pulmonary inflammatory reactions to aspirated SWCNTs. The induced vitamin E-deficiency (a 90-fold depletion of α -tocopherol in the lung) resulted in a significant decline of other antioxidants as well as in accumulation of lipid peroxidation products. A more severe decrease of pulmonary antioxidants was detected in SWCNT treated vitamin E-deficient mice as compared to controls. Exposure of vitamin E-sufficient mice to SWCNTs markedly shifted the ratio of low to high molecular weight forms of extra-cellular SOD (EC-SOD) such that approximately 3-4 times greater amounts of this neutrophil-associated enzyme were present. This effect was enhanced in vitamin E-deficient animals. Lowered levels of antioxidants in vitamin E-deficient mice were associated with a higher sensitivity to SWCNT-induced acute inflammation (PMNs number, released LDH, protein content, pro-inflammatory cytokines level) as well as pro-fibrotic pathways (TGF- β elevation and collagen deposition). Given that pulmonary levels of vitamin E can be manipulated through diet, its effects on SWCNT responses may be of practical importance in optimizing protective anti-inflammatory strategies.

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180 LUNG EXTRACELLULAR THIOCYANATE LEVELS ARE MODULATED BY CFTR AND CAN AMELIORATE MYELOPEROXIDASE-MEDIATED INJURY.

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The cystic fibrosis transmembrane conductance regulator (CFTR) has been shown to transport a wide variety of anions into the lung epithelial lining fluid (ELF). We wanted to examine the effect of defective CFTR on thiocyanate levels in the lung ELF and adaptive responses to *Pseudomonas aeruginosa* (PA) challenge. Wild type and CFTR KO mice were challenged intratracheally with PA, and on the 3rd day of infection bronchoalveolar lavage fluid was obtained and analyzed for thiocyanate levels using a colorimetric assay. Basal levels of lung ELF thiocyanate were similar in both wild type and CFTR KO mice. PA infection increased the levels of lung ELF thiocyanate 4-fold in the wild type but only 2-fold in the CFTR KO mice. In separate studies, we measured the basal thiocyanate levels in human lung epithelial cell lines sufficient (C38) and deficient (IB3) in CFTR grown in transwells. CFTR sufficient cells had significantly higher basal levels of thiocyanate in apical compartment as compared to CFTR deficient cells. To test for the functional importance of thiocyanate, we exposed human lung epithelial (A549) cells to a MPO system that generates hypochlorous acid in presence of different concentrations of thiocyanate. Thiocyanate significantly decreased the MPO-induced cytotoxicity in dose-dependent manner. These data suggest that CFTR is partially responsible for thiocyanate transport in the apical compartment. Furthermore, our data also indicates that the lung adapts to infectious agents with elevated ELF thiocyanate levels which may protect the lung against MPO released from activated neutrophils. Individuals with cystic fibrosis may lack the ability to adequately adapt to lung infections resulting excessive injury. (This work was supported in part through funding by NIH HL75523).

181 A ROLE FOR GLUTATHIONE TRANSPORT IN THE ATTENUATION OF OXIDATIVE INJURY BY HYPERTONIC SALINE.

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The lung is continually exposed to environmental agents and pathogens. The lung epithelial lining fluid (ELF) is first to encounter these agents and GSH is a major antioxidant found in this apical fluid. The cystic fibrosis transmembrane conductance regulator protein (CFTR) is the only known GSH transporter that maintains

lung ELF GSH levels. Cystic fibrosis (CF) patient have low levels of GSH in their ELF and have copious and viscous mucus. Hypertonic saline is used in CF patient to help clear mucous and improve lung function however the mechanism(s) by which it does so are poorly understood. The purpose of these studies was to examine whether hypertonic (3.0%) saline can modulate apical GSH levels and protect lung epithelial cells against the oxidant, t-butyl hydroperoxide (t-BOOH). We used human lung epithelial cell lines sufficient (C38) and deficient (IB3) in CFTR. The CFTR deficient IB3 cells have 40% lower basal levels of apical GSH as compare to CFTR sufficient C38 cells. Cells were exposed separately and in combination to 3% saline, and t-BOOH (100 μ M) for 48 hours. The CFTR deficient IB3 cells were much more sensitive to t-BOOH-mediated oxidative injury as measured by lactate dehydrogenase (LDH) release. Hypertonic saline exposure was associated with an increase in apical GSH levels in both CFTR sufficient and deficient cells and decreased t-BOOH-mediated oxidative injury in both cells lines. Hypertonic saline increased GSH in the apical compartment, which appeared to be largely CFTR mediated. This data suggests that CFTR and GSH adaptive responses play an important role in lung's reaction to oxidants. We propose that factors, which interfere with the lung's capability to mount and maintain an adequate adaptive apical GSH response, may compromise and sensitize the lung to oxidative injury. (Supported in part from NIH grant HL075523).

182 DIFFERENTIAL MODULATION OF STRESS SIGNALING PATHWAYS BY CADMIUM IN CULTURED MOUSE LUNG FIBROBLASTS HETEROZYGOUS FOR GPX4.

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Cadmium is a widely dispersed environmental and occupational toxicant and has been associated with mutagenic, carcinogenic, teratogenic and neurotoxic endpoints. Inhalation exposure to cadmium has been associated with airway inflammation, pulmonary edema, emphysema, and cancer. Cadmium causes oxidative stress in cells by formation of reactive oxygen species (ROS), induction of lipid peroxidation, binding to protein thiols, and alteration of intracellular glutathione status. Glutathione peroxidase 4 (GPx4) is a member of the family of selenium dependent enzymes that catalyze the reduction of peroxides. GPx4 specifically reduces mitochondrial membrane-bound phospholipid hydroperoxides in situ and thus, protects against membrane peroxidative damage. Mouse lung fibroblasts deficient in GPx4 have increased susceptibility to cadmium-induced cytotoxicity. In the present study cultured mouse lung fibroblasts from wild-type (+/+) and GPx4 heterozygous (+/-) mice were exposed to cadmium chloride (CdCl₂), and cell extracts were analyzed for stress (p38, JNK) and survival (Akt) pathway activation by Western blot analyses. Levels of phosphorylated p38 and JNK were increased following CdCl₂ treatment in GPx4+/+ cells, but less so in GPx4+/- cells. Phosphorylated Akt levels, though decreased slightly, remained high in GPx4+/+ cells, but dropped to only 10% or less of control in GPx4+/- cells. However, GPx4+/- cells exhibited an attenuated survival response, as measured by the ratio of survival pathway activation (Akt) to stress pathway activation (p38 and JNK). These results implicate the involvement of survival and stress signaling pathways in cadmium-induced cytotoxicity, and the potential involvement of these pathways in defining the differential susceptibility to cadmium-induced stress in cells deficient in GPx4.

183 DIESEL EXHAUST PARTICLE-INDUCED OXIDANT INJURY AND CELLULAR RESPONSES IN WILD TYPE AND INDUCIBLE NITRIC OXIDE SYNTHASE-DEFICIENT (iNOS KO) MICE: ROLES OF PARTICLE CORE AND ADSORBED ORGANICS.

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Studies have shown that diesel exhaust particle (DEP) exposure induces lung inflammation and injury, which are mediated through reactive oxygen/nitrogen species (ROS/RNS) generation by alveolar macrophages (AM). The present study examines the differential roles of DEP particle core, represented by carbon black (CB), and DEP organic extract (DEPE) in ROS generation and nitric oxide (NO) production through inducible nitric oxide synthase (iNOS)-related cellular responses using C57B/6J wild type (WT) and iNOS knockout (iNOS KO) mice. Mice (8-10 weeks old) were exposed to saline, CB (35 mg/kg), or DEPE by aspiration and sacrificed at 1, 3 and 7 days post-exposure. AM were isolated by bronchoalveolar lavage (BAL). CB, but not DEPE, significantly induced neutrophil infiltration in both WT and iNOS KO mice. CB-exposed AM also exhibited enhanced hydrogen peroxide and superoxide anion generation, peaking at 3 d post exposure. This CB-induced oxidative stress was correlated with mitochondrial damage, involving reduction of mitochondrial mass and membrane potential in

AM from both WT and iNOS KO mice. These results suggest that NO did not play a major role in modifying CB-induced mitochondrial damage in AM. In contrast, the organic component of DEP, DEPE, only slightly induced ROS generation and did not significantly affect mitochondrial function in AM. Measurement of macrophage ATP content showed that CB and DEPE did not significantly affect ATP levels in either WT or iNOS KO mice. This is due to the fact that mitochondrial oxidative phosphorylation pathway is not the major energy source for AM. In summary, this study shows that CB, but not DEPE, plays a major role in DEP-induced pulmonary inflammation and AM mitochondrial dysfunction by a mechanism independent of NO. (The findings and conclusions in this abstract have not been formally disseminated by the NIOSH and should not be construed to represent any agency determination or policy.)

184 BLOCKADE OF HMGB1 INHIBITS HYPEROXIA-INDUCED PRO-INFLAMMATORY LUNG INJURY.

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Prolonged exposure to hyperoxia results in acute lung injury (ALI) with markedly elevated levels of proinflammatory cytokines and infiltrated leukocytes in lungs. However, the mechanisms underlying hyperoxia-induced proinflammatory ALI remain poorly understood. Here we report our studies on determining the role of high mobility group box protein 1 (HMGB1), a newly discovered proinflammatory cytokine, in hyperoxic lung injury. Exposure of adult mice to >99% oxygen increased the accumulation of HMGB1 in the bronchoalveolar lavage fluid, which preceded the onset of severe lung injury. Neutralizing anti-HMGB1 antibodies, administered to mice 24 hours prior to hyperoxic exposure, significantly mitigated ALI, indicated by the levels of the wet/dry ratio, lung permeability and total leukocyte infiltration. This protection was also observed when the treatment with HMGB1 inhibitors was delayed until after the onset of the oxidative stress. In addition, ethyl pyruvate, a stable aliphatic antioxidant, not only inhibited HMGB1 secretion from hyperoxic macrophages, but also mitigated hyperoxic lung injury when administered post hyperoxic exposure. The contribution of HMGB1 to the initiation of hyperoxic lung injury was further confirmed using purified recombinant HMGB1 (rHMGB1) protein instilled intratracheally. Administration of rHMGB1 caused a marked increase in the levels of leukocyte infiltration into the lung of these mice compared to those that were treated with the same amounts of non-specific peptide ($31 \pm 4.8 \times 10^4$ vs. $14 \pm 3.4 \times 10^4$, $p < 0.05$). Taken together, these results indicate that HMGB1 plays a critical role in mediating hyperoxic lung injury through the recruitment of leukocytes into the lung, and support the potential clinical application of HMGB1 inhibitors as therapeutic interventions against oxidative lung injury in patients receiving hyperoxia.

185 METABONOMIC SIGNATURES OF SILICA NANOPARTICLE RESPIRATORY TRACT TOXICITY IN NORMAL AND DISEASED ANIMALS.

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Characterizing the hazard potential of the significant number of nanomaterials that have been or will be produced is one of the most noteworthy challenges faced by the regulatory, research, and producer communities. Ultrafine (aerodynamic diameter dae, 0.1 μ m) and fine (dae, 2.5 μ m) particulate matter is associated with decreased lung function in child and adult asthmatic populations and increased cardiovascular morbidity and mortality in the general population. One weakness of occupational studies is they generally do not consider effects on sensitive populations. The Wistar-Kyoto (WKY) derived spontaneously hypertensive (SH) rat is a useful model of cardiovascular disease with underlying chronic pulmonary injury, inflammation, and oxidative stress. Metabonomic analysis involves the quantitation of the multivariate response of an organism to a pathological event. To provide a metabonomic comparison of the biological response to nanoparticle exposure between the normal (WKY) and sensitive (SH) rat, male rats were given a single dose of amorphous silica (16 nm) at either 0.12 or 5 mg/kg via intratracheal instillation. Bronchoalveolar lavage fluid (BALF) and blood were collected 24 hours after exposure. Screening of small molecule biomarkers less than 1400 atomic mass units was accomplished using LC-ESI-MS. Comparison of peak areas from spectra of blood and BALF samples showed clear differences in endogenous levels of compounds associated with oxidative stress between nanoparticle exposed and control animals of both strains. More subtle changes in peak areas of endogenous compounds were observed in comparison of BALF and blood collected from hypertensive versus normotensive animals. This study indicates that non-invasive monitoring of readily available biofluids using mass spectrometry holds promise as a rapid methodology for tracking metabonomic changes and identification of biomarkers of nanomaterial induced respiratory and cardiovascular toxicity.

186 POST-TRANSLATIONAL MODIFICATION OF SURFACTANT PROTEIN-D IN OZONE-INDUCED LUNG INJURY: AN INFLAMMATORY SIGNAL.

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Ozone is a ubiquitous urban air pollutant known to induce damage to the lower lungs. This is associated with an accumulation of macrophages in the tissue and the release of reactive nitrogen intermediates (RNIs) which we have previously demonstrated contribute to tissue injury. However, the molecular mechanisms by which RNIs induce cell death and injury are unclear. Surfactant Protein-D (SP-D), a pulmonary collectin, possesses both pro and anti-inflammatory functions; these functions are controlled via two cysteine residues in the hydrophobic tail. Here we examined whether SP-D was a target for NO-mediated modification within a mouse model of ozone-induced injury. Mice were exposed to air or ozone (0.8 ppm) for 3 hr. Bronchoalveolar lavage (BAL) fluid was collected 0-72 hr later. Utilizing a biotin-switch method we identified that SP-D is nitrosylated to form SNO-SP-D. SNO-modification peaked 24-48 hr following exposure and resolved by 72 hr. SNO-modification was accompanied by disruption of the multimeric structure of SP-D, as shown by native gel-electrophoresis. In vitro these modifications can be replicated within BAL from control animals or recombinant protein by transnitrosation with S-nitrosocysteine. Utilizing a modified Boydin chamber we showed that SNO-SP-D, but not control SP-D can induce RAW 264.7 macrophage chemotaxis. Further, while SP-D suppresses calcium influx in response to bradykinin in A549 lung epithelial cells, this was not observed with SNO-SP-D. These studies suggest that nitrosylation of SP-D may be a mechanism whereby RNIs induce pulmonary injury and inflammation. Supported by NIH grants, ES004738, ES005022, ES007148, and HL074115

187 MASS SPECTROMETRIC ANALYSIS OF PHOSPHOLIPID MOLECULAR SPECIES AND THEIR HYDROPEROXIDES IN APOPTOTIC NEURONS AND INJURED RAT BRAIN.

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We applied oxidative lipidomics approach (that includes quantitative HPLC fluorescence-based assessment of lipid hydroperoxides) and mass spectrometry (MS) (electrospray ionization, ESI-MS and matrix-assisted laser desorption ionization, MALDI-MS) to characterize individual molecular species of phospholipid classes and their hydroperoxides in neurons and brain. In in vitro experiments, rat primary cortical neurons were triggered to apoptosis by staurosporine (STS). Traumatic brain injury (TBI) was employed as an in vivo model in which mitochondrial fraction from rat brain cortex was utilized for phospholipid analysis. In both models MS analysis revealed the formation of several oxygenation products of two major anionic phospholipids - phosphatidylserine (PS) and cardiolipin (CL). Selective oxidation of CL and PS with highly oxidizable molecular species C22:5 and C22:6 were detected. We identified one major molecular cluster for PS and at least 12 different molecular species of CL. Detailed analysis of doubly-charged peak of CL ions at m/z 790 after TBI demonstrated that it corresponds to several CL species containing fatty acid residues with m/z 281 (C18:1), 283 (C18:0), 327 (C22:6), and 359 (C22:6+OOH). Notably, two most abundant phospholipids, phosphatidylcholine and phosphatidylethanolamine, did not reveal any oxidation products induced by either STS in neurons or after TBI in brain in spite of the presence of polyunsaturated molecular species. Thus, phospholipid oxidation products can be successfully detected and characterized in vitro and in vivo by MS analysis. Support: AHA0535365N, NIH HL70755, AI068021, PA Dept of Health, DAMD 17-01-2-0038.

188 STUDIES OF O₂ TOXICITY IN RAT CORTICO- HIPPOCAMPAL TISSUE SLICES.

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Brain O₂ levels can vary from near hypoxia during ischemia, to hyperoxia during hyperbaric oxygen (HBO) events, to episodic changes in sleep apnea. We are studying brain sensitivity to O₂ using measures of reactive oxygen species (ROS), electrophysiology, and NMR-based metabolomics. Rat cortico-hippocampal slices were incubated in artificial CSF with 0.95 ATA O₂ (atmospheres absolute) for 1h, then

transferred to a pressure chamber at 36 °C for 1h at either: 0.40 ATA (n=6), 0.95 ATA (n=6), or 4.5 ATA O₂ (HBO, n=6). Slices were rinsed, blotted dry, and frozen in liq N₂. Aqueous and lipid extracts were prepared and analyzed using a Varian INOVA 600 NMR. Previous work shows ROS increases as normobaric O₂ increases, and excitability is reduced at 0.40 vs 0.95 ATA O₂. To examine the metabolic effects, 1H and 31P spectra of aqueous extracts were analyzed for lactate, N-acetylaspartate (NAA), creatine (Cr), phosphocreatine (PCr), ATP, and inorganic phosphate (Pi). These reflect neuronal viability (NAA/Cr ratio) and cellular energy status. Principal Component Analysis (PCA) of 1H aqueous spectra and 13C lipid spectra showed data clustering in the scores plot and clear separation for the 0.40 ATA O₂ vs HBO groups. The PCr/ATP ratios vs [O₂] show a positive linear correlation (r²=0.95), and the value for 0.95 ATA (2.2±0.2) compares favorably with values in vivo. PCr/ATP at 0.40 ATA (1.4±0.2) may indicate early signs of metabolic stress. But ATP/Pi and NAA/Cr ratios at 0.40 vs 0.95 ATA were not different suggesting that cells are not yet energy compromised and show no significant loss of neurons. Thus the reduced excitability observed at 0.40 vs 0.95 ATA O₂ may be normal, and activity at 0.95 ATA may reflect a hyperoxic state. Indeed, HBO shows >2-fold higher PCr/ATP (3.5±0.2) and 2-fold lower lactate/Cr (0.71±0.1) vs 0.40 ATA, suggesting that the cellular energy charge may be augmented under high O₂.

189 DFP-INDUCED OXIDATIVE INJURY AND DENDRITIC DAMAGE IN RAT BRAIN.

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Increasing evidence suggests that in addition to excessive cholinergic stimulation, organophosphates, such as DFP (diisopropylphosphorofluoridate) induce glutamatergic activation, inflammatory responses as well as generation of reactive oxygen (ROS) and nitrogen (RNS) species, leading to neuronal degeneration. In order to establish the relevance of these mechanisms to structural neuronal injury, we studied multiple effectors of DFP exposure, which are critical in causing oxidative damage. Rats treated with DFP developed onset of toxicity signs within 10-15 min, which progressed to maximal severity of seizures and fasciculations within 60 min. At this time point, biomarkers of ROS, F₂-isoprostanes (F₂-IsoPs) and F₄-neuroprostanes (F₄-NeuroPs) were significantly increased (p<0.001) to 196% and 756% of control, respectively. Severe seizures also induced 2-5 fold increase in citrulline, a marker of RNS in discrete brain regions (cortex, amygdala, and hippocampus) but no changes in cerebral pro-inflammatory prostaglandins E₂ (PGE₂) were seen at 1 h following DFP compared to control (1.71 ng/g tissue). Significant elevations in PGE₂ levels were seen at a later time point (6 h) with the highest increase (340%) at 24 h. Quantitative morphometric analysis of pyramidal neurons of the hippocampal CA1 region 1 h following DFP exposure revealed significant decreases (p<0.01) in dendritic lengths and spine density to 64% and 60% of control, respectively. These findings support the involvement of ROS, RNS and PGE₂ as mediators of seizure-induced neuronal injury. (Supported by NIH P30 ES000267)

190 ANGIOTENSIN II-INDUCED RENAL OXIDATIVE STRESS IN CHRONIC ALCOHOL-INDUCED HYPERTENSION.

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The link between chronic alcohol consumption and the prevalence of hypertension is well established in clinical as well as experimental studies. However, molecular mechanisms implicated with alcohol-induced increases in blood pressure (BP) remain elusive. The aim of this study was to investigate the relationship of chronic ethanol-induced increase in blood pressure (BP) with angiotensin II-induced renal oxidative injury response in rats. Male Fisher rats were divided into two groups of seven animals each and treated as follows: (1) Control (5% sucrose, orally) daily for 12 weeks; (2) ethanol (4 g kg⁻¹, orally) daily for 12 weeks. The BP (systolic, diastolic and mean) was recorded every week through tail-cuff method. The animals were sacrificed after 12 weeks blood and kidney were isolated and analyzed for angiotensin II levels and oxidative stress parameters. The results show that the systolic and mean BP was significantly elevated 6-12 weeks after ethanol ingestion, whereas diastolic BP was significantly elevated 8-12 weeks post ethanol ingestion. The increased BP was related to elevated angiotensin II levels in the tissues of alcohol treated group compared to control. The renal oxidative stress parameters such as NADPH oxidase activity, ratio of oxidized and reduced glutathione (GSSG/GSH) and lipid peroxidation significantly increased in alcohol group compared to control. The renal antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) were significantly depressed in alcohol-treated rats compared to control. The study concluded that chronic ethanol ingestion induces elevations in systolic, diastolic and mean BP which are correlated with elevated tissue angiotensin II levels. Elevated angiotensin II levels induced superoxide production via activation of NADPH oxidase activity causing increased

lipid peroxidation and depletion of antioxidants (glutathione levels and antioxidant enzyme activities) leading to oxidative renal injury in rats (supported in part by NIH grant #2 G12 RR03050-19).

191 PREVENTION OF HYPEROXIA-INDUCED RETINOPATHIES AND ABNORMAL NEOVASCULARIZATION IN THE NEWBORN RAT BY RETINOIC ACID.

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Supplemental oxygen administration to premature infants contributes to the development of retinopathy of prematurity (ROP). In this study, we tested the hypothesis that neonatal exposure of rats to a combination of all-trans retinoic acid (RA) and hyperoxia would alleviate retinopathy and abnormal neovascularization compared to those exposed to hyperoxia alone. Newborn Fisher 344 rats were maintained in room air or exposed to hyperoxia ([gt] than 95% O₂) for 7 days. Some animals were treated i.p. with RA (1 mg/kg) or vehicle (saline), once daily for the first 5 days of hyperoxic exposures. Animals were sacrificed at selected time points after termination of hyperoxia. Retinal histopathology and vascular densities of flat mounted retinas were assessed. mRNA expression of VEGF and its receptors (VEGFR-1/sFLT-1 and VEGFR-2), and HIF-1 α was determined by real time RT-PCR. Immediately after 7 days of exposure to hyperoxia or to hyperoxia + RA, we observed constricted retinal vessels, compared to air breathing animals. Seven to thirty days after termination of hyperoxia, the animals displayed formation of abnormal retinal vessels and capillaries, whereas animals given RA+ hyperoxia showed significantly lesser extent of neovascularization. At these time points, VEGF mRNA expression in the hyperoxia group was much higher than that of the RA+ hyperoxia group. On the other hand, the expression of VEGFR-1 and sFLT-1, but not VEGFR2, was differentially regulated by RA + hyperoxia, compared to those animals exposed to oxygen alone. EMSA and supershift experiments suggested that CREB plays an important role in the modulation of VEGFR-1 by RA. In conclusion, our results suggest that RA protects retinas from oxygen-induced retinopathy with abnormal neovascularization, and that modulation of VEGFR-1 via CREB contributes to the retinoprotective effects of RA. Future studies to elucidate the mechanisms of RA action could lead to the potential use of RA in infants with the risk of developing ROP.

192 MITOCHONDRIAL THIOREDOXIN-2 OVEREXPRESSION INHIBITS OXIDATIVE DAMAGE IN AGED MICE.

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Numerous studies have shown an increase in oxidative stress biomarkers and damage as a consequence of aging. While antioxidant supplementation does not show beneficial effects in slowing the aging process, a decrease in mitochondrial reactive oxygen species (ROS) inversely correlates with lifespan duration. Thioredoxin-2 (Trx2) is an antioxidant system particular to the mitochondria and is capable of participating in the elimination of ROS. To evaluate the effects of mitochondrial health and aging, we generated a Trx2 transgenic (Trx2 Tg) mouse and measured parameters of oxidative stress in young mice (~3 months) and old mice (~24 months). For measurement of reactive oxygen metabolites, a dROMs (reactive oxygen metabolites) assay was performed. There was not a significant difference between young wild-type and Trx2 Tg mice. While both old wild-type and Trx2 Tg mice showed an increase of ROMs with age compared to respective young mice, old wild-type mice showed an increase of nearly 130% while the old Trx2 Tg mice showed a 58% increase. These differences approached statistical significance (p=0.07). Plasma nitrotyrosine (N-Tyr) and protein carbonyls (PC) were determined by mass spectrometric and colorimetric assays, respectively. Plasma N-Tyr concentrations between young wild-type and Trx2 Tg mice were not different but plasma N-Tyr was significantly less (approximately 50%) in old Trx2 Tg mice as compared to old Wild-type mice. Plasma PC showed a similar pattern, where significant differences were observed between old wild-type and Trx2 Tg mice measuring an approximate difference of 55%. Plasma redox status was measured via HPLC and showed no significant differences in the glutathione/glutathione disulfide redox potential (Eh) between any of the groups. Significant oxidation of the cysteine/cystine Eh was observed and appears to be a consequence of a significant decrease in plasma cysteine concentrations. In summary, the overexpression of Trx2 protects against oxidative damage that occurs during aging and may contribute to increase in lifespan.

193 INDUCTION OF OXIDATIVE STRESS AND OXIDATIVE DNA DAMAGE IN RATS BY ACRYLONITRILE.

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Acrylonitrile, widely used in the manufacture of plastics, acrylic fibers, and synthetic rubber, resulted in a dose-related increase in gliomas in rats following chronic exposure. Studies have provided evidence supporting that the mechanism for the induction of brain tumors by acrylonitrile involves the induction of oxidative stress. The present study examined the ability of acrylonitrile to induce oxidative stress and DNA damage in the rat. Male Sprague-Dawley rats were treated with 0, 3, 30, 100 ppm acrylonitrile in drinking water for 28 days; and in a time-response study, rats were treated with 0 or 200 ppm acrylonitrile in drinking water for 14 and 28 days. Direct DNA strand breakage in white blood cells was measured using the alkaline Comet assay. Oxidized DNA damage was evaluated using the formamidopyrimidine-modified Comet assay and HPLC with electrochemical detection in white blood cells and brain cortex, respectively. No increase in direct DNA strand breaks were observed in white blood cells from any of the treated rats, however, oxidative DNA damage was increased in white blood cells in a dose- and time-dependent manner (up to 3.8 fold over control). Similarly, dose- and time-dependent increases in the level of 8-hydroxy-2'-deoxyguanosine in brain cortexes (up to 3.9 fold over control) and hydroxyl free radicals in plasma (up to 9.8 fold over control) were observed in rats treated with acrylonitrile. A slight but significant decrease in the GSH/GSSG ratio was seen in the brain cortexes of rats receiving 30, 100, 200 ppm acrylonitrile in drinking water for 28 days, and 200 ppm for 14 days. These results show that ACN dose not produce direct damage to DNA, but does cause oxidative stress and oxidative DNA damage in rats, and supports that oxidative DNA damage may be involved in acrylonitrile-induced tumorigenicity.

194 WHICH RISK FACTOR IS IMPORTANT FOR INCINERATOR WORKERS: EXPOSURE TO DIOXINS OR POLYCYCLIC AROMATIC HYDROCARBONS?

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Incinerator workers are well known to be at the risk of exposure to dioxins and the cancer is considered to be a critical outcome. On the other hand combustion of carbon materials produces polycyclic aromatic hydrocarbons (PAH) as well as dioxins and PAH are also well known as mutagens or carcinogens. We intended to evaluate the effects on oxidative stress level among workers exposed to dioxins or PAH. We studied workers from four factories, which were three municipal solid waste incinerators (MSWI) A, B, and C and one industrial waste incinerator D. Subjects were 97 male, aged from 22 to 64 and mean 44.7. Blood were collected from cubital vein before the work shift and 7 polychlorinated debenzo-para-dioxins, 10 polychlorinated dibenzofurans, and 12 non-ortho and mono-ortho coplanar polychlorinated biphenyls were measured. Urine samples were collected before and end of the work shift and 1-hydroxypyrene (1-OHP), 2-naphthol (2-N), 8-hydroxy-2'-deoxyguanosine (8-OH-dG) and creatinine were measured. 1-OHP was highest for factory A workers, 2-N was highest for factory B workers, dioxins were highest for factory D workers and 8-OH-dG was highest for factory B. These results suggested that oxidative stress was highest for workers of factory B who were exposed to rather high naphthalene and rather low pyrene. Dioxin levels did not influence the oxidative stress level. Factory B is ordinary MSWI and workers have not dealt with industrial chemicals or house building materials. Emitted PAH from the incinerator must be produced mainly from the combustion of municipal solid waste. Although low molecular PAH were considered not to have significant effect to carcinogenicity and the increased exposure of factory B workers to naphthalene does not directly relate to increased oxidative stress level, the relation needs to be examined further.

195 HMGB1 IN HYPEROXIA-INDUCED IMPAIRMENT OF PSEUDOMONAS AERUGINOSA PHAGOCYTOSIS.

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Pseudomonas aeruginosa (PA) is a leading cause of nosocomial pneumonia in patients receiving mechanical ventilation with hyperoxia. Alveolar macrophages are instrumental in bacterial clearance in the lung. Previously, we have shown that hyperoxic exposure ($\geq 95\%$ O₂) results in a significant impairment of phagocytosis of PA in both alveolar macrophages that were isolated from hyperoxic mice and in cultured macrophages. Since patients are weaned from these high concentrations of oxygen at the earliest possibility, we tested whether the magnitude of the impairment of PA phagocytosis depends on the concentrations of oxygen. RAW 264.7

cells were exposed to different concentrations of oxygen, ranging from 95% down to 40%. Hyperoxia ($\geq 95\%$ O₂)-induced cell proliferation-arrest was diminished when the concentration of oxygen was 65% or lower. However, this differential effect on cell proliferation was not reflected in the macrophage's ability to phagocytose PA. Exposure to $\geq 40\%$ O₂ induced similar extents of PA phagocytosis impairment. Corresponding to the reduction in PA phagocytosis, similar levels of HMGB1, a newly discovered cytokine and an important contributor to the pathogenesis of hyperoxic acute lung injury, were accumulated in the cultured media of RAW cells exposed to various concentrations of hyperoxia. To test whether HMGB1 contributes to hyperoxia-induced reduction of PA phagocytosis, RAW cells were exposed to recombinant HMGB1. Compared to RAW cells treated with a control peptide or trypsinized HMGB1, recombinant HMGB1 caused a reduction of PA phagocytosis at a similar level as those exposed to hyperoxia. These data suggest that HMGB1 plays a critical role in hyperoxia-induced impairment of PA clearance.

196 A DIFFERENTIAL EFFECT OF HYDROGEN PEROXIDE ON THE ABILITY OF MACROPHAGES TO PHAGOCYTOSE MICRO-ORGANISMS.

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Prolonged exposure to hyperoxia during mechanical ventilation causes acute proinflammatory responses. Nosocomial pneumonia in patients receiving mechanical ventilation is a related problem and one of the major causes for mortality and morbidity in Intensive Care Units. Hydrogen peroxide, generated by phagocytes during inflammation to kill the invading microorganisms, impairs the macrophage's ability to phagocytose. However, hydrogen peroxide exhibits differential effects on cell proliferation and cell death in a concentration-dependent manner. In this study, we tested whether the impairment of macrophage phagocytosis by hydrogen peroxide depends on its concentration in cultured macrophages. RAW 264.7 cells were exposed to various concentrations of hydrogen peroxide from 1mM to 1 μ M for up to 3 hours. Consistent with the previous studies, hydrogen peroxide significantly reduced macrophage phagocytosis of microbeads at concentrations higher than 500 μ M. In addition, loss of mitochondria dehydrogenase activity, as evidenced by the MTT assay, and reduced total actin polymerization were observed in these cells. In contrast, the ability of RAW cells to phagocytose was significantly ($>50\%$) increased when the cells were treated with <200 μ M hydrogen peroxide. Furthermore, actin polymerization was markedly more pronounced in cells treated with low concentrations of hydrogen peroxide. These results suggest that a moderate amount of hydrogen peroxide can stimulate actin polymerization and improve phagocytosis in macrophages although high concentrations of hydrogen peroxide are detrimental to macrophage functions.

197 IN VITRO TOXICITY AND OXIDATIVE STRESS STUDY WITH NANO- VS. FINE-SIZED ZNO PARTICLES IN HUMAN LUNG CANCER CELLS.

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Cytotoxicity of 71 nm ZnO nanoparticles and 0.48 μ m ZnO fine-sized particles was investigated in cultured human bronchoalveolar carcinoma-derived cells. The physicochemical properties of these two nanoparticles were well characterized using TEM, XRD, and BET. The results demonstrated that exposure to both particles at 10-14 μ g/mL dosage levels for 24-hr decreased cell viability in a dose-dependent manner. Dosage levels less than 8 μ g/mL resulted in no cell viability while dosage levels higher than 18 μ g/mL caused approximately 95% cell viability reduction. The 71 nm ZnO nanoparticles (IC₅₀=13.3 μ g/mL) showed only slightly higher cytotoxicity than that of 0.48 μ m ZnO particles (IC₅₀=13.9 μ g/mL). In the subsequent mechanistic study, both particles were used to determine levels of cellular oxidative stress. Indicators of oxidative stress and cytotoxicity, including total reactive oxygen species (ROS), glutathione, malondialdehyde, and lactate dehydrogenase, were assessed quantitatively. Significant oxidative stress was observed after cells had been exposed to 10-14 μ g/mL of 71 nm or 0.48 μ m ZnO particles for 24-hr, as indicated by elevated cellular ROS levels and reduced GSH. Elevated malondialdehyde levels indicated lipid peroxidation, a consequence of elevated oxidative stress due to ZnO exposure. The increased lactate dehydrogenase levels in the cultured medium indicated cell membrane damage ($p < 0.05$). In addition, results from single cell gel electrophoresis (Comet Assay) showed DNA damage was induced by ZnO particles. In general, the extent of oxidative stress induced by 71 nm ZnO nanoparticles was higher than that of 0.48 μ m ZnO particles.

198 THE MOUSE CLARA CELL AS A TARGET FOR STYRENE-INDUCED TOXICITY.

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Styrene is widely used in the manufacturing industry. In mice and rats, it is both hepatotoxic and pneumotoxic. It causes lung tumors in mice but not in rats. The Clara cell is the main target for the toxicity of styrene and its metabolites, and it also has the highest activity in the lung for styrene metabolism. Therefore, adverse effects of styrene and its metabolites on Clara cells isolated from CD-1 mice were investigated. Cytotoxicities due to styrene and its metabolites were compared in vitro in Clara cells at concentrations ranging from 0.1 to 10 mM. The cytotoxicity of styrene was greater in vitro than that of its metabolites styrene oxide (SO) (racemic, R- and S-) and 4-vinylphenol (4VP) in contrast with what has been observed in vivo in previous studies on hepatotoxicity and pneumotoxicity. Levels of the antioxidant glutathione were measured in mouse Clara cells following treatment with styrene and its metabolites in vitro and in vivo at doses of 600 mg/kg for styrene, 300 mg/kg for SO, and 100 mg/kg for 4VP. Decreases in glutathione concentrations were seen even at doses which did not cause the death of mouse Clara cells. Decreases in Clara cell glutathione were observed 3 hours after treatment with all compounds and were significant with racemic SO and R-SO. At 12 hours, rebound effects were seen for all compounds, with all but R-SO rebounding above controls. Reactive oxygen species were measured in vitro in Clara cells using 2',7'-dichlorodihydrofluorescein diacetate, with small but significant increases observed 3 hours after treatment with racemic SO, S-SO, R-SO, and styrene. Using agarose gel electrophoresis, DNA fragmentation was observed 3 hours after treatment of mice with racemic SO and R-SO. These studies suggest that the in vitro cytotoxicities of styrene and its metabolites do not strictly follow in vivo effects and that the toxicity of styrene and its metabolites to Clara cells may be related to oxidative stress. (This work was supported in part by a grant from the Styrene Information and Research Center.)

199 MYCOPLASMA PNEUMONIAE INFECTION INCREASES OXIDATIVE STRESS AND INHIBITS THE LUNG'S GLUTATHIONE ADAPTIVE RESPONSE TO CIGARETTE SMOKE IN IN VIVO AND IN VITRO.

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The lung epithelial lining fluid (ELF) serves as the first line of defense against environmental agents (EA) and potential pathogens. It has been speculated that antioxidants play important roles in protecting the lung against these agents. Cigarette smoke (CS) is known to elevate the levels of glutathione (GSH) in the ELF, although it is unclear what role this plays in the lung's ability to adapt to the damaging effects of CS. Little is currently known about how the lung regulates the levels of these antioxidants in the ELF. Recent studies suggest that the cystic fibrosis transmembrane conductance regulator protein (CFTR) is involved in the maintenance of the high levels of GSH in the ELF. Lung pathogens such as Mycoplasma pneumoniae (Mp) have been shown to exacerbate airway inflammation associated with asthma. The purpose of these studies was to examine the role of CFTR in adaptive responses following CS and Mp exposures both in vitro and in vivo. Human lung epithelial cell lines sufficient (C38) and deficient (IB3) in CFTR were exposed separately and in combination to cigarette smoke extract (CSE) and Mp for 48 hours. CSE elevated the extracellular levels of GSH in IB3 & C38 cells as compared to controls and these increases were blocked in the Mp treatment groups. BALB/c mice were exposed separately and in combination to CS and Mp for 2 and 4 months. CS exposure resulted in a 3.5-fold elevation in ELF GSH levels, which was completely blocked in the Mp exposed mice. In addition, the CS/Mp exposed mice had elevated oxidized glutathione (GSSG) and dramatic change in their ratio of GSH/GSSG ratios. Furthermore, CS/Mp exposed mice also had high levels of DNA oxidation as measured by 8OHdG. These studies suggest Mp infection may interfere with the lung's ability to respond appropriately, resulting in lung inflammation and injury. The role of GSH transporter(s) requires further studies to better understand these mechanism(s). (Supported in part from NIH grant HL075523)

200 INDUCTION OF OXIDATIVE STRESS BY URANIUM IN LUNG EPITHELIAL CELLS.

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Uranium compounds are widely used in the nuclear fuel cycle, antitank weapons, tank armor, and also as a pigment to color ceramics and glass. Effective management of waste uranium compounds is necessary to prevent exposure to avoid adverse health effects on the population. Health risks associated with uranium exposure includes kidney disease and respiratory disorders. In addition several published

results have shown uranium or depleted uranium causes DNA damage, mutagenicity, cancer and neurological defects. Oxidative stress induces DNA damage and also changes in related signaling cascade. Therefore to evaluate the role of oxidative stress induced by uranium in rat lung epithelial cells was studied. The study shows uranium induced significant oxidative stress in rat lung epithelial cells followed by concomitant decrease in the antioxidant potential in the cells. Treatment with uranium to the cells decreased cell proliferation after 72h in culture. The decrease in cell proliferation was attributed to loss of total glutathione and superoxide dismutase in presence of uranium. The supplementation of antioxidants counteracted the oxidative stress induced by uranium. In presence of NAC, GSH and vitamin E, uranium induced oxidative stress was considerably decreased suggesting loss of cellular antioxidants. Thus the results indicate that oxidative stress is induced by uranium in the rat lung epithelial cells. This work was supported by NSF-HRD 0401587; NIH RR03045-19 (GR).

201 ATTENUATION OF CADMIUM-INDUCED TOXICITY BY MONOISOAMYL 2, 3-DIMERCAPTOSUCCINATE ON CRL-1439 NORMAL RAT LIVER CELLS.

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Cadmium (Cd) is a non-essential heavy metal that is found in environment and industry. Cadmium causes severe human health problems, and also causes deleterious effects on antioxidant enzymes. Monoisoamyl 2,3-dimercaptosuccinate (MiADMS) is an ester of DMSA containing two sulfhydryl (SH) groups. MiADMS is potent chelator of cadmium and also provides an antioxidant function by removing cadmium from the site of deleterious oxidation reactions. Therefore, we studied the protective effect of MiADMS on cadmium induced oxidative stress by evaluating the activities of the antioxidant enzymes catalase, glutathione reductase (GR), and glutathione peroxidase (GPx) in cultured normal rat liver CRL-1439 cells. The cells were treated with 150µM cadmium chloride and co-treated with 300µM MiADMS at the same time, 2-hrs post or 4-hrs post for 24 hrs. Cd concentration of 150µM decreased cell viability by 61.9% compared to untreated control cells. MiADMS increased cell viability by 45.3%, 42%, and 26.25% in the same time, 2-hrs post, and 4-hrs post treatments, respectively. In the presence of 150 µM Cd, GR, catalase, and GPx activities decreased by 87.6%, 82.1% and 18.2% respectively compared to the untreated control. GR activity was increased by 110.4%, 144.7%, and 66.2% in MiADMS treated cells, respectively. Catalase activity was also increased by 53.85%, 82.55%, and 71.55% in MiADMS treated cells, respectively. GPx activity was increased by 24.3% and 5.2% in same time and 2-hrs post MiADMS treated cells, while in 4-hrs post MiADMS treated cells activity decreased by 74.35%. These results show that MiADMS protects the cells by increasing the activity of GR and catalase enzymes, while GPx showed a slight increase in activity.

202 ATTENUATION OF CADMIUM-INDUCED TOXICITY BY CO-TREATMENT OF N-ACETYL-CYSTEINE AND CADMIUM ON CRL-1439 RAT LIVER CELLS.

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Cadmium (Cd), a ubiquitous heavy metal is listed among the top seven of the 275 most hazardous substances in the environment, and it is classified as a Type I carcinogen. Human exposures to cadmium are usually through inhalation, dermal or oral routes. Although, the quantity of cadmium exposure is usually low, accumulation and long half-life of cadmium in animals and humans are responsible for the initial high health effects of cadmium. N-acetyl-cysteine (NAC), a sulfur-containing amino acid and precursor of glutathione is used as an antioxidant to protect the cells from oxidative stress. Therefore, we studied the protective effect of NAC on cadmium-induced oxidative stress in cultured normal rat liver CRL-1439 cells by evaluating the activities of antioxidant enzymes such as catalase, glutathione reductase (GR), and glutathione peroxidase (GPx). Cd concentration of 150 µM decreased cell viability by 57% compared to untreated control cells. Cells that were pre-treated with 5 mM NAC for 2-hour, or post treated for 2-hour, or co-treated with 150 µM Cd exhibited improved viability by 36%, 43%, and 34.8% respectively. There is was no catalase activity detected in the 150 µM Cd treated cells compared to untreated cells. Catalase activity increased by 39.7%, 33.8% and

13.5% respectively in pre, same, and post NAC treatment than 150 µM Cd treated cells. GR and GPx activity decreased by 43.9% and 21.7% respectively in the presence of 150 µM Cd compared to untreated cells. Interestingly, in the presence of NAC, GR activity for each treatment increased by 34.9%, 34.4%, and 43.4%, respectively. In the presence of NAC, GPx activity increased by 66.3%, 61.6%, and 66% respectively. These results clearly show NAC protects cells by increasing the activities of antioxidative enzymes.

203 ALLOXAN REDOX CYCLING IS NOT REQUIRED FOR INHIBITION OF GLUCOSE-STIMULATED OXYGEN FLUX IN ISOLATED PANCREATIC BETA ISLETS.

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Alloxan (1,3-diazinane-2,4,5,6-tetrone) is commonly used as a model to induce type II diabetes in rodents. In addition to inhibiting the glucose responsiveness of beta islet cells, it has been suggested that alloxan undergoes redox cycling. We speculate that this leads to the generation of cytotoxic reactive oxygen intermediates (ROI), and that this contributes to its diabetogenic actions. For these studies, self-referencing oxygen microensors were used to measure oxygen flux from intact islets isolated from female CD1 mice. Menadione (100 µM), a non-diabetogenic redox cycling agent was used as a control. We found that menadione caused a rapid increase (2-4 fold) in oxygen utilization by islets. In contrast, alloxan (1 mM) had no effect on basal oxygen consumption suggesting that it was not redox cycling. However, both menadione and alloxan were able to inhibit the glucose-stimulated respiratory burst in intact beta islets. Using purified cytochrome P450 reductase in an enzymatic system for redox cycling that generates hydrogen peroxide, we found that menadione effectively stimulated production of ROI, while alloxan had no effect on cytochrome P450 reductase basal activity. Unlike menadione, alloxan non-enzymatically generated ROI in the presence of cellular reducing agents, including NADPH, cysteine and glutathione in a process that utilizes molecular oxygen. However, this redox cycling activity is unlikely to occur in islets since alloxan treatment was not associated with alterations in oxygen utilization in beta islets. Taken together, these data indicate that alloxan and menadione alter islet cell functioning by distinct mechanisms and that inhibition of the glucose-stimulated respiratory burst alone is not sufficient for islet cell toxicity leading to diabetes. Supported by NIH grants ES05022, CA093798, P41 RR001395, and CA100994.

204 DIFFERENTIAL EFFECTS OF CA²⁺-INDEPENDENT PHOSPHOLIPASE A₂ INHIBITION ON OXIDANT-INDUCED CELL DEATH IN PRIMARY NEOCORTICAL CELLS.

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Our previous studies demonstrated that inhibition of Ca²⁺-independent phospholipase A₂ (iPLA₂), a phospholipid metabolizing enzyme, increased oxidant-induced (hydrogen peroxide (H₂O₂), and tert-butylhydroperoxide (TBHP)) cell death in human glioblastoma (A172) cells. The goal of this study was to determine the effect of iPLA₂ inhibition on oxidant-induced cell death in primary cultures of mouse neocortical neurons. Immunoblot analysis demonstrated that these neurons expressed both cytosolic (iPLA₂β) and microsomal (iPLA₂γ) isoforms of iPLA₂, and that these isoforms were inhibited by bromoenol lactone (BEL), an iPLA₂ selective inhibitor. Exposure of neurons to H₂O₂ (0-1000 µM) and TBHP (0-800 µM) induced concentration-dependent increases in cell death as determined by cell morphology (36 hr) and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) staining (24 hr). To examine the mechanism of cell death, adenosine 5'-triphosphate (ATP) levels and production of reactive oxygen species (ROS) were measured using bioluminescent and dichlorofluorescein assays, respectively. Both oxidants induced concentration-dependent decreases in ATP levels (50-75%) at 24 hr. H₂O₂ treatment did not induce ROS production compared to control, while TBHP treatment induced a 200% increase after 2 hr. Treatment of cells with 2.5 µM BEL prior to exposure to 500 µM H₂O₂ increased ATP levels (~30%), increased MTT staining (~10%), but did not increase ROS formation in comparison to H₂O₂-treated cells. In contrast, treatment of neurons with BEL prior to exposure to 100 µM TBHP did not prevent ATP depletion, decrease MTT staining or alter the formation of ROS. Collectively, these data demonstrate that both iPLA₂ isoforms are expressed in primary cultures of neocortical neurons. iPLA₂ inhibition appeared to protect against H₂O₂-induced neuronal cell death but not TBHP-induced cell death. This suggests the novel hypothesis that inhibition of iPLA₂ plays differential roles in cell death induced by different oxidants.

205 ROLE OF REACTIVE OXYGEN SPECIES, NFKAPPAB AND CASPASE-3 IN PESTICIDE-INDUCED NEURONAL CELL CYTOTOXICITY.

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A number of epidemiological studies have demonstrated a strong association between the incidence of Parkinson's disease and factors that increase the likelihood of exposure to pesticides. We and others have demonstrated that exposure to endosulfan and zineb causes dopaminergic system dysfunction in vivo. Reactive oxygen species (ROS) has been implicated in neurotoxicity, based on its role in the cascade of biochemical changes that lead to dopaminergic neuronal cell death. We, therefore, examined the role of ROS in pesticides (endosulfan and zineb)-induced neurotoxicity in human neuroblastoma cells (SH-SY5Y) in culture. The generation of intracellular hydrogen peroxide and superoxide anion was monitored using spectrofluorometric analysis in combination with dichlorofluorescein diacetate and hydroethidine dyes, respectively. SH-SY5Y cells exposed to pesticides (50-200 μ M) were found to generate both superoxide anion and hydrogen peroxide in a dose- and time-dependent manner. Mixtures of the pesticides significantly enhanced the production of these reactive oxygen species compared to individual pesticide exposures. Exposure to these pesticides individually or in mixtures decreased the activities of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase in SH-SY5Y cells. Additionally, these pesticides augmented lipid peroxide formation in these cells. While both pesticides individually (at 100 μ M) increased caspase-3 activity, cells exposed to a mixture of the pesticides exhibited significantly low levels of this protein, probably due to excessive necrotic cell death. Furthermore, exposure to these pesticides increased nuclear transcription factor NFkappaB activity. Taken together, these findings suggest that the cytotoxicity of endosulfan and zineb, both individually and in mixtures may, at least in part, be associated with the generation of reactive oxygen species with concomitant increased expression of NFkappaB.

206 QUINONE-THIOETHER MEDIATED STRESS RESPONSES IN HUMAN PROXIMAL TUBULAR EPITHELIAL CELLS.

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Hydroquinone (HQ) is a rodent carcinogen and a potential human carcinogen. HQ is metabolized in the liver to 2,3,5-tris-(glutathion-S-yl)HQ (TGHQ), a redox active and potent toxic quinone- thioether. The kidney is a known target organ of HQ toxicity and is critical for TGHQ biotransformation. We are utilizing an immortalized human proximal tubular epithelial cell line, HK-2, to serve as an in vitro model for the human exposure to TGHQ. Consistent with data obtained in a porcine renal cell model (LLC-PK1), TGHQ generated reactive oxygen species (ROS) in HK-2 cells. Catalase abolished ROS production and efficiently prevented TGHQ-mediated cell death, as determined by leakage of LDH from cells. No molecular markers of apoptosis, annexin V - propidium iodide staining, were observed following TGHQ exposure, indicating that HK-2 cells undergo necrotic cell death. The mitogen-activated protein kinases (MAPKs), including ERK, JNK and p38, and another serine/threonine kinase, Akt, were all activated by TGHQ. In addition, HK-2 cells were more susceptible to TGHQ when maintained in Keratinocyte medium with EGF and bovine pituitary extract, than when cultured in DMEM-F12 medium with 10% FBS. HK-2 cells have similar doubling times and express the same level of γ -glutamyl transpeptidase in both culture media. However, ROS generation was more rapid and greater in cells cultured in Keratinocyte medium. Current studies focus on dissecting the cellular signaling pathways leading to TGHQ-mediated oncotic cell death. Moreover, elucidating the factors contributing to differences in sensitivity of HK-2 in response to TGHQ will be useful for designing mechanism-based strategies to attenuate TGHQ-mediated cell death. (RO1 DK59491, P30 ES 06694)

207 MITOCHONDRIA TARGETED HEMI-GRAMICIDIN S CONJUGATED NITROXIDES INHIBIT APOPTOSIS AND CARDIOLIPIN OXIDATION IN MOUSE EMBRYONIC CELLS.

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Reactive oxygen species (ROS) generated by disrupted electron transport in mitochondria are involved in irradiation induced intrinsic apoptosis. Removal of superoxide, a major form of ROS in mitochondria, has been suggested as an effective intervention strategy against irradiation. Although nitroxides have been proven as ideal candidates for their unique electron scavenging features and recycling ability, they are not effective in ameliorating oxidative stress due to the poor accumulation in mitochondria. Here we evaluated the effect of a hemi-gramicidin S conjugate of 4-amino-TEMPO (4-AT), (GS-TEMPO), on γ -irradiation induced mouse embryonic cell apoptosis. We report that GS-TEMPO accumulated in cells and mitochondria orders of magnitude more efficiently than 4-AT. Treatment of cells with GS-TEMPO, either pre- or post- (1h) irradiation, inhibited irradiation induced cytochrome c release, caspase-3 activation, and phosphatidylserine externalization. Further studies revealed that GS-TEMPO suppressed irradiation induced superoxide generation and accumulation of cardiolipin hydroperoxides. Finally, the comparative study of GS-TEMPO derivatives and Monte-Carlo simulations of GS-TEMPO in model lipid membranes indicated that the conservation of the DPhe-Pro reverse turn in hemi-GS analogs ensure the specific positioning of the nitroxide moiety at the mitochondrial membrane interface and maximize their protective effects. In conclusion, GS-TEMPO can be effectively delivered into mitochondria and act as radioprotectors preventing irradiation induced mitochondrial ROS production, oxidative damage and apoptosis. Supported by NIAID U19-AI068021

intervention strategy against irradiation. Although nitroxides have been proven as ideal candidates for their unique electron scavenging features and recycling ability, they are not effective in ameliorating oxidative stress due to the poor accumulation in mitochondria. Here we evaluated the effect of a hemi-gramicidin S conjugate of 4-amino-TEMPO (4-AT), (GS-TEMPO), on γ -irradiation induced mouse embryonic cell apoptosis. We report that GS-TEMPO accumulated in cells and mitochondria orders of magnitude more efficiently than 4-AT. Treatment of cells with GS-TEMPO, either pre- or post- (1h) irradiation, inhibited irradiation induced cytochrome c release, caspase-3 activation, and phosphatidylserine externalization. Further studies revealed that GS-TEMPO suppressed irradiation induced superoxide generation and accumulation of cardiolipin hydroperoxides. Finally, the comparative study of GS-TEMPO derivatives and Monte-Carlo simulations of GS-TEMPO in model lipid membranes indicated that the conservation of the DPhe-Pro reverse turn in hemi-GS analogs ensure the specific positioning of the nitroxide moiety at the mitochondrial membrane interface and maximize their protective effects. In conclusion, GS-TEMPO can be effectively delivered into mitochondria and act as radioprotectors preventing irradiation induced mitochondrial ROS production, oxidative damage and apoptosis. Supported by NIAID U19-AI068021

208 NAD(P)H:QUINONE OXIDOREDUCTASE 1 (NQO1)-MEDIATED INDUCTION OF OXIDATIVE STRESS AND DNA DAMAGE BY LAVENDAMYCIN ANTI-TUMOR AGENTS.

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Lavendamylicins are quinone antitumor agents with structural similarity to streptonigrin, a redox cycling substrate for the two-electron reductase, NQO1. Therefore, we hypothesized that efficient lavendamylicin substrates for NQO1 could also undergo redox cycling with concomitant production of reactive oxygen species (ROS) resulting in the formation of the biological marker of oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine (8-oxo-2dG) and depletion of reduced glutathione (GSH). NQO1-rich BE-NQ and NQO1-deficient BE human colon adenocarcinoma cells were treated with MB-353 (good substrate) and MB-323 (poor substrate) to investigate 8-oxo-2dG generation. An HPLC-based analysis was utilized to determine levels of 8-oxo-2dG, 2'-deoxyguanosine (2-dG) and the molar ratio, 8-oxo-2dG (fmol)/2-dG (nmol). Among MB-353 treated cells, a significant increase in the molar ratio of 8-oxo-2dG to 2-dG was observed only in BE-NQ cells suggesting a NQO1-mediated selective induction of oxidative DNA damage in BE-NQ cells by MB-353. No significant increase in the corresponding molar ratio was observed in either cell line treated with MB-323 implying that NQO1-mediated induction of oxidative DNA damage in BE-NQ cells was specific to good NQO1 substrates. Our laboratory previously determined that bioactivation of MB-353 by NQO1 caused selective depletion of GSH in NQO1-rich cells. To determine whether GSH depletion was specific to good substrates of NQO1, we measured levels of GSH in BE-NQ and BE cells after treatment with MB-323, the poor substrate. No difference was observed in GSH levels in either cell line treated with MB-323 suggesting that GSH depletion in BE-NQ cells was specific to good lavendamylicin substrates. Overall, only the good lavendamylicin substrate, MB-353, caused significant oxidative stress and DNA damage in NQO1-rich cells. Supported by NIH grants RR17670 (HDB) and CA74245 (MB).

209 ERYTHROPOIETIN PROTECTS CELLS FROM OXIDATIVE STRESS BY REDUCING OXIDATIVE DNA DAMAGE.

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Erythropoietin (EPO) was, at one time, considered only as an erythropoietic lineage-specific regulator. The production of EPO is under the influence of a complex molecular signal detection system influenced by the partial oxygen pressure. More recently, EPO has been shown to exhibit multifunctional properties and its receptor has been found not only on erythropoietic cells, but also on cells from many different tissues and organs. Erythropoietin is a proliferation factor for primitive erythropoietic cells, but a differentiation/survival factor for late erythroid cells due to its anti-apoptotic function. We now demonstrate that EPO also exhibits anti-oxidative effects. Fresh, human bone marrow cells were incubated in the absence or presence of 0.1, 1 and 10U/ml human recombinant EPO. At the time cultures were prepared and after 24h incubation, the cells were incubated in the absence or presence of 1mM methylene blue (MeB), which, when illuminated with bright light, induces oxidative DNA damage (ODD) and the production of 8-oxoguanine residues. Additional controls included the absence or presence of light. After incubation, oxidative DNA damage was detected by OxyFLOW™, a flow cytometric detection system that uses a fluorescence isothiocyanate (FITC)-conjugated 8-oxoguanine – specific binding protein to measure changes in median fluorescence intensity. The results indicate that at 0h, EPO reduced ODD, but not in a dose dependent manner. In contrast, 10U/ml EPO significantly reduced ODD by more

than 40% compared to cells without EPO. These results demonstrate that EPO has an anti-oxidative function by acting to reduce oxidative DNA damage. This may occur in a number ways, e.g. iron store depletion, increase in glutathione peroxidase. The net result is protection against free radicals and oxidative stress for a large number of cell types that express the EPO receptor.

210 AMINOGLUTETHIMIDE METABOLISM BY MYELOPEROXIDASE RESULTS IN PROTEIN FREE RADICAL FORMATION THAT CORRELATES WITH CASPASE ACTIVATION AND PROTEASOME INHIBITION: A POSSIBLE MECHANISM OF AGRANULOCYTOSIS.

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Aminoglutethimide (Cytadren®, AG) is a drug used for estrogen-dependent breast cancer, adrenal gland tumors, and also as an adjuvant to prostate tumor treatment. Agranulocytosis, an unpredictable loss of neutrophils, occurs in 1% of patients undergoing AG administration. The loss of neutrophils increases the susceptibility of the patient to bacterial infection and sepsis. We hypothesize that AG may induce this harmful side-effect by metabolism to a reactive metabolite catalyzed by myeloperoxidase (MPO), an enzyme found within neutrophils. Using purified human MPO, we have found that MPO metabolism of AG resulted in protein free radical formation on MPO itself. The protein free radical was identified by using DMPO to trap the unstable radical, which was detected using an anti-DMPO antibody. The formation of the protein free radical was likely mediated by a phenyl radical metabolite of AG (detected by electron paramagnetic resonance spectroscopy). HL-60 cells, which express MPO, were used to determine if protein free radical detection also occurred in living cells. We found that protein free radical formation occurred in a time- and drug-dependent manner when cells were treated with AG and a H₂O₂-generating system. The absence of H₂O₂ did not result in protein free radical formation. Cytotoxicity, assessed using MTT, was greatest with 5 mM AG treatment. However, Caspase 3/7 activity was greatest with 1 mM AG. Interestingly, 1 mM AG also produced the most intense protein radical formation as determined by anti-DMPO western blot analysis. To determine the effect of this protein modification, we determined proteasomal activity as a possible mechanism of AG-induced cytotoxicity. Both 1 and 5 mM AG resulted in a significant inhibition of proteasomal activity both with and without the presence of DMPO. We speculate that that proteasomal inhibition may be a signal for apoptosis as a result of AG treatment, and could be a possible mechanism for AG-induced agranulocytosis.

211 IMMUNO-SPIN TRAPPING OF DNA RADICALS: A SENSITIVE DETECTION OF OXIDATIVELY GENERATED GENTOTOXIC DAMAGE.

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Reactive oxygen species can directly or indirectly affect the structure and function of biomolecules. Most of the techniques used to study oxidatively generated damage to the DNA are based on the detection of final oxidation products such as 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG). However, 8-oxo-dG can be repaired, excreted, and/or artifactually generated during sample work-up and analysis. We thought that by detecting DNA radicals it must be possible to achieve an early and more sensitive and specific characterization of the oxidatively generated damage to the genome than detecting final products of DNA oxidation. To detect DNA radicals we have developed immuno-spin trapping (IST) that involves three main steps: i) trapping, in situ and in real time, DNA radicals with DMPO, thus forming DMPO-DNA nitron adducts (nitron adducts); ii) purification of nitron adducts; and iii) analysis of nitron adducts by heterogeneous immunoassays using antibodies against DMPO. The nitron spin trap DMPO possesses low toxicity and excellent pharmacokinetics in cells and whole animals. Indeed, DMPO accesses the cell and traps, in situ and in real time, DNA radicals in the nucleus and/or mitochondria of cells under oxidative stress forming DMPO-DNA radical adducts (radical adducts). However, the analysis of the unstable radicals and radical adducts, which only can be achieved by electron spin resonance, is difficult in biological systems due to the time required to isolate the DNA from the biological matrix relative to their decay. Radical adducts decay to nitron adducts, in which DMPO is stably bound to the DNA facilitating their extraction and analysis. Applications of IST to investigate genotoxic damage to the DNA are empowered by its sensitivity, specificity, affordability, and reliability. Theoretical and practical considerations for the successful IST analysis of DNA radicals in toxicological models are presented.

212 THE MOLECULAR MECHANISM OF NRF2 ACTIVATION BY SODIUM ARSENITE AND MONOMETHYL ARSONOUS ACID.

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Epidemiology studies have correlated arsenic exposure to various human diseases including cancer of the skin, lung, and bladder. Following uptake, inorganic arsenic is converted primarily in the liver to methylated metabolites, including monomethylarsonous acid [MMA(III)], which has been found to be 20x more toxic than arsenite and able to transform human cells. Both arsenite and MMA(III) have been found to generate an oxidative response even at environmentally relevant, low levels of exposure. The transcription factor Nrf2, through the antioxidant responsive element (ARE) in the regulatory regions of its downstream genes, regulates a cellular defense response against environmental toxicants and carcinogens. Degradation of Nrf2 is blocked and Nrf2-dependent genes are expressed in cells under oxidative stress. In this study, the induction of Nrf2 by arsenite and MMA(III) was examined. Steady state levels of Nrf2 and its transcriptional activity were increased in a breast cell line (MDA-MB-231) at concentrations as low as 2 µM for sodium arsenite and 250 nM for MMA(III), as evaluated by immunoblot analysis and the ARE-luciferase reporter gene assay. Elevated levels of the endogenous Nrf2 protein in response to sodium arsenite and MMA (III) were also observed in a human urothelium cell line. It appears both arsenite and MMA(III) exposures trigger induction of Nrf2 at low concentrations in both of these human cell lines. The mechanism of this induction is under investigation. (NIEHS 1R01ES015010-01 and NIEHS 04940)

213 MULTIPLEXED FLUORESCENT METHODS FOR MEASURING OXIDATIVE MODIFICATIONS OF PROTEIN/NON-PROTEIN THIOLS IN THE LUNG: APPLICATION TO THE STUDY OF CYTOTOXIC AROMATIC HYDROCARBONS.

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Oxidative stress caused by agents undergoing redox cycling or which are metabolized to electrophilic metabolites may alter both protein and non protein thiols. These processes have been implicated as key events in cellular toxicity. Naphthalene (NA) and 1-nitronaphthalene (NN) are metabolized to glutathione depleting metabolites in the lung and the formation of these metabolites has been associated with Clara cell toxicity. In these studies we examined alterations in murine pulmonary epithelial protein thiols (PSH) and the formation of more oxidized proteins (PSSP, PSSG, PSOH, PSO3) in response to diethylmaleate, a well known glutathione depleting agent. Reduced and oxidized protein thiols were measured using a multiplexed fluorescent two-dimensional gel electrophoresis labeling method developed by our laboratory. Briefly, proteins selectively isolated from lung epithelium using a method modified from Wheelock et al. (Am. J. Physiology, LCOMP 286: L399, 2004) were labeled with the sulfhydryl reactive maleimide dye Cy-3, excess label removed, disulfides were reduced and labeled with Cy-5 maleimide. Proteins were separated by 1 or 2D gel electrophoresis to examine loss of protein sulfhydryl associated with sulfhydryl oxidation. Preliminary validation studies with purified proteins containing both free SH and disulfide bridges demonstrated that the method yields data which accurately mirrors the number of free SH and PSSP in the protein. Treatment with diethylmaleate decreases PSH and increases the number of protein disulfides relative to control. Additional work to determine the identity of susceptible proteins is currently underway. We conclude that our method provides an assessment of the status of individual cellular proteins that may reveal key mechanistic features associated with the cytotoxic responses of aromatic hydrocarbons. Supported by NIEHS 04311, 04699, and T32ES07059.

214 TAQMAN LOW DENSITY ARRAYS: TESTING THE POTENTIAL FOR USE AS A HIGHER THROUGHPUT MOLECULAR TOXICOLOGY SCREEN FOR OXIDATIVE STRESS.

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Reactive oxygen species are highly toxic by-products of aerobic respiration and can lead to oxidation and damage of cellular constituents. This damage has been implicated in the cause of a number of diseases including Parkinson's, diabetes, Hepatitis B and cancer, hence there is a need for earlier detection of oxidative stress in drug development. The aim of this work was to design and test the feasibility of a higher throughput molecular toxicology screen for oxidative stress.

There are currently a large number of biochemical assays available for the measurement of oxidative stress, however these are all relatively low throughput. Multiplex gene expression analysis, using TaqMan low density arrays (TLDA), could potentially enable increased throughput, coupled with greater insight into the mechanism of oxidative stress. The following work describes the study of 2 cell lines namely HepG2 and Hela cells, treated with a known oxidant Diethyl Maleate (DEM) and non-oxidant Oubain, for 24 hours. The cells were then evaluated using a routinely used viability assay and GSH depletion assay before gene expression analysis.

Standard TaqMan qRT-PCR and TLDA were employed to analyse gene expression. A total of 23 genes were selected by review of the literature and knowledge of a previous study performed in HepG2 cells (Morgan et al, 2002). HepG2 gene expression data from this study showed high concordance with the investigation of 7 genes, by Morgan et al. Data from both cell lines suggest the 23 genes chosen are capable of differentiating between an oxidant and non oxidant to some extent. In summary, the preliminary data generated, has shown that it may be feasible to differentiate compounds based on a set of genes related to oxidative stress. Also a comparison of the standard TaqMan assay and the TLDA has demonstrated a consistency in data generated and thus the potential to use TLDA in higher throughput molecular screens for toxicology.

215 ANALYSIS OF LIPID ALDEHYDE MODIFICATION OF PEROXIREDOXIN 6 USING MASS SPECTROMETRY.

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Peroxiredoxins are a class of antioxidant enzyme that possess peroxidase activity. These enzymes are placed into two classes, 1-cys or 2-cys, based on the number of conserved cysteine residues in their active sites. Peroxiredoxin 6 (Prx6) belongs to the 1-cys class (TRENDS in Biochemical Sciences. 28(1): 32-40). This specific peroxiredoxin has been shown to protect the lung from paraquat induced oxidative stress using wild type and Prx6 ^{-/-} knockout mice (Journal of Biological Chemistry. 278(27): 25179-25190). Collectively, these reports demonstrate that Prx6 has the ability to protect the cell from oxidative damage by scavenging hydrogen peroxide. Preliminary in vivo data has shown that PRX6 is adducted by 4-hydroxynonenal (4-HNE) in the livers of rats chronically fed an ethanol containing diet; therefore, the goal of this study was to identify specific aldehyde modifications of peroxiredoxin 6 (PRX6) using various mass spectrometry techniques. This analysis was carried out using both matrix-assisted laser desorption/ionization – time of flight mass spectrometry (MALDI-TOF) along with liquid chromatography and tandem mass spectrometry (LC-MS/MS). Molecular modeling technologies were also employed to investigate active site accessibility, solvent accessible residues and changes in conformation due to aldehyde adduction. After incubations with various concentrations of 4-HNE, Prx6 was analyzed for adducts using MALDI-TOF and tryptic peptides were analyzed via MALDI-TOF and LC-MS/MS. Analysis of the intact protein and peptides demonstrated multiple sites of modification, including the active site, Cys47. Also, analysis of solvent accessible residues utilizing the molecular modeling software showed that virtually all lysine residues and Cys47 are accessible to solvent, while the ring structures of the three histidine residues are buried within the globular protein. These observations support previous activity data that demonstrated a concentration-dependent loss of peroxidase activity due to aldehyde adduction and cross-linking.

216 STRESS PATHWAY-BASED REPORTER ASSAYS TO ASSESS TOXICITY OF ENVIRONMENTAL CHEMICALS.

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There is an increasing need for assays for the rapid and efficient assessment of toxicities of large numbers of environmental chemicals. To meet this need, we are developing cell-based reporter assays that measure the activation of key molecular stress pathways. We are using promoter regions from various stress-responsive genes such as heme oxygenase I (HO), heat shock protein 70 (hsp70), and nuclear factor-kappa B, etc. with the ultimate goal of building and characterizing a panel of reporter assays that can be multiplexed within the same cell line for use in high-throughput chemical screening. Our preliminary work towards this goal with the heme oxygenase 1- reporter shows that several toxic compounds can activate HO expression of a in a dose-dependent manner. Cadmium sulfate significantly activated HO expression at a LOAEL concentration of 250 nM, compared to hsp70, which was activated at a LOAEL concentration of 10 uM, indicating the differential sensitivities of these two stress pathways. Less toxic metallic compounds such as copper sulfate did not appreciably affect HO activity at identical concentrations. Additionally, HO expression was induced by a polychlorinated biphenyl (PCB) mixture, Aroclor 1254, at a LOAEL concentration of 10 uM indicating the broad responsiveness of the HO-1 promoter to metal and non-metal stressors. The data

presented will show that in vitro stress reporter assays can discriminate toxic compounds at sub-lethal concentrations, provide a quantitative method to assess cellular toxicity and infer mechanism(s) of toxicity. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy).

217 GENETIC PATHWAY ANALYSIS FOR RISK ASSESSMENT OF EXPOSURE TO OXIDATIVE STRESSORS.

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A broad spectrum of drugs and environmental agents exert toxicity by the induction of oxidative stress in target cells. Oxidative stress is also implicated in the etiology of complex diseases such as cancer, diabetes, and the neurodegenerative syndromes. Therefore, the identification of genetic risk factors for susceptibility to oxidative stress and the corresponding development of pathway-targeted drugs represent a key area of focus for mechanism-based strategies in personalized medicine. To support these strategies, we have previously reported development of a high throughput Bioscreen assay to quantify cellular oxidative stress responses in a conserved yeast genetic model. In our Bioscreen assay, a quantitative cell growth / death profile is collected in the presence or absence of a dose series of hydrogen peroxide. Comparison of this Bioscreen stress profile for a wild type yeast strain to those obtained from a whole-genome collection of knockout mutant strains allows us to identify conserved genetic risk factors that confer either susceptibility or resistance to oxidative stressors. To further validate our assay system, we demonstrate that genetic ablation of either of the two major anti-oxidant defense pathways, glutathione or the peroxiredoxin family, results in hypersensitivity to low doses of hydrogen peroxide. Hydrogen peroxide induces apoptotic cell death in mammalian systems. We similarly observe that ablation of either of the conserved caspase "executioner" or the AIF death signaling pathways confers resistance to the hydrogen peroxide-induced apoptotic death response in the Bioscreen assay. In contrast, we observe that ablation of the autophagy / lysosomal stress response confers sensitivity to hydrogen peroxide. Significantly, we report for the first time that ablation of the yeast ortholog of the human beclin1 tumor suppressor / autophagy gene also sensitizes a cell to oxidative stress. In conclusion, our Bioscreen assay allows the separation and quantitative evaluation of the individual component pathways that mediate oxidative stress-induced toxicity.

218 α -SYNUCLEIN INTERACTS WITH VMAT2 TO REGULATE VMAT2 ACTIVITY.

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Mutations in α -synuclein (ASYN) are associated with familial forms of Parkinson Disease (PD). Although ASYN is expressed throughout the brain, dopaminergic neurons are preferentially affected in PD. This may be due to the ability of dopamine (DA) to readily oxidize in the cytosolic environment. Oxidized DA is believed to stabilize a toxic protofibril aggregate form of ASYN. Therefore, DA neurons' ability to prevent protofibril-induced toxicity may rely on the ability to protect DA from oxidation. The vesicular monoamine transporter 2 (VMAT2) is responsible for the transport of cytosolic DA into synaptic vesicles for sequestration and subsequent release at synaptic terminals. Since the storage of DA also protects DA from the oxidative environment of the cytosol, factors responsible for the maintenance of normal VMAT2 activity may be necessary to protect dopaminergic neurons from cell death. In order to understand how ASYN promotes dopaminergic cell death, we must first understand ASYN's cellular function. One possible role for ASYN in dopaminergic neurons is the maintenance of DA homeostasis. Recently, we have identified an interaction between ASYN and the C-terminus of VMAT2 by yeast two-hybrid assay. We also show that this interaction leads to functional changes in VMAT2 activity in transiently transfected SHSY5Y cells. Coexpression of VMAT2 and either human wild-type or the disease-associated mutant A53T ASYN increases tetraabenazine sensitive-VMAT2 sequestration of 3H-DA in cell lysates by 33% and 77%, respectively as compared to empty vector control and VMAT2 cotransfected cells. These data suggest that one function of ASYN may be to maintain proper storage of DA via regulation of VMAT2.

219 4-HYDROXYNONENAL COVALENTLY MODIFIES TUBULIN AND INHIBITS MICROTUBULE ASSEMBLY.

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Chronic ethanol consumption causes oxidative stress and lipid peroxidation in the liver, which is associated with the accumulation of lipid droplets in hepatocytes, a condition known as steatosis. The mechanisms by which steatosis occurs remain

elusive. Intact microtubules are critical to a number of cellular transport processes, including the secretion of triglycerides as VLDL particles. Disruption of the microtubule cytoskeleton in hepatocytes could contribute to steatosis by reducing the secretion of triglycerides. Our in vitro experiments show that the lipid peroxidation product 4-hydroxynonenal (4-HNE) inhibits polymerization of purified tubulin in a dose-dependent manner. The inhibitory effect of 4-HNE on tubulin polymerization is greater than the effect of acetaldehyde, the proximate metabolite of ethanol, but less than the effect of the cysteine-specific modifier N-ethylmaleimide (NEM). Immunoblotting experiments have shown that 4-HNE Michael adducts can form on intact microtubules as well as on the tubulin heterodimer. Treatment of tubulin dimers with 4-HNE in vitro causes the formation of high molecular weight cross-links. 4-HNE does not induce depolymerization of microtubules, whereas cysteine specific modification of tubulin results in a modest degree of depolymerization. The microtubule binding and ATP hydrolysis of the anterograde motor protein kinesin to taxol-stabilized microtubules is not affected by 4-HNE. Mass spectrometry data indicate that 4-HNE adducts of tubulin occur on cysteine residues, as well as on other nucleophilic amino acids, suggesting cysteine-lysine cross-links between alpha and beta tubulin subunits. Because microtubules are dynamic structures in the cell, impairment of polymerization by 4-HNE may reduce microtubule-based lipid transport, and could contribute significantly to the accumulation of lipid droplets observed in alcoholic livers.

220 CO INTERACTS WITH CYTOCHROME C/CARDIOLIPIN COMPLEXES AND INHIBITS THEIR PEROXIDASE ACTIVITY: A POSSIBLE PROTECTIVE ROLE IN APOPTOSIS.

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CO has been shown to protect cells against apoptosis in vivo and in vitro through yet to be identified pathways. A number of biological effects of CO are associated with its binding hemo-proteins including the reduced cytochrome c oxidase in mitochondria. Cytochrome c (cyt c) is another abundant heme-containing protein of the inter-membrane space of mitochondria. The hexa-coordinate organization of heme-iron effectively accommodates electron-transporting function of cyt c, however, hinders its binding and chemical interaction with small molecules thus precluding its peroxidase activity. Recently, we demonstrated that interaction of cyt c with a mitochondria-specific phospholipid, cardiolipin (CL), induced protein unfolding accompanied by an exchange and loss of axial ligands of heme iron resulting in an increased accessibility for H₂O₂ and appearance of the peroxidase activity. The latter caused selective CL oxidation during apoptosis. Using optical spectroscopy and measurements of tryptophan fluorescence we now demonstrate CO binding with ferrous heme in cyt c/CL complexes. In triple CL/cyt c/CO complexes, the heme is likely less accessible for the interaction with H₂O₂ or lipid hydroperoxides as evidenced by EPR-detectable delayed and inhibited oxidation of ascorbate and etoposide. We speculate that CO contributes to regulation of the peroxidase activity of cyt c/CL in mitochondria, particularly during apoptosis-associated oxidative stress. Supported by NIH HL070807.

221 GAMMA-GLUTAMYL-CYSTEINE SYNTHETASE OPTIMIZATION AND USE IN WILDLIFE CHEMICAL EFFECTS ASSESSMENTS.

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Gamma-Glutamylcysteine synthetase (GCS), catalyzes the binding of glutamate and cysteine, the first and rate-limiting step of glutathione (GSH) biosynthesis. In this study, tissues from Sprague Dawley rats, deer mice and mallard ducks were used to characterize GCS activity and responsiveness to chemical exposure. Varying glutamic acid concentrations from 5mM to 80mM did not affect the GCS activities, whereas cysteine concentrations from 2.5mM to 40mM influenced GCS activities substantially. The optimal cysteine concentrations for GCS activities were tissue and species dependent. Generally, the maximum and linear range of hepatic GCS activities of each species was obtained between 10- and 30- fold dilution. The reaction rates of GCS decreased with longer time with an optimum reaction period of 30 minutes. Intra-assay variation of GCS activity demonstrated coefficients of variation ranging from 5.0% to 8.0%. Deer mice GCS activities differed among tissues with the highest activity in liver, followed by kidney, brain and heart sequentially. Carbon tetrachloride decreased both hepatic total GSH concentration and GCS activity. In kidney, however, GCS activities were induced with increased GSH (reduced form). Mixed-metal-dosed deer mice had increased GSH forms. Duck studies with mixed-metals will also be described.

222 IRON-CATALYZED OXIDATIONS OF TRYPTOPHAN RESIDUES IN W-E SEQUENCES OF APOB-100 IN LOW DENSITY LIPOPROTEINS.

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To test the hypothesis that iron-catalyzed oxidations of LDL in vitro produce diagnostic biomarkers of oxidation of the apoprotein that could be applied to studies of LDL oxidation in vivo, LDL were oxidized in 200 μM Fe+2, 60 μM EDTA, 5 mM ascorbic acid at 37°C for up to 40 h. Following delipidation and trypsin digestion, the peptides were separated by HPLC, with four peaks detected at 365 nm, whereas none were observed in peptides from unoxidized LDL. Treatment of Fe-oxidized LDL with 2,4-dinitrophenylhydrazine prior to trypsinization and HPLC did not yield peptides detected at A365 nm. The peptides were purified by three consecutive buffer systems (ammonium acetate, phosphate, and 0.1% TFA). The four peptides were identified by MALDI-Q-TOF, as YH(W+4)EHTGLTLR (AA: 4061-4071), IYSL(W+4)EHSTK (AA:3532-3541), IVQILP(W+4)EQNEQVK (AA: 550-563), and FEGLQE(W+4)EGK (AA: 3937-3946), respectively, with (W+4) signifying (W plus 4 Da) in place of the W residues in the unoxidized protein. The mass gains and the absorbances at 365 nm indicate kynurenines, which were found in trypsin-releasable peptides that are thought to be motifs exposed on the surface of LDL particles. All four peptides thus characterized also share the sequence of WE, with glutamate residues immediately C-terminal to W. Of the 4 peptides identified in the present studies with Fe, 2 were among the 9 characterized in our previous studies with Cu oxidation. The results demonstrate marked differences in products from different methods of LDL oxidation in vitro and suggest potential biomarkers of iron-mediated oxidation for studies in vivo. The preferential oxidation of W residues in WE sequences suggest contributions from the C-proximate glutamate residues in chelation of the iron species, thereby influencing site selectivities of oxidation.

223 TYROSINE NITRATION AND OXIDATION OF MNSOD: COMPETITION AND TOXICOLOGICAL CONSEQUENCES.

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Mitochondria are the major source of superoxide radicals and other reactive oxygen species produced mostly by components of disrupted respiratory chains. Extremely high reactivity of superoxide with NO and resulting production of a potent and toxic oxidant, peroxynitrite are prevented by MnSOD, a unique enzyme of the mitochondrial matrix. Sufficiency of catalytically competent MnSOD is essential for integrity of mitochondria and cell survival. However, nitration of catalytically important tyrosine residues leads to inactivation of the enzyme. Tyrosine residues in MnSOD may also be involved in the formation of intra- or inter-molecular di-tyrosine crosslinks leading to protein oligomerization whose ability to support enzymatic activity is not well characterized. Using recombinant protein, rat brain and liver mitochondria as well as HL-60 cells we found that nitration and dimerization of tyrosine residues represent two competing pathways of stress-mediated modification of MnSOD. Oxidative stress mediated di-tyrosine formation induces covalent oligomerization of MnSOD which does not substantially affect its catalytic activity. Moreover, the oligomerized enzymatic complexes are resistant to unfolding and protein degradation. In contrast, nitration of tyrosines leads to loss of dismutase activity, protein unfolding, and confers a new catalytic quality on the enzyme, a peroxidase activity. Further, nitration prevents di-tyrosine formation and thus oligomerization of MnSOD. These results are discussed in the context of tetrameric organization and interactions of 36 tyrosine residues in catalytically active hMnSOD with emphasis on crystallographic data indicating that Tyr45 and Tyr166 are within contact distances with respective Tyr45 and Tyr166 of adjacent subunits of MnSOD.

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224 THE EFFECTS OF NITRATION USING PEROXYNITRITE ON PROTEIN FUNCTION AND DEGRADATION.

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Ethanol induces oxidative stress in the liver, which can negatively impact cell function and bring about hepatic injury. Ethanol metabolism increases mitochondrial release of superoxide, which reacts with nitric oxide to form peroxynitrite.

Peroxynitrite can injure the cell by its ability to react with proteins and alter structure, which could disable proteins from performing their function and alter their susceptibility to degradation. In order to determine whether protein nitration enhances or inhibits a protein's ability to undergo degradation, we performed *in vitro* experiments to determine whether nitration of proteins with peroxynitrite affects their susceptibility to degradation by the proteasome, an important cellular protease, which regulates protein homeostasis. Preparations of both highly purified lysozyme, and HepG2 cell lysate, which was radiolabeled with [^3H]leucine, were nitrated to varying degrees. Nitration was performed by increasing the molar ratio of peroxynitrite to lysozyme and radiolabeled proteins 0, 1, 2, 4, 8, 16 times. Proteolytic susceptibility was measured by incubating nitrated lysozyme or radiolabeled proteins with either trypsin, pronase, or purified rat liver proteasome. As nitration of lysozyme increased, its catalytic activity decreased by three-fold at 16x peroxynitrite. The, proteolytic susceptibility of lysozyme to degradation by the proteasome generally increased by as much as three-fold as nitration of lysozyme increased. Although some nitrated radiolabeled cellular proteins showed a two-fold increase in proteolytic susceptibility by the proteasome compared with its un-nitrated control, the results were not always related to the degree of nitration. We conclude that peroxynitrite altered the structure of lysozyme and those of radiolabeled cellular proteins by the formation of nitrotyrosine adducts, which decreased the ability of nitrated proteins to function properly and generally enhanced their susceptibility to degradation by the proteasome.

225 INHIBITION OF NADPH OXIDASE ACTIVITY SUPPRESSES GROWTH AND PROMOTES DIFFERENTIATION OF B16 MELANOMA CELLS.

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Oxidative stress has been recognized as a mediator for cell proliferation and implicated in playing an important role in cancer development. Oxidative stress is accompanied by overproduction of reactive oxygen species. The activity of DADPH oxidase, an enzyme capable of generating superoxide radicals, is increased in malignant skin keratinocytes after a tumor promoter treatment. Herein we demonstrated that inhibition of DADPH oxidase activity by diphenyleneiodonium (DPI) suppressed free radical production, inhibited cell growth while promoted cell differentiation of B16 melanoma cells as indicated by cell morphology and increased production of melanin. Biochemical studies revealed that pERK expression was induced whereas PKC α expression was reduced by DPI treatment, suggesting ERK and PKC α may play opposite roles in the event. In addition, the protein levels of tumor suppressor p53 were suppressed by DPI, suggesting that p53 is activated by oxidative stress and it may negatively regulate differentiation in melanoma cells. Taken together, these results suggest three folds of the effects of inhibiting NADPH oxidase activity in melanoma cells: halting cell proliferation, promoting cell differentiation, and suppressing the tumor suppressor p53.

226 DESIGN, SYNTHESIS AND APPLICATION OF A HYDRAZIDE-FUNCTIONALIZED ISOTOPE-CODED AFFINITY TAG (HICAT) FOR THE IDENTIFICATION AND QUANTIFICATION OF OXYLIPID PROTEIN CONJUGATES.

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Reactive oxygen species (ROS) chemically modify a variety of biological molecules, including polyunsaturated acyl chains of membrane phospholipids to generate a variety of α , β -unsaturated acyl aldehydic intermediates, such as keto aldehydes, 2-alkenals, and 4-hydroxy-2-alkenals. Especially, 4-hydroxy-2-alkenals such as 4-hydroxy-2-nonenal (HNE) and 4-hydroxy-2-hexenal (HHE) are prominent aldehydes generated during peroxidation. HNE has a high stability and reactivity, and is largely responsible for many types of cellular damage associated with oxidative stress. HNE-induced modification was found to occur mainly on histidine, cysteine, and lysine residues via a Michael addition preserving the aldehyde functionality on the targeted protein, rather than by Schiff's base formation. Therefore, the purpose of this study was to develop an analytical method which can assist us to identify and quantify the oxylipid-damaged mitochondrial proteins on a proteome-wide scale. In this study, we are developing a mass spectrometry-based quantitative proteomics approach using hydrazide-functionalized isotope-coded affinity tag. We designed the isotopomers, $^{12}\text{C}_6$ -HICAT and $^{13}\text{C}_6$ -HICAT, as the 'light' and the 'heavy' labeling reagents. To demonstrate the feasibility of using the HICAT probes to selectively target and potentially quantify carbonylated proteins, we de-

scribe the modification of thioredoxin (Trx) with HNE, followed by $^{12}\text{C}_6$ and $^{13}\text{C}_6$ -HICAT labeling and the selective affinity enrichment from tryptic digests of HNE-conjugated Trx. The HICAT-labeled peptides were identified and quantified by MALDI tandem mass spectrometry. Successful results also have been obtained from *in vitro* treated HNE mitochondrial adducts, showing the potential of HICAT as a new tool in proteome wide screens for identifying and quantifying LPO-modified proteins.

227 CHARACTERIZATION OF RAT ALANINE AMINOTRANSFERASE ISOENZYMES ALT1 AND ALT 2.

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ALT has been used as an indicator of hepatocellular damage and is an important enzyme involved in gluconeogenesis and amino acid metabolism and thus may be an indicator of metabolic adaptation and not just hepatocellular damage. The purpose of this study was to clone and sequence rat ALT 1 and 2 (rALT1 and rALT2) genes as well as to produce and characterize recombinant ALT proteins to better understand their role in metabolic processes. Full length rALT1 cDNA was amplified by PCR from a liver cDNA library and rALT2 was obtained from IMAGE clone 7113147 of the Mammalian Genome Collection. ALT isoenzymes are well conserved between rodents in that rALT1 and rALT2 share 97% and 98% homology at the amino acid level with their murine counterparts. The amino-terminus of ALT2 peptide is 28-amino acids longer than ALT1 which represents a putative mitochondrial targeting signal sequence. Northern analysis of normal Sprague-Dawley rat tissues showed ALT1 mRNA was highly expressed in liver, colon and moderate levels in small intestine, brown fat, heart and skeletal muscle. ALT2 mRNA was expressed in high levels in skeletal muscle and liver and moderate levels in brain. Recombinant ALT1 and ALT2 enzymes were expressed and purified from SF9 insect cells as His-tag fusion proteins using nickel affinity chromatography, followed by the removal of His tag for functional analysis. rALT1 and rALT2 had molecular masses of 56,613 and 58,507 Da respectively. The Km values for rALT1 with the substrates L-alanine and 2-oxoglutarate were: 48 mM and 11.3mM respectively with a Vmax of 10.5 $\mu\text{mol/min per mg protein}$. The Km values for rALT2 with the substrates L-alanine and 2-oxoglutarate were: 13.3 mM and 54.1 mM, respectively with a Vmax of 7.2 $\mu\text{mol/min per mg protein}$. Functional and mass based assays are in development to measure these isoenzymes in tissues and blood and will lead to a more effective use of these markers in safety risk assessment.

228 INHIBITION OF THE EPIDERMAL PERIPHERAL NEUROPATHY BIOMARKER PGP9.5 IN DIFFERENTIATED PC12 NEURONAL CELLS BY PROTEASOME INHIBITION.

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Anti cancer therapy is a common cause of peripheral neuropathy in the clinic. pgp 9.5 immunohistochemistry on epidermal biopsies represents an important tool in peripheral neuropathy diagnosis in patients. pgp 9.5 is an ubiquitin protein ligase/hydrolase representing about 2% of the total protein in neuronal cells and is used as a marker for quantifying free nerve endings in epidermal tissues during neuropathy assessment in patients. In a gene expression study using differentiated PC12 neuronal cells an experimental proteasome inhibitor (EPI) down regulated the UCHL1 gene coding for pgp9.5 protein in a concentration dependent manner without affecting protein levels. However, proteasome inhibition resulted in the appearance of ladder-type pattern in Western blots, indicating post-translational modifications of pgp9.5 (ubiquitination). This observation prompted us to analyze the intracellular localization of pgp9.5 in cultured rat dorsal root ganglion. Following proteasome inhibition in this system, pgp9.5 translocated and accumulated in the perinuclear areas resulting in weakened signals in the neurites. In conclusion, EPI induced down regulation of pgp9.5 gene expression, had no effect on total protein, but induced changes in pgp9.5 intracellular localisation. These findings indicate a potential complexity factor of intraepidermal free nerve ending quantification. Thus, in parallel with intraepidermal free nerve end counting for proteasome inhibitor-induced peripheral neuropathy an assessment of the direct effect on pgp9.5 gene and protein expression should be included.

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COMPARISON OF COMMERCIAL CARDIAC TROPONIN (CTN) ASSAYS FOR EVALUATION OF MYOCARDIAL INJURY IN THE LABORATORY RAT, DOG AND CYNOMOLGUS & RHESUS MONKEY, AND KINETIC PROPERTIES OF SERUM CTN IN RATS WITH ACUTE CARDIOTOXICITY.

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Cardiac troponins have valuable utility as biomarkers that bridge monitoring for myocardial injury in laboratory animals to humans. The HESI Troponins Expert Working Group sponsored studies to advance the analytical qualification of assays for, and biological characterization of serum cTn in animals. In these studies, commercial assays (n=9) were compared for cross-reactivity, linearity, precision and lower limits of detection for measurement of serum cTn I and T in common laboratory species. In addition, in vivo time-course evaluation of serum cTn in rats administered an acutely cardiotoxic dose of isoproterenol (0.1 or 4 mg/kg SC) was compared with concurrent cardiac histopathology. Results of the analytical study showed inherent differences among assays in cross-reactivity, precision and dynamic range with cTn-positive serum from the test species. Results also indicated that some assays were suboptimal for measurement of serum cTn in select species. In the in vivo study, good correlation in kinetics of serum cTn I and T in the animals occurred with 3 different assays, although sensitivity and dynamic range varied. Increases in serum cTn occurred coincident with early histologic changes of myofiber injury at 0.5 hrs post dose; however, more progressive histologic changes were seen at later intervals (24-48 hrs) when cTn values were in decline. Sensitivity of cTn over traditional serum markers of myocardial injury was also shown in this model. These results support the need for adequate qualification of each cTn assay for each species, and further define the utility of cTn as a cardiac injury biomarker.

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DRAMATIC REDUCTION OF GLUCOCORTICOID RECEPTOR EXPRESSION IN RAT AND HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) TREATED WITH THE HEAT SHOCK PROTEIN (HSP)90 INHIBITOR 17-DIMETHYLAMINOETHYLAMINO-17-DEMETHOXYGELDANAMYCIN (17-DMAG) *IN VITRO*.

D. Zhou, S. Qin, X. Zhang, L. Li, J. Ye, X. Zhang, W. Ying and J. Barsoum. *Biology, Synta Pharmaceuticals Corp., Lexington, MA.* Sponsor: *E. Kitayama.*

Hsp90 is an important chaperone protein that regulates the folding of key signaling proteins and maintains their function. One of the Hsp90 client proteins is the glucocorticoid receptor (GR) that plays multiple critical roles in development, glucose homeostasis, metabolism, proliferation, and anti-inflammatory responses. Although inhibition of Hsp90 promotes degradation of its multiple oncogenic client proteins and demonstrates anti-tumor activity, the effects of inhibition of Hsp90 on GR expression in normal cells have not been fully investigated. In this study, we evaluated the expression of GR in rat and human PBMCs after treatment with the Hsp90 inhibitor, 17-DMAG, in vitro. The fresh isolated rat and human PBMCs were treated with 17-DMAG at 0, 1, 5, 25, and 100 nM for 2 or 16 hours. Immunoblot analysis was performed to measure and compare the levels of the GR protein expression. We found that 17-DMAG caused a dose-dependent decline in GR expression when compared with the vehicle after normalization to gapdh levels in both rat and human PBMCs. There were 3- and 6-fold reductions in rat PBMCs 16 hours after the treatment at 25 nM and 100 nM, respectively. However, GR protein expression was not significantly affected after a 2-hour treatment. In human PBMCs, GR protein levels were decreased 2-fold after a 16-hour treatment at 25 nM, and further reduced 5-fold at 100 nM. In both rat and human PBMCs, 17-DMAG only affected the GR α , not β , isoform. In conclusion, our data show a dose- and time-dependent degradation of GRs in both rat and human PBMCs after the treatment with 17-DMAG. The changes could impact GR function in tumor and normal cells. The results suggest that the GR α isoform, one of the Hsp90 client proteins affected by 17-DMAG, could be used as a biomarker to monitor the activities or toxicities of Hsp90 inhibition.

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FAT-SOLUBLE VITAMIN ANALYSES BY LC/MS IN THE TISSUES OF RATS AND DOGS.

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Fat-soluble vitamins are lipophilic molecules stored in liver and fatty tissue. Depletion of fat-soluble vitamins has been observed during weight loss, use of some drug therapies, or with specific diseases and clinically results in conditions such as

coagulopathy, decreased infection resistance, and peripheral neuropathy. During the drug development process, information concerning fat-soluble vitamin levels has become increasingly valuable for understanding the potential involvement of changes in vitamin levels and toxicity. An assay to measure vitamins A, D, E and K in rat and dog tissues has been developed. Extraction of the fat-soluble vitamins from the tissue was performed by combining ~ 10 mg of tissue, hexane, and internal standard with subsequent processing on the Fastprep bench top homogenizer. The analytes of interest were separated from matrix components on a Gemini C18 (2.0 x 50 mm) HPLC column with isocratic elution and a total runtime of 5 minutes. The vitamins were detected on a triple quadrupole mass spectrometer coupled with atmospheric pressure photoionization. The linear range for all four vitamins was 1 to 500 ng vitamin/mg wet tissue. Inter- and intra-assay precision was within 22 % and 20 %, respectively, for all of the vitamins. The assay was applied to measure fat soluble vitamins in dog and rat peripheral nerve and adipose tissue. The combination of the Fastprep homogenization system with the hexane extraction increased sample throughput (5-fold) and decreased sample preparation time (4-fold) over assays previously used for vitamin tissue analyses.

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VALIDATION OF THREE ENZYME-LINKED IMMUNOSORBENT ASSAYS FOR THE QUANTITATIVE DETERMINATION OF TOTAL IGG, IGM AND IGE ANTIBODIES IN CYNOMOLGUS MONKEY SERUM.

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The validation of three enzyme-linked immunosorbent assays (ELISA) for the quantitative determination of Total IgG, IgM and IgE antibodies in Cynomolgus monkey serum was performed in order to monitor the Cynomolgus monkey total immunoglobulin concentration during the course of toxicology studies. Validation parameters included selectivity, intra- and inter-batch precision and accuracy, prozone effect, parallelism and stability. The validated concentration range for all three methods is from 3.91 ng/mL to 200.00 ng/mL. The methods were successful at detecting IgG, IgM and IgE antibodies when spiked in ten different lots of diluted Cynomolgus monkey serum at concentrations between QC1 and QC2 while taking the endogenous concentrations of IgG, IgM or IgE into account. The intra and inter precision and accuracy met the acceptance criteria. No assay deficiency was detected when samples spiked at a high concentration of IgG, IgM or IgE antibodies were analyzed. The parallelism between the standard curve and an ex-vivo sample was demonstrated for sample dilutions of up to 1/320000 and 1/128000 for the IgG and IgM methods respectively. Parallelism could not be assessed for IgE since no Cynomolgus monkey serum sample with a suitably high concentration of IgE was available. IgG, IgM and IgE antibodies in Cynomolgus monkey serum samples were shown to be stable at room temperature for approximately 6 hours, at approximately 4°C for approximately 24 hours and after 4 freeze-thaw cycles at approximately -80°C. IgG, IgM and IgE antibodies in Cynomolgus monkey serum samples were shown to be stable when stored for up to 124, 102 and 160 days respectively, at approximately -80°C. The quantitation of Total IgG, IgM and IgE antibodies using these validated ELISA methods will be valuable endpoints for toxicology studies.

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COMPETITIVE ELISA FOR FATTY ACID ETHYL ESTERS AS BIOLOGICAL MARKERS FOR THE DIAGNOSIS OF FETAL ALCOHOL SYNDROME.

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Fetal alcohol syndrome (FAS) is a serious disease that can affect children born to mothers who have consumed alcohol during their pregnancy and is best treated with early diagnosis. One of the consequences to children with this disorder is that they have altered fatty acid metabolism. 13-Hydroxyoctadeca-9,11-dienoic acid (13-HODE), a metabolite of linoleic acid, was converted to an ethyl ester with lipase from *Candida antarctica* and ethanol. The ethyl ester was converted to a succinate derivative via its C13 hydroxyl group in the presence of succinic anhydride. Identification of the ethyl ester of 13-HODE and the succinate derivative was achieved with gas chromatography/mass spectrometry of the trimethylsilyl derivatives. The succinate product was conjugated to keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). The KLH conjugate was then used to immunize a goat. IgG was isolated from the KLH-immunized goat serum and used in im-

munoassays with the BSA-conjugate. Western blot analysis revealed that, whereas the IgG did not bind to BSA, the IgG strongly bound to BSA conjugated with ethanol-esterified 13-HODE. A competitive ELISA using a plate coated with the IgG was carried out with various concentrations of BSA conjugated with ethanol-esterified 13-HODE and horseradish peroxidase (HRP) conjugated with ethanol-esterified 13-HODE. A negative control for this ELISA was BSA without conjugated ethanol-esterified 13-HODE. Whereas BSA did not compete for its binding to the IgG with HRP conjugated with ethanol-esterified 13-HODE, BSA conjugated with ethanol-esterified 13-HODE competed with the HRP conjugate in a dose-dependent manner. This result demonstrated that the IgG recognized the ethanol-conjugated fatty acids in Western blot analysis and competitive ELISA. (Supported by NIAAA SBIR Phase I R43 AA014535).

234 A POTENTIAL BIOMARKER FOR DEOXYNIVALENOL INTOXICATION AND EXPOSURE: PLASMA HAPTOGLOBIN.

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Deoxynivalenol (DON) is a foodborne mycotoxin contaminated in cereal crops such as wheat, barley, oats and etc. DON induces systemic health problems and reduces immune functions in both human and farm animals but there have not been convincing markers for diagnosis or exposure assessment until now. The present study was aimed to find out sensitive biomarkers for the determination of DON intoxication and exposure. B6C3F1 male mice, 8 weeks old, were exposed orally with 0.83, 2.5 and 7.5 mg/kg bw DON for 8 days and plasma was collected at the day of sacrifice. Protein expression patterns were examined using SELDI-TOF (Surface Enhanced Laser Desorption/Ionization) Mass Spectrometer. 9.7 kDa copper immobilized protein and 11.7 kDa hydrophobic protein were significantly increased and 17.4 kDa weak cationic protein was decreased by DON with dose-dependent manner. Among those proteins, 11.7 kDa hydrophobic plasma protein captured on H50 chip was expressed the most sensitively. This protein was purified by the methyl ceramic Hyper D F column optimized with 1M ammonium sulfate, 50mM sodium phosphate pH 7.0 for adsorption and with 50mM sodium phosphate pH 7.0 for desorption. The purified 11.7 kDa protein was identified as haptoglobin precursor by peptide mapping using LC/Q-TOF and MALDI-TOF and confirmed by western blotting and ELISA.

To confirm the specificity of haptoglobin as a biomarker for DON intoxication or exposure, we measured the amount of haptoglobin in plasma of rat orally administered with other mycotoxins (Aflatoxin 1.0 mg/kg bw, and Zearalenone 240 mg/kg bw for 8days) using ELISA. Haptoglobin was increased only by DON 7.5 mg/kg bw while decreased by Zearalenone 240 mg/kg bw compared to control and not detected by Aflatoxin 1.0 mg/kg bw.

These results show that plasma haptoglobin could be a potential biomarker for DON exposure and intoxication.

235 BIOMARKER LEVELS IN FEMALE F-344 RAT TISSUES AFTER 28-DAY ORAL TREATMENT WITH SR16157.

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SR16157 is a potent novel dual-acting breast cancer therapy agent that inhibits both estrogen biosynthesis directly and the estrogen receptor by its metabolite, SR16137. In a GLP toxicity study, rats received SR16157 by daily gavage for 28 days at 10, 33 or 100 mpk (150, 300, 600 mg/m²) with 14 days recovery. Blood was collected to determine plasma drug levels of SR16157 and SR16137, the metabolite released after SR16157 binds to estrone sulfatase (ES). Liver and uterus were flash frozen at necropsy and evaluated for estrone, estradiol, and estrone sulfate levels, and ES activity (liver only). Mortality was observed at 100 mpk by Day 12. ES activity in the liver was completely inhibited after 28 days at 10 and 33 mpk/d SR16157, or after 14 days of 100 mpk/d SR16157. At recovery, ES activity was 23%, 7%, and 4% of control at 10, 33, and 100 mg/kg, respectively. Estrone, estrone sulfate, and estradiol levels in the liver and uterus were unremarkable from controls and do not appear to be reliable biomarkers of SR16157 activity. Repeated administration resulted in increased exposure to SR16157, based on AUC and C_{max}, suggesting accumulation. SR16137 was also detected in plasma and its AUC was ~2-4% of SR16157 AUC. Histopathology findings included changes in liver (fatty changes in all treated groups; hyperplasia at 100 mpk/d), kidney nephrosis, atrophy in thymus and reproductive organs, and lymphoid depletion; cholesterol

and triglyceride changes were likely due to marked changes in body weight and food consumption of treated animals. In conclusion, daily oral gavage administration of SR16157 for 28 days in female F-344 rats was associated with dose-related changes in body weight, food consumption, clinical observations, clinical chemistry parameters, toxicokinetics, and histology. ES inhibition is a very sensitive indicator of the pharmacologic action of SR16157. Work supported by NCI Contract N01-CM-42203.

236 IDENTIFICATION OF PROBABLE BIOMARKERS FOR PHOSPHODIESTERASE INHIBITOR INDUCED VASCULAR INJURY USING TOXICOGENOMICS.

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Phosphodiesterase inhibitors are used for erectile dysfunction (ED) and have the potential for other clinical indications such as asthma and COPD. Vascular injury induced by phosphodiesterase inhibitors is a frequent observation in preclinical toxicology studies. The deficiency in understanding of its mechanism of toxicity as well as the lack of available biomarkers presents obstacles for development of this class of compounds. To develop probable biomarkers of toxicity, we treated rats with Minoxidil, SCH 351591 (a PDE4 inhibitor) and Vardenafil (a PDE5 inhibitor), and examined gene expression changes upon compound treatment using the Affymetrix GeneChip platform. The biomarker candidates identified through microarray were confirmed for their expression changes using RT-PCR. A set of probable biomarkers is proposed for their potential application in toxicity assessment in preclinical drug development. Some of these probable biomarkers are expressed in peripheral blood cells, suggesting potentials of non-invasive assays for clinical applications.

237 A SURVEY OF PROBABLE NEPHROTOXICITY BIOMARKERS FROM THE LITERATURE THROUGH GENE EXPRESSION ANALYSIS.

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Nephrotoxicity is a recurring impediment in drug development. The existing biomarkers of nephrotoxicity serum creatinine and blood urea nitrogen (BUN) are not sensitive enough to indicate early renal injury. In an effort to search for better nephrotoxicity biomarkers, we did a literature survey and identified 49 probable biomarker candidates. These candidates were screened for their kidney gene expression changes in rats following treatments of either nephrotoxins (n=4) or hepatotoxins (n=3) using RT-PCR technology. A set of genes emerged from this screen as probable biomarkers whose expression is regulated significantly by nephrotoxins but not by hepatotoxins. The range of gene expression changes ranges from several fold to thousands compared with control. The genes displaying most expression changes upon treatment of nephrotoxins include kidney injury molecule 1 (KIM1), lipocalin 2 (LCN2) and osteopontin (SPP1). The identification of this gene set offers a new tool in risk assessment for nephrotoxicity.

238 IDENTIFICATION OF OXIDATIVELY-MODIFIED PROTEINS IN CEREBROSPINAL FLUID BY A TARGETED PROTEOMICS APPROACH.

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A targeted proteomics approach was employed for the identification of oxidatively-modified proteins in human cerebrospinal fluid (CSF) using an aldehyde reactive probe, N^ε-aminooxymethylcarbonylhydrazino-D-biotin (ARP). Ten proteins in 2-dimensional (2D) gels of CSF from healthy and Alzheimer's disease (AD) subjects have been identified with MALDI-MS/MS analysis. The proteins were serotransferrin, serum albumin, hemopepin, Ig gamma chain C region, transthyretin, prostaglandin D-synthase, α -2 microglobulin, Ig kappa chain C region, α -1-antitrypsin and adducin gamma. Some proteins, such as serum albumin, serotransferrin and prostaglandin D-synthase were found in several spots indicating extensive post-translational processing. There was an overlap of adducin gamma with serotransferrin in the 2D gels. Avidin affinity stained blots of CSF protein samples revealed that 6 of the 10 proteins reacted with ARP. Protein spots that were identified in 2D gel

spots and corresponded to the ARP-reactive protein spots were serotransferrin, serum albumin, transthyretin, prostaglandin D-synthase, α -1-antitrypsin, and aducin gamma.

The use of ARP coupled with mass spectrometry represents a novel approach for the identification of carbonylated CSF proteins. The identified proteins might potentially lead to the development of novel markers for AD and other neurodegenerative diseases.

239 IN SITU BREAST CANCER METASTASIS CHARACTERIZATION BY MALDI MASS SPECTROMETRY.

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Breast cancer frequently metastasizes to bone, causing bone destruction, hypercalcemia, fracture and pain. To examine the mechanisms responsible for breast cancer metastasis and growth in bone, and study the tumors in their metastatic microenvironment, tumorigenic bone sections from nude mice inoculated with MDA-231 human breast cancer cells were analyzed by direct-tissue protein profiling using matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS). This technique allows "in situ" mass spectrometry analysis to be performed directly on tissue sections, and protein profiles are generated from discrete tissue subregions such as bone, marrow, tumor and muscle. The described approach is applicable to a variety of studies including monitoring small molecule localization within tissues and identifying key biomarkers indicative of disease state, tissue response to exposure or therapy, or patient outcome.

Tissue from five control mice and five mice containing breast cancer metastases were harvested, processed and analyzed, including tumor-bearing bone, tumor-bearing adrenal gland, muscle, liver and kidney. To optimize mineralized tissue preparation techniques, specimens were either fresh frozen or fixed in 70% ethanol and decalcified, sectioned, and analyzed by MALDI MS. The resulting protein profiles were evaluated. Comparable protein patterns were generated for the two tissue preparation methods, with the fixation/decalcification procedure giving better histology and the fresh frozen preparation yielding enhanced tumor protein patterns. The fresh frozen sample preparation method was then applied to characterizing breast cancer metastasis to bone and adrenal gland. Comparison of the protein patterns from tumor metastatic regions demonstrated greater than 20 protein signals with potential differential expression between the tumor and surrounding non-tumor regions as well as between tumor metastases in different tissues.

240 URINARY PROTEOMIC-BASED BIOMARKER DEVELOPMENT FOR EVALUATION OF OCCUPATIONAL EXPOSURE USING SELDI-TOF MASS SPECTROMETRY.

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The utility of four types of Ciphergen ProteinChips were evaluated by comparing protein profiles in control, and in in vitro acrylamide or glycidamide exposed urine using Surface Enhanced Laser Desorption Ionization Time of Flight (SELDI-TOF) mass spectrometry. Acrylamide (CAS 79-06-1), a widely used industrial chemical, which also may be formed in thermally processed food, can produce peripheral neurotoxicity and reproductive effects. Acrylamide has been classified as a probable human carcinogen. Changes in the levels of potential protein biomarkers in serum and urine have been reported in exposure to toxic agents, and bioactivation of acrylamide to glycidamide (5694-00-8), a reactive epoxide, suggests that urinary protein levels would be affected in acrylamide exposure. This work was designed to identify molecular biomarkers in human urine for use to assess acrylamide exposure or detect early disease onset. Urinary proteins were processed on NP20, CM10, H50, and IMAC30 chips having normal phase, cationic exchange, hydrophobic, and ionic surface chemistries, respectively. The number of peaks and mean peak intensity varied significantly with IMAC30 > CM10 > NP20 > H50. Various factors were optimized during the development of binding protocols for each chip, including surface pretreatment, sample binding time and washing methods. The data from this work suggest that the detection and further characterization of urinary protein levels using SELDI-TOF mass spectrometry can provide specific proteomic-based biomarkers of exposure for acrylamide.

Disclaimers: Mention of company names and/or products does not constitute endorsement by the Centers for Disease Control and Prevention (CDC). The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy.

241 IDENTIFICATION OF PROTEIN EXPRESSION PROFILES AFTER TREATMENT WITH THE HEAT SHOCK PROTEIN (HSP)90 INHIBITOR, 17-DIMETHYLAMINOETHYLAMINO-17-DEMETHOXYGELDANAMYCIN (17-DMAG) IN HUMAN AND RAT PBMCs USING THE SELDI PROTEIN CHIP SYSTEM.

S. Qin, D. Zhou, X. Zhang, L. Li, J. Ye, X. Zhang and J. Barsoum. Synta Pharmaceuticals Corp., Lexington, MA. Sponsor: E. Kitayama.

Proteomic techniques hold the promise to improve efficiency of protein profiling and biomarker identification for treatment-responsive proteins. In this study, we use a surface-enhanced laser desorption/ionization time-of-flight mass spectroscopy (SELDI TOF-MS) system to identify protein profiles in normal PBMCs treated with Hsp90 inhibitor in vitro. The fresh human and rat PBMCs were isolated and treated with 17-DMAG overnight at different concentrations from 1 to 100 nM. The cells were harvested and proteins were extracted, quantified, and then applied to weak cation exchange (CM10) and strong anion exchange (Q10) ProteinChip® arrays for protein profiling by SELDI-TOF MS. Ciphergen Express and Biomarker Patterns Software package was used to compare the protein profiles from different samples acquired from different protein chips. When the peak intensity was calculated as a fold increase or decrease vs. control in a range from 1-13 kDa, 49 protein picks were identified in rat and human PBMCs, which all relevant to the treatment of 17-DMAG. Among those, 29 (16 from Q10 and 13 from CM 10 chips) proteins identified in the treatment groups were differentially by 3 folds or greater relative to the vehicle control with a trend of dose-dependency. Overall, the number of protein picks identified in the rat was higher than that in the human PBMCs based on the peak intensity. Our results suggest that SELDI-TOF-MS has the potential to identify a protein profile that distinguishes action of the testing compounds vs. vehicle controls in both rat and human PBMCs. These protein expression profiles determined by SELDI TOF-MS can provide a new resolution to identify drug-responsive biomarkers and potentially lead to a better understanding of mechanisms related to the activity/toxicity of Hsp90 inhibitors.

242 DEVELOPMENT OF A PROTEOME DATABASE FOR QUANTITATIVE ANALYSIS OF MACROPHAGE RESPONSES TO NANOMATERIALS AND ENVIRONMENTAL PARTICULATES.

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The increase in industrial use of engineered nanoparticles and the link between particulate matter exposure and cardiovascular and respiratory effects in humans has created a need to understand the biological pathways stimulated by these agents. Identification of protein biomarkers of exposure to particulates and nanomaterials may aid in early detection of toxicity and highlight differential mechanisms of action. Toward this goal, we have established a reference macrophage proteome database for comparison of responses to different activating stimuli, which can be applied for future quantitative comparisons. We used a global proteomic approach to identify proteins in the extracellular compartment following treatment with particulate, biological, and biochemical stimuli. Conditioned medium from RAW 264.7 cells treated with urban particulate matter (UPM), crystalline silica, lipopolysaccharide (LPS), phorbol ester (PMA), or serum-free media (control) was analyzed by LC-MS/MS following tryptic digestion. We confidently identified 623 proteins, which is a significant increase in coverage compared to previous 2-D gel based studies of the extracellular proteome. Based on spectral counts, we found proteins that were differentially detected compared to control for UPM (80), silica (84) LPS (87), and PMA (117), as well as several proteins that were unique to each stimulus. We identified proteins associated with inflammatory (74), proteolytic (55), and apoptotic (15) processes among other biological pathways. Several of the proteins responses identified by MS/MS have been validated by orthogonal protein microarray analysis. This study established a reference database that is being applied for high-throughput investigations of macrophage responses to an array of nanomaterials using quantitative mass spectrometry-based approaches.

243 METABOLOMIC DETERMINATION OF BIOMARKER CANDIDATES OF PHOSPHOLIPIDOSIS USING CAPILLARY ELECTROPHORESIS TIME-OF-FLIGHT MASS SPECTROMETRY.

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We conducted a metabolomic analysis based on capillary electrophoresis time-of-flight mass spectrometry (CE-TOF-MS) to find biomarker candidates of phospholipidosis (PLD). SD rats received oral doses of a vehicle, amiodarone or MPC1 (a

Mitsubishi Pharma Compound) for two weeks. PLD was evaluated by histopathology and lung phospholipids (PL) contents. Amiodarone (150mg/kg) induced microscopically visible PLD in the lungs with 2.1-fold increase in PL content in the organ. On the other hand, MPC1 had no effect on the histopathology with 1.4-fold increase in the PL contents, demonstrating MPC1 induced milder PLD compared with amiodarone. CE-TOF-MS metabolomic analysis was conducted on urine and blood plasma collected after 2-weeks of treatment. Thousands of peaks detected by each assay were aligned from sample to sample to facilitate differential analysis between groups. Application of filtering with correlation coefficient to the lung PL contents as well as statistical significance between groups successfully extracted potentially valuable peaks from huge data sets. Tandem mass spectrometry with CE-Q-TOFMS clarified the identities of these peaks by confirmation of the exact molecular weight, migration time and MS/MS fragmentation pattern. Metabolites identified included phenylacetylglycine (PAG), a benchmark metabolite reported to be altered by PLD. Comparison with PAG showed that many of the metabolites newly discovered had superior property as biomarkers of PLD, especially in detection of milder PLD in terms of statistical significance and magnitude of changes. Moreover, the metabolomic analysis detected metabolic shift of a particular metabolic pathway, which exhibited more pronounced detection of PLD than each single metabolite. The results demonstrated the power of CE-TOF-MS based differential metabolome analysis to find multiple biomarker candidates simultaneously.

244 USE OF METABONOMICS TO DEFINE MECHANISMS OF TOXICITY: CONSORTIUM FOR METABONOMIC TOXICOLOGY-2 (COMET-2) PROJECT.

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COMET-1 was formed to develop a comprehensive database of toxicologically important changes in metabolic patterns determined by NMR analysis of rat urine and serum. Although metabonomics is a powerful approach for evaluating toxicity, its application to determining mechanisms of toxicity and ultimately human risk remains limited. COMET-2 was developed to apply metabonomic data (now both NMR and MS) to defining mechanisms of toxicity and assessing risk factors for toxicity. Two projects are underway. 2-Bromoethanamine (BEA), an acute papillary toxicant, induces renal injury in rats within 24 hours (>150 mg/kg) but the mechanism is not fully established. In initial COMET-2 studies, BEA markedly increased the urinary excretion of glutaric acid in rats, suggesting possible inhibition of glutaryl CoA dehydrogenase. This was observed at both non-toxic (< 50 mg/kg) and toxic (>150 mg/kg) doses indicating a lack of concordance with histopathologic changes. In contrast, metabonomic data indicate correlative changes in deoxynucleoside and N-methylnicotinic acid pathways, providing new mechanistic leads. The second project is to understand the metabolic basis of inter-individual variability in response to toxicant exposure using the highly variable hepatotoxicity of galactosamine (Gal) as an exemplar. At a dose of 415 mg/kg, ca 75% of rats showed hepatotoxicity ranging from minimal to marked necrosis, while 25% showed no toxicity. A mechanistic understanding of the basis for this variation is a current objective of COMET-2. Ultimately, results of these studies will be used to develop and test hypotheses regarding toxic mechanisms to position the utility of metabonomic data in investigative toxicology and risk assessment.

245 METABONOMIC ANALYSIS BY 1H-NMR SPECTROSCOPY OF CONTROL URINES OF DIFFERENT TOXICOLOGICAL ANIMAL MODELS.

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Metabonomic studies investigate a wide range of low-molecular mass endogenous metabolites present in biological samples and the influence of administered compounds on these profiles. However, the concentration of many urinary endogenous metabolites fluctuates as a result of normal physiological variation. The number of control samples analysed per individual metabonomic study is limited. To have a broader database and to better distinguish between physiological and compound induced variation, control urine samples of different toxicological species were analysed. Standard 1H-NMR spectroscopy and Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) were utilised to determine the influence of species, gender and age. Overnight urine samples from male and female rats, minipigs, beagle dogs and cynomolgus monkeys were

analysed. The results of these studies showed that many physiological conditions influence the composition of urine samples. A clear distinction between the four species was noted. Gender differences could easily be detected in rats and minipigs, but not in dogs or cynomolgus monkeys according to PCA analysis of urinary metabolic profiles. In rats, discrimination between male and female urines was due to the amount of citrate present, with females having far higher levels than males. The poster further discusses the influence of age on endogenous metabolic profiles. A broader understanding is necessary to avoid misinterpreting the natural fluctuation of endogenous metabolites as potential markers of toxicity.

246 NMR-BASED METABONOMIC EVALUATION OF GALACTOSAMINE HEPATOTOXICITY AND THE PROTECTIVE ROLE OF GLYCINE.

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Galactosamine (GalN) has been shown to alter gut permeability and increase bacterial translocation leading to endotoxemia which is believed to be primarily responsible for the hepatic effect. It has been postulated that glycine protects against GalN toxicity as it counteracts the activation of Kupffer cells and prevents cytokine release. Rats were acclimatised to a 0 or 5% glycine diet for 6 days and subsequently administered vehicle, GalN (500 mg/kg), glycine (via the diet), or both GalN and glycine. Urine was collected over 12 days prior to administration of GalN and for 24 hours thereafter. The metabolic profiles of biofluids and tissues were determined using NMR methodologies. The multivariate classification method, orthogonal-projection on latent structures (O-PLS), which pre-filters classification-irrelevant variation from data, was applied to model the NMR spectral data. O-PLS loadings enabled the urinary and serum metabolites that discriminated between control and treated classes to be determined. The urinary metabolic effects of GalN treatment were increased levels of N-acetylglucosamine together with decreased levels of 2-oxoglutarate, isoleucine, leucine, valine, deoxycytidine, N-methylnicotinamide and creatinine. The corresponding serum data revealed elevated levels of tyrosine, 3-hydroxybutyrate, TMAO, lactate, phenylalanine and reduced levels of lipid triglyceride and glucose. The metabolic effects of treatment with both GalN and glycine were significantly different; N-acetylglucosamine urinary levels were not elevated and the serum profile remained very close to that of controls. Histopathological data and clinical chemistry measurements confirmed the protective effect of glycine. These novel data highlight the applicability of NMR-based metabonomics in elucidating the metabolic consequences of toxicity and protection from toxicity, and lead to understanding a mechanism of toxicity.

247 METABOLOMIC RESPONSE PROFILE FOR SURGERY AND RADIATION.

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The goal of the study was to determine small molecule biochemical markers for both surgery and radiation treatment. There were four treatment groups: untreated mice; surgery treated mice; surgery treated mice with livers exposed to low radiation [10 grays (gy)]; and surgery-treated mice with livers exposed to high radiation [50 grays (gy)]. 24 hours later, blood samples were taken from the mice and complete biochemical profiles were obtained. The relative concentrations of over 150 small molecules were measured using GC and LC mass spectrometry. Statistical analysis consisted of looking for specific compounds with treatment effects and looking for sets of compounds indicative of changes in biochemical pathway response. The results found two sets of natural, small molecules, e.g., citric acid, were discovered that allowed for classification of the treatments, surgery effects, and radiation effects.

248 METABONOMICS EVALUATION OF URINE FROM RATS DOSED WITH ACETAMINOPHEN USING NMR AND UPLC/MS.

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Urinary metabolic perturbations associated with acetaminophen induced hepatotoxicity were investigated using NMR and ultra performance liquid chromatography-mass spectrometry (UPLC/MS). Single low and high doses of acetaminophen

were administered to male Sprague-Dawley rats in groups of 10. Pre-dose urine samples and urine samples from days 1 to 7 were collected for each rat and monitored by NMR and UPLC/MS. The PCA clustering of NMR and UPLC/MS spectra were shown to be associated with the measured clinical chemistry and histopathology findings. Many of the metabolic changes observed were due to dietary changes in the animals that received the high dose of acetaminophen. Both phenylalanine and pantothenic acid, which are derived from the diet, were decreased after the high dose of acetaminophen was administered. Furthermore, concentrations of ferulic acid and glycine both decreased as expected since they both play a role in detoxifying the reactive intermediates of acetaminophen. General biomolecules, including hippurate, phenylacetylglutamine, kynurenic acid, N-acetylsuccinyl, citrate, α -ketoglutaric acid, pipercolonic acid and other unknown metabolites were also observed as altered components in urine. As such, metabolomics based on NMR and UPLC/MS analysis has provided complimentary information concerning acetaminophen-induced liver injury.

249 EVALUATING DRUG-INDUCED HEMOLYSIS EFFECTS USING A WHOLE BLOOD-LIVER SLICE CO-CULTURE MODEL.

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In this study, we demonstrate the utility of an in vitro model to determine drug induced effects on the hemolysis of red blood cells (rbc) and the expression of liver genes involved in heme turnover. After pre-incubation in media alone, precision-cut rat liver slices are transferred to fresh media mixed with whole blood plus compound or vehicle for up to 72 hr. The release of hemoglobin from rbc and levels of ATP and GSH in rbc and liver slices are quantitated over the culture time. Additionally, changes in the expression of liver genes involved in heme metabolism (e.g., heme-binding protein 23, heme-oxygenase 1, and ferroportin 1) are determined by quantitative RT-PCR. One test compound, aniline, which is known to require metabolism to induce hemolysis, causes hemolysis in a time- and concentration- dependent manner. For example, at 48 hr, a single dose of 10 and 25 mM, resulted in 30% and 70% hemolysis respectively. Similarly, another test compound, methimazole, administered as a single dose up to 1.5 mM for up to 72 hr, induces up to 60% hemolysis in a time- and concentration- dependent manner. Preceding hemolysis, levels of GSH in the blood declined, while ATP levels remained stable. The expression of heme metabolism related liver genes positively correlated with hemolysis. In the absence of a liver slice but in the presence of aniline or methimazole, the percent hemolysis is similar to cultures with no drug exposure, <5%. This study demonstrates that the whole blood-liver slice co-culture model is a useful tool for the investigation of liver generated drug metabolites leading to hemolysis. Furthermore, the model can be extended across species, including human, for the characterization of species susceptibility to drug-induced hemolysis and the identification of the metabolic pathways involved.

250 NEWBORN MICE EXPOSED TO HYPEROXIA EXHIBIT INCREASES IN LIPID OXIDATION PRODUCTS AND CYTOSOLIC PHOSPHOLIPASE A2.

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Respiratory failure, lung inflammation, and alterations in lung development are serious clinical complications associated hyperoxic lung injury in premature infants. We tested the hypothesis that exposure to hyperoxia is associated with increases in lipid inflammatory mediators and the enzymes involved in their formation and that these increases would be associated with changes in lung concentrations of nuclear receptors PPAR α and/or γ . Newborn mice were placed in either room air (RA) or >95% O₂ for 1, 3, or 7 days and subsequently lung tissues were harvested for lipid and protein analyses. Histological analysis indicated no detectable lung injury in these mice through 7 days of exposure. In the phospholipid fractions of lung tissues, levels of 5-HETE, 8/12-HETE, and 11-HETE were greater in the O₂ exposed animals than the RA controls at all days tested (5-HETE: day1, 472+65 vs 275+71; day 3, 235+64 vs 129+43; day 7 235+64 vs 119+26 pmol/g tissue), (8/12-HETE: day1, 123+46 vs 77+21; day 3, 61+8 vs 34+8; day 7, 73+26 vs 39+14 pmol/g tissue), (11-HETE: day1, 231+25 vs 93+11; day 3, 127+11 vs 95+23; day 7, 124+24 vs 91+26 pmol/g tissue). Western blots indicated that cPLA2 levels were greater in the O₂ exposed mice than RA controls by day 7 but 15-lipoxygenase (15-LO) levels were dramatically less at 7 days in O₂, than day 1 or 3 in O₂ or in all RA controls. Neither PPAR α nor PPAR γ protein levels were different in the tissues from oxygen treated animals than tissues from the room air controls. However, PPAR γ protein levels indicated an age dependent decrease in expressions in both the treated and control tissues. Our data indicate that increases in specific lipid oxidation products are detectable as early as 1 day of exposure and may be relevant in the course of lung injury. Levels of cPLA2 were increased by 7 days of hy-

peroxia and are likely to contribute to the increases in oxidized lipids. Levels of 15-LO were decreased and may be associated with both inflammatory resolution and growth in the newborn lung.

251 TOTAL HOMOCYSTEINE CHANGES IN ADULT MALE MICE AFTER EMBRYONIC EXPOSURE TO 5-AZA-2'-DEOXYCYTIDINE (5-AZA-CdR): ROLE OF EPIGENETICS.

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Homocysteine is considered a risk factor marker for several pathologies, mainly cardiovascular diseases. It is produced by intracellular demethylation of the essential amino acid methionine. Its remethylation ensures the provision of S-adenosylmethionine, the main methyl group donor in the body. DNA methylation, the most understood epigenetic mechanism, controls at least in part gene expression during embryonic and post-natal life. To investigate if an embryonic exposure to 5-AZA-2'-Deoxycytidine (5-AZA-CdR) – a demethylating agent- affects the total homocysteine levels in postnatal stages. CD-1 mice at gestation day (GD) 10 were administered one dose of 1 mg/kg of 5-AZA-CdR via intra peritoneal injection. Blood samples from male offspring were collected at post natal days (PD) 70 and 154 by cardiac puncture. Levels of total homocysteine were analyzed using the Microplate STE-Based Assay of homocysteine. Total homocysteine levels for control and exposed mice were 14.19 (+/- 2.17) and 13.4635 (+/- 1.29) at PD 70 and 16.5369 (+/-1.46) and 16.0117 (+/-1.47) at PD 154. While the ANOVA (0.95 confidence) did not identify statistical differences among experimental groups, there was a trend in which embryonic exposed mice exhibited lower levels of total homocysteine. Further studies are needed to elucidate the effects of altered methylation during embryogenesis on homocysteine levels during adult life and the subsequent implications.

252 MULTIPLEXING FLUORESCENT-BASED OXYGEN AND PH SENSING FOR THE ANALYSIS OF MITOCHONDRIAL FUNCTION AND DYSFUNCTION.

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Mitochondrial dysfunction is a major mechanism of drug-induced toxicity. Our goal is to identify NCEs that exhibit the capacity to perturb mitochondrial function early within the drug discovery process. Recently, we have demonstrated in the suitability of the MitoXpress™ approach for HTS analysis of mitochondrial respiration in (Toxicological Sciences, 2006, 92(1):186-200). Here, we present a multiplexed approach to obtain an even more detailed and complete picture of cellular metabolism via parallel monitoring of aerobic and non-aerobic metabolism. Knowledge of the effect of NCE treatment on both glycolysis and oxidative phosphorylation allows inferences to be drawn regarding the site of metabolic insult; allowing glycolytic inhibition to be delineated from direct mitochondrial effects, increasing the confidence with which mitochondrial toxicity can be detected. Currently this is achieved in our laboratory through screening in HepG2 cells which are either anaerobically or aerobically poised, respectively (The Toxicologist 2005). However, dictating metabolic poise via substrate restriction is not feasible for all cell lines, many of which are grown in proprietary culture media. In an attempt to adapt the measurement of extracellular acidification to a convenient, high throughput assay, capable of multiplexing with the Luxcel MitoXpress™ oxygen sensing assay, we evaluated the use of new pH sensitive fluorescent probes. Data to be presented includes the spectral characterisation of both O₂ and pH probes in the presence of each other and assessment of probe performance in multiplexed assay

253 ASSESSMENT OF DRUG-INDUCED MITOCHONDRIAL TOXICITY IN DRUG SAFETY.

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Mitochondrial integrity is essential for viability of aerobically poised cells; when mitochondria fail, the cell dies. The evidence is accumulating that many ethical drugs have deleterious mitochondrial effects that are manifest as tissue toxicity in liver, cardiovascular system, skeletal muscle, nervous system, and kidneys, among others. In some cases, such as antiviral therapy, toxicity results from coincident interference with mitochondrial replication. In others, however, the drug acutely and directly undermines mitochondrial function. Importantly, not all drugs within a class have mitochondrial liabilities, indicating that the therapeutic objective can be accomplished while circumventing cytotoxicity due to mitochondrial impairment.

We present here mitochondrial assessments of several families of drugs used for treatment of diabetes and hyperlipidemia, including statins, thiazolidinediones, and fibrates. Respiration of isolated rat liver mitochondria during State 2 (oxidizable substrate) and State 3 (substrate plus ADP) was monitored using a fluorescent probe that reports PO₂. Of the 6 statins evaluated, 4 uncoupled State 2 respiration, and also inhibited OXPHOS. Of the 6 thiazolidinediones, 3 profoundly uncoupled State 2 respiration, 2 showed intermediate uncoupling, and one was without effect. However, all 6 thiazolidinediones inhibited State 3 respiration. Of the 5 fibrates examined, 1 showed modest uncoupling of State 2 respiration, and 2 modestly inhibited State 3. Because these drugs are frequently co-prescribed, we also evaluated their mitochondrial effects when combined at half the previously tested concentration. In several cases, although neither drug alone elicited a mitochondrial response, the combination additively uncoupled or inhibited respiration, suggesting that some combinations are more likely to yield drug-induced mitochondrial side effects.

254 CURRENT DEVELOPMENTS IN REAL-TIME FOURIER TRANSFORM INFRARED SPECTROSCOPIC ANALYSIS OF THE CELLULAR RESPONSE TO NANOMATERIALS.

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There is considerable uncertainty surrounding the toxicity of nanomaterials and associated mechanisms of action. Cost effective technologies that can provide real-time screening of nanomaterials and provide information regarding the mode of action are needed to reduce this uncertainty. Here we are investigating the application of Fourier Transform Infrared (FTIR) spectroscopy as a tool to screen the cellular response to nanomaterials rapidly. Initial efforts have focused on the activation of macrophage cells (RAW 264.7) by lipopolysaccharide (LPS) as a model proinflammatory stimulus, a mode of action that is widely implicated in nanomaterial toxicity. LPS induces a proinflammatory state in RAW 264.7 cells, characterized by up-regulation of cyclooxygenase-2 (Cox-2) and inducible nitric oxide synthase (iNOS). FTIR data on LPS-treated macrophages indicated a marked increase in a peak detected at wave number 1652, characteristic of the protein carbonyl bond. Pretreatment of RAW 264.7 cells with indomethacin, a Cox-2 inhibitor, fully suppressed the increase in this peak, suggesting a link between this spectroscopic change and Cox-2 activity. Additionally, we overexpressed Cox-2 in RAW 264.7 to explore this link further. Treatment of Cox-2 expressing cells with arachidonic acid, the substrate for Cox-2, was associated with an increase in the peak occurring at the same wave number 1652, providing additional evidence to this link. Dehydration experiments were investigated to reduce interference by water revealing additional FTIR observable features masked in live cell experiments. Treatment of RAW 264.7 cells with multiwalled carbon nanotubes (MWNs) resulted in a reproducible change in a peak observed at wave number 1083. These data illustrate the most recent advances in the application of FTIR spectroscopy to interrogate the cellular response to nanomaterials. This research was supported by U.S. DOE funding (Contract DE-AC05-76RL01830).

255 DEVELOPEMENT OF A CIGARETTE SMOKE MODEL OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) IN THE MOUSE.

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The purpose of this study and its preceding work was to develop a model that adequately replicated several of the physiological and molecular characteristics of COPD that could be used to evaluate the efficacy of new chemical entities to delay disease onset. Changes in gene expression, inflammatory biomarker levels and lung remodeling were evaluated in smoke sensitive A/J mice. Mice were exposed to vehicle, 4% smoke, 4% smoke + LPS (known for inducing neutrophilia) or 4% smoke + rolipram (a PDE4 inhibitor shown to decrease the influx of inflammatory cells to the site of inflammation) for 2 hours daily, over a 3 month period. Mild changes in lung pathology, increased alveolar airspace along with chronic macrophage infiltration were noted in smoke and smoke + LPS treated groups; whilst macrophage infiltration was decreased in Rolipram treated animals. Bronchoalveolar lavage fluid cytokine levels indicated increased levels of MDC, MCP-1 and MPO, consistent macrophage accumulation. However changes in TNF α , INF γ , IL10, IL18, KC (Gro- α /CXCL1), normally associated with COPD were not present. Lung tissue expression showed significant upregulation of 186 probe sets, which included oncogenes and cardiac myosin genes, possible indicators of cancer and heart disease. A comparison of gene expression changes observed in 5 human patient microarray/SAGE studies identified 32 genes with similar upregulation. However no changes in tissue expression were noted for Metalloproteases, Serpine or RTP801 which would normally be altered in COPD. The data indicates that although the

model does not fully mimic COPD, some common characteristics, i.e alveolar enlargement and chronic macrophage inflammation were noted. The next stage of this model development will be to increase the total smoke concentration and/or exposure period, in order to upregulate expression of other markers commonly associated with COPD.

256 DEVELOPMENT AND APPLICATION IN A NEPHROTOXICITY MODEL OF A NOVEL ENZYME IMMUNOASSAY FOR THE QUANTIFICATION OF URINARY CLUSTERIN IN RATS.

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Clusterin / Apolipoprotein J (ApoJ) is a secreted glycoprotein found in numerous mammalian tissues and biological fluids. Although clusterin has been implicated in numerous biological processes, the localised over-expression of clusterin at sites of tissue damage or stress suggests that clusterin acts as a molecular chaperone displaying cytoprotective characteristics. The marked induction of clusterin in several renal disease states, such as glomerulonephritis, cystic renal disease and renal tubular injury suggests the possibility that urinary clusterin could be a useful biomarker of nephrotoxicity.

Biotrin International has developed a novel enzyme immunoassay (EIA) for the detection and quantification of urinary clusterin levels in rats. Here, we present the development and performance characteristics of this EIA together with data from a study on urinary clusterin release in laboratory rats during and after treatment with the non-steroidal anti-inflammatory drug, indomethacin (9 rats, 10mg/kg body weight/day over a period of 4 days). For all animals treated, the post-treatment urinary levels of clusterin were significantly higher than pre-treatment levels. These preliminary findings corroborate results from previous nephrotoxicity studies and support the use of urinary clusterin as a biomarker of nephrotoxicity.

257 A CHEMOGENOMIC ANALYSIS OF THE DRUG-DRUG INTERACTION BETWEEN STATINS AND FIBRATES.

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The incidence of HMG-CoA reductase inhibitor (statin) associated rhabdomyolysis increases with co-administration of a PPAR α agonist (fibrate) due to drug-drug interaction (DDI) between these two classes of lipid lowering agents. This DDI was initially considered to be a pharmacodynamic effect since both classes of drugs can cause myopathy when administered alone; however, recently gemfibrozil was reported to increase the plasma concentration of cerivastatin by 5-fold. We have analyzed drug-induced gene expression changes in liver and skeletal muscle tissues of statin or fibrate-treated rats and found that the DDI may alternatively be attributed to insufficient hepatic glucose output resulting from synergistic down-regulation of key genes involved in hepatic gluconeogenesis, PEPCK and glucose-6-phosphatase, by fibrates and statins, respectively. The state of glucose-insufficiency in skeletal muscles of statin-treated (MTD) animals was reflected in i) the up-regulation of PDK4 - known to be induced both under conditions of starvation and by PPAR α agonists and serving to facilitate entry of acetyl-CoA derived from β -oxidation into the TCA cycle, and ii) increased expression of mitochondrial HMG-CoA synthase, the rate limiting enzyme for ketogenesis. Thus, in skeletal muscles, statins and fibrates synergistically increase the consumption of fatty acids. This leads to an hypothesis that under conditions where the individual's circulating serum triglyceride level is low, the triglyceride and fatty acid store in muscle could be depleted leading to muscle protein break-down, increased serum creatine kinase, and rhabdomyolysis. We have also found that fibrates suppress drug-metabolizing enzymes and transporters such as CYP2C, CYP3A, PAPSS2 and ABCC2 that are involved in the metabolism and excretion of the statins, potentially leading to increased plasma concentration of co-administered statins. In conclusion, chemogenomic analysis allowed us to develop a mechanistic understanding of the DDI between fibrates and statins and postulate testable molecular hypotheses for these DDIs.

258 MUSCLE CREATINE DEPLETION EXACERBATES CERIVASTATIN-MEDIATED TOXICITY.

D. F. Wells, C. M. Rohde, L. A. Egnash, L. C. Robosky, M. L. Manning, S. A. Roys, M. D. Reily and D. G. Robertson. *Pfizer, Ann Arbor, MI.*

One clinically noted side effect of statin therapy is an adverse effect on muscle, ranging from aches and weakness to rhabdomyolysis. In an effort to discover new biomarkers of myotoxicity, a metabonomics evaluation of statin-mediated myotoxicity was conducted. Female Sprague-Dawley rats (n=5-6/gp) were given daily oral

doses of 0, 0.03, 0.1, 0.3, and 1 mg/kg cerivastatin (CVA) for 14 consecutive days. On Day 15, rats given 1 mg/kg CVA exhibited increased serum creatine kinase (CK) and transaminase levels as well as liver and muscle histopathology, primarily in Type 2 (fast-twitch) muscles. Correspondingly, urinary creatine levels increased while creatinine levels decreased. As creatine plays a key role in energy utilization in Type 2 muscles, we investigated whether CVA was affecting muscle creatine levels. In a separate study, rats (n=6/gp) were fed Certified Rodent Diet 5002 (5002), a 1% β -guanidinopropionic acid (β -GPA)-supplemented diet, or a 2 % creatine-supplemented diet and then dosed orally with 0 or 1 mg/kg CVA daily for 14 days. β -GPA is a creatine analog that is commercially available as a health-food supplement and competes with creatine for uptake into muscle, thus depleting muscle creatine levels. Dietary creatine supplementation did not ameliorate the myotoxicity associated with CVA treatment. However, dietary β -GPA supplementation exacerbated CVA toxicity (moribundity) relative to rats administered CVA alone, although the toxicity was not necessarily via muscle or liver damage. This effect may in part be due to an increase CVA exposure levels (2-fold) in the presence of β -GPA. Muscle extracts of rats fed 5002 and dosed with CVA exhibited a marked decrease in muscle creatine content as did animals treated with β -GPA. These data suggest that CVA affects muscle creatine levels and that decreases in tissue creatine exacerbates CVA toxicity, but depletion of creatine in itself is not sufficient to induce muscle toxicity.

259 AIN-93G DIET EXACERBATES CERIVASTATIN-MEDIATED MYOTOXICITY.

C. M. Rohde, D. F. Wells, L. C. Robosky, L. A. Egnash, M. L. Manning, S. A. Roys, M. D. Reily and D. G. Robertson. *Metabonomic Evaluation Group, Pfizer, Inc., Ann Arbor, MI.*

While looking for new biomarkers of cerivastatin (CVA)-mediated myotoxicity, a novel dietary interaction was observed in female rats being fed AIN-93G, a defined, casein-based diet, and orally dosed with 0 or 1 mg/kg/day CVA. Markers of muscle and liver toxicity were markedly increased relative to effects seen in previous study where rats were given 1 mg/kg CVA and fed Certified Rodent Diet 5002. The AIN-93G study had to be terminated two days early due to moribundity and mortality, with only 3 rats surviving to Day 13. To confirm this dietary interaction, a second study was performed. Rats (n=6/gp) were fed 5002 or AIN-93G and orally dosed with 0 or 1 mg/kg CVA once daily for 11 days. All rats fed 5002 and dosed with CVA survived to Day 11. Except for one rat, all the animals in this group (5/6) showed no indications of CVA-mediated toxicity. One rat (1/6) had high serum creatine kinase (CK) and transaminase levels as well as signs of minimal muscle necrosis. In comparison, rats that received AIN-93G and CVA had markedly increased clinical and clinical pathologic evidence of toxicity. One rat (1/6) in this group died just prior to study termination on Day 11. All of the surviving rats in this group exhibited signs of CVA-mediated myotoxicity, including maximal serum CK levels (32000 U/L), transaminase levels and early indications of muscle necrosis. The markedly increased severity of CVA-induced myotoxicity could not be explained by an effect of AIN-93G on CVA exposure levels, as exposures (AUC) were comparable in rats fed the two diets. In conclusion, the AIN-93G diet exacerbated CVA-mediated myotoxicity by a mechanism that remains to be elucidated.

260 INVESTIGATION OF THE EFFECTS OF A LIPOPHILIC ANTI-INFLAMMATORY AGENT ON BILE ACID TRANSPORTER PROTEIN FUNCTION.

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Several molecules affect bile acid excretion patterns *in vivo* however the mechanisms by which these effects are mediated are not fully understood. We investigated these mechanisms using a lipophilic anti-inflammatory compound (logP=3.29) from a series of molecules that we have shown to modify biliary excretion patterns in the rat. We have investigated the effects of the compound on bile acid transporter biology *in vitro* as a possible explanation for the observed *in vivo* effects. We have used *in vitro* rat hepatocyte sandwich cultures to study the effects of the compound on the canalicular efflux of cholesteryl lysyl fluorescein (CLF) by the bile salt efflux protein (Bsep), the multidrug resistance protein 2 (Mrp2)-mediated canalicular efflux of carboxydichlorofluorescein (CDF), and the basolateral uptake of [^{3} H]-taurocholate via the sodium taurocholate co-transporter protein (Ntcp). The compound decreased the efflux of CLF by Bsep, increased the Mrp2-mediated efflux of CDF and slightly increased the uptake of [^{3} H]-taurocholate via Ntcp. Further studies, using immunocytochemistry techniques, indicated that transporter expression and the distribution of Bsep and Mrp2 in hepatocyte cultures was also modified following exposure. These data suggest that subtle effects on hepatocyte transporter biology may underpin the observed modification in bile acid excretion patterns seen with other compounds in this series *in vivo*.

261 BERBERINE AND BERBAMINE MODULATE P-GLYCOPROTEIN.

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Oregon Grape Root (OGR), *Mahonia aquifolia*, is native to the west coast of North America, and is also known by the names Mountain Grape and Holly-Leaved Barberry. Berberine as an alkaloid found in the OGR as well as berbamine which have been reported to have significant anticancer activity. The hypothesis of our study is that berberine and berbamine can modulate P-glycoprotein (P-gp), thereby, altering the bioavailability of drugs that are substrates of P-gp, such as cyclosporine A (CSA) and digoxin using Caco-2 and MDCKII-MDR1 drug transport models. Cytotoxicity was evaluated by measuring LDH levels and by assessing mitochondrial function using the MTT assay. Cells were exposed to various concentrations of OGR extract, berberine or berbamine for 0 - 4 hr. There were no significant increases in LDH leakage or decreases in MTT reduction for OGR extract at 4 h. However, there was a significant increase in LDH leakage and decrease in MTT reduction as early as 2 h for 300 μ M berbamine and 4 h for 150 μ M berberine (p<0.05). [^{3} H]-CSA/[^{3} H]-digoxin (0.5 μ M) transport experiments were performed with and without co-exposure of OGR extract, berberine or berbamine. OGR extract inhibited the efflux of CSA and digoxin in a dose-dependent manner with a significant effect at 0.1 mg/ml. Ten μ M berberine and 30 μ M berbamine significantly reduced the apparent permeability coefficient's (P_{app}) efflux of CSA ($3.31 \pm 0.32 \times 10^{-6}$ to $1.79 \pm 0.40 \times 10^{-6}$ cm/sec and $2.18 \pm 0.46 \times 10^{-6}$ cm/sec for berberine and berbamine, respectively), while there was no measurable effect of berberine with digoxin. In the MDCKII-MDR1 cells, 10 μ M berberine and 30 μ M berbamine were capable of inhibiting the efflux of CSA (from $1.31 \pm 0.19 \times 10^{-5}$ to $5.34 \pm 0.16 \times 10^{-6}$ cm/sec and $2.55 \pm 0.11 \times 10^{-6}$ cm/sec, respectively) and digoxin (from $3.30 \pm 0.13 \times 10^{-6}$ to $1.56 \pm 0.43 \times 10^{-6}$ cm/sec and $1.71 \pm 0.66 \times 10^{-6}$ cm/sec, respectively). Thus, these data suggest that OGR extract, berberine and berbamine can inhibit P-gp thereby increasing the bioavailability of drugs that are substrates of P-gp.

262 COMBINATION TOXICITY STUDIES: SCIENTIFIC NECESSITY OR A REGULATORY REQUIREMENT? A CASE STUDY WITH DARUNAVIR-BOOSTED RITONAVIR.

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The need for nonclinical safety evaluation for combination therapy of non co-formulated products is unclear. The treatment of HIV-infection, unlike many other diseases, necessitates multi-drug therapy to prevent viral replication. Darunavir is a protease inhibitor used for the treatment of HIV-infection and like most drugs in its class it is used in the clinic in-combination with ritonavir to increase its oral bioavailability. In order to assess the pharmacokinetics and the safety of each molecular entity alone and in-combination, two 6-month repeated dose toxicity studies were conducted in rats. Animals were dosed orally with darunavir alone at 20, 100 and 500 mg/kg and in a separate study with darunavir/ritonavir at 0/75, 20/50, 100/50, 500/75 and 1000/75 mg/kg. Observations were made for clinical signs, body weight, food consumption, blood and urine chemistry and animals were subjected to necropsy and histopathological examination. Blood samples were taken for toxicokinetic evaluation. Systemic exposure to darunavir decreased with repeated administration due to metabolic autoinduction. The enhancement of systemic exposure of darunavir by ritonavir was limited. Exposure to ritonavir was decreased in rats when administered with darunavir. The target systems identified with darunavir were the hematopoietic system, the blood coagulation system, liver and thyroid. No new target organs of toxicity were identified when darunavir was combined with ritonavir. To conclude, combination toxicology studies allow for the assessment of nonclinical safety of drugs clinically used in-combination and thus fulfill the recommendations of regulatory authorities by providing a comprehensive data-package for drug approval. However, the scientific value of such studies can be limited especially for compounds that exhibit a different pharmacokinetic interaction in animals relative to humans.

263 ADAPTIVE RENAL WEIGHT CHANGES IN DOGS FOLLOWING IV ADMINISTRATION OF THE NOVEL NEURO-PROTECTANT DRUG-NEU2000-K.

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Increase in renal weights in pre-clinical toxicity studies should be scrutinized carefully for accurately defining the toxicity of the drug.

Intravenous (slow bolus) administration of Neu2000-K for 28-days at 25, 50, 125 mg/kg/day in Beagle dogs, resulted in increases in absolute and relative weights of kidney.

The parameters examined to identify the plausible cause of increased renal weights following Neu2000-K administration were: serum chemistry parameters, micropathology, special histochemical staining of kidney sections (lipofuscin and Prussian blue) and transmission electron microscopy (EM).

Microscopic pathology assessment revealed test-article related findings (brown pigmentation of renal tubular epithelial cells, minimal to moderate severity) in the kidney of dogs at 50 and 125 mg/kg/day, no microscopic lesions were observed at 25 mg/kg/day. No alterations were observed in any of the serum chemistry parameters determined during the study. The results of special staining histology procedures revealed that the brown pigment in the proximal tubular epithelium was due to the accumulation of iron positive material (interpreted as hemosiderin, a metabolic product of hemoglobin break-down). Transmission EM evaluation corroborated the findings of special staining and confirmed that the pigment present in the renal tubule was consistent with hemosiderin. In addition, EM evaluation also revealed occasional presence of small lipid droplets which were interpreted as lipofuscin (residual bodies). Based on these observations, increase in renal weights at 50 and 125 mg/kg may be due to pigment/droplet accumulation in the proximal tubular epithelium and the numerical increase in kidney weight, at the 25 mg/kg/day is an adaptive response of the renal tissue following repeated IV dosing with the test-article. Overall, observed increased renal weights at all the dose levels did not induce any alterations in serum chemistry parameters indicating that, the renal function was not affected.

264 HEMATOLOGICAL EFFECTS OF PLURONIC F127 AND HYDROXYPROPYL METHYLCELLULOSE UTILIZED AS DELIVERY VEHICLES FOR HEMATOPOIETIC GROWTH FACTORS.

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Pluronic and cellulosic polymers have been utilized experimentally as injectable drug delivery vehicles to provide a single dose format for pharmaceuticals that typically require multiple dosing to be pharmacologically active. A combination of Pluronic F127 and hydroxypropyl methylcellulose (HPMC) polymers have been used to deliver the hematopoietic growth factors, fms-like tyrosine kinase 3 ligand (Flt3L) and granulocyte colony stimulating factor (G-CSF), so that efficacy was achieved after single dose administration to mice. The goal of the current study was to evaluate the activity of F127 and HPMC vehicles, administered in combination or alone, on hematological parameters. In this regard, potential acute adverse actions or contribution to expected drug effect by these vehicles would be addressed. Mice received a single subcutaneous injection of F127/HPMC, F127, or HPMC vehicles or F127/HPMC + G-CSF and hematological parameters were assessed from 1 to 5 days after injection. F127/HPMC + G-CSF increased neutrophil numbers while decreasing lymphocyte and bone marrow nucleated cell numbers. In addition, the numbers of splenic nucleated cells were increased, a common effect following high dose G-CSF in mice. These effects differed significantly ($p < 0.05$) from non-injected control mice or mice injected with buffer or F127/HPMC vehicles. A single administration of F127 and HPMC vehicles, alone or in various combinations, did not significantly alter numbers of neutrophils, lymphocytes, spleen cells, or bone marrow cells compared to controls. Furthermore, F127 and HPMC vehicles neither significantly affected body weight nor numbers of red blood cells. Therefore, F127 and HPMC did not induce acute adverse effects on these hematological endpoints nor generate drug-related responses. Assessment of additional toxicological and hematopoietic endpoints, including hematopoietic stem cell numbers and function, would allow further critical evaluation of the safety profile of these polymers.

265 QUANTITATIVE ANALYSIS OF OPIOID DRUGS AND PEPTIDES IN HUMAN PLASMA BY MALDI-LINEAR ION TRAP MASS SPECTROMETRY (LITMS).

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Rapid quantitative analysis of 5 opioid drugs, morphine, codeine, oxycodone, fentanyl, hydromorphone and 4 opioid peptides, Met-enkephalin, Leu-enkephalin, alpha neo endorphins and beta neoendorphin in plasma was performed by MALDI-Linear ion Trap Mass Spectrometry (LITMS). Identification of opioid drugs and peptides was traditionally performed by gas chromatography-mass spectrometry (GC/MS) and liquid chromatography-mass spectrometry (LC/MS) techniques. However, these processes require chromatographic separation followed by mass spectrometry identification which are tedious and time consuming. This

paper describes a quick quantification method using meso-tetrakis (pentafluorophenyl) porphyrin as MALDI matrix for opioid drugs and α -cyano-4-hydroxycinnamic acid (CHCA) and dihydroxy benzoic acid (DHB) for peptides. Porphyrin (MW 974.57) was identified as the preferred matrix for small molecules due to a lack of interfering matrix peaks at lower mass range (m/z 100-700). Spiked plasma samples were processed by solid phase extraction (SPE) using Bond Elute Certify cartridge. Validation of the methodology was demonstrated for each compound in plasma by selective reaction monitoring method. Quantitative analyses were performed through the use of a deuterium labelled analog of each analyte as an internal standard. Linearity was observed for the analysis of 5 opioid drugs (5-1000 ng/ml) and for peptides (10-1000 ng/ml) in plasma with a limit of detection ranging from 1-4 ng/ml. In general, the observed concentrations showed good linearity and reproducibility with coefficients of variation of within 15%. This is the first time MALDI-Ion Trap MS has been reported as a potential means for quantification of opioid drugs and peptides. We conclude that MALDI-Ion Trap MS improved sensitivity, speed and throughput for complex mixture analysis of opioid drugs and peptides in human plasma and is ideally suited as a reliable and complementary technique to GC/MS and LC/MS.

266 A MODEL OF POST-OPERATIVE AND OPIOIDS INDUCED ILEUS FOR ASSESSMENT OF GASTRO INTESTINAL TRANSIT IN RATS.

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Post-operative ileus (POI) is a transient impairment of bowel motility. The pathophysiology of POI is multifactorial and causes include, but are not limited to, surgical manipulation of the bowel, post-surgical inflammation and a generalized stress response. The use of postoperative opioids also appears to contribute to ileus. In order to better comprehend the invasive association of abdominal surgery and use of opioids with POI, this study was conducted to investigate the effects of exposure of abdominal contents to manual handling (specifically the cecum) and of exposure to morphine. POI was quantified by measurement of gastric emptying through dilution of a phenol red dye. A first group of Sprague-Dawley rats was anesthetized with isoflurane gas and the abdomen opened. Half of the animals had the cecum exteriorized and gently patted for 1 minute, while the second half were exposed to surgery, but with no cecum manipulation. Another group of rats was administered a subcutaneous dose of either 1 or 4 mg/kg of morphine. A group of rats was administered the phenol red alone and served as a control. Phenol red served as a gastrointestinal marker and was administered orally post-surgery or post morphine injection, and was assayed colorimetrically 30 minutes post administration. Increases in stomach content of phenol red in cecum-manipulated and non-manipulated surgical groups were approximately 19% and 31%, respectively, when compared to control group (absorbance of 1.9 and 2.1, respectively vs 1.6 for control). The stomach content of phenol red in the cecum-manipulated group was approximately 9.5% lower than the non-manipulated group. Exposure to morphine alone significantly reduced the stomach motility and equivalent increases of approximately 88% in stomach content were seen at both dose levels (absorbance of 3.0). These responses confirm that POI is a response to surgery and administration of opioids and its magnitude is not unilaterally dependent on the degree of surgical manipulation or dose dependent on the exposure of opioids.

267 TRANSPORT KINETICS OF THENORPHINE INTO RAT BRAIN BY THE BLOOD-BRAIN BARRIER.

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Thenorphine is a newly developed partial agonist of μ -opioid receptor. Recent pharmacological studies have shown that the drug may be of a great potential for treatment of opioids addiction in clinics. However, few pharmacokinetic and toxicokinetic studies have been performed to elucidate brain transport properties of thenorphine. In the current study, we used an in situ brain perfusion technique coupled with the capillary depletion method to investigate the transport kinetics of thenorphine into rat brain. Wistar rats of both sexes were divided into 3 dosage groups, each of which was perfused with 12.5, 25, and 50 μ g/mL of thenorphine, respectively, for 2, 5, 8, or 10 min. At the end of perfusion, the brain was harvested and homogenized, and the cerebrocapillary was removed. The concentrations of thenorphine in the brain parenchyma were then extracted and analyzed by HPLC. Data showed that thenorphine could be detected in brain parenchymal fractions, suggesting the capability of the drug in passing across the blood-brain barrier. Concentrations of thenorphine in brain parenchyma increased with the perfusion time and were positively associated with thenorphine concentrations in the perfusate. The influx transfer coefficient (K_{in}) values for thenorphine at three perfusion concentrations were 0.162, 0.207 and 0.205 mL/min/g, respectively. Our results demonstrate that thenorphine can pass across the blood-brain barrier to reach its brain targets. Since the influx values does not change with the increase of the

thenorphine concentration in the perfusate, the transport of thenorphine across the blood-brain barrier appears to be rate-limited by yet-unknown mechanism(s), but not likely a passive diffusion. These data may help better design the drug for treatment of opioid addiction.

268 CORRELATION OF RENAL TOXICITY OF A NOVEL ANTI-INFLAMMATORY COMPOUND IN CYNOMOLGUS MONKEYS WITH PRIMARY CULTURES OF KIDNEY PROXIMAL TUBULAR CELLS AND TRANSFECTED CELL LINES.

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PF1, an exploratory drug candidate, demonstrated nephrotoxicity at the lowest dose of 50 mg/kg, QD for 14 days in cynomolgus monkeys. In the mid and high dose groups (300 and 750 mg/kg, QD) monkeys were euthanized after 7 days of dosing due to significant clinical signs and indication of renal failure detected by increased serum creatinine level. In this study, the observed toxicity was found to be qualitatively comparable to nephrotoxicity observed with the two previous drug candidates (PF2 and PF3). Based on the severity of nephrotoxicity, PF1 ranked between the other two compounds: PF2 induced mortality at all doses (up to 1000 mg/kg, BID) from 3 to 6 days of dosing and PF3 elicited mild renal toxicity at all doses (up to 2000 mg/kg, QD). Primary human and monkey renal proximal tubular cell cultures with functional drug metabolizing enzymes and transporters were developed and evaluated as an in vitro tool to predict nephrotoxicity. A positive correlation between in vitro model and vivo toxicity findings in monkeys was established using LDH-release as the toxicity marker. Based on these results, several related compounds were screened in hOAT1 and hOAT3 transfected cell lines to evaluate their interaction potential with these transporters. PF2 was a strong hOAT3 substrate with an IC₅₀ of 18 μ M in this in vitro model. This presentation will discuss the results of this study and highlight the potential role of the organic anion uptake transporters in the observed renal toxicities of these compounds.

269 AN ANIMAL MODEL FOR SELECTION OF CETP INHIBITORS LACKING BLOOD PRESSURE CHANGES BASED ON EXPERIENCE WITH TORCETRAPIB.

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Following the very successful control of low density lipoproteins in dyslipidemia with HMG CoA reductase inhibitors ("statins"), the next step in therapeutic intervention in this area is considered to be elevation of HDL cholesterol, the "good cholesterol". Inhibition of the cholesteryl ester transfer protein (CETP) is considered a promising target to achieve this goal and the compound torcetrapib is in late stage clinical trials. However, torcetrapib has been reported to increase blood pressure in man. This finding triggered concerns that this may be a target-associated finding and would consequently pose a toxicity issue for other CETP inhibitors. Hence, an animal model was designed to investigate the blood pressure changes observed in man with torcetrapib. Mean arterial (MAP), systolic arterial (SAP) and diastolic arterial (DAP) pressures, and heart rate (HR) were measured by telemetry in normotensive and spontaneously hypertensive (SH) rats treated with CETP inhibitors from different chemical classes (Torcetrapib 10, 40, 80 mg/kg/day; RO4607381 (JTT-705) 100, 300, 500 mg/kg/day). In SH rats, torcetrapib dose-dependently increased MAP, SAP, and DAP. Torcetrapib induced significant MAP increases (approx. +7 to +11 mmHg) over the 24h recording period, which were greater and more persistent in hypertensive than in normotensive rats. In both cases HR did not change significantly. Interestingly, the other CETP inhibitor RO4607381 (JTT-705) neither changed BP nor HR. These results show that SH rats are a relevant model for assessing the potential for CETP-inhibitor-related BP changes and suggest that torcetrapib-induced hypertensive effects do not appear to be a class effect of CETP inhibition; may not be observed with other CETP inhibitors and is not linked to CETP inhibition (since rats do not express CETP).

270 INTRAVENOUS CARDIOVASCULAR SAFETY PHARMACOLOGY STUDY IN DOGS ON A PDE4 INHIBITOR (TOFIMILAST).

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Tofimilast is PDE4 inhibitor that is being studied for the treatment of asthma/COPD. In this study, 12 dogs received a single 6 hour IV infusion of tofimilast, 4 dogs received vehicle only and 4 dogs were administered norepinephrine

over a 5 hour IV infusion and served as the positive control. Dogs were anesthetized with morphine/chloralose and were mechanically ventilated with room air so that arterial blood gasses remained within physiological limits. Dogs were instrumented to monitor physiological variables of cardiovascular function. Markers of myocardial injury were measured from samples collected at the coronary sinus and peripheral venous blood. Myocardial oxygen consumption were either measured or calculated from intravascular pressures and echocardiographic images. Coronary arterial to coronary sinus oxygen differences were measured to determine oxygen demand. Systemic exposure of tofimilast was measured from plasma samples collected during the study. Gross examination and histopathologic evaluation of the heart was performed on each dog in the study.

There were no gender differences in exposure to tofimilast. Control and low dose dogs did not exhibit an imbalance in myocardial oxygen consumption, myocardial injury or signs of cardiotoxicity. High dose tofimilast dogs had increases in myocardial oxygen consumption indices without evidence of cardiotoxicity. There were no drug-related histopathological changes in the hearts of the tofimilast treated dogs. Norepinephrine exposed dogs exhibited a greater increase in myocardial oxygen consumption with corresponding increases in potassium, troponin I and T in both coronary sinus and peripheral venous blood. Three of 4 norepinephrine exposed dogs had slight to mild degeneration of the subendomyocardial and papillary muscles of the left ventricle and septum.

These data demonstrated that the hemodynamic effects of tofimilast when administered IV are mild and related to the pharmacology of the test article.

271 ARIPIRAZOLE: 18-MONTH ORAL INVESTIGATIVE RETINAL STUDY IN FEMALE RATS.

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The study investigated whether the increased incidence and severity of retinal degeneration seen in albino Sprague-Dawley (SD) rats treated with aripiprazole (Abilify®), a dopamine D2 partial agonist marketed for treatment of schizophrenia and related disorders) was a consequence of increased light exposure or a direct effect of aripiprazole. Aripiprazole was administered orally at 0 or 60 mg/kg/day (75 rats/group) to Long-Evans (LE) and SD female rats (strains with and without photoprotective ocular melanin, respectively) for up to 18 months followed by a 3-month recovery period. Study endpoints included clinical observations; electroretinogram (ERG) measurements; and histopathologic, morphometric, and ultrastructural retinal evaluations. Predose hyperactivity was seen in drug-treated SD and LE rats after 3 months and persisted to the end of the study. Time-dependent, light-induced retinal injury was noted in treated SD rats and included ultrastructural findings of condensed photoreceptor nuclei in outer nuclear layer (ONL) and vesiculated photoreceptor disk membranes in the outer segments at 3 months, qualitative/quantitative microscopic findings of decreased ONL thickness beginning centrally at 6 months, and progressive degeneration of central and peripheral retina through the recovery period. Progressive ERG abnormalities (decreased a/b-wave amplitude and increased implicit times) were also noted in treated SD rats beginning at 12 months. Treated LE rats with systemic drug exposure comparable to those in treated albino SD rats had no evidence of light-induced retinal injury, demonstrating the photoprotective effect of ocular melanin. These data indicate the exacerbation of light-induced retinal degeneration in treated albino SD rats was likely a consequence of pharmacologically mediated hyperactivity during the animal room light phase, resulting in increased light exposure, rather than a direct effect of aripiprazole on the retina. Importantly, this finding of retinal degeneration in treated SD rats has no established relevance to humans.

272 THE USE OF DIETARY ADMINISTRATION FOR TOXICITY EVALUATION OF THE NOVEL ANTI-HIV DRUG DARUNAVIR.

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Darunavir (Prezista TM) is a novel protease inhibitor that is used for the treatment of HIV-infected patients. It has a limited solubility and a high metabolic first-pass effect following oral administration in animals and humans. These characteristics lead to low drug bioavailability and dosing limitations when assessing safety in non-clinical species. In order to increase systemic exposure to darunavir and evaluate its safety profile at high doses, a repeated-dose toxicity study was performed via dietary administration in rats. The results obtained were compared to findings observed with gavage administration. Rats were treated daily with darunavir via oral gavage in polyethylene glycol (vehicle) at 20, 100 and 500 mg/kg (n= 10/sex/group) and in a separate study via dietary admixture at 260, 1040 and 2600 mg/kg (n= 20/sex/group), for up to 3 months. Observations were made for clinical signs, body weight, food consumption, blood and urine biochemistry and animals were sub-

jected to necropsy and histopathological examination. Blood samples were also taken for toxicokinetic evaluation. The toxicity profile of darunavir was similar following both routes of administration. Target organs identified were the hematopoietic system (increase in red blood cell turnover), the coagulation system (prolongation in clotting time), liver and the thyroid (cellular hypertrophy as a result of enzyme induction). Systemic exposure to darunavir was similar following both routes of administration. Dietary administration did not increase darunavir exposure in spite of the higher daily doses given to animals relative to oral gavage. This indicates that dietary administration has a limited use in enhancing systemic exposure and assessing the safety of drugs such as darunavir, as high metabolic clearance remains the main limiting factor preventing the achievement of high plasma levels regardless of the method of drug administration.

273 EFFECTS OF A LONG-ACTING GROWTH HORMONE RELEASING FACTOR ANALOGUE, CJC-1295, ON MALE FERTILITY.

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CJC-1295 is a synthetic analogue of growth hormone releasing factor (GRF) which stimulates the pulsatile release of growth hormone and the secretion of insulin-like growth factor 1 (IGF-1). By applying the Drug Affinity Complex (DACTM) technology to GRF, the peptide selectively and covalently binds to circulating albumin after subcutaneous (SC) administration, thus prolonging its $t_{1/2}$ from 10-12 min to approximately one week in humans. The fertility and general reproduction toxicity effects of CJC-1295 were evaluated in male CD rats (25/group) following every other day (q2d) SC administration at dose levels of 0, 0.5, 3, or 16 mg/kg/q2d. Treatment of the male rats began 28 days before cohabitation with untreated female rats and continued for a total of 49-50 days. There were no CJC-1295-related effects on survival. Clinical findings were limited to injection site irritation at 16 mg/kg/q2d, and increased body weights or body weight gain, and increased food consumption at ≥ 3 mg/kg/q2d. Increases in cauda epididymal sperm count and sperm density, and reductions in the absolute and relative weights of the seminal vesicles were noted at ≥ 3 mg/kg/q2d at the end of the treatment and recovery periods. Irrespective of the noted changes, treatment with CJC-1295 did not affect any mating or fertility parameters and the embryonic viability was not affected up to the highest dosage tested. Thus, the No-Observed-Adverse-Effect Level (NOAEL) for general and male reproductive toxicity was determined to be ≥ 16 mg/kg/q2d of CJC-1295.

274 TC-2696-12: PRE-CLINICAL SAFETY ASSESSMENT OF A SELECTIVE $\alpha 4\beta 2$ NEURONAL NICOTINIC RECEPTOR AGONIST.

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TC-2696-12 is currently under development in Phase I clinical trials for the treatment of acute post-operative pain. To support human safety, preclinical toxicology studies were conducted: in mice, rats, and dogs in acute oral and intravenous studies; in rats and dogs in 14-day range finding and in 28-day definitive subacute studies; in 3 genotoxicity bioassays; and in safety pharmacology studies evaluating 5 functional tests. The above assays provide sufficient support for the compound's further development:

- Absence of GI toxicity after repeated administration in toxicology studies
- Non specific functional changes in safety pharmacology studies
- No major pathologic findings in definitive toxicity studies at high dosages and blood levels
- Absence of genotoxicity
- Favorable TK profile in animals.

In safety-evaluation studies, animals tolerated high oral doses and the majority of the side effects were an extension of the compound's pharmacologic action: in the rat: ataxia, hyperactivity, rapid respiration, and circling; and in the dog: tremors and emesis, which is a commonly observed finding in the dog model with nicotinic compounds.

The only functional changes were:

- Increased alkaline phosphates or alanine aminotransferase and liver weights in rats, primarily at the high oral doses of 50 mg/kg and greater, but without definitive histopathologic alterations in the 14 - and 28 - day studies
- Increased blood pressure and bradycardia in dogs at single oral doses of 3 mg/kg and higher in safety pharmacology studies, but not observed when evaluated in the repeat dose subacute 14 - and 28 - day studies. There were no changes in ocular, ECG's, gross necropsy, or histopathology at any dose level in the definitive 28 - day studies.

TK evaluation showed rapid absorption; peak plasma levels at about one hour and dose proportional C_{max} and AUC plasma levels. Clinical Phase I studies are currently ongoing and TC-2696-12 is well tolerated at single doses up to and including 150 mg and at 10 day multiple dosing at 25 and 50 mg.

275 POTENT ANTI-INFLAMMATORY EFFECTS OF NOVEL QUINOLINEDIONE DERIVATIVES, OQ1 AND OQ21, MEDIATED BY DUAL INHIBITION OF INOS AND COX-2.

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Induction of nitric oxide synthase and cyclooxygenase-2 (COX-2) and resultant increased productions NO and prostaglandins have been suggested as the key components in inflammatory processes, contributing to progression and maintenance of various inflammatory diseases. Dual inhibitory effects against nitric oxide (NO) synthesis and inflammatory prostaglandin, prostaglandin E₂ (PGE₂) production by novel quinolinedione derivatives, 6-(4-fluorophenyl)-amino-5,8-quinolinedione (OQ1) and 6-(2,3,4-trifluorophenyl)-amino-5,8-quinolinedione (OQ21) were found in lipopolysaccharide (LPS)-stimulated macrophage cell line, RAW264.7. NO production was inhibited by OQ1 and OQ21 by affecting the expression of inducible nitric oxide synthase (iNOS) as well as iNOS activity itself. LPS-induced expression of iNOS was down-regulated by OQ1 and OQ21 in concentration-dependent manners as determined by western blot analysis of iNOS. Down regulation of iNOS was mediated by the blockade of NF κ B activation pathway as shown by attenuation of LPS-induced I κ B degradation in western blot analysis. De novo synthesis and cellular accumulation of PGE₂ were also suppressed in a concentration dependent manner by OQ1 and OQ21. Western blot analysis and enzymatic assay results suggested that enzymatic activity was inhibited by OQ1 and OQ21 without any suppressive effect on COX-2 expression. Consistent with these in vitro anti-inflammatory effects, OQ1 showed significant anti-inflammatory effects in in vivo topical inflammation model, 12-O-tetradecanoylphorbol 13-acetate (TPA)-induced mouse ear edema. OQ1 derivatives with dual inhibitory effects against COX-2 activity and iNOS expression will provide a powerful therapeutic tool for the cure of chronic inflammatory diseases, which has been resistant to conventional drug therapies.

276 DETERMINATION OF PHOSPHOLIPIDOSIS IN HUMAN CELL CULTURES USING A FLUORESCENTLY TAGGED PHOSPHOLIPID PROBE.

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Several cationic amphiphilic drugs (CADs) cause local or systemic phospholipidosis after exposure in preclinical species and humans. Phospholipidosis is characterized by the accumulation of CADs, phospholipids, and concentric lamellar bodies within cells. The objective of this study was to develop a human white blood cell line model for detecting phospholipidosis. This is particularly important since most of the phospholipid accumulation in humans is observed in the lymphatic tissues and immune cells, and these cells are routinely monitored. To select a suitable cell type, we examined the phospholipidosis-detection sensitivity of three human cell lines, Jurkat (human T-lymphocyte-derived cell line), THP-1 (human monocyte-derived cell line), and C3 (microglial) cells, with four CADs (amiodorone, amitriptyline, perhexiline, and fluoxetine) as phospholipidosis inducers and a negative control (tetracycline). We used 96-well microplate cell-culture method for detecting phospholipidosis, using a fluorescently tagged phospholipid, N-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (NBD-PE). Drug concentrations were used in a range from 1 to 100 μ M. After 24 h incubations, NBD-PE uptake was quantified in controls and treated cells. The NBD-PE fluorescence intensity was normalized to that of ATP content, used as an indicator of viable cells, to obtain the uptake of NBD-PE in the living cells. Phospholipid accumulation was observed in all the three cell lines at higher concentrations (30 and 100 μ M) of the four CADs, but due to cytotoxicity at these concentrations, these are false positive results. Interestingly, only in the THP-1 cells significant phospholipidosis (~ 2 to 5 fold) was observed at non-cytotoxic concentrations of the four drugs compared to the controls. Thus, these results indicate that the THP-1 model can be used as an in vitro screen for testing the phospholipidogenic potential of drugs and compounds on human cells.

277 CJC-1134-PC, A LONG-ACTING EXENDIN-4 ANALOGUE, IS WELL TOLERATED IN RATS AND MONKEYS FOR UP TO 28 DAYS.

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CJC-1134-PC is being developed for the control of Type II diabetes using ConjuChem's DACTM technology by the in vitro conjugation of modified exendin-4 peptide to recombinant human albumin (rHA). In doing so, it prolongs the $t_{1/2}$

and activity of the peptide. The toxicity of CJC-1134-PC was evaluated following subcutaneous (SC) 28-day repeat dosing at 0, 1, 5 or 25 mg/kg/day in rats (10/sex/group) and at 0, 0.4, 2 or 10 mg/kg/day in monkeys (3/sex/group), with 2 weeks recovery. All animals survived to study termination and there were no treatment-related effects on ophthalmology, coagulation, clinical chemistry or urinalysis in either species, or on ECG in monkeys. Treatment-related clinical signs were reversible in both species, including decreased activity and fecal output in rats (≥ 5 mg/kg/day), and decreased activity, hunched posture (≥ 2 mg/kg/day), and decreased fecal output (≥ 0.4 mg/kg/day) in monkeys. Transient, dose-dependent decreases in food consumption in both species correlated with decreases in body weight gain at ≥ 1 mg/kg/day in rats and body weight loss at ≥ 0.4 mg/kg/day in monkeys. Mild reversible decreases in erythrocyte counts, Hb and Hct were seen in male (≥ 2 mg/kg/day) and female (10 mg/kg/day) monkeys. Compared to controls, decreased thymus weight was noted in females (10 mg/kg/day) and decreased thymus to body weight ratio was seen in both sexes (≥ 0.4 mg/kg/day), correlating to stress-related lymphoid depletion in spleens and thymus glands, which were reversible. The $t_{1/2}$ of CJC-1134-PC was ~ 35 -67 h in rats and ~ 79 h in monkeys, which is similar to the $t_{1/2}$ of albumin of their respective species. Treatment with CJC-1134-PC produced antibodies in both species predominantly against the human albumin portion of CJC-1134-PC with lesser reactivity to the peptide portion. The NOAEL was established to be 5 mg/kg/day in rats and 2 mg/kg/day in monkeys. In conclusion, SC administration of CJC-1134-PC to rats and monkeys up to 28 days was well tolerated with expected pharmacologically-related effects.

278 KSR AS-214231 (NSC-731442) TARGET ORGAN TOXICITY AND ITS REVERSIBILITY IN CYNOMOLGUS MONKEYS FOLLOWING 14 DAYS OF CONTINUOUS INTRAVENOUS DOSING (CIV).

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KSR AS-214231 (KSR), an antisense phosphorothioate oligonucleotide that inhibits KSR1 thereby abrogating of RAS-mediated tumorigenesis, may be useful in the treatment of pancreatic and/or lung cancer. A toxicity study using Cynomolgus monkeys was conducted to identify target organs and whether identified toxicities were reversible. Male and female Cynomolgus monkeys were dosed by CIV at 14 and 28 mg/kg/day (280 and 560 $\mu\text{g}/\text{m}^2/\text{day}$) for 14 days followed by a 14-day recovery period. Control animals were infused with vehicle (PBS). There were no drug-related clinical observations and no effects on body weight, coagulation, or clinical chemistry endpoints. Reversible hematological findings attributed to KSR included increased total white cell, neutrophil, monocyte, and/or lymphocyte counts. KSR appeared to elevate complement breakdown product Bb, indicating complement activation. KSR plasma concentrations were generally stable over the infusion period, and increased in a dose-related manner, suggesting that at the doses tested, the disposition of KSR was linear. Target organs included the kidneys, liver, and lymph nodes. Microscopic findings were generally dose-related and included accumulation of basophilic granules in renal tubular epithelium and hyperplasia of granule-bearing macrophages in liver sinusoids and lymph nodes. The granules were interpreted to be KSR. After a 14-day recovery period, all microscopic findings were still present. A no-observed effect level (NOEL) could not be determined. However, the maximum tolerated dose (MTD) was 28 mg/kg/day. (Supported by N01-CM-42200)

279 SEXUAL MATURITY AND AGE OF ANIMALS IN PRE-CLINICAL STUDIES TO SUPPORT 'FIRST IN MAN' STUDIES.

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The poster discusses the problems associated with using sexually immature animals to screen for toxicity in repeat dose preclinical pharmaceutical studies prior to first human exposure. The issues relate to the inability of assessing testicular toxicity in immature animals and the difficulties of identifying test article related changes in peripubertal animals. The age of sexual maturity for all routinely used rodent and non-rodent species is discussed but, by way of example, the poster focuses upon the complications of using sexually immature dogs. Of the species routinely used in preclinical toxicity studies, dogs and monkeys cause the most concern because these species are frequently immature or peripubertal at the end of the study. Male dogs mature anywhere between 7-12 months of age but it is not uncommon to start studies with dogs 4-6 months of age. Therefore, depending on study duration, dogs coming to necropsy at the end of the study may be immature, peripubertal, fully mature or a mixture of all three. If dogs are immature testicular toxicity cannot be

identified since there are no germ cells present. Dogs that are peripubertal cannot be distinguished from mature dogs that are undergoing spermatogenic degeneration. As small group sizes of only 3/group are routinely used it is quite likely that dogs of different maturity status will be unequally distributed in the control and treated groups. Examples will be provided to show how these confounding factors can result in generation of false positive, false negative or uninterpretable results. In accordance with guidance from the Society of Toxicologic Pathologists, it is strongly recommended that rodents be at least 10 weeks, and dogs be at least 10 months of age at the end of a study. In the case of primates, where males do not mature until >4 yrs of age, supply problems dictate that most studies are conducted with totally immature animals. It should be appreciated that adverse effects on spermatogenesis will not be detected in this age of animal.

280 A 1-MONTH TOXICITY STUDY IN DOGS TREATED WITH NAVEGLITAZAR (LY519818), A NON-TZD GAMMA-WEIGHTED PPAR ALPHA, GAMMA DUAL AGONIST.

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Peroxisome proliferator-activated receptor (PPAR) gamma agonists [e.g., thiazolidinediones (TZDs) such as pioglitazone and rosiglitazone] are insulin sensitizers used clinically to treat Type 2 diabetes while PPAR alpha agonists (e.g., fenofibrate) are beneficial in controlling dyslipidemia. This study evaluated the toxicity of naveglitazar when given orally (capsules) to beagle dogs ($n=3/\text{sex}/\text{group}$) at doses of 100, 30, 3, 0.3, or 0 (vehicle control) mg/kg/d for 1 month. Clinical signs of toxicity (abnormal feces, thinness, sporadic emesis), body weight loss, and decreased food consumption occurred in a dose-related fashion. Despite the body weight effects, physical and neurological exams were normal. Target organs for toxicity included the heart, liver, hematopoietic system, and male reproductive tissues. Cardiac effects included ECG changes (QT prolongation), elevated heart weights, and an increase in pericardial fluid. Liver changes included increased CYP4A activity (without significant changes in peroxisomal beta-oxidation activity and with an overall decrease in total cytochrome P450 content), increased liver weights, variable increases in ALT (and AST in some cases) with no clear dose- or duration-dependence, and a variety of histologic changes that did not explain the transaminase elevations. These adverse liver findings are not consistent with human clinical experience with the marketed TZDs, supporting the view that dogs are not predictive for humans regarding target organ toxicities with this class of drugs. Hematologic changes (decreased hematocrit, reticulocytopenia, neutropenia, and/or thrombocytopenia) were likely due, at least in part, to plasma volume expansion. Additional hematopoietic effects included bone marrow hypocellularity and splenic extramedullary hematopoiesis. Decreased testes and prostate weights, testicular degeneration, decreased spermatogenesis, and/or prostatic atrophy were noted at 30 and 100 mg/kg, but may have been secondary to inanition and weight loss. The NOAEL was 0.3 mg/kg.

281 SAFETY EVALUATION OF SYSTEMIC HEDGEHOG PATHWAY INHIBITION.

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Hedgehog (Hh) signaling is an important regulator of normal development and tissue homeostasis. Mutations in the Hh pathway are common in human basal cell carcinomas (BCC), resulting in pathway activation and uncontrolled basal cell proliferation. To support development of a topical therapeutic for BCC, the safety of a Hh pathway antagonist, Cur-61414, was evaluated in rodent and non-rodent species. Mini-pigs were selected as the non-rodent species since their skin is morphologically similar to human skin. Topical application of Cur-61414 was well tolerated and resulted in minimal systemic exposure in mini-pigs (reported previously). To evaluate the systemic effects of Hh inhibition, Cur-61414 was administered intravenously (IV) to rodents and mini-pigs in single- and multiple-dose toxicity studies. The maximum tolerated IV dose was 66, 35, and 5 mg/kg for mice, rats, and mini-pigs, respectively. Effects after single-dose IV administration of >17 mg/kg to rodents included decreased activity, labored breathing, and/or injection site irritation. Transient decreases in heart rate occurred after IV dosing of ≥ 25 mg Cur-61414/kg/day for 7 or 28 days in rats and 6.6 mg Cur-61414/kg in a dose escalation study in mini-pigs; however, no heart weight changes or microscopic abnormalities were observed. Cur-61414 was generally well tolerated at IV doses up to 2.5 and 5 mg/kg/day in rats and mini-pigs treated for 28 days, respectively. Moreover, in mini-pigs, no effects on heart rate or other electrocardiogram parameters, blood pressure, or changes in troponins T and I or creatine kinase levels were detected. In vitro screening, 84% inhibition of the L-type calcium channel was observed with 3 μM Cur-61414, levels achieved in vivo, suggesting that heart rate

decreases observed following Cur-61414 administration may be related to calcium channel inhibition rather than Hh pathway antagonism. In summary, Cur-61414 was well tolerated systemically in rodents and mini-pigs at therapeutically relevant doses. These findings are consistent with a limited role of Hh signaling in most normal adult tissues.

282 TOXICITY OF AN ADENOVIRUS VECTOR (AD-KD3) ADMINISTERED INTRAVENOUSLY TO SYRIAN HAMSTERS.

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The objective of this study was to assess the toxicity of a replication-competent adenovirus vector (Ad-KD3), compared to wild-type adenovirus (WT-Ad5), in male Syrian hamsters. Ad-KD3 is derived from the human serotype 5 adenovirus, and is being investigated as a potential treatment for cancer. Hamsters were given single jugular vein doses of Ad-KD3 (3.0×10^9 , 3.0×10^{10} , or 1.9×10^{12} viral particles (vp)/kg), WT-Ad5 (1.9×10^{12} vp/kg), or vehicle. Necropsy, clinical and microscopic pathology evaluations were performed on Days 2, 7, and 29. All animals survived to scheduled necropsy, and no clinical signs of toxicity were noted. Animals in the highest Ad-KD3 dose group had a mild decrease in body weight on Day 7. Hematology changes associated with administration of Ad-KD3 or WT-Ad5 included increases in neutrophil, WBC, monocyte, and platelet counts; decreases in HCT values; and decreases in lymphocyte and reticulocyte counts. Clinical chemistry changes associated with administration of Ad-KD3 or WT-Ad5 included decreases in total protein and albumin values and increases in ALT, ALP, and GGT values. Microscopic liver changes seen on Day 2 in animals treated with WT-Ad5 or Ad-KD3 included hepatocellular viral intranuclear inclusion bodies, hepatocellular karyomegaly, cytoplasmic alteration, and bile stasis; these changes were attributed to virus administration. Other potential virus-related lesions, observed at the dosing site on Day 7 in animals treated with WT-Ad5 or with the low or middle doses of Ad-KD3, included venous endothelial cell hypertrophy and venous inflammation and chronic inflammation of overlying tissues. Overall, adverse clinical and microscopic effects were similar in animals receiving equivalent doses of WT-Ad5 and Ad-KD3. With few exceptions, none of the effects observed on Days 2 or 7 were evident on Day 29. Under the conditions of this study, the No Observed Adverse Effect Level for Ad-KD3 was considered to be 3.0×10^{10} vp/kg. Supported by NCI Contract N01-CM-42201

283 DATA EVALUATION OF PRE-CLINICAL INCURRED SAMPLE REPEAT ANALYSIS.

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As a result of discussions at the 2006 Quantitative Bioanalytical Methods Validation and Implementation: Best Practices for Chromatographic and Ligand Binding Assays (Crystal City III), we reviewed pre-clinical studies that included reanalysis of incurred samples which were conducted both in our laboratories and in contract bioanalytical laboratories from 2000 through 2006. We determined the frequency of change for reassay results, the magnitude of those changes, the effect on TK parameters, and overall effect on study results from those changes. Data from two separate Pfizer sites, Ann Arbor (AA) and Groton (GR) were analyzed. From AA, 363 total samples were reassayed (3% of total study samples) and 90% (327 of 363) changed by <10-fold in concentration, while from GR, 96% (292 of 303) changed by <10-fold. From AA, 69% (147 of 214) of drug in control, pre-dose and/or reassayed in error samples confirmed the original concentration. From GR, 92% (91 of 99) confirmed that quantifiable concentrations of drug were in the control or pre-dose samples. The remaining 204 samples from GR (TK outliers) resulted in 195 of the 204 samples (96%) changed by <10-fold in concentration. The impact of the GR data that changed upon reanalysis was further evaluated for impact on TK parameters. Because some profiles contained reanalyzed samples from multiple time points, TK parameters for only 30 profiles were recalculated for C_{max} and AUC estimates. In the 30 profiles, C_{max} changed by <15% in 29 of 30 profiles (97%). In addition, AUC values changed by <15% in 28 of 30 profiles (93%). In conclusion, in general the data obtained after reanalysis did not differ substantially from the original data, thus confirming that reanalysis of incurred samples will yield similar bioanalytical results. Further, the overall impact on the TK parameters used to estimate exposure for pre-clinical safety studies was minimal and did not change the overall interpretation of the TK data or the pre-clinical toxicology or safety pharmacology study.

284 EFFECT OF TRICYCLIC COMPOUNDS ON BASAL AND SECRETAGOGUE-INDUCED AMYLASE RELEASE FROM ISOLATED MOUSE PANCREATIC ACINI.

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Cyproheptadine and structurally-related compounds such as cyclizine and chlorcyclizine have been shown in previous studies to cause pancreatic islet cell toxicity. Whether these tricyclic compounds also induce toxicity of pancreatic acini or alter digestive enzyme secretion has not been investigated. We examined the effects of these three compounds on basal amylase release and release in response to carbachol and to cholecystokinin octapeptide (CCK8) from isolated mouse pancreatic acini. In the absence of tricyclics, both carbachol and CCK8 caused a dose-dependent increase in amylase secretion which was maximal for carbachol at a concentration of 1 μ M and for CCK8 at 0.01 nM. In the absence of secretagogues, cyproheptadine did not appreciably alter basal amylase release at concentrations below 0.1 mM. At concentration above 0.1 mM, cyproheptadine caused massive release of amylase from acini which was accompanied by a nearly 100% cellular uptake of trypan blue. Cyproheptadine abolished carbachol-stimulated amylase release with an IC₅₀ of 60 nM, consistent with earlier reports of its antimuscarinic properties. Cyclizine and chlorcyclizine also inhibited carbachol-induced amylase release, with IC₅₀'s of 2.5 μ M and 5.4 μ M, respectively. As with cyproheptadine, at concentrations above 0.1 mM, chlorcyclizine caused massive amylase release and near total trypan blue uptake, although cyclizine did not. By contrast to its effects on carbachol-stimulated amylase release, 0.1 mM cyproheptadine slightly reduced amylase release stimulated by CCK8, while cyclizine and chlorcyclizine at the same concentration were somewhat more effective inhibitors. The present results suggest that these tricyclic compounds have inhibitory effects on pancreatic exocrine as well as endocrine secretion and that these effects may be caused by combination of receptor-mediated signaling pathways and more generalized mechanisms of cellular toxicity.

285 MINIMALIST SAMPLING STRATEGIES FOR TOXICOKINETICS: A RETROSPECTIVE ANALYSIS – PART 2.

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As part of the regulatory process for IND and NDA submissions to the FDA, ICH Guidelines require that non-clinical safety studies characterize the Toxicokinetics (TK) of all NCE's at the dose range used during the non-clinical safety assessment program. The purpose of this retrospective analysis was to determine whether four sampling time points could adequately estimate exposure (C_{max} and AUC). TK data from more than 150 legacy studies (dose range-finding, multiple dose, bid dosing, and large animal cardiovascular safety pharmacology), comprising more than 1000 data sets were included in the analyses. Two different approaches were used: 1) Four optimized time points were chosen for each compound after evaluating full profiles and early TK data. 2) Four pre-defined, standardized time points (1, 4, 7, and 24 hr) were used for all studies. TK parameters were calculated and the data were compared to the original TK data. The recalculated parameters were considered similar to the original parameters if they were within $\pm 15\%$ of the original estimates. The data from this analysis indicated that C_{max} was adequately described in 92% of the data sets analyzed using four optimized time points, and in 74% of the data sets using the four standardized time points. AUC was adequately described in 84% of the data sets analyzed using either approach. However, a number of profiles were eliminated from the analysis because the time points collected were too dissimilar to those used in the standardized approach, therefore the comparison overestimated how well using 4 standardized time points described the TK parameters. In conclusion, four optimal time points accurately described C_{max} and AUC for most studies. Four standardized time points accurately estimated AUC in 84% of the studies, however it failed to adequately estimate C_{max} in over 25% of the studies. Neither strategy adequately described TK parameters following bid dosing, shifting T_{max}, or when T_{max} differs for different analytes (i.e. parent/metabolites).

286 POTENTIAL ROLE OF THE PLASMA MEMBRANE H⁺-ATPASE IN THE ANTI-MICROBIAL PROPERTIES OF EBSELEN AND ITS 3-PYRIDINYL ANALOG.

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Ebselen (2-phenyl-1,2-benzisoxselenazol-3(2H)-one) is a selenium-containing antioxidant demonstrating anti-inflammatory and cytoprotective properties in mammalian cells and cytotoxicity in lower organisms. In the present study, the antimicrobial activity of this compound and its analog,

2-(3-pyridinyl)-1,2-benziselenazol-3(2H)-one, was evaluated. *Saccharomyces* sp. were found to be more sensitive to ebselen (IC₅₀ ~3 µM) than to the 3-pyridinyl analog (IC₅₀ ~16 µM). Wash-out experiments and scanning electron microscopic analysis revealed that the fungicidal effects of ebselen were irreversible. Previous work in the lab found that ebselen inhibits medium acidification by several *Saccharomyces* strains. Medium acidification by yeast is achieved by the action of the plasma membrane H⁺-ATPase pumps PMA1 and PMA2, respectively. Of the two pumps, PMA1 is more highly expressed and is more critical to yeast survival. To ascertain how ebselen blocks medium acidification, its effect on PMA1 was examined. Ebselen inhibited medium acidification by PMA1-proficient yeast in a concentration-dependent manner without altering the ATPase activity of the pump. Additional studies using PMA1 (+/-) and PMA1 (+/+) yeast cells revealed the mutant to be more sensitive to a treatment with ebselen than the wild type. Because of its propensity to react with free thiol groups in mammalian cells, the inhibition of medium acidification by ebselen may be the result of binding to one or more cysteine residues on PMA1. To this end, an *in vitro* interaction of ebselen and free cysteine was detected by thin-layer chromatography, inferring that inhibition of proton pumping via this ATPase may be the result of ebselen binding to critical cysteine residues in the protein structure. Taken together, the data suggest that the fungicidal action of ebselen to be due, at least in part, to interference with the proton translocating function, and not with the ATPase activity, of the plasma membrane H⁺-ATPase.

287 DEVELOPMENT AND VALIDATION OF A PCR-BASED ASSAY FOR THE DETECTION OF ADENOVIRAL INTEGRATION INTO HOST GENOME.

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Genomic integration studies are warranted when gene therapy products are detected in the germ-line or when a vector has potential to integrate. Although adenoviruses (Ad) generally do not integrate into the host genome a few studies report Ad integration at very low frequency. In addition, efforts are being made to develop integrating Ad that could provide long-term gene expression. We have developed and validated a sensitive method for the detection of integrated Ad in mouse tissues in support of preclinical studies.

Detection of integrated Ad was accomplished by differential amplification of integrated and free particles in genomic DNA. The entire genome was amplified using primers specific for repetitive sequences (mouse B1 elements) along with a single Ad-specific primer. This specifically amplifies integrated but not free Ad. A quantitative PCR assay (QPCR) was used to measure the total number of Ad particles both before and after the B1 PCR step. The post-B1/pre-B1 ratio determines if any integrated particles are present. In theory, a ratio of 1 or below indicates that no integrated particles are present. In practice, a cut-off of 1.4 was determined to account for the variability observed within integration-negative samples. A spiked positive control Ad integrant was used with each sample to control for potential PCR inhibition. The assay achieved 100% specificity for samples containing up to 700,000 free Ad particles/µg of DNA. The assay allowed the detection of as few as 5.3x10⁻⁷ integration events per gene, even in the presence of free Ad particles. This is 20 times lower than the spontaneous mutation rate observed in mammalian cells. This validated method was successfully applied to preclinical studies. It shows great sensitivity and specificity. Furthermore, this assay would be easily applicable to other gene transfer products, viral or non-viral, and to other toxicology species.

288 A MODEL OF POST-OPERATIVE AND OPIOIDS INDUCED ILEUS FOR ASSESSMENT OF GASTRO INTESTINAL TRANSIT IN RATS.

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Post-operative ileus (POI) is a transient impairment of bowel motility. The pathophysiology of POI is multifactorial and causes include, but are not limited to, surgical manipulation of the bowel, post-surgical inflammation and a generalized stress response. The use of postoperative opioids also appears to contribute to ileus. In order to better comprehend the invasive association of abdominal surgery and use of opioids with POI, this study was conducted to investigate the effects of exposure of abdominal contents to manual handling (specifically the cecum) and of exposure to morphine. POI was quantified by measurement of gastric emptying through dilution of a phenol red dye. A first group of Sprague-Dawley rats was anesthetized with isoflurane gas and the abdomen opened. Half of the animals had the cecum exteriorized and gently patted for 1 minute, while the second half were exposed to surgery, but with no cecum manipulation. Another group of rats was administered a subcutaneous dose of either 1 or 4 mg/kg of morphine. A group of rats was administered the phenol red alone and served as a control. Phenol red served as a gastrointestinal marker and was administered orally post-surgery or post morphine in-

jection, and was assayed colorimetrically 30 minutes post administration. Increases in stomach content of phenol red in cecum-manipulated and non-manipulated surgical groups were approximately 19% and 31%, respectively, when compared to control group (absorbance of 1.9 and 2.1, respectively vs 1.6 for control). The stomach content of phenol red in the cecum-manipulated group was approximately 9.5% lower than the non-manipulated group. Exposure to morphine alone significantly reduced the stomach motility and equivalent increases of approximately 88% in stomach content were seen at both dose levels (absorbance of 3.0). These responses confirm that POI is a response to surgery and administration of opioids and its magnitude is not unilaterally dependent on the degree of surgical manipulation or dose dependent on the exposure of opioids.

289 FOUR WEEK ORAL TOXICITY STUDY OF THE RETINOIC ACID DERIVATIVE 9-CIS-UAB-30, IN FEMALE DOGS.

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The purpose of this study was to assess the toxicity of 9-cis-UAB-30 [(2E,4E,6Z,8E)-8-(3',4'-Dihydro-1'(2'H)-naphthalen-1'-ylidene)-3,7-dimethyl-2,4,6-octatrienoic acid] in female dogs following four weeks of daily oral treatment by capsule administration at doses of 0, 10, 30 and 100 mg/kg/day. No mortalities occurred and no treatment-related clinical signs were seen. No treatment-related changes in body weights, food consumption, clinical pathology parameters, ophthalmology or electrocardiology were seen. Total body weight gains were dose-dependently decreased, although not significantly, in the dose groups administered 9-cis-UAB-30 compared to the control group. No treatment-related changes in organ weights, gross pathology or histopathology were seen. Thymus weights were dose-dependently decreased, although not significantly, in groups administered 9-cis-UAB-30. An increase in kidney weights was seen in the 10 mg/kg/day dose group. These changes were considered sporadic and not biologically meaningful. These data indicate that administration of 9-cis-UAB-30 to female dogs did not result in treatment-related toxicity. Blood was collected for pharmacokinetics from all dogs at 0, 1, 2, 4, 8, and 24 hours post-dosing. The value of AUC_{0-∞} did have an increase with each increasing dose, but not proportionally to the dose administered. The value of AUC₀₋₂₄ increased approximately in proportion to the dose when dose groups of 30 mg/kg/day and 100 mg/kg/day were numerically compared. The values of AUC_{0-∞} ranged between 5250.0 ng*hr/mL and 13421.3 ng*hr/mL for the different dose groups. The half-life ranged between 2.68 and 4.01 hours. Based on the results obtained from this study, the no-observed adverse effect level (NOAEL) was at least 100 mg/kg/day for 9-cis-UAB-30.

290 THE LACK OF GENOTOXICITY IN AN UNPREDICTED CASE OF RAT LIVER CARCINOGENICITY.

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RO-450 is an alpha adrenoreceptor agonist developed for urinary incontinence that represents an interesting example of a rat hepatocarcinogen with no clear genotoxic signals of carcinogenesis. The data described herein detail the efforts to elucidate the potential genotoxicity of this compound. Non-GLP genotoxicity testing conducted during early compound development resulted in negative results (micro ames and the *in vitro* micronucleus test) with subsequent GLP genotoxicity also testing negative (ames, human lymphocyte chromosome aberration test, and *in vivo* mouse micronucleus test). A 2-year carcinogenicity study was conducted using Sprague-Dawley rats treated with RO-450 once daily at dose levels of 0, 5, 50, or 100 mg/kg/day. Treatment for 2 years was directly related to the development of hepatocellular adenomas (male and female, trend test p-values <0.001) and carcinomas in male rats (p-value = 0.008). A no-effect level for hepatocellular neoplastic changes was not achieved in male rats. Clinical development of RO-450 was halted but subsequent work has focused on identifying whether the mechanism by which hepatocellular neoplastic transformation occurs is via a genotoxic mechanism. RO-450 tested negative in the *in vitro* PPAR, *in vivo* rat bone marrow MNT, *in vivo* kidney and liver rat comet assay, and SHE cell assays. The weight of evidence of the existing data indicates that the increased incidences of rodent hepatocellular adenomas and carcinomas are likely not related to a genotoxic mechanism. Re-evaluation of short term studies indicates that only negligible changes in liver weight and hepatocellular pathological changes occur following RO-450 dosing. Hepatocellular

focal lesions however were identified in all dose groups during a 6- month repeat dose study suggesting an early indicator of the hepatocellular carcinogenicity finding. Investigations are currently focused on identifying RO-450-specific changes in shorter term studies that might yield insight into the nonogenotoxic mechanism by which hepatocellular neoplasia develop.

291 REDOX REGULATION OF GAMMA-IRRADIATION INDUCED APOPTOSIS BY CYTOCHROME C-CARDIOLIPIN COMPLEX IN HELA CELLS.

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In apoptosis, cytochrome c (cyt c) released from mitochondria into the cytosol, is central to apoptosome formation and caspase activation. Recently, we established that cyt c participates in apoptosis acting as a cardiolipin (CL)-specific peroxidase. The CL oxidation products thus formed are essential for the release of pro-apoptotic factors from mitochondria. While assembly of apoptosomes requires only minimal amounts of cyt c, catalysis of CL oxidation is strongly dependent on its content and availability for CL in mitochondria. Thus we hypothesized, the concentration of cyt c can be a determinant of cell's sensitivity to irradiation induced apoptosis. To test the hypothesis, we used siRNA approach to engineer HeLa cells with a lowered content of cyt c (14% of its amount in parental cells), HeLa 1.2 cells. γ -irradiation (in doses up to 40 Gy) induced apoptosis - as revealed by caspase-3/7 activation and phosphatidylserine externalization - was proportional to the cyt c content in HeLa 1.2 cells. Using a fluorescence HPLC-based Amplex Red assay and ESI-MS analysis, we found that irradiation caused selective accumulation of CL hydroperoxides. HeLa 1.2 cells responded by a lower irradiation-induced accumulation of CL oxidation products. No release of a pro-apoptotic factor Smac/DIABLO was detected in cyt c-deficient cells after irradiation, whereas Bax translocation was the same as in HeLa cells. Combined, our results demonstrate that cyt c is an important catalyst of CL peroxidation, critical to the execution of apoptotic program. This redox catalytic role of cyt c in irradiation induced apoptosis can be useful for the development of new radioprotectors and radiosensitizers. Supported by NIH NIAID U19 AI068021, NIH HL70775 and The Human Frontier Science Program.

292 PEROXIDASE COMPLEXES OF CYTOCHROME C WITH ANIONIC LIPIDS: STRUCTURAL PRE-REQUISITES, MECHANISMS, AND CYTOTOXIC EFFECTS.

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Interaction of cytochrome c (cyt c) with a mitochondria-specific cardiolipin (CL) confers peroxidase activity on the protein resulting in selective CL oxidation and release of proapoptotic factors. Because the complex cyt c/CL is stabilized by a combination of electrostatic and hydrophobic interactions, we determined the extent to which other anionic lipids - phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylinositolphosphates (PIP), and phosphatidylcholine (PC) as a control - are effective in inducing the peroxidase activity of cyt c. EPR spectroscopy of nitrosylated cyt c, optical spectroscopy and measurements of tryptophan fluorescence demonstrated that cyt c interaction with anionic lipids induced protein unfolding accompanied by an exchange and loss of axial ligands of heme iron so that cyt c heme became more accessible for the interaction with small molecules like NO or H₂O₂. Using several peroxidase substrates we showed that all anionic lipids activated peroxidase activity of cyt c in a dose-dependent manner with the efficiency decreasing in the row: CL>PA>PIP>PIP₂>PS. Recombination of protein-derived radicals formed in peroxidase reaction caused oligomerization of cyt c and formation of protein-lipid aggregates detectable by PAGE and Western blotting. In line with this, Western blotting revealed the formation of cyt c aggregates after its incubation in the presence of H₂O₂ with membrane (but not cytosolic S-100) fraction of brain homogenates. Oxidation products of anionic lipids were detected after induction of apoptosis in HeLa cells and mouse embryonic wild type cells but not in cyt c-deficient cells. Overall, our study identified anionic lipids as physiologically relevant regulators of peroxidase activity of cyt c in mitochondria and other cell compartments, particularly during apoptosis. Supported by NIH U19 AI068021, HL 070807, NIOSH OH008282, AHA0535365N, PA Dept. of Health SAP 4100027294.

293 FREE FATTY ACIDS FORM PEROXIDASE COMPLEXES WITH CYT C: ROLE IN MITOCHONDRIAL OXIDATIVE STRESS AND DAMAGE.

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Recently, we reported that negatively charged phospholipids- cardiolipin, phosphatidylserine-form strong complexes with cyt c in which the hemoprotein acts as a potent peroxidase capable of selective oxidation of respective phospholipids (Kagan et al., *Nature Chem. Biol.*, 2005). Both electrostatic and hydrophobic interactions were found essential for the complexes formation. Free fatty acids (FFAs) carry both a negatively charged carboxy-group and long chain hydrophobic moiety. Because FFA accumulation occurs in membranes and biofluids in many dyslipidemias and other disease conditions, we hypothesized that they can stimulate peroxidase activity of cyt c. Here we report, that oleic acid effectively stimulated peroxidase activity of cyt c as evidenced by oxidation of three typical peroxidase substrates: Amplex Red, etoposide, and luminol. The peroxidase activation of cyt c occurred only at ratios of oleic acid/cyt c exceeding 50:1. Generation of characteristic protein-derived radicals was confirmed by EPR spectroscopy of cyt c/oleic acid incubated with H₂O₂. Oligomerization of cyt c/FFA complexes was demonstrated by Western blotting. By utilization of immuno-spin trapping technique, we found the production of protein-immobilized DMPO nitron adducts in the PAGE bands corresponding to oligomeric forms of cyt c. Two lines of evidence suggest interaction of cyt c with oleic acid unfolds the protein resulting in a greater accessibility of its heme: 1) in low temperature EPR spectra of heme-nitrosylated oleic acid/cyt c complexes, a signal of penta-coordinate heme-iron was detectable, and 2) increased fluorescence of tryptophan quenched in native enzyme by the heme moiety. Increased levels of cyt c-associated peroxidase activity were found in isolated mitochondria and in cells incubated with oleic acid suggesting that the complexes may function as a peroxidase in vivo. Supported by grants from NIH U19 AI068021, NIOSH OH008282, AHA0535365N, Pennsylvania Department of Health SAP 4100027294.

294 OXIDIZED CARDIOLIPIN AS A NEW BIOMARKER OF GAMMA-IRRADIATION-INDUCED APOPTOSIS.

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Recently, we demonstrated that cytochrome c acts as a cardiolipin (CL) - specific peroxidase in mitochondria early in apoptosis and that CL oxidation products are required for the release of proapoptotic factors. Accordingly, gamma-irradiation initiates apoptotic response in HeLa cells and CL oxidation is critical to the execution of apoptotic program in these cells. We hypothesized that accumulation of oxidized CL can potentially be used in vivo as an early biomarker of apoptosis initiated by total body irradiation (TBI). As intestines are one of sensitive targets of irradiation, we studied phospholipid oxidation in guts isolated from control and irradiated mice. To this end C57BL/6NHsd female mice were subjected to 10 and 15 Gy of TBI and sacrificed 24 h thereafter. We found that TBI caused a dose-dependent apoptosis as revealed by caspase-3/7 activation. Moreover, a significant dose-dependent decrease in sphingomyelin content was detected in guts of TBI exposed mice thus confirming activation of sphingomyelinase typically involved in ceramide-dependent apoptotic pathway. TBI induced apoptosis was accompanied by oxidative stress as evidenced by depletion of two major intracellular antioxidants - ascorbate and GSH. Using our new protocol, oxidative lipidomics, we characterized and quantitatively assessed the formation of hydroperoxides of different classes of phospholipids and found that only CL, a mitochondria specific phospholipids (but not other phospholipids) undergo significant oxidation in mouse guts after TBI. Thus, early accumulation of CL hydroperoxides can be used in vivo as a biomarker of TBI induced apoptosis, useful for the development of new radioprotectors and radiosensitizers. Supported by NIH NIAID U19 AI068021, NIH HL70755.

295 UNMASKING PEROXIDASE ACTIVITY OF CYTOCHROME C IN MITOCHONDRIA: ROLE OF CARDIOLIPIN.

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Redox properties of cyt c are critical to its normal electron transport function in mitochondria, as well as to its participation in apoptotic signaling via the peroxidase oxidation of two anionic phospholipids, cardiolipin (CL) and phosphatidylserine

(PS). Peroxidation of CL contributes to the permeabilization of mitochondrial membranes, and subsequent cyt c release into the cytosol. How cyt c changes its role from an electron shuttle to a peroxidase is currently unknown. Previous work has established that upon interaction with CL, cyt c loses its tertiary structure, and its peroxidase activity dramatically increases. During apoptosis, the fraction of CL-bound cyt c markedly increases. Consequently, we have hypothesized that binding of CL to cyt c acts as a switch that turns off its electron transport function, while simultaneously turning on its peroxidase catalytic function. The redox behavior of cyt c bound to CL-containing membranes was studied using direct voltammetry of cyt c adsorbed on alkanethiol monolayers and equilibrium redox titrations in the presence and absence of CL. The effects of CL binding on the redox potential of cyt c in liver and brain mitochondria, as well as on the regulation of electron transport activity in the mitochondrial electron transport chain, were examined by EPR and UV-Vis spectroscopy. The data shows that binding of cyt c to CL causes: 1) significant (-350-400 mV) negative shift of the redox potential of cyt c; 2) inhibition of cyt c reduction in mitochondria, 3) interruption of mitochondrial electron transport; and 4) inability to oxidize superoxide and ascorbate. These findings suggest that CL acts as a switch and regulates cyt c's mitochondrial redox functions. This work was supported by NSF (CHE-0415457), NIH (HL 70755, U19 AI 068021), NIOSH (OH 008282), The Human Frontier Science Program and PA Department of Health SAP 4100027294.

296 P38 MAP KINASE MEDIATES APOPTOSIS THROUGH PHOSPHORYLATION OF BIMEL AT SER65 AFTER SODIUM ARSENITE TREATMENT.

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The stress activated c-Jun N-terminal protein kinase (JNK) and p38 mitogen-activated protein (MAP) kinase (p38) regulate apoptosis induced by several forms of cellular insults. Potential targets for these kinases include members of the Bcl-2 family proteins which mediate apoptosis generated through the mitochondria-initiated, intrinsic cell death pathway. Indeed, the activities of several Bcl-2 family proteins, both pro- and anti-apoptotic, are controlled by JNK phosphorylation. For example, the pro-apoptotic activity of BimEL, a member of the Bcl-2 family, is stimulated by JNK phosphorylation at Ser65. In contrast, there is no reported evidence that p38-induced apoptosis is due to direct phosphorylation of Bcl-2 family proteins. Here we report evidence that sodium arsenite-induced apoptosis in PC12 cells may be due to direct phosphorylation of BimEL at Ser65 by p38. This conclusion is supported by data showing that ectopic expression of a wild-type, but not a non-phosphorylatable S65A mutant of BimEL, potentiates sodium arsenite-induced apoptosis and experiments showing direct phosphorylation of BimEL at Ser65 by p38 in vitro. Furthermore, sodium arsenite induced BimEL phosphorylation at Ser65, which was blocked by p38 inhibition. This study provides the first example whereby p38 induces apoptosis by phosphorylating a member of the Bcl-2 family, and illustrates that phosphorylation of BimEL on Ser65 may be a common regulatory point for cell death induced by both JNK and p38 pathways.

297 APOPTOSIS INDUCED BY LOW CONCENTRATIONS OF DOMOIC ACID IS MEDIATED BY THE P38 AND JNK MAP KINASE PATHWAYS IN MOUSE CEREBELLAR GRANULE NEURONS.

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In cerebellar granule neurons (CGN) domoic acid (DomA) induces neuronal cell death, either by apoptosis or by necrosis, depending on its concentration. Necrotic damage predominates in response to concentrations above 0.1 μ M while apoptotic cell death (assessed by Hoechst staining) is evident after exposure to lower concentration of DomA (0.1 μ M). The AMPA/kainate receptor antagonist NBQX, but not the NMDA receptor antagonist MK-801, prevented DomA-induced apoptosis. To evaluate the role of oxidative stress in DomA-induced apoptosis, experiments were carried out in CGN from wild-type mice [Gclm (+/+)] and mice lacking the modifier subunit of glutamate-cysteine ligase [Gclm (-/-)]; the latter have very low levels of GSH, and were found to be more sensitive to DomA-induced apoptosis. The antioxidants catalase and GSH ethylester (GSHEE) completely prevented DomA-induced apoptosis, and DomA caused an increase in reactive oxygen species in CGN.

The p38 MAP kinase and the c-Jun NH2-terminal protein kinase (JNK) have been shown to be preferentially activated by oxidative stress. The p38 inhibitor SB203580 and a JNK inhibitor (SP600125) protected CGNs from DomA-induced apoptosis. DomA increased p38 MAP kinase and JNK phosphorylation and this effect was more pronounced in Gclm (-/-) CGNs. This early event was fol-

lowed by an increase in caspase-3 activity as assessed by both fluorescence microscope and activity assay. The ability of NBQX, catalase and GSHEE to prevent DomA-induced phosphorylation of JNK and p38 MAP kinase suggests that the activation of these kinases is mediated by oxidative stress through the AMPA/kainate receptor. These data indicate the importance of oxidative stress-activated JNK and p38 MAP kinase pathways in DomA-induced apoptosis. (Supported by ES012762/NSF-OCE-0434087, ES 07033, and ES 04696).

298 THE ROLE OF THE IKK KINASE (IKK) IN ARSENIC TOXICITY.

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The role of the IKK kinase (IKK) in arsenic toxicity

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Abstract:

Arsenic is a widely spread environmental agent that exerts diverse toxicity in humans. In cultured cells, high concentrations (>5 μ M) arsenic induces cell death. The present study is to investigate the role of the IKK kinase (IKK) in arsenic toxicity using cells deficient in the α , β and γ subunit of the IKK complex. Cells were exposed to sodium arsenite at various concentrations (0.01 - 200 μ M) and times (30 min to 48 hr). Cell death was determined by measuring lactate dehydrogenase (LDH) release and nuclei condensation. Cellular reactive oxygen species (ROS) accumulation was measured by the CM-H2DCFDA and the activation of the c-Jun N-terminal kinases (JNKs) and NF- κ B pathways was monitored by Western Blot analyses. We found that arsenic at 50 μ M did not activate NF- κ B; however, it caused apoptosis of the IKK α - and IKK β -null, but not of the IKK γ -null and wild type cells, at 6 hour of exposure. In the IKK α - and IKK β -null cells, arsenic induced higher levels of ROS accumulation and JNK activity. Pretreatment of the IKK β -null cells with ROS scavengers, vitamin E and N-acetylcysteine, and a JNK inhibitor, SP600125, significantly decreased arsenic-induced apoptosis. These results suggest that the IKK α and IKK β , but not IKK γ , offer protection against arsenic-induced oxidative and apoptotic damage. Because all the IKK-null cells were impaired in IKK α degradation and NF- κ B nuclear translocation, we suggest that the protective role of IKK α and IKK β against arsenic toxicity cannot be simply explained by the IKK-NF- κ B pathway activation and that other IKK downstream effectors may be involved. -Supported by NIH ES 11798

299 SPLICING VARIANTS OF HUMAN FKBP8 GENE IN OXIDANT-INDUCED APOPTOSIS.

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Regulation of apoptosis involves the interaction between Bcl-2 family proteins and components of other signaling pathways. FKBP8 is a member of the FK506 binding proteins and is a potent inhibitor of apoptosis by targeting Bcl-2 and Bcl-XL to mitochondria. The current study investigates the transcriptional regulation of the fkbp8 gene and the potential functional roles of the splicing variants in response to oxidative stress. Human fkbp8 gene is composed of 9 exons located on chromosome 19p12. The full length mRNA was cloned and sequence verified by RACE PCR. In both cultured human neuroblastoma cells and retinal pigment epithelial cells, two splicing variants were identified. The alternative splicing resulted in loss of three exons but otherwise did not cause a frame shift in the coding sequence. The ratio between the long and short forms was regulated by exposure to oxidants and other environmental toxicants. Overexpression of the long form with an N-terminal FLAG tag showed mitochondrial localization and protection against apoptosis. In contrast, the short form was found to have distinct subcellular distribution and function. The data suggest that FKBP8 is a potential sensor protein to oxidative stress and its alternative splicing is a potential mechanism regulating oxidant-induced apoptosis. (Supported by NIH grant ES 014668, American Health Association Foundation, and Research to Prevent Blindness, Inc.)

300 CYANIDE INDUCES MITOCHONDRIAL DYSFUNCTION AND DEATH IN MESENCEPHALIC DOPAMINERGIC CELLS EXPRESSING BNIP3.

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BNIP3 is a member of the Bcl-2 family proteins that displays pro-apoptotic activity. BNIP3 has been linked to both apoptotic and necrotic death involving mitochondrial dysfunctions. In this study we demonstrate that forced over-expression of

BNIP3 by transient transfection (BNIP3⁺) induced significant apoptotic death in an immortalized mesencephalic dopaminergic cell line (N27 cells) as determined by cell viability and TUNEL assays. Cyanide, a neurotoxicant that produces mitochondrial dysfunction and death of brain cells, did not induce apoptosis or alter constitutive BNIP3 expression in wild type N27 cells. In BNIP3⁺ cells exposure to cyanide (400 μ M) for 24 h a marked increase in BNIP3 expression and apoptotic cell death was noted. KCN-induced death in BNIP3⁺ cells was caspase independent since an increase in cellular caspase activity was not detected or pretreatment with the pan caspase inhibitor z-VADfmk did not alter the BNIP3-mediated death. On the other hand, cell death was accompanied by a reduction of mitochondrial membrane potential ($\Delta\Psi_m$) that was enhanced by KCN. Knockdown of BNIP3 by RNAi or suppression of BNIP3 activity by a transmembrane-deleted dominant-negative mutant (BNIP3 Δ TM) markedly reduced the level of KCN-induced cell death. Similarly pretreatment with cyclosporine A, an inhibitor of the mitochondrial pore transition blocked the reduction of $\Delta\Psi_m$ and decreased cell death. It is concluded that a high expression level of BNIP3 can mediate apoptotic death in a mesencephalic cell line through mitochondrial dysfunction without involving the caspase cell death cascade. The cytotoxic effect mediated by BNIP3 is potentiated by KCN through upregulating the protein expression level. (Supported in part by NIH grant ES04140)

301 IDENTIFICATION AND CHARACTERIZATION OF FACTORS INVOLVED IN DELAYED EFFECTS OF RADIATION.

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Evidence from in vitro tissue culture studies, in vivo animal models, and radiotherapy patients, indicated that a variety of biological effects, including genomic instability, occur outside the radiation field and to the progeny of irradiated cells. Media conditioned by the growth of transformed GM10115 cells, made genomically unstable by radiation exposure, is toxic to the parental cell line, the Death Inducing Effect (DIE). We hypothesized that the transformed cells release (through protein shedding and/or secretion) one or more factors into the media that cause stable cells to die primarily through apoptotic processes. We are using biochemical approaches to determine the nature of the factor(s) responsible for DIE activity. Our results indicate that the DIE activity can be diluted 2-4 fold without loss of activity. Dialysis using 2- and 10-KDa pore sizes retained DIE activity indicating that the factor(s) are large molecules and unable to escape through the pores. When DIE media was treated with proteases, DIE activity was reduced. Similarly DIE activity was unaffected by temperatures as low as -20°C; however, activity was mostly lost when media was boiled. While not yet completed, these biochemical studies are consistent with DIE factors being proteins and likely derived from either shedding or secretory processes. Current efforts are focused on implementing mass spectrometry based proteomics approaches that will allow us to identify proteins in DIE medium and determine whether they are up- or down-regulated relative to controls.

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302 SUITABILITY OF A CYNOMOLGUS MONKEY KIDNEY CELL LINE, JTC-12, AS A MODEL TO INVESTIGATE LIGAND-MEDIATED APOPTOSIS.

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Cynomolgus monkeys (Cyno) are used in toxicological studies to determine toxic effects of therapeutics. Unfortunately there is limited availability of in-vitro cell assay systems from non-human primates (NHP) for use in studies of drug candidates. Typically, in-vitro experiments are carried out in primary cells, most often hepatocytes, derived from suitable NHP models including Cyno monkey. However, hepatocytes from primates are subject to limited availability and their inability to proliferate limits applicability to discrete therapeutic areas. We investigated the utility of a cyno-derived cell line, JTC-12, for potential application in lead optimization studies for drugs that act by inducing apoptosis. Tumor necrosis factor related apoptosis-inducing ligand (TRAIL) is a TNF family member that interacts with at least four membrane receptors that all belong to the TNF receptor family. TRAIL receptor 1 (TRAIL-R1 or DR4) and TRAIL receptor 2 (TRAIL-R2, DR5, TRICK2, or KILLER) have cytoplasmic death domains and activate caspases in most tumorigenic but not normal cell lines. Two other receptors, TRAIL-R3 (DcR1) and TRAIL-R4 (DcR2), have truncated death domains and are considered "decoy" receptors as they do not activate caspases. We evaluated the response of

JTC-12 cells to commercially available TRAIL reagents compared to a human tumorigenic colon derived cell line, Colo205. In addition, we evaluated the response of the JTC-12 cells with the addition of a sensitizer, actinomycin D. Experiments confirmed that JTC-12 cells do respond to TRAIL reagents by activating caspase cascades though not to the same extent as the Colo205 cells. The addition of a sensitizer increases the induction of caspase 3/7 with a subsequent reduction in cell viability. Thus, JTC-12 cells may be used to evaluate candidate therapeutics for TRAIL or TNF-mediated toxicities.

303 KINETICS OF NASAL INFLAMMATION AND NEUROTOXICITY IN MICE EXPOSED TO MACROCYCLIC TRICHOHECENE MYCOTOXIN.

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Inhaled macrocyclic trichothecene (MTMs) mycotoxins may cause airway inflammation and neurotoxicity. Histopathology after exposure to roridin A (RA), a representative MTM, was related to apoptotic and inflammatory gene expression in murine nasal airways. C57Bl/6 mice were intranasally instilled with RA (500 μ g/kg bw) in 50 μ l saline or saline alone (controls) and sacrificed 6, 12, 24, or 72 h post instillation (PI). Both nose and brain were subjected to histopathologic and mRNA analyses. RA-induced apoptosis of olfactory sensory neurons (OSNs) resulted in marked atrophy of olfactory epithelium (OE) that peaked at 72 h PI. Elevated expression of proapoptotic genes Fas, PKR, p53, Bax, and CAD, as well as caspase-3 activation, preceded microscopic detection of OSN apoptosis and OE atrophy. Concomitantly, there was elevated mRNA expression of proinflammatory cytokines (TNF-alpha, IL-6, IL-1) and the chemokine MIP-2. Elevated mRNA expressions of IL-6 and MIP-2 were also detected in the olfactory bulb of the brain from 6 to 24 h PI. Simultaneous exposure to E. coli lipopolysaccharide (LPS; 500 ng/kg bw) and a lower dose of RA (250 μ g/kg bw) amplified nasal inflammation and apoptosis of OSNs induced by RA or LPS alone. Thus, a single intranasal exposure of RA induces both inflammation and loss of OSNs in the nose and brain of mice. RA-induced lesions were similar in character and severity to those previously reported in mice that were intranasally exposed to satratoxin G, an MTM found in *Stachybotrys chartarum*. These findings suggest that MTMs may have common cellular and molecular mechanisms of toxicity.

304 BEHAVIORAL DEFICITS AND NEURONAL APOPTOSIS FOLLOWING EARLY VPA EXPOSURE IN A MOUSE MODEL OF AUTISM.

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Previous studies have demonstrated neurobehavioral deficits following P14 administration of 400mg/kg of sodium valproate (VPA) to BALB/c mice. Our objective is to create a histopathological correlate for the behavioral deficits seen in mice following treatment. The time course of VPA-induced hippocampal and cerebellar apoptosis was examined using TUNEL staining. Mice were examined from P5 through P40 using a developmental behavioral battery consisting of motor, cognitive and social tests. VPA- and saline-injected mice were sacrificed at several time points following treatment, and their brains paraffin embedded and analyzed for apoptosis. VPA caused deficits in a number of neurobehavioral tests, most notably a reduction in play behavior starting on day 30, 16 days after the P14 injection. VPA-injected pups exhibited apoptotic cells in both the cerebellar and hippocampal regions with peak effects at 12 and 24 hrs, respectively (reflecting up to a 30-fold increase in apoptotic cells in cerebellum and a 10-fold increase in hippocampus over baseline). Cerebellar and hippocampal apoptosis provides an excellent biomarker for the behavioral deficits associated with VPA treatment. Both the behavioral deficits and damaged regions have been associated with autism.

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305 BISPHENOL A INDUCES APOPTOSIS IN MOUSE HIPPOCAMPAL (HT-22) CELLS: ROLE OF CALCIUM-INDUCED REACTIVE OXYGEN SPECIES, MITOGEN-ACTIVATED PROTEIN KINASE AND NUCLEAR FACTOR- κ B.

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Bisphenol A [BPA, 2, 2-bis-(4 hydroxyphenyl) propane], known to be xenoestrogen, is used in production of water bottles, baby bottles, cans, and teeth suture materials. Xenoestrogen causes adverse effects on the reproductive functions of human

and animals, similar to estrogen by binding estrogen receptor. In this report, we studied neurotoxicity of BPA and underlying mechanisms of action in mouse hippocampal cell lines (HT-22 cells). BPA treated HT-22 cells showed higher cell viability than control at below 100 μ M of BPA, but lower cell viability at over 100 μ M. BPA induced apoptotic cell death indicated by staining with Hoechst 33258, co-staining with Annexin V/propidium iodide, and activation of caspase 3. BPA-induced intracellular calcium modulated reactive oxygen species (ROS) production, which resulted in apoptotic cell death. Calcium antagonists abolished ROS production. BPA activated the phosphorylation of extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK), and nuclear translocation of nuclear factor (NF)- κ B. Pretreatment with specific inhibitors for Ca, ROS, ERK, and JNK decreased BPA-induced cell death; however inhibitor for NF- κ B increased BPA-induced cell death. Results suggest that calcium, ROS, ERK, and JNK involve in BPA-induced apoptotic cell death in HT-22 cells. On the contrary, NF- κ B cascade is activated for survival signaling after BPA treatment. This work was supported by Brain Korea 21.

306 IMMUNOLOGICAL ALTERATIONS IN CHICKS FOLLOWING IN OVO EXPOSURE TO A BROMINATED FLAME RETARDANT MIXTURE: DE-71.

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Polybrominated diphenyl ethers (PBDEs) are one of five major classes of currently used brominated flame retardants (BFRs). This study examined the effects of the commercial PentaBDE mixture DE-71 in white leghorn hatchlings on various developmental parameters and aspects of the immune system, including antibody responses, lymphocyte proliferation, PHA-skin test responses, white blood cell differential counts, and plasma lysozyme activity. DE-71 was dissolved in DMSO and added to Safflower oil at 10% to yield the dose concentrations. Gestation day (GD) 0 eggs were injected with either safflower oil/10%DMSO or DE-71 in safflower oil/10% DMSO at 0, 1, 10 or 25 mg/kg egg weight, and the chicks were grown to post hatch day (PHD) 14. Hatch rate was not effected by DE-71 treatment. A decrease in crown-rump length was observed but only at the 10 mg/kg egg weight treatment. Body mass, brain mass were not altered by DE-71 treatment while liver mass was increased only in the 19 mg/kg egg weight treatment. No significant differences in organ cellularity or mass were observed in the spleen, thymus or bursa. Lymphocyte proliferation was not altered by DE-71 treatment. Plasma antibody titers, as measured by hemagglutination, were not significantly altered by DE-71 treatment. Plasma lysozyme activity was significantly increased compared to control in the 10 mg DE-71/kg egg. The most striking response was suppression of the PHA skin response at the 10 and 25 mg/kg egg weight treatments. These data suggest alterations in cell-mediated immunity may occur following in ovo exposure to PDBEs in avian species and further studies should continue to characterize this in wild species.

307 IMMUNOLOGICAL ALTERATIONS IN CHICKS FOLLOWING IN OVO EXPOSURE TO PERFLUOROOCTANE SULFONATE (PFOS).

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Perfluorinated hydrocarbons have been manufactured for over 40 years and have numerous applications in industry. Studies show that these perfluorinated compounds cause various toxicological effects; however, effects on immune function have not been addressed at length. This study examined the effects of PFOS in white leghorn hatchlings on various developmental parameters and aspects of the immune system, including antibody responses, lymphocyte proliferation, PHA-skin test responses, T-cell immunophenotypes, and plasma lysozyme activity following in ovo exposure. PFOS was dissolved in DMSO and added to Safflower oil at 10% to yield the dose concentrations. Gestation day (GD) 0 eggs were injected with either safflower oil/10%DMSO or PFOS in safflower oil/10%DMSO at 0, 1, 2.5 or 5 mg/kg egg weight, and the chicks were grown to post hatch day (PHD) 14. No decrease in hatch rate was observed in the PFOS treated eggs. Following in ovo exposure chicks exhibited increased liver and spleen weights at all treatment levels and at the 5 mg/kg treatment, body length (crown-rump length) was increased compared to control. For all three treatment groups, plasma IgG titers were increased compared to control as was lysozyme activity while no effect was seen in the cell mediated response in the PHA skin test. This is the first study to assess developmental and immune modulation following PFOS exposure in an avian species. These data indicates that immune alterations can occur in birds following in ovo

exposure to environmentally relevant concentrations of PFOS and demonstrates the need for further research on the effects of perfluorinated compounds on immune function.

308 IMMUNOLOGICAL ALTERATIONS IN WESTERN FENCE LIZARDS FOLLOWING ORAL EXPOSURE TO PFOS.

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Perfluorinated hydrocarbons have been manufactured for over 40 years and have numerous industrial applications. This group of compounds has recently generated considerable interest as some of these compounds (i.e., perfluorooctane sulfonate, PFOS) are ubiquitously persistent in the environment and widely detectable in blood samples of both wildlife and humans. Studies have shown that these perfluorinated compounds cause various toxicological effects; however, potential impacts on immune function have not been adequately addressed nor have effects in reptilian species been studied. In this preliminary study, adult male western fence lizards, an ideally small and environmentally sensitive species, were exposed to PFOS orally for 28 days (0, 1, 10, 100, 250 mg/kg total dose). Plasma, liver and spleen PFOS concentrations, T- and B-cell proliferation, the PFC response, hemagglutination, and lysozyme activity were assessed. The calculated LD50 (Trimmed Spearman-Kärber) in this experiment was 178.4 (95% CI: 162.9, 195.4). SRBC-specific IgM responses were increased compared to control in the 1 mg/kg treatment in both the hemagglutination assay and the PFC response. However, the PFC response was suppressed compared to control in the 250 mg/kg treatment group. Plasma lysozyme activity and T-cell proliferation were not significantly altered by any PFOS treatment. B-cell proliferation was suppressed compared to control at the 250 mg/kg treatment. Whether these changes in immune function are a direct effect of PFOS exposure on the immune system or a secondary effect related to other systemic toxicity remains to be investigated. Overall, this data indicates that immune alterations can occur in reptiles following exposure to PFOS and demonstrates the need for further research on the effects of perfluorinated compounds at more environmentally relevant concentrations on immune function.

309 PFOS-INDUCED IMMUNE MODULATION IN B6C3F1 MICE FOLLOWING ORAL EXPOSURE.

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Perfluorinated hydrocarbons have been manufactured for over 40 years and have numerous applications in industry. This group of compounds (i.e., perfluorooctane sulfonate (PFOS)) have recently generated much interest as they are persistent in the environment and detectable in blood samples of both wildlife and humans. Studies show that these perfluorinated compounds cause various toxicological effects; however, effects on immune function have not been fully characterized. We have previously shown the PFC response is suppressed in adult B6C3F1 mice. This expanded study includes assessment of splenic and thymic T-cell populations, hemagglutination, and plasma lysozyme activity. Adult male and female B6C3F1 mice were exposed to PFOS orally for 28 days (0, 0.005, 0.05, 0.1, 0.5, 1, 5 mg/kg total dose). The PFC cell response, as we have shown previously, was suppressed in males beginning at 0.05 mg/kg while female mice were affected beginning at 0.5 mg/kg. Numbers of splenic CD4+ and CD8+ cells were increased in female mice at all treatment levels while CD4+/CD8+ and CD4-/CD8- cells were increased beginning at 0.05 mg/kg. Splenic CD8+ and CD4-/CD8- cells in males were increased while CD4+/CD8+ lymphocytes were decreased in the 0.1, 0.5, 1, and 5 mg/kg treatments. Furthermore, CD4+ cells were significantly decreased in male following 0.1, 0.5 and 5 mg/kg exposures. In females, thymic CD8+ cells were increased in the 1 and 5 mg/kg treatments, while no alterations in thymic CD4/8 lymphocyte subpopulations were observed in males. Plasma lysozyme activity was not altered compared to controls in male mice but was significantly increased in the females at 5 mg/kg. These data suggests that at environmentally relevant ranges PFOS alters immunological function.

310 AMELIORATING POTENTIAL OF EMBLICA OFFICINALIS ON LEAD-INDUCED IMMUNOSUPPRESSION AND OXIDATIVE STRESS IN CHICKENS.

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Lead(Pb) is one of the major industrial pollutants, which adversely affects animal health. They act as inducers of oxidative stress & disrupt the normal immune system of the body making animals prone to many disease processes. There are certain

medicinal plants known to resist diseases in human by reducing oxidative stress or stimulating immune system. The present study evaluates the ameliorating potential of emblica officinalis on lead induced toxicity in chickens. 50% ethanolic extract of *E. officinalis* fruit (EFE) was assessed for its effect on Pb-treated chickens. Six groups of day old chicks were used in this trial. Group I birds served as control. Group II birds received EFE @ 150mg/kg /day. In Group III to VI, day-3 birds were allowed to receive Pb-treated feed @ 660ppm. The birds of group IV & V were treated with EFE @ 50 and 150 mg/kg/day, respectively in drinking water. Thiamine hydrochloride (100 mg/kg/day) was given to group VI birds. Immunological & oxidative profiles were recorded after 5 & 8 weeks of feeding trial. Immunological parameters like Macrophage function test (MFT), lymphocyte stimulation test (LST), DTH & antibody (Ab) titre (ELISA) were measured. For oxidative profile malondialdehyde, catalase and reduced glutathione was measured in blood & tissue samples. Pb significantly decreased the value of MFT, LST and DTH and Ab titre against RD-vaccine birds. EFE and thiamine significantly increased the value of ELISA, MFT, LST & skin thickness (DTH) in normal & Pb treated broilers after 5 weeks of trial. The 8 weeks of Pb resulted in increase in lipid peroxidation and decrease in the activity of GSH and catalase in RBC, liver & brain. EFE & thiamine normalized the levels in Pb treated birds. As compared to Pb-treated group, there was reduction in blood Pb level in EFE and thiamine treated groups. It can be concluded that EFE have the potential to provide protection in broilers from lead-induced alterations in the immunological and oxidative profiles.

311 DELTA-9-TETRAHYDROCANNABINOL MODULATES LEVELS OF VIRAL H1, HOST IMMUNE CELL POPULATIONS, AND AIRWAY MORPHOLOGY IN A MODEL OF HOST-RESISTANCE TO INFLUENZA.

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Delta-9-tetrahydrocannabinol (THC) is recognized for its role in modulating host-immune responses, in particular those mediated by T lymphocytes. Accordingly, the present study was designed to examine the effects of THC on: 1) immune cell recruitment and release of soluble mediators in response to a challenge with a non-lethal dose of influenza virus A/PR/8 (PR8) in the lower airways, and 2) associated changes in the morphology of the epithelium lining the main axial airway. Three separate studies were conducted wherein C57BL/6 mice were gavaged with one of three different concentrations of THC (25, 50, or 75 mg/kg) or corn oil for 5 consecutive days. On day three, mice were intranasally instilled with 50 plaque forming units of virus or vehicle, saline, 4 hours prior to THC exposure, and then sacrificed 7 days post-infection (dpi). In these studies, THC increased hemagglutinin 1 mRNA in a dose-dependent manner above PR8 controls. Retrievable populations of inflammatory cell types in bronchoalveolar lavage fluid, in particular monocytes and CD4+ and CD8+ T cells, were attenuated with THC treatment. Likewise, inflammation scores obtained from histopathology were suppressed by THC by 10 dpi. THC had little to no effect on protein levels of chemokines and cytokines. However, THC influenced epithelial morphology associated with inflammation at 10 dpi by decreasing mucous cell metaplasia (MCM), which was not correlated with levels of neutrophil-derived elastase. Collectively, the findings in this study suggest that THC exposure increased viral burden through modulation of the host-immune response while decreasing the overall inflammatory response. (Supported in part by NIH DA007908 and MSU Foundation SPG)

312 CHARACTERIZATION OF THE HOST IMMUNE RESPONSE TO AN INFLUENZA CHALLENGE IN CB1/CB2 RECEPTOR-NULL MICE AND C57BL/6 WILD TYPE MICE TREATED WITH DELTA-9-TETRAHYDROCANNABINOL (THC).

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It has been previously shown that THC modulates host-immune responses to influenza infection in a concentration-dependent manner. Therefore, we hypothesized that THC exerted these modulatory effects through a cannabinoid receptor-dependent mechanism. Accordingly, the present study was designed to examine the effects of THC on host-immune responses to an influenza infection in wild-type (WT) mice as compared to cannabinoid receptor (CB1/CB2) knockout (KO) mice. Mice received THC (75 mg/kg) and/or corn oil (CO) by oral gavage. On the third

day of exposure, mice were intranasally instilled with 50 plaque forming units (pfu) of virus or vehicle, saline (SAL), 4 hours prior to THC exposure, and then sacrificed at 7 days post-infection (dpi). KO mice exhibited marked decreases in the numbers of retrievable monocytes and neutrophils in bronchoalveolar lavage fluid (BALF) with THC treatment. Additionally, background CD4+ T cells were elevated in BALF from KO mice. KO mice receiving both THC and PR8 had increased levels of CD4+ T cells. No effect of THC was observed with PR8 treatment as compared to PR8 controls for CD8+ T cells. Additionally, no difference was observed between strains. The levels of soluble chemokines (MCP-1) and cytokines (TNF- α and IFN- γ) in the BALF were elevated with PR8 treatment alone in KO mice as compared to WT. Interestingly, THC treatment resulted in enhanced numbers of mucin-positive epithelial cells lining the main axial airway in KO mice that was not associated with inflammation. Collectively, this study suggests that THC, in part, modulated host-immune responses to PR8 in a CB1 and CB2 receptor-independent manner. (Supported in part by NIH DA007908 and MSU Foundation SPG)

313 TWO SELECTIVE CB2 CANNABINOID RECEPTOR AGONISTS DO NOT EXHIBIT IMMUNOTOXICITY IN RAT KLH ELISA AND HOST RESISTANCE STUDIES.

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Selective agonists of the CB2 cannabinoid receptor have demonstrated potent activity in various animal models of pain, including acute antinociception, inflammatory and neuropathic pain. CB2 cannabinoid receptors are peripherally located and found primarily on immune cells, although their role in immune function is not clear. Studies were undertaken to evaluate the potential immunotoxic effects of two potent and selective CB2 agonists, AM1241 and MAK1400. Both compounds were tested in the rat Keyhole Limpet Hemocyanin (KLH) ELISA Assay; AM1241 was also evaluated in the Rat Influenza Host Resistance Model. All studies were conducted in female F344 rats. Compounds were administered by intraperitoneal injection. Doses selected (AM1241: 0.3-3.0 mg/kg; MAK1400: 0.2-6 mg/kg) were within to several-fold higher than the range found to be effective in various pain models. Compounds were administered once daily by intraperitoneal injection in all studies. In the KLH assay, compounds were administered for 14 days. Reconstituted KLH was injected intraperitoneally on Day 9 and KLH-specific IgM determined on Day 14. Cyclophosphamide was included as a positive control. In the Host Resistance Study, AM1241 was administered for up to 28 days. Animals were infected intranasally with rat-adapted influenza virus after 7 days of AM1241 administration; lung infectious virus titer was determined 1, 3, 6, 8 and 21 days after infection. Dexamethasone was included as a positive control. No overt toxicity (mortality, clinical signs, body weight effects) was observed in any study. There were no effects of either compound on spleen weight or KLH-specific IgM production in the KLH ELISA studies. AM1241 did not affect viral clearance in the Host Resistance Study. Results of these studies indicate that repeated administration of selective CB2 receptor agonists does not adversely impact immune system function in rats.

314 EXPRESSION LEVELS OF I κ B α PLAY AN IMPORTANT ROLE IN 3'IGH RR ACTIVATION.

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Transcriptional regulation of the immunoglobulin heavy chain (IgH) gene involves several regulatory elements including the 3'IGH regulatory region (3'IGH RR). The 3'IGH RR is composed of four distinct enhancers (hs3A, hs1.2, hs3B, hs4) and contains DNA binding sites for several transcription factors including NF- κ B/Rel proteins. A recently identified DNA binding motif the dioxin responsive element (DRE) may also contribute to the regulation of the 3'IGH RR. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a known disrupter of B cell differentiation, induces binding of the aryl hydrocarbon receptor (AhR) nuclear complex to a DRE motif within the hs1.2 and hs4 enhancers. Interestingly, TCDD profoundly inhibits the activation of the 3'IGH RR by the B cell activator lipopolysaccharide (LPS) but enhances LPS-induced activation of the hs4 enhancer. Within the hs4 enhancer, the DRE overlaps an NF- κ B/Rel binding motif (κ B) and site directed mutational analysis demonstrated a cooperative interaction between proteins binding to these motifs. The objective of the current studies was to evaluate the expression of NF- κ B/Rel proteins including their negative regulator I κ B α following LPS stimulation and TCDD treatment and to compare these results to 3'IGH RR activity under the same treatment conditions. For these studies we utilized the CH12.LX B cell line and its variant, CH12.I κ B α AA, that expresses an inducible I κ B α super repressor (I κ B α AA). Western blot analysis demonstrated a marked decrease in endogenous I κ B α with a reciprocal increase in nuclear RelA in the CH12.LX cells one hour after LPS stimulation. In the CH12.I κ B α AA cells endogenous I κ B α was also

decreased by one hour after LPS stimulation and maximal expression of the IkB α AA could be induced by one hour. Furthermore, expression of the IkB α AA partially attenuated the LPS-induced activation of the 3'IgH RR and the activation of the h δ 4 following LPS and TCDD cotreatment. These results suggest that NF- κ B/Rel proteins are at least partially responsible for 3'IgH RR modulation by LPS and TCDD. (Supported by Boonshoft School of Medicine, WSU)

315 MIXTURES EXPOSURE WITH PHOSPHORO-ORGANIC PESTICIDES AND RADIONUCLIDES (89-SR, 144-CE) AND CHANGE OF PHAGOCITARY ACTIVITY OF NEUTROPHILS IN RATS.

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Organophosphorous pesticides (PhO) represent one of the most widely used classes of pesticides. Development of the nuclear energy determines a migration of radionuclides (RA) in the environment. The both factors are with high potential for human exposure and respectively immune system. We investigated the interaction of the PhO and the RA with the organisms of rats. The study was carried out on the changes in the liver function of rats and the kinetics of 89-Sr and 144-Ce after treating with PhO. The chemical and radiation factors were applied one by one or in combination between them. Chronic administration (during 4 months) of RA and PhO in the rats line Wistar perorally (89-Sr) and intraperitoneally (144-Ce) were introduced in two concentrations (370kBq and 3,6kBq for the two isotopes) each in combination with the PhO, introduced in an amount of 1/60 and 1/20 out of a lethal dose LD50/30. With a 30-day period have traced the kinetics of RA in the femur, liver and gastrointestinal tract of the experimental and the control animals and determined the phagocitary activity of neutrophils. The results obtained show that a certain tendency is observed to smaller quantities in the bones and liver and longer retaining in the gastrointestinal tract of 89-Sr and 144-Ce, introduced in the rats in combination with the PhO compared to the control groups. It was established that the effect of radiation and non-radiation factors in chronic treatment increased, expressed in inhibition of the phagocitary activity of neutrophils. This effects may become grave in doses causing approximately equal biological changes. These recent data are beginning to shape risk assessment. They will be used to regulate and provide assurance of health and environment protection, and more appropriate perspective to human risk assessment.

316 NEUTROPHIL DEPLETION DELAYS OR ALTERS AIRWAY EPITHELIAL REPAIR FROM 1-NITRONAPHTHALENE INDUCED LUNG INJURY.

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Neutrophils play an important role in airway epithelial repair. In response to oxidant injury, neutrophils have been shown to have a beneficial role through clearance of damaged cells. However, the role of neutrophils in repair from bioactivated toxicants is unclear. 1-Nitronaphthalene (INN) is a bioactivated toxicant that causes a significant inflammatory response in the rat lung. Earlier studies have shown that airway epithelial repair appears to be complete 4 days post INN treatment in neutrophil-sufficient rats. Airway epithelial cell composition is still altered in neutrophil-depleted rats at 4 days post INN treatment. Neutrophil-depleted rats had greater mass of nonciliated cells than neutrophil-sufficient rats and control rats. Nonciliated cells are known to be progenitor cells of ciliated cells and we believe that this altered epithelial cell composition is due to delayed repair of airway epithelial cells. In order to test this hypothesis, we compared proliferation between neutrophil-sufficient and neutrophil-depleted rats at 4 days post INN treatment. To deplete circulating neutrophils, rats were injected with an anti-neutrophil antibody 14 hours before INN exposure. Proliferation, as determined by bromodeoxyuridine incorporation, was quantified between neutrophil-depleted and neutrophil-sufficient rats in 2 different airway generations. In proximal and distal airway epithelium of neutrophil-sufficient rats, the labeling index was 2.67 and 0.69 respectively, indicating that cellular proliferation was near control levels. However, in the proximal and distal airway epithelium of neutrophil-depleted rats, the labeling index was 13.00 and 9.87 respectively. These data combined with the morphological observations of incomplete epithelium suggest that repair is delayed or altered following INN-induced lung injury without the presence of neutrophils. Supported in part by NIH grants ES 004311, ES06700, ES04699 and USEPA grant RD-8324 1401-0. This abstract does not reflect EPA policy.

317 1-BROMOPROPANE-INDUCED INDUCIBLE NITRIC OXIDE SYNTHASE AND PROINFLAMMATORY CYTOKINES EXPRESSION IN MACROPHAGES.

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1-Bromopropane (1-BP) is an isomeric chemical of 2-bromopropane (2-BP). 2-BP was used as an alternative to chlorofluorocarbons in a Korean electronics factory and caused reproductive and hematopoietic disorders in male and female workers. After identification of 2-BP toxicity, 1-BP was introduced to the workplace as a new alternative to ozone-depleting solvents. But, the influence of 1-BP on cytokine production or the functions of macrophages is unclear. This study examined the effects of 1-BP on the production of nitric oxide (NO) and the proinflammatory cytokines, and analyzed the molecular mechanism in mouse macrophages. The addition of 1-BP to macrophages induced the NO and proinflammatory cytokines (IL-1 β , IL-6, and TNF- α) and expression levels of inducible nitric oxide synthase (iNOS) and these cytokine genes in a dose-dependent manner. The NF- κ B sites were identified in the promoter of the iNOS and proinflammatory cytokines genes. The transient transfection and electrophoretic mobility shift assays revealed that the NF- κ B transcription factor mediated the 1-BP-induced increase in the iNOS and proinflammatory cytokines expression levels. Overall, this study provides evidence showing that 1-BP has inflammatory potential that is previously unrecognized immunomodulating activity.

318 DEVELOPMENTAL IMMUNOTOXICOLOGY OF A COMMERCIAL POLYBROMODIPHENYLETHER (PBDE) MIXTURE.

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Polybromodiphenylethers (PBDEs) are used as flame retardants in a wide variety of textiles and plastics and are found at ppb levels in human blood, milk and tissues. To assess the impact of these chemicals on the developing immune system, adult (F0) rats (8/sex/group) were administered PBDEs (DE-71 technical mixture) by oral gavage at doses of 0, 0.5, 5 or 25 mg/kg body weight/day for 10 weeks, after which the rats were bred. Oral exposure of F0 rats continued through breeding, gestation and lactation. Weanling (F1) rats (13-18 rats/sex/group) received corresponding doses of the DE-71 mixture by gavage until post-natal day 42, at which time pups from each dose group were sacrificed to assess clinical, hematological and histopathological changes. Although dose-dependent increases in apoptotic lymphocytes and tingible macrophages were evident in the thymus and mesenteric lymph nodes of F1 males and females, there were no significant changes in numbers of circulating white blood cells, including lymphocytes and lymphocyte subsets. There were no histopathological changes in spleen from F1 pups as a result of PBDE exposure; however, tritiated thymidine incorporation into spleen lymphocytes was elevated in a dose-dependent manner in unstimulated and mitogen-stimulated cultures. To assess the functional effects of immune changes a further group of F1 rats was challenged with the T-dependent antigen keyhole limpet hemocyanin (KLH). Humoral (anti-KLH IgG) responses were not significantly altered by PBDE exposure. Delayed type hypersensitivity (DTH) responses to footpad injections of heat-inactivated KLH were significantly elevated in female rats exposed to 0.5 mg PBDE/kg bwt/day, but not in rats exposed to 5 or 25 mg PBDE/kg bwt/day. While the dichotomy between PBDE-related increases in immune function in conjunction with histopathological changes indicative of immunosuppression remains to be clarified, the results point to the potential for PBDEs to modulate immune function in developing animals.

319 ROLE OF GLUTATHIONE CONJUGATION IN THE HEPATOTOXICITY AND IMMUNOTOXICITY INDUCED BY 1, 3-DIBROMOPROPANE IN FEMALE BALB/C MICE.

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The effects of 1,3-dibromopropane (1,3-DBP) on the hepatotoxicity and immunotoxicity were investigated in male ICR mice. 1,3-DBP is a clear, colorless to yellow liquid used in industry and employed to stabilize wool. In this study, based on the LC/ESI-MS analyses, S-bromopropyl glutathione (GSH) were observed at m/z 427.9 and 429.9 in the positive ESI spectrum with a retention time of 5.29 and 5.23 min, respectively. Following treatment of mice with 150, 300 and 600 mg/kg of 1,3-DBP once for 12 h, the amounts of S-bromopropyl GSH was detected maximally in liver homogenates at the 600 mg/kg and the hepatic GSH level was significantly decreased. In a time course study, the production of S-bromopropyl GSH was detected maximally 6 h after the treatment and the hepatic GSH level de-

creased at 6 and 12 h. Oral 1,3-DBP significantly suppressed the numbers of antibody-forming cells in response to a T-dependent antigen in a dose-dependent manner. The formation of GSH conjugate with 1,3-DBP might be related to the increased oxidative stress following the depletion of GSH in liver, by which it leads to serious hepatotoxicity and immunotoxicity in female BALB/c mice. (Supported by a grant from KFDA and a BK 21 grant)

320 ROLE OF GLUTATHIONE CONJUGATION IN 1-BROMOBUTANE-INDUCED HEPATOTOXICITY AND IMMUNOTOXICITY IN FEMALE BALB/C MICE.

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1-Bromobutane (1-BB) was used as an alkylating reagent or a material of dehalogenation study by bacteria. In the present study, the hepatotoxic and immunotoxic effects of 1-BB were investigated in female BALB/c mice. The animals were treated once orally with 375, 750 and 1500 mg/kg of 1-BB in corn oil for a dose response study or treated orally with 1500 mg/kg of 1-BB for 6, 12, 24 and 48 hr for a time course study. The hepatic contents of GSH were significantly reduced by 1-BB at the highest dose and the splenic contents of GSH were slightly reduced by 1-BB in a dose-dependent manner. The S-butyl GSH was identified in livers following the treatment with 1-BB by liquid chromatography-electrospray ionization tandem mass spectrometry. When the production of conjugates from 1-BB was investigated in livers following an oral treatment with 1500 mg/kg of 1-BB for 6, 12, 24 and 48 hr, the GSH conjugates were detected maximally 6 hr after the treatment. The hepatic contents of malondialdehyde were significantly increased at 1500 mg/kg of 1-BB, 12 and 24 hr after the treatment. An oral 1-BB treatment significantly suppressed the antibody response to a T-dependent antigen and the production of splenic intracellular IL-2 in response to Con A in dose-dependent manners. Our present results suggested that 1-BB could cause hepatotoxicity and immunotoxicity due to the depletion of GSH content following the formation of GSH conjugates with 1-BB in female BALB/c mice. (Supported by the grant from National Institute of Toxicological Research, KFDA and the BK 21 grant)

321 DEVELOPMENTAL AND EARLY-LIFE EXPOSURE OF AUTOIMMUNE-PRONE MRL+/+ MICE TO TRICHLOROETHYLENE ALTERS CENTRAL AND PERIPHERAL IMMUNE FUNCTION.

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Trichloroethylene (TCE) is a widespread environmental toxicant known to promote CD4+ T lymphocyte activation and autoimmunity in adult MRL+/+ mice. Because developing tissues may be more sensitive to toxicant exposure, it was hypothesized that continuous TCE exposure beginning at the onset of gestation might induce even more pronounced CD4+ T cell effects and exacerbate the development of autoimmunity in MRL+/+ mice. In the current study, MRL+/+ mice were exposed to occupationally-relevant doses of TCE from conception until adulthood. The CD4+ T cell effects in the thymus and periphery were evaluated. Serum antibody levels were also tested as indicators of early signs of autoimmunity. TCE exposure altered the number of thymocyte subsets, and reduced the capacity of the most immature thymocytes to undergo apoptosis *in vitro*. In the periphery, T cell IFN- γ production was monitored in the blood prior to sacrifice by intracellular cytokine staining and flow cytometry. TCE induced a dose-dependent increase in T cell IFN- γ as early as 4-5 weeks of age. In addition, the serum levels of anti-histone autoantibodies (autoabs) and total IgG2a were significantly elevated in the TCE-exposed offspring. The data illustrated that occupationally-relevant doses of TCE administered throughout development until adulthood affected central and peripheral immune function in association with early signs of autoimmunity. It is possible that early-life exposure to TCE may alter some aspect of self tolerance in the thymus, leading to autoimmune disease later in life.

322 AUTO-IMMUNE RESPONSE IN REFRIGERATION MECHANICS EXPOSED TO HALOGENATED HYDROCARBONS.

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Hydrofluorocarbons (HFC) and hydrochlorofluorocarbons (HCFC) have replaced the chlorofluorocarbons (CFC) in e.g. refrigeration installations and air-conditioning systems. During the substitution period, refrigeration mechanics reported

symptoms like asthma, influenza-like reactions, and joint troubles. These symptoms resemble those of chronic inflammatory diseases with an autoimmune component. Exposure to gases similar to the new refrigerants (e.g. halothane) may result in development of antibodies against proteins modified by their reactive metabolites as well as auto-antibodies against CYP2E1, which is the main enzyme metabolizing these compounds. The aim of this study was to determine if occupational exposure to halogenated hydrocarbons used as refrigerants generates autoimmune responses against CYP2E1.

Venous blood was collected from 44 Swedish refrigeration mechanics who were occupationally exposed to halogenated hydrocarbons. Thirty of these men had asthma, joint- or influenza-like symptoms and the remaining 14 individuals were free from such symptoms. Unexposed, healthy Swedish (n=35) and Italian (n=26) persons were used as controls. The anti-CYP2E1 reactivity in serum was measured by ELISA. Non-parametric tests were used for group comparisons (Kruskal Wallis, Wilcoxon rank-sum) and correlations (Spearman's Rho). The significance level was set to 0.05. The study was approved by Swedish and Italian ethics committees.

No increase in CYP2E1 antibody titer was seen among refrigeration mechanics with symptoms, neither compared with healthy controls, nor with asymptomatic workers. Further, no relation between antibody titer and severity of symptoms, smoking or age was detected.

In conclusion, no autoimmune response against CYP2E1 was detected in refrigeration mechanics exposed to halogenated hydrocarbons.

323 DEOXYNIVALENOL IMPAIRS RESISTANCE TO RESPIRATORY REOVIRUS INFECTION AND PROMOTES VIRAL BRONCHOPNEUMONIA.

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The capacity of the trichothecene mycotoxin deoxynivalenol (DON), a frequent contaminant of cereal grains, to interfere with the murine respiratory reovirus infection was assessed. Balb/c female mice (5 wk old) were orally gavaged with DON (10 mg/kg body weight) or saline vehicle and then intranasally instilled with reovirus serotype 1, strain Lang (T1/L). Both viral titers and reovirus L2 gene expression were 10-fold higher in lungs of DON-treated mice than in saline controls at 10 d post-instillation (PI). As little as 2 mg/kg bw DON impaired viral clearance. DON amplified reovirus-induced IFN-beta and IFN-gamma mRNA responses in lung, however, the toxin suppressed mRNA expression for IFN-alpha beta receptor (IFNAR) and IFN-gamma receptor (IFNGR). Decreased IFNAR expression corresponded to impaired induction of two Type 1 IFN-dependent antiviral genes, double-stranded RNA protein kinase (PKR) and oligoadenylate synthase 2 (OAS2). Respiratory reovirus infection caused a mild bronchopneumonia in mice which was markedly exacerbated by DON as evidenced by severe inflammatory cell infiltration, marked alveolar damage and a higher volume density of intraepithelial mucous substances in pulmonary airways. DON also enhanced viral-induced elevations of protein, MCP-1, TNF-alpha and inflammatory cells in the bronchoalveolar lavage fluid (BALF). Furthermore, DON exposure upregulated induction of reovirus-specific IgA in BALF, fecal pellets and serum. DON's effect on BALF IgA was preceded by elevated IL-6 expression and secretion in the lung. Accordingly, the results suggest that DON compromised resistance to respiratory viral infection.

324 THE RED BLOOD CELL PROTEOME IS ALTERED BY PRIMING WITH 2-BUTOXYETHANOL.

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Administration of a low priming dose of 2-butoxyethanol (BE, 500 mg/kg, po) 7 days prior to a LD90 dose (1500 mg BE/kg, po) offers protection against the lethal dose-induced hemolysis and death in female Sprague Dawley rats due to efficient replacement of RBCs with new resilient red blood cells (RBCs). *In vitro* incubation of RBCs isolated from vehicle-treated (distilled water, 5 ml/kg, po, V-RBCs) and primed rats (500 mg BE/kg, 5 ml/kg, po, P-RBCs) on day 7 with 2-butoxyacetic acid (1 to 10 mM BAA, hematotoxic metabolite of BE) over a time course (1 to 4 h) revealed resiliency of P-RBCs to hemolysis as compared to V-RBCs (Sivarao *et al. Arch. Toxicol.* 69:526-532, 1995). The objective of the present work was to test the hypothesis that the resiliency of P-RBCs to hemolysis is due to the appearance of survival proteins in P-RBCs. The cytosolic and membrane fractions were prepared from V-RBCs and P-RBCs. The fractions were labeled with Cy3 and Cy5 fluorescent dyes and subjected to 2-dimensional differential gel electrophoresis (2-DIGE) to perform protein profiling studies on V-RBCs versus P-RBCs. Owing to

high amount of hemoglobin, cytosolic samples had to be sub-fractionated into three different fractions (I, II, and III) by size exclusion chromatography. While the protein profiling in P-RBCs cytosolic fraction III is underway, separation and quantitation of other fractions revealed 11 membrane proteins, 9 (fraction I) and 25 (fraction II) cytosolic proteins increased significantly (≥ 2.5 fold) in P-RBCs as compared to V-RBCs. Our preliminary studies with membrane proteins suggest that the increased protein spots might be identified as ankyrin, adducin, anion exchange, 4.1 or 4.2 proteins. Identification of the increased proteins by LC-MS/MS and their functional categorization is in progress.

325 LUNG INFLAMMATION AND CARDIOVASCULAR OUTCOMES - WHOLE BLOOD GENE EXPRESSION STUDIES IN A LIPOPOLYSACCHARIDE (LPS) PHARYNGEAL ASPIRATION MOUSE MODEL.

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Recent animal and epidemiological studies suggest that pulmonary inflammation (e.g. ultrafine particulates) can trigger negative cardiovascular outcomes although the mechanism(s) resulting in systemic effects are not well established. Expression profiling of blood samples is becoming a useful tool for disease screening and identification of new biomarkers. In this study, it was hypothesized that lung inflammation results in activation of systemic gene expression related to cardiovascular disease. Prior to analyzing models of occupational exposure, pharyngeal aspiration of LPS was characterized with intraperitoneal (IP) injection as a positive control. C57BL/6 mice were treated with LPS (0.1mg/kg) and sacrificed 2hr post exposure. Whole blood and tissues were harvested for analysis of mRNA transcripts related to inflammation and coagulation. Expectedly, IP LPS resulted in a marked inflammatory response in the blood, heart and lung and pharyngeal aspiration of LPS resulted in a significant lung inflammation. Interestingly, whole blood showed similar gene expression induction of macrophage-inflammatory protein-2 (MIP-2) and tumor necrosis factor alpha (TNF α) following LPS by pharyngeal aspiration or IP injection. Extrapulmonary tissues were mostly unaffected by instillation of LPS into the lung. In conjunction with inflammation, changes in tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1) have been associated with increased risk of cardiovascular disease. Neither TF nor PAI-1 gene expression was elevated in blood following IP LPS although PAI-1 was strongly induced in the heart and lung. Following pharyngeal aspiration of LPS, both TF and PAI-1 were significantly increased in the lung and whole blood but not in the heart. In conclusion, lung inflammation results in activation of inflammatory and coagulation biomarkers associated with cardiovascular diseases and blood gene expression studies can identify biomarkers which predict cardiovascular outcomes in occupational-related respiratory exposures.

326 OVINE MATERNAL STRESS DURING LATE PREGNANCY ALTERS OFFSPRING FEBRILE RESPONSIVENESS TO SYSTEMIC ENDOTOXIN CHALLENGE LATER IN LIFE.

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Epigenetic programming of the neuroendocrine-immune axis during fetal development may contribute to variation in the febrile response and subsequently influence an individual's resistance to pathogenic infection later in life. In this study gender-specific changes in the ovine febrile response to systemic *Escherichia coli* lipopolysaccharide (LPS) were examined over time using infrared imaging, and an assessment was carried out to determine if maternal inflammatory stress experienced during late pregnancy influences febrile responsiveness of the offspring later in life. Pregnant sheep were allocated to 3 treatment groups and challenged with saline, or *E. coli* LPS (400 ng/kg x 3 consecutive days, 1.2 μ g/kg) commencing on gestation day 135. Offspring of these stressed sheep were challenged with 400 ng/kg of LPS at six months of age and infrared energy emitted from the ear and eye was measured hourly for 6 hours post challenge to determine tissue temperatures; these measurements were compared with rectal temperatures. As expected, eye and rectal temperatures increased and then returned to basal levels within 6 hours of the LPS challenge. Ear temperature measurements on the other hand, decreased rapidly and then increased such that basal measurements were exceeded. This response was more pronounced in the females and is likely attributed to peripheral vasoconstriction. Female eye and rectal temperature was also higher than male offspring during LPS challenge, and the change in eye temperature was significantly different between the two different female LPS treatment groups. Overall, these results demonstrate that prenatal stress alters the ovine febrile response to LPS later in life, females have higher body temperatures than males, and infrared imaging is sufficiently sensitive to detect these subtle changes in body temperature.

327 EXPOSURE OF MICE TO CIGARETTE SMOKE *IN UTERO* INDUCES AIRWAY HYPER-RESPONSIVENESS (AHR) IN EXPOSED OFFSPRING LATER IN LIFE.

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More than a million infants are born each year in the U.S. after *in utero* exposure to maternal cigarette smoke (CS). Accumulating epidemiologic data suggest that exposure to CS *in utero* poses a substantial risk for respiratory disease/asthma in the next generation. While these findings carry extensive clinical consequences, few studies are available to provide insight into the possible mechanisms by which these effects might occur. Thus, a toxicologic study was performed to test the hypothesis that prenatal exposure to CS from maternal smoking persistently enhances airway reactivity (a defining feature of asthma) and related biologic parameters in the exposed offspring, and that these effects were sex-dependent. Pregnant CD1 mice were exposed daily (by inhalation) for 4 hr/d (5 d/wk) from gestational day 4 to parturition to mainstream CS at a concentration equivalent to smoking <1 pack of cigarettes per day (PM = 16 mg/m³; CO = $\leq$ 25 ppm). In the absence of effects on maternal weight gain, litter size or offspring sex ratio, prenatal exposure to mainstream CS significantly enhanced airway reactivity (compared to the sex/age-matched, air exposed controls) in 5-wk-old female (but, not male) offspring in response to bronchoprovocation challenge with acetylcholine (ACH); AHR was observed in the absence of any pulmonary inflammation, lung cell damage, overt pulmonary pathology, or elevated serum IgE levels. In contrast, airways of prenatally exposed older female offspring (4-mo-old) were significantly less responsive to ACH challenge (compared to controls). These studies suggest that: prenatal exposure to CS has long-term effects on offspring respiratory health; effects of prenatal CS exposure on offspring airway function are gender-dependent; mechanisms other than airway inflammation may underlie changes in offspring bronchial reactivity in response to *in utero* CS exposure; and, even modest smoking during pregnancy may increase the offspring's risk of respiratory dysfunction later in life. Funded by IFSH.

328 AGE-RELATED CHANGES IN ADRENAL RESPONSIVENESS, GRANULOCYTE TRAFFICKING, AND SERUM IL-6 CONCENTRATIONS IN FEMALE SHEEP SYSTEMICALLY CHALLENGED WITH *ESCHERICHIA COLI* LIPOPOLYSACCHARIDE.

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The production of inflammatory mediators has been associated with senescence of both the neuroendocrine and immune systems and may contribute to a number of age-related diseases. In this study, this process of "inflamm-aging" was investigated using an ovine model of acute systemic inflammation. Seven mature ewes (4-5 years old) and 7 ewe lambs (6 months old) were challenged intravenously with 400 ng/kg *Escherichia coli* lipopolysaccharide (LPS). Temperature measurements and venous blood samples were obtained at baseline, and 2, 4, 6, and 24 hours post-LPS administration to assess changes in serum interleukin-6 (IL-6), cortisol, and haptoglobin concentrations, the percentage of blood granulocytes, and biochemical markers of hepatotoxicity and liver function. Basal IL-6 concentrations in the adult sheep were dramatically higher than those of the younger animals, and the response to LPS challenge was significantly different over time between age groups. Serum cortisol concentrations increased in both age groups in response to LPS challenge however, the response was significantly greater in the adult versus the young animals. In both age groups the percentage of blood granulocytes decreased 2 hours, and increased 6 hours post LPS challenge. The 2-hour decrease however, was significantly more pronounced in the young versus adult sheep. Significant differences between the age groups were not observed for either haptoglobin, or temperature, and there was no evidence of hepatotoxicity in either group of animals. Overall, these results indicate that inflamm-aging also occurs in ruminants, and that the sheep may be useful large animal model for studying the ageing neuroendocrine and immune systems.

329 EFFECTS OF LATENT *IN UTERO* AND PERINATAL TCDD EXPOSURE IN POST-NATAL C57BL/6 AND SNF1 MURINE OFFSPRING.

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TCDD [2,3,7,8-tetrachlorodibenzo-p-dioxin] and its congeners have a long and well-documented history of causing damage from both acute and chronic exposure. However, the potential risks associated with latent *in utero* and perinatal exposure

to dioxin have not been investigated. In this prefatory study, we evaluated the latent effect of TCDD exposure in utero on select immune parameters of murine offspring on postnatal day 1 (PND1), postnatal day 14 (PND14), and postnatal day 21 (PND 21). C57BL/6 mice, a known TCDD-ArH receptor sensitive strain, and SNF1 mice, an autoimmune disease predisposed strain, were used in this study. In an ongoing study, murine mothers (C57BL/6 and SWR) were acutely exposed to either corn oil vehicle or TCDD (C57BL/6, 5 & 10 µg/kg; SNF1, 40 & 80 µg/kg) on gestation day 12. Post-parturition, the murine mothers nursed the first litter for 21-25 days after which the pups were weaned. At approximately 32 days post-exposure, TCDD-exposed C57BL/6 and SWR mothers were rebred. The pups from these litters were randomly evaluated on PND 1, PND 14, and PND 21. Endpoints examined included body weight, organ weight/body weight ratio, splenic cellularity, thymic cellularity, hepatic cellularity, splenic cell surface marker expression, thymic cell surface marker expression and hepatic cell surface marker expression. Data from this preliminary study suggest that latent exposure as previously defined increases the incidence of immune dysregulation in both C57BL/6 and SNF1 mice.

330 COMPARISON OF ON AND OFF ROAD DIESEL EXHAUST SOURCES ON SUSCEPTIBILITY TO INFLUENZA INFECTION IN MICE.

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Diesel exhaust particulate (DEP) is a major component of urban air pollution and its influence on susceptibility to respiratory infection is of concern. DEP is emitted from various types and ages of engine and the toxicity is dependent upon fuel, and combustion conditions which influence the physico-chemical characteristics of the emission. The purpose of this study was to evaluate the effects of an on- and off-road source of DEP exposure on the severity of acute influenza infection in mice. BALB/c mice were exposed (4 hrs/day, 5 days) to air or 0.5 mg/m³ DEP from engines powering a compressor (C-DEP) or a diesel truck (T-DEP). One hour after the final diesel exposure, mice were intra-tracheally instilled with 10 HA units of influenza A/Bangkok/1/79 virus. 18 hours later mice were euthanized and lung tissue was assessed by RT-PCR for mRNA expression of viral hemagglutinin antigen, IFN-β, IL-6, and surfactant protein A and D (SP-A and SP-D). Lung injury was measured by differential cell counts and the production of LDH, microalbumin, and protein in lung lavages (BAL). Exposure to 0.5 mg/m³ DEP from either source in conjunction with infection caused increased protein levels and neutrophil numbers as well as enhanced IFN-β expression relative to air/flu controls. Mice exposed to C-DEP had significantly higher viral HA mRNA suggesting greater viral proliferation that was not seen with mice exposed to T-DEP. This effect was also associated with decreased expression of SP-A and SP-D, which is an important in defense against respiratory infections. We conclude that while exposure to the on road (T-DEP) emission produced additive effects of lung injury during influenza infection, exposure to the DEP from the off road source (C-DEP) also enhanced susceptibility to infection in association with decreased production of SP-A and SP-D. Further studies will clarify the physico-chemical elements associated with this effect. (Funded by EPA CT829470. This abstract does not reflect EPA policy).

331 ENHANCEMENT OF CONTACT HYPERSENSITIVITY BY EXPOSURE OF TETRABROMOBIPHENOL A IN MICE.

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Increase of allergic patients has become a social problem. We have paid attention to the contact hypersensitivity among the allergic reaction, and have thought that exposure of some chemicals enhances the reaction. We screened the enhancing effect of chemicals, such as fire retardants, on contact dermatitis. Test chemical was painted on the back of mice everyday for 14 days. On the day 7, the mice sensitized by painting of 2,4-dinitrofluorobenzene (DNFB) on the shaved abdomen, then on the day 14 DNFB was painted on the right ear just one hour after the last painting of test chemical. The ear thickness was measured with an engineer's micrometer. We found that tetrabromobisphenol A (TBBPA) enhanced the contact hypersensitivity. To investigate the mechanism, the female BALB/c mice were challenged by TBBPA 25 µg/body/day for a week before and after sensitization. Lymph node cells and liver from mice were collected. We carried out antigen-specific T cell proliferation assay and the proliferation was significantly enhanced in TBBPA-treated mice than in the vehicle-treated. The IFN-γ production from lymph node cells was increased in the TBBPA-treated mice in comparison with the vehicle-treated, but no difference was observed on the IL-10 production. Concentrations of glutathione in the ears and

the livers were increased in the TBBPA-treated mice in comparison with the vehicle-treated. From these results, TBBPA exposure via skin affect immunocytes and possibly exacerbates contact hypersensitivity reaction.

332 TRICHLOROETHENE-MEDIATED AUTO-IMMUNE RESPONSE: ROLE OF LIPID PEROXIDATION-DERIVED ALDEHYDES.

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Lipid peroxidation is implicated in the pathogenesis of various autoimmune diseases. Lipid peroxidation-derived aldehydes (LPDAs) such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE) are highly reactive and bind proteins covalently, but their potential to elicit autoimmune responses and to contribute to disease pathogenesis remains unclear. To investigate the role of lipid peroxidation in the induction and/or exacerbation of autoimmune responses, 6-week-old autoimmune-prone female MRL +/+ mice were treated with trichloroethene (TCE; 10 mmol/kg, i.p., once a week), an environmental contaminant known to induce lipid peroxidation, for 4 weeks. Sera from TCE-treated mice showed significant levels of antibodies against MDA- and HNE-adducted proteins along with anti-nuclear antibodies. This suggested that TCE exposure not only caused increased lipid peroxidation, but also accelerated autoimmune response. Furthermore, stimulation of cultured splenic lymphocytes, isolated from both control and TCE treated mice, with MDA-adducted mouse serum albumin (MDA-MSA) or HNE-MSA adducts for 72 h showed significant increases in the percentage of activated CD4+ T cells in TCE-treated mice, as analyzed by flow cytometry. Also, splenic lymphocytes from TCE-treated mice released more IFN-γ and IL-2 into cultures when stimulated with MDA-MSA or HNE-MSA, suggesting a Th1 T cell activation. Thus, our data suggest a role for lipid peroxidation-derived aldehydes in TCE-mediated autoimmune responses, and involvement of Th1 T cell activation. Supported by NIH ES013510.

333 LOW MERCURY BURDENS ATTENUATE BCR MEDIATED SIGNAL TRANSDUCTION.

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The ubiquitous environmental heavy metal contaminant mercury (Hg) is a potent immunomodulator that has been implicated as a factor contributing to autoimmune disease in animal models as well as in humans. The mechanisms whereby Hg initiates autoimmune responses, especially at the biochemical/molecular level, remain poorly understood. Signal strength through clonotypic antigen-specific receptors present on developing lymphocytes is a key determinant that shapes the immune repertoire and establishes tolerance to self antigens. Strength of signals through the antigen specific receptors on B lymphocytes (i.e., the BCR) determines many of the decisions that B cells have to make including whether, or not, they proliferate, differentiate or survive. Recent work has established a relationship between impaired BCR signal strength and autoimmune disease. In previous studies we have shown that in mouse WEHI-231 B cells, non-cytotoxic concentrations of inorganic mercury (iHg) interfered with BCR-mediated growth control, suggesting that BCR signal strength was impaired by iHg. ERK 1,2 MAPK is responsible for the activation of several transcription factors in B cells. Phosphorylation of ERK serves as an essential node of signal integration for the BCR. Thus, the magnitude of ERK activation serves as an operational metric for BCR signal strength. Using Western blotting and phosphospecific flow cytometry, we now show that the kinetics and magnitude of BCR mediated activation of ERK MAP kinase are markedly attenuated in WEHI-231 cells and splenic B cells that have been exposed to low and non-toxic burdens of iHg. However, iHg does not seem to act directly on ERK MAP kinase but rather on an upstream element of the BCR signal transduction pathway, above the level of the key protein tyrosine kinase Syk. Preliminary data suggest that the site of action of iHg may very well be localized on the plasma membrane. These findings support a connection between iHg and attenuated BCR signal strength in the etiology of autoimmune disease.

334 TIMING IS EVERYTHING: LOW DOSE INORGANIC MERCURY BEFORE THE TRIGGERING EVENT INCREASES SEVERITY AND FREQUENCY OF AUTO-IMMUNE MYOCARDITIS IN MICE.

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Mercury is a widespread environmental contaminant with neurologic and immunotoxic impacts observed at low dose exposures. Inorganic mercury (iHg) has a range of immunotoxic effects, including both immune suppression and induction

of autoimmunity. Epidemiological studies have demonstrated an association between Hg exposure and risks of autoimmune disease. In some rodent strains, iHg at relatively high doses can induce lupus-like disease; much lower doses can accelerate lupus-like disease in nonsusceptible mice that are challenged with an appropriate trigger. We have utilized this model in studies of low doses of iHg and induced autoimmune cardiac disease using coxsackievirus B3 (CVB3) in BALB/c mice. CVB3 is a cause of dilated cardiomyopathy (DCM) in human populations and in mice, induces a biphasic cardiac disease which is characterized by a primary acute phase, peaking at day 12 post-infection (pi), and a chronic phase, peaking at about day 35 pi, often with accompanying DCM. In these studies, the animals were treated with iHg either before infection (200 ug/kg, every other day for 15 days) or after CVB3 infection, in the interim (every other day for 7 days) between the acute and chronic phases. We found that only pre-treatment exacerbated the chronic disease. With iHg pre-treatment the percentage of mice with dilated cardiomyopathy (DCM) was increased; however, this increased pathology was not accompanied by changes in heart cytokine profiles. In contrast, iHg exposure following infection did not change the incidence or severity of disease, but increased IL-12 and IL-4 in the heart. These results suggest that Hg lowers the threshold of activation for autoreactive cells before encountering autoantigen (presumably the "trigger" event in this induced autoimmune disease).

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335 GLOBAL GENE EXPRESSION PROFILING OF MACROPHAGES IN RESPONSE TO D-PENICILLAMINE TREATMENT.

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Rationale: At present idiosyncratic drug reactions are impossible to predict largely because the mechanisms involved are unknown. The previous studies in our lab have demonstrated the involvement of macrophages in pathogenesis of D-Penicillamine-induced autoimmune disease in Brown Norway rats. Meanwhile, literature evidence has implicated a reversible imine bond formation between carbonyls and amines on APCs and T-cells respectively in T-cell activation.

Hypothesis: D-penicillamine binds irreversibly with aldehyde groups activating macrophages, which in some patients and rats might lead to the autoimmune reaction. **Experimental Procedures:** Brown Norway rats (n=3) were given 150 mg/kg of D-Penicillamine by oral gavage while the control group (n=3) received the same volume of water. At 6 h post-dosage, spleen macrophages were purified by using an immunomagnetic separation column and monoclonal antibody CD163, and total RNA was isolated. Microarray analysis was performed using the rat expression array 230 2.0. Quantitative real time PCR was used to confirm the gene expression changes detected by microarrays.

Results: All three D-Penicillamine treated rats were separately compared to each individual control rat. The criteria for defining whether genes were up- or down-regulated were if at least comparisons between 2 treated rats with 2 control rats were different and the fold change for up and down-regulation was no less than 1.2 and no more than 0.7. A comparison of the observed changes of genes and the literature macrophage activation markers lead us to focus on 20 genes. Out of these 20 genes, 14 associated with inflammatory reactions, recruitment of immune cells, and regulation and polarization of macrophages, natural killer cells, and T helper cells were chosen for additional testing by RT-PCR and about 95% of the observed changes were confirmed.

Conclusions: Microarray data in this study demonstrated very early activation of macrophages by D-Penicillamine treatment, which suggests that macrophages play a critical role in initiation of immune responses to D-Penicillamine leading to autoimmune disease.

336 ARSENITE INDUCES CD14 AND HLA-DR STRONG POSITIVE DENDRITIC MACROPHAGE (ARSENITE-INDUCED MACROPHAGE; AS-MP) GENERATION WITH GM-CSF FROM HUMAN PERIPHERAL BLOOD MONOCYTES.

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A trivalent inorganic arsenic, arsenite, has been recently developed as a potent therapeutic agent for acute promyelocytic leukemia (APL), while, it is also causing chronic poisoning through the consumption of contaminated well water. The total peripheral blood arsenic concentrations of two APL patients who underwent complete remission by intravenous injection of arsenite and chronic arsenic poisoning patients who had inflammatory-like immune responses are less than 1 μM , thus, nM concentrations may be very importance to induce both therapeutic and chronic

toxic effects by arsenite. We demonstrate in this study the effect of nM concentrations of arsenite on human peripheral blood monocytes. Arsenite significantly inhibited the cytokine-induced *in vitro* normal maturation of monocytes into macrophage species at nM levels. Surprisingly, arsenite induced the generation of a unique nonadherent dendritic macrophages (arsenite-induced macrophages; As-Mp) which strongly expressed CD14 and HLA-DR at nM levels together with GM-CSF. They were responded more strongly to bacterial lipopolysaccharides by inflammatory cytokine, TNF α and IL-1 α , release and more effective at inducing T cell responses as compared to normal macrophages induced by GM-CSF alone. On the other hand, As-Mp expressed lower levels of cell membrane adhesion molecules, such as CD11b and CD54, and phagocytic ability was less efficiently. We also demonstrated that the optimum amount of cellular reactive oxygen species induced by nM arsenite might play an important role in this As-Mp generation. As-Mp could be still responded to M-CSF and GM-CSF and were differentiated into partially abnormal adherent macrophages. This work may have implications in both the chemotherapeutic use and in chronic poisoning of this remarkable metalloind. Ref.; Sakurai *et al. Int. Immunopharmac.*, 4(13), 1661-1673 (2004); 6(2), 304-315 (2006); Sakurai *et al. Toxicol. Appl. Pharmacol.*, 203(2), 145-153 (2005).

337 DISTINCT EICOSANOID RESPONSE OF LIVER AND LUNG MACROPHAGES TO ACUTE ENDOTOXEMIA: ROLE OF TOLL-LIKE RECEPTOR 4.

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Exposure to excessive quantities of bacterially-derived endotoxin (ETX) is associated with injury to the liver and the lung. Macrophages (MP) play a key role in the pathogenic response to ETX by releasing excessive quantities of inflammatory mediators including reactive oxygen and nitrogen intermediates and eicosanoids. In the present studies, we examined the role of TLR-4 in the regulation of MP expression of prostaglandin synthase (cyclooxygenase [COX]-1 and COX-2) and the prostaglandin-catabolizing enzyme, 15-hydroxyprostaglandin dehydrogenase (PGDH) during acute endotoxemia. Treatment of control C3H/OuJ with ETX (3 mg/kg) was associated with a significant increase in the number of MP recovered from both the liver and the lung. In the lung this occurred within 12 hr, while in the liver it was delayed for 24 hr. This was not observed in TLR-4 mutant C3H/HeJ mice. Acute endotoxemia also resulted in a dramatic upregulation (140-fold) in COX-2 expression in liver MP from control, but not in TLR-4 mutant mice. This was maximum within 3 hr and persisted, although at reduced levels for at least 24 hr. No significant effects of ETX on expression of COX-1 were observed in either mouse strain. ETX-induced increases in COX-2 in liver MP were correlated with increases in PGE2 and PGE1. This was also evident within 3 hr and persisted for at least 24 hr. In contrast, PGDH was down-regulated in liver MP from both control and TLR-4 mutant mice. In control mice this persisted for 24 hr. When compared to liver MP, lung MP were relatively unresponsive to ETX. These results demonstrate that functional TLR-4 is essential for hepatic MP responses to ETX. Moreover, the effects of TLR-4 are mediated in part via regulation of prostaglandin levels in the liver. Supported by NIH ES004738, ES005022, ES007148, GM03410 and APERG.

338 EVALUATION OF THE EFFECTS OF ORGANOCHLORINE PESTICIDES ON MACROPHAGE FUNCTIONS.

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Macrophages play important roles in immunological responses, especially in removal of cell debris, antigen presentation, and cytokine secretion. Defects in macrophage function are related to many disorders, including autoimmune disease. Organochlorine pesticides can accelerate autoimmunity in the classic (NZB x NZW)F1 [NZBWF1] mouse model of systemic lupus erythematosus, but their effects on macrophage function have not been well studied. To determine whether there may be a class effect, we examined five organochlorine pesticides (chlordecone, dieldrin, DDE, toxaphene and methoxychlor) for their effects on a series of macrophage functions using the RAW 264.7 macrophage cell line. Cell proliferation and apoptosis rates were measured by CFSE and annexin-V/7AAD staining, respectively, while cytokine secretion was determined by ELISA. Macrophage clearance of apoptotic cells was conducted using dexamethasone-induced apoptotic thymocytes from BALB/c mice as targets. Furthermore, *ex vivo* studies were conducted with peritoneal macrophages obtained from two-month-old ovariectomized NZBWF1 mice treated with chlordecone sustained release pellets for six weeks. All five pesticides at 1 μM and 10 μM concentrations reduced RAW cell proliferation (after 72 hrs) and apoptosis (48 hours). Chlordecone, dieldrin, DDE and toxaphene treatment all caused a concentration-dependent augmentation in TNF- α secretion by RAW cells. One μM chlordecone, DDE and toxaphene reduced RAW cell clearance of apoptotic cells. *In vivo* exposure to chlordecone increased the

expression of MHC class II molecules on peritoneal macrophages and caused a dose-dependent decrease in clearance of apoptotic cells, a source of self antigen. Taken together, these results indicate organochlorine pesticides can affect macrophage functions, and suggest a role in the augmentation of autoimmune responses in NZBWF1 mice through adverse effects on tolerogenic, autoantigen clearance mechanisms. (Supported by NIH ES07375)

339 METALLOTHIONEIN GENE DOSE AFFECTS LISTERIOSIS IN MICE.

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Metallothioneins (MTs) are small cysteine-rich proteins with a wide range of functions including ROS scavenging, chemokine activity, immunomodulation, essential cation management and metal sequestration. MT-I and MT-II are the most widely studied isoforms. C57/BL6 wild-type mice (WT), congenic MT-I/II knock-out mice (MTKO), and congenic MT-I transgenic mice (MTTG) were used to evaluate the effects of MT gene dose on antioxidant, chemotactic, and immunomodulatory activities. *Listeria monocytogenes* is a Gram-positive bacterium that is pathogenic in all mammals, and is commonly used as a model of mammalian immune response to infection. Immunocompromised individuals, neonates, and women in their third trimester are particularly vulnerable to listeriosis. Here we have used an in vivo model of listeriosis to evaluate the effects of MT gene dose on the course of *Listeria* infection in mice. Our results suggest that either over-expression of MT or a lack of functional MT I and II will significantly alter the course of intravenous listerial infection. Both MTTG and MTKO mice showed significantly lower *Listeria* burdens in both liver and spleen three days post-inoculation. A time course study suggested that this difference is most prominent in the first 48 hours of infection, after which *Listeria* clearance occurs at comparable rates in all three strains. Lymphocytes from MTKO mice exhibited both increased levels of surface thiols and increased apoptosis compared to WT and MTTG mice. DHR fluorescence assays revealed that lymphocytes from the MTTG mice exhibit an increased oxidative burst compared to both WT and MTKO strains. Macrophages from both MTTG and MTKO mice exhibited increased oxidative burst compared to WT, suggesting a possible mechanism by which these strains maintain a lower *Listeria* burden in this model of infection. These results indicate that MT gene dose can have dramatic influences on the course of disease. * co-first authors. Supported by R01 ES07408.

340 METALLOTHIONEIN GENE DOSE INFLUENCES T-DEPENDENT HUMORAL IMMUNITY IN MICE EXPOSED TO ZN AND CD.

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Metallothionein (MT) is a stress response protein with significant influences on immune activities. To understand the immunomodulatory function of MT and the role of MT in heavy metal detoxification, we employed three C57BL/6J congenic strains of mice in these studies: wild-type (WT) mice, MT transgenic (TgN) mice and MT knockout (KO) mice. In vivo ovalbumin (OVA) challenge of each strain fed water, with or without 100 uMCdCl₂ or 100 uM ZnCl₂ revealed (1) at 4 weeks, TgN mice express higher levels of MT mRNA irrespective of treatment but only cadmium induces significantly higher levels of MT protein in liver; (2) KO mice exhibit increased anti-OVA antibody compared to WT controls in the latter stages of the 45 day experiment and KO mice had more anti-OVA secreting splenocytes; (3) cadmium suppressed anti-OVA antibody (IgG1 isotype) levels in both KO and TgN mice, while zinc only suppressed anti-OVA antibody production in TgN mice; (4) neither metal exposure (at the exposure level in this experiment) affected the anti-OVA response in WT mice; (5) TgN mice have less splenocytes compared with their counterparts; (6) there are significantly less B cells among the splenocytes of TgN mice compared to both wild-type and KO mice, and metal-treatment did not change the subpopulation distribution of the splenocytes. In vitro cytokine profiles from stimulated splenocytes revealed that: (1) KO splenocytes produced higher levels of IL-4 and lower levels of IFN- γ compared to WT and TgN splenocytes; (2) TgN splenocytes produced lower levels IL-2, IL-4, and IFN- γ compared to WT splenocytes; (3) the higher dosage of CdCl₂ (25 uM) used in these in vitro cultures decreased IL-2, IL-4 levels in all three strains, but the lower (6.25 uM) dosage of CdCl₂ decreased IL-2, IL-4, and IFN- γ only in KO splenocytes. Taken together, this data suggests that KO mice express a Th2 cytokine profile that favors B cell class switching to the IgG1 isotype, and that splenocytes from KO mice are more sensitive to cadmium treatment. (NIEHS grant R01 ES07408).(*=co-first authors)

341 DIFFERENCES IN THE APOPTOTIC RESPONSE IN ACUTE AND CHRONIC SILICOSIS.

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Silicosis is characterized by lung granulomas and generally develops by chronic occupational inhalation of crystalline silica. Chronic silicosis has a long asymptomatic latency and, similarly, Lewis rats, exposed chronically to occupationally realistic doses of silica inhalation, show limited lung inflammation and injury, and delayed granuloma formation. Acute silicosis, a relatively rare form of the disease, usually resulting from massive accidental exposures, and is associated with severe lung inflammation, apoptosis, and tissue injury. While the cellular and molecular mechanisms of silicosis are largely unknown, inferences from animal models of acute silicosis are commonly extrapolated to human silicosis. Using the rat model of chronic silicosis (6.2 mg/m³, 5 days/wk for 6 wk), and single intratracheal instillation of 35 mg silica/rat for acute exposure, we present evidence that acute and chronic silicosis are mechanistically distinct diseases. Thus, unlike acute exposure, chronic silica inhalation produces a strong anti-apoptotic response, characterized by dramatic increases in Bcl-2 and procaspase-3, and loss of Jun-N-terminal Kinase in bronchoalveolar lavage cells. Lungs exhibit total lack of TUNEL staining and Bcl-2 is highly exaggerated in the epithelioid macrophages of developing granulomas. Changes in Bcl-2 are associated with increased protein expression of NF κ B-p50, but not the p65 subunit. Moreover Bcl-3, which regulates Bcl-2 expression through interaction with NF κ B-p-50, shows strong nuclear localization. Thus, unlike acute silicosis, apoptosis is not a critical event in chronic silicosis, and NF κ B-p50/Bcl-3 interaction may play an important role in inducing the anti-apoptotic phenotype in chronic silicosis.

342 GENE EXPRESSION PROFILING OF XENOBIOTIC METABOLIZING ENZYMES (XMEs) IN THE AGING MALE FISHER RAT.

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Detoxification and elimination of xenobiotics is a major function of the liver and is important in maintaining the metabolic homeostasis of the organism. The degree to which aging affects hepatic metabolism is not known. The expression of XMEs, in part, determines the fate of the xenobiotic and whether exposure will result in toxicity. This project was designed to examine the changes in XMEs during the aging process in male Fisher rats. Gene expression profiles for XMEs in male Fisher rats (6, 11, 18, 24 months of age) were generated using Affymetrix Rat 230 2.0 arrays. Four animals per age group were profiled. Principal component analysis showed a clear age-dependent separation in expression profiles between 6 and 24 month hepatic transcripts. Differentially expressed genes (DEG) were identified using the following algorithm: background correction was performed using MAS5 followed by a quantile normalization, perfect match adjustment, median polish, Loess normalization and Cyber T statistics. 1135 genes were found to be significantly altered in a 24 versus 6 month comparison, and 155 genes were significantly altered in a 18 versus 6 month comparison. No significant gene changes were observed between 11 and 6 month old rats. In the 24 versus 6 month comparison, we found 23 phase I, 10 phase II, and 20 phase III metabolism genes significantly altered. In the 18 versus 6 month comparison, we found 3 phase I and 2 phase III metabolism genes significantly altered. qRT-PCR was performed to confirm altered expression. These data confirm an age-dependent change in XME gene expression in male Fisher rats. This information can be used to adapt pharmacokinetic models to reflect age-dependent differences in xenobiotic metabolism. (This abstract does not necessarily reflect US EPA policy).

343 EVALUATION OF PATHWAY-FOCUSED PCR ARRAY FOR EXPRESSION PROFILING OF DRUG METABOLIZING GENES.

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Side effects and toxicities of drugs are the main reasons for the termination of drug development and withdrawal of approved drugs. Drug metabolizing enzymes are an important battery of genes involved in drug-induced toxicities. In this study, the performance of the pathway-focused PCR arrays for expression profiling of 84 human drug metabolizing genes were evaluated for intra-, inter-laboratory and cross-platform comparability by using two reference RNA samples, Universal Human Reference RNA and Human Brain Reference RNA established in the MicroArray Quality Control project. For each of the two laboratories, the experiment was conducted in five technical replicates for each RNA sample and repeated

three months later. Therefore, data from 40 PCR arrays were generated. The reproducibility of PCR arrays was measured by the Pearson correlation coefficients of ΔCT and $\Delta\Delta CT$, and the overlap of differentially expressed genes (DEGs). The intra- and inter-laboratory correlation coefficients of ΔCT were 0.995 and 0.976, and the intra- and inter-laboratory correlation coefficients of $\Delta\Delta CT$ were 0.987 and 0.9744. The overlap between the two DEGs from the repeated experiments within a laboratory was about 90%, and over 90% of the DEGs generated in two laboratories were in common. The data from SYBR Green based PCR arrays were also compared with those from TaqMan assays generated in MAQC project, and there was a high level of concordance between PCR arrays and TaqMan. Finally, the PCR arrays were validated by observing the expected gene expression changes in biological samples resulting from dosing primary human hepatocytes with known drug metabolizing enzymes inducers. In conclusion, the high throughput PCR arrays can be used as a reliable tool for the quantification of expression levels of drug metabolizing genes.

344 TISSUE DISTRIBUTION, GENDER DIFFERENCE, ONTOGENY, AND CHEMICAL INDUCTION OF MULTIPLE DRUG RESISTANCE GENES (MDR1A, MDR1B, MDR2) IN MICE.

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Multi-drug resistance (Mdr) transporters are ABC transporters that efflux amphipathic cations from cells, and thus aids in protecting tissues from xenobiotics. Unfortunately, Mdr transporters also efflux cancer chemotherapeutic drugs from some tumor cells, resulting in drug resistance. There are two groups of Mdr genes in mice: group I includes Mdr1a and Mdr1b, which transport xenobiotics; whereas group II consists of Mdr2 only, which excretes phospholipid into bile. Little is known about the regulation of Mdr genes in vivo. Therefore the purpose of this study was to determine the regulation of three Mdr genes by tissue, age, gender, and chemicals. The data indicate that Mdr1a mRNA is mainly expressed in the gastrointestinal tract, Mdr1b mRNA is highest in ovary and placenta, and Mdr2 mRNA is mainly expressed in liver. Gender differences in expression of the three Mdr transporters were observed in several tissues: Mdr1a in kidney; Mdr1b in lung, kidney, and brain; and Mdr2 in lung. The ontogeny of mouse Mdr1a in duodenum and brain, as well as Mdr1b in brain, kidney, and liver, all share a similar pattern, which is low mRNA expression at birth, which gradually increases to mature levels at about 30 days of age. The developmental pattern of Mdr2 is different, in that there is a marked up-regulation at birth, a return to low levels by 5 days of age, and then an increase to mature levels with a similar pattern of the other transporters. None of the three Mdr mRNA levels were significantly up-regulated by all three chemicals in any class of selected microsomal enzyme inducers. Moreover, down-regulation was produced in Mdr1b by Nrf2 ligands, and in Mdr2 by AhR ligands. In conclusion, the expression of Mdr transporters in mice is tightly regulated by age and gender, the distribution of which indicates a tissue-specific pattern, and Mdr genes are not readily up-regulated by xenobiotics. (Supported by NIH grants ES-09649 and ES-09716)

345 HUMAN SULT2A1 IS TRANSCRIPTIONALLY REGULATED BY THE NUCLEAR RECEPTOR CONSTITUTIVE ANDROSTANE RECEPTOR (CAR).

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Sulfotransferase (SULT) catalyzed sulfation regulates hormone activity and detoxifies xenobiotic toxicants. Human dehydroepiandrosterone sulfotransferase (hSULT2A1) is a major SULT catalyzing the sulfation of hydroxysteroids and xenobiotic alcohols. In our previous studies, we have shown that the anti-folate drug methotrexate (MTX) can up-regulate several major human SULTs. To determine the mechanisms controlling the regulation of hSULT2A1, we cloned the 5'-flanking region of hSULT2A1 and investigated its transcriptional regulation using Caco-2 cell line based reporter gene assay. Nuclear receptor co-transfection indicates that CAR and RXR α were involved in the transcriptional regulation of hSULT2A1. RNA interference experiments further prove the positive role of CAR in hSULT2A1 regulation. Progressive promoter deletion, DNA sequence alignment, and site directed promoter deletion suggest that an imperfect inverted repeat DNA motif, IR2 (-186AGCTCAGATGACCC-173), within the hSULT2A1 5'-flanking region mediates the hSULT2A1 induction. Furthermore, electrophoretic mobility shift assay and super shift assay have been exploited to characterize the interactions of CAR and RXR α with the IR2 element. In summary, we identify an IR2 DNA cis-element located at -186/-173 of hSULT2A1 promoter region. The IR2 element mediates the MTX induction of hSULT2A1 by interacting with CAR and RXR α .

346 INDUCTION OF HUMAN SULFOTRANSFERASES BY ISOFLAVONES IN HEP G2 CELLS.

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Sulfotransferases (SULTs) are phase II drug-metabolizing enzymes. Their induction by hormones and endogenous molecules is relatively well known, although xenobiotic induction of SULTs has not been well studied. Isoflavones are naturally occurring phytoestrogens that mainly exist in soy food products. They have been described as health-promoting, disease-preventing dietary supplements, and have activity as cancer preventive agents. Recently, isoflavones have been reported interacting with nuclear receptors, including those that have been well known to mediate the induction of drug metabolizing enzymes. In the present investigation, isoflavones are shown to be xenobiotic inducers of human SULTs in transformed human liver (Hep G2) cells. Following isoflavone (Genistein, Biochanin A and Daidzein) treatments, two isoforms of SULTs were induced in Hep G2 cells. Enzyme assay, Western blot, reverse-transcription polymerase chain reaction (RT-PCR) and Real-Time RT-PCR results demonstrated that protein and mRNA expressions of human simple phenol sulfotransferase (P-PST, hSULT1A1) and human dehydroepiandrosterone sulfotransferase (DHEA-ST, hSULT2A1) were significantly induced in Hep G2 cells. Inductions of hSULT1A1 and hSULT2A1 in Hep G2 cells by these isoflavones were time- and dose- dependent. Enzyme activity and Western blot results were in good agreement with RT-PCR and Real-Time RT-PCR results, suggesting that the induction is at the gene transcription level. These isoflavones are the first nutritionally related phytoestrogens to show induction of SULTs.

347 INVESTIGATION OF NEW SULFUR DONORS FOR CYANIDE ANTAGONISM.

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This study focuses on developing effective cyanide (CN) antidotal systems with selected sulfur donors (SDs) having superior efficacy and pharmacokinetic characteristics to sodium thiosulfate (TS). TS is the standard antidote for CN intoxication. It is recognized that utilization of SDs that serve as substrates for sulfurtransferases (STase) (e.g. rhodanese, mercaptopyruvateSTase, TS reductase) is inefficient. Our approach is based on the idea of converting CN to the less toxic thiocyanate before it reaches the target organs.

Stealth liposomes and polymeric nanocapsules have been chosen to provide protective environments for the enzymes employed to destroy CN in the body. Sulfane sulfur containing molecules with three major sources a) endogenous (e.g. sulfur containing amino acids), b) naturally occurring plant components (e.g. allyl-, diallyl-, alkyl disulfides, and trisulfides; allylmercaptocysteine, ajoene), and c) synthetic sulfur donors designed from SAR studies have been chosen as SDs for present studies. The in vitro investigations, based on a) determining kinetic constants (KM and Vmax) for enzymatic reactions between the SDs and CN, b) cytochrome c oxidase inhibitions by CN in the presence of SDs with an STase and/or nitrites, and c) lipophilicity studies serve as tools to predict the in vivo efficacy. The in vivo efficacy studies are focused on determining LD50 values for CN and brain cytochrome c oxidase activity and inhibition.

Alkyl thiosulfonates (R-SO₂-S-) with longer alkyl chains showed greater lipophilicity, chemical reactivity, and in vivo efficacy than their methyl- and ethyl- counterparts. Butane thiosulfonate in combination with encapsulated rhodanese and sodium nitrite showed a 14 x LD50 protection against cyanide intoxication, while TS with sodium nitrite protect only 6 x LD50 on Balb/C mice.

Previous and ongoing studies show the potential usefulness of enzyme encapsulation in developing antidotal therapeutic agents.

348 THE REACTION KINETICS OF PARAOXONASE BOUND TO AN HPLC STATIONARY PHASE.

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The purpose of this project was to determine the enzyme kinetics of a purified organophosphorus acid anhydride (paraoxon hydrolase, E.C. 3.1.1.3). During this process, we developed a simple and more precise HPLC based method for determining enzyme kinetics at steady state. We then compared this HPLC method to the traditional spectrophotometric method for determining enzyme kinetics. First, the enzyme was eluted onto an anion exchange HPLC column under conditions that allowed the enzyme to bind reversibly. Enzyme substrate, paraoxon, in dilute aqueous solution was eluted past the bound enzyme at determined concentrations. The enzyme hydrolyzed the substrate to the products 4-nitrophenol and diethyl phosphate. The concentration of the 4-nitrophenol determined at 400 nm in the effluent was constant with constant flow. From these 4-nitrophenol concentrations

at different flow rates, the reaction rates for the bound enzyme were determined by multiplying the product concentration ($\mu\text{mol/mL}$) by the flow (mL/sec). The reaction rates determined by this method demonstrated Michaelis-Menten behavior (linear double reciprocal plot, correlation coefficient 0.97). The K_m of the reaction was $0.421 \pm 0.007 \text{ mM}^{-1}$. The V_{max} was $0.0585 \pm 0.004 \mu\text{mol/sec/mL}$. The values for these parameters determined by a traditional spectrophotometric method were similar but the HPLC method achieved a 10-fold increase in the precision of measurement. This method of enzyme kinetic analysis offers the benefits of a true reaction steady state (change in concentration of the reactive intermediate is 0 at constant flow), the elimination of product inhibition (product is carried away from the enzyme by the effluent), rapid analysis times and the potential for simulating many different enzyme environments not obtainable in stirred solutions.

349 EXAMINATION OF THE PROPOSED "SIDE DOOR" IN THE XENOBIOTIC METABOLIZING ENZYME CARBOXYL ESTERASE.

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Carboxylesterases (CEs) are involved in the metabolism of a wide range of endobiotics and xenobiotics containing ester bonds, including several classes of pesticides. Recently, the structure of a mammalian CE suggested that a side door was located adjacent to the active site. The side door has been proposed to act as an entrance/exit pore for substrates and products, with four amino acid residues identified as "gate keepers" to this side door. The purpose of this study was to investigate the role of one of these residues, leucine(L) 362, in the CE-catalyzed hydrolysis of several esterified substrates. Recombinant CE enzymes (wild-type and L362 site-specific mutants) were purified and tested for their hydrolytic activity toward simple esters and both pyrethroid and organophosphate insecticides. Mutation of position L362 into various residues decreased k_{cat} values by 1.5–3-fold compared to wild type when the kinetics of 4-nitrophenyl valerate hydrolysis was examined. In contrast, the same L362 mutants enhanced the hydrolysis rate of 4-methylumbelliferyl acetate. In this instance, the mutant enzymes exhibited k_{cat} values that were increased by 1.2–2-fold compared to wild type, which suggested that the mutant proteins were correctly folded. All mutant CEs exhibited decreased k_{cat} values (between 3.2- and 7-fold) when hydrolysis of the pyrethroid insecticide *trans*-permethrin was examined. CEs covalently react with organophosphate oxons and are inhibited by these compounds. Reactions of enzymes with chlorpyrifos oxon demonstrated that mutant enzymes were less sensitive to inhibition compared to wild type, as indicated by 3–5-fold increases in the half-maximal inhibitory concentration (IC_{50}) values. Taken together, our results demonstrate that the side door residues of CEs can influence the rate of catalysis and degree of inhibition by interactions that are dependent upon the charge of the residue at position 362 and the structure of the substrate. [Supported by NIH P20RR017661]

350 EVALUATION OF THE CARBOXYL ESTERASE PHENOTYPE IN HUMAN LIVER.

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Carboxylesterases (CEs) of the serine hydrolase superfamily are xenobiotic metabolizing enzymes that hydrolyze esterified substrates to alcohol and carboxylic acid products. Two CE isoforms are expressed in human liver and are termed hCE1 and hCE2. Human liver microsomes (HLMs) are often used in reaction phenotyping studies to discern interindividual variability in xenobiotic metabolism. We have begun studies to evaluate the 'CE phenotype' of human liver samples using traditional western blotting and hydrolytic assays to ascertain both the levels of hCE1 and hCE2 protein and their hydrolytic activities. We have further extended these studies using an 'activity-based' fluorophosphonate fluorescent probe (FP-TMR), which covalently reacts with catalytically-active serine residues of serine hydrolases but not with inactive enzymes. Quantitative immunoblotting of pooled HLMs using antibodies raised against hCE1 or hCE2 demonstrated that the amount of hCE1 protein expressed in liver was far more abundant than hCE2 protein (46-fold, $64.4 \pm 16.5 \mu\text{g}$ hCE1/mg microsomal protein compared to $1.4 \pm 0.2 \mu\text{g}$ hCE2/mg microsomal protein). Moreover, the FP-TMR probe robustly labeled the hCE1 proteins in HLMs, while hCE2 proteins were labeled weakly. The hCE1 enzyme was by far the most active serine hydrolase labeled in the HLMs studied. Furthermore, differences were observed in the extent of hCE1-specific labeling by FP-TMR between individual HLM samples; however, the differential labeling by FP-TMR appeared to be specific to hCE1 since another robustly active serine hydrolase in the same HLMs (arylacetamide deacetylase) exhibited a similar degree of labeling in all individuals. We are currently identifying the cause of the differential hCE1 activities among the individual HLMs. Identification of the factors that lead

to altered CE activities in HLMs will be important to characterize since several therapeutic drugs and toxicants are metabolized by these enzymes. [Supported by NIH P20RR017661]

351 DOSE-DEPENDENT PANCREATIC AND HEPATIC INJURY BY ETHANOL IN HEPATIC ALCOHOL DEHYDROGENASE-DEFICIENT DEER MICE.

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Alcoholic pancreatitis and liver disease are the two major health problems associated with chronic alcohol abuse and cause high morbidity and mortality among alcoholic patients. The mechanisms of both the diseases are not well understood. In previous study, we found a greater pancreatic and hepatic injury in hepatic ADH-deficient (ADH-) vs. hepatic ADH-normal (ADH+) deer mice fed 4% ethanol in Lieber-DeCarli liquid diet. Therefore, present study was undertaken to evaluate the dose-dependent ethanol-induced pancreatic and hepatic injury in ADH- and ADH+ deer mice fed 1, 2 and 3.5% ethanol in Lieber-DeCarli liquid diet for 2 months. Control animals of both strains were provided an isocaloric liquid diet. A total of 6/22 animals died in the ADH- deer mice vs. none in ADH+ deer mice fed 3.5% ethanol during the 2 months of ethanol exposure. However, no deaths occurred in groups of deer mice fed 1 and 2% ethanol. All the animals were sacrificed on day 60 and toxicity and histological changes for pancreata and livers were evaluated. Dose-dependent increases in hepatic and pancreatic injury markers were observed in both strains, but the increases were significantly higher in ethanol-fed ADH- vs. ADH+ deer mice. Similarly, histological and electron microscopic changes such as vacuolization, apoptosis and endoplasmic swelling were more prominent in pancreata and livers of ADH- vs. ADH+ deer mice fed 3.5% ethanol. Overall, the pancreatic and hepatic toxicity of ethanol in the ADH- deer mice appears to be dose-dependent and indicates the role of ethanol metabolism in ethanol-induced pancreatic and hepatic pathologies. This work was supported by NIH grant AA13171.

352 DOSE-DEPENDENT METABOLISM OF ETHANOL AND RELATED PANCREATIC INJURY IN HEPATIC ALCOHOL DEHYDROGENASE-DEFICIENT DEER MICE: SIGNIFICANCE OF FATTY ACID ETHYL ESTERS.

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Impaired hepatic alcohol-dehydrogenase (ADH) is an important metabolic condition during chronic alcohol abuse that facilitates nonoxidative metabolism of ethanol to fatty acid ethyl esters (FAEEs). Inhibition of hepatic ADH by 4-methyl pyrazole is reported to induce nonoxidative metabolism of ethanol to FAEEs and cause pancreatitis-like injury in rat model. Pancreatic injury and cytotoxic effects of FAEEs are also reported in vitro and in vivo. However, events leading from early pancreatic injury to pancreatitis are not well known. In the present study, we studied a dose-dependent biosynthesis of FAEEs in the pancreata of hepatic ADH-deficient (ADH-) deer mice and compared with that in the pancreata of hepatic ADH-normal (ADH+) deer mice fed Lieber-DeCarli liquid diet containing 1, 2 and 3.5% ethanol for 2 months. Individual and total pancreatic FAEEs analyzed in the pancreata of both strains of deer mice fed 1 and 2% ethanol in the liquid diet showed negligible or trace amount of individual and total FAEEs. However, the levels were significantly high in the pancreata of ADH- vs. ADH+ deer mice fed 3.5% ethanol. Ethyl esters of oleic (18:1), linoleic (18:2) and arachidonic (20:4) fatty acids were very prominent and significantly high in the pancreata of ADH- deer mice fed 3.5% ethanol. The pancreatic injury assessed by measuring trypsinogen activation peptide and histology was also observed only in the 3.5% ethanol-fed ADH+ deer mice. These results suggest a dose-dependent formation of FAEEs and 18:1, 18:2 and 20:4 FAEEs could be reliable markers of pancreatic injury and alcohol exposure in chronic alcohol abusers. This work was supported by NIH grant AA13171.

353 EFFECT OF CAFFEINE AND ALCOHOL ON THE TOXICITY AND METABOLISM OF METHACRYLONITRILE (MEAN) IN MALE SPRAGUE-DAWLEY RATS.

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We have studied the relationship between the toxicity of MeAN and its metabolism to cyanide using normal rats and those pre-treated with caffeine or alcohol or both. One group of experimental rats received an oral dose of 6 % MeAN solution in corn oil (equivalent to 0.5 LD50). Other three groups of rats were pre-treated with alcohol (2 ml of 50 % solution in water), caffeine (1 ml of 2 % solution in water) or both alcohol and caffeine twelve hours before receiving MeAN dose. The rats

were closely observed for known signs of toxicity of nitriles. Six and twelve hours after the treatments the rats were sacrificed under ether anesthesia for collection of blood, urine, liver, kidney and brain. The concentrations of cyanide, thiocyanate and glutathione (GSH) were determined in blood, liver, kidney and brain. In the rats treated with MeAN alone and those pre-treated with alcohol and alcohol + caffeine the GSH concentrations decreased in liver, brain and kidney by 23-36 %. In the rats pre-treated with caffeine alone the concentrations of GSH were not significantly different from controls. In the rats treated with MeAN alone and those pre-treated with alcohol and alcohol + caffeine the cyanide and thiocyanate concentrations increased in the blood and other organs up to 2-4 fold whereas in rats pre-treated with caffeine alone the concentrations of cyanide and thiocyanate were not significantly different from controls. These results suggest that caffeine inhibited and alcohol enhanced metabolism of MeAN resulting in a significant decrease in organ GSH and increase in cyanide concentration in blood and organs. When rats were pre-treated with both caffeine and alcohol, caffeine could not provide this protection. The opposite effects of caffeine and alcohol may be through opposite modulation of microsomal activity. (Supported by NIH South Texas Doctoral Bridge Program Grant # 2 R25GM50080).

354 GENDER AND SPECIES DIFFERENCES IN DNA DAMAGE PRODUCED BY THE CARCINOGEN 2-ACETYLAMINOFLUORENE IN RAT LIVER AND CULTURED RAT HEPATOCYTES AND IN CULTURED HUMAN HEPATOCYTES.

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The potent DNA-reactive hepatocarcinogen 2-acetylaminofluorene (AAF) is more carcinogenic to the livers of male rats than female rats of several strains. To elucidate further the basis for rat gender differences, DNA reactivity of AAF was studied in livers and cultured hepatocytes of both genders of Sprague-Dawley rats and cultured human hepatocytes of both genders. Rats were administered oral doses of AAF at 3 mg/kg for up to 8 weeks. This dose produces liver neoplasms in male F344 rats by 36 weeks, when given 3 times per week for 8 weeks. The levels of adducts were 25.3 ± 1.54 in 10^8 normal nucleotides in males and 4.3 ± 1.4 in 10^8 in females, comprising 3-(deoxyguanosin-*N*²-yl)-2-acetylaminofluorene and *N*-(deoxyguanosin-8-yl)-2-aminofluorene together with other minor adducts. In cultured rat hepatocytes exposed to AAF at a concentration of about 100 μ M, the levels of DNA adducts formed in male hepatocytes were 14 times greater than that in female hepatocytes and the level of DNA repair synthesis was greater. In contrast, in human hepatocytes of both genders, AAF at the same concentration, produced similar levels of adducts and DNA repair synthesis, which were intermediate between those of male and female rats. Thus, the greater susceptibility of male rats to AAF-induced liver neoplasia is due at least in part to greater levels of genotoxicity. Moreover, it would appear that livers of both human genders are not as susceptible as those of male SD rats to the formation of AAF DNA adducts and hence cancer susceptibility to a carcinogen of this type would be anticipated to be lesser.

355 COMBUSTION-DERIVED PAHS CONCENTRATE IN LIPID DROPLETS AND UPREGULATE BIOTRANSFORMATION ENZYMES IN HUMAN RESPIRATORY EPITHELIAL CELLS.

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Cytoplasmic lipid droplets sequester lipids for subsequent cell activities (membrane synthesis and eicosanoid production). Here we report that these droplets concentrate hydrophobic components from combustion-derived ultrafine particles and this coincides with up-regulation of AhR-associated genes. Butadiene soot (BDS) generated from the combustion of 1,3-butadiene is a model mixture and a real-life example of a petrochemical product of incomplete combustion. BDS is a metals-poor, organic-rich mixture of carbonaceous particles to which numerous polynuclear aromatic hydrocarbon (PAH) species are adsorbed. We previously described the characteristics of BDS (EHP, 109: 965-71, 2001; *ibid*, 113: 956-63, 2005): 1) Fresh BDS particles are in the ultrafine respirable range (PM 0.1); 2) A wide range of PAHs, including several carcinogens, is adsorbed to the surface of the particles; 3) Cultured human bronchial epithelial cells (BEAS-2B) exposed to BDS exhibit a similar spectrum of PAHs as the soot particles. Cells exposed to BDS in culture display a time- and concentration-dependent fluorescence pattern that begins as diffuse blue fluorescence and progresses to punctate, perinuclear fluorescence. Here, we show that the punctate blue BDS-associated fluorescence and a red fluorescent cholesterol analog, cholesteryl-BODIPY, co-localize in BEAS-2B cells. Deconvolution of fluorescence microscopy images of BDS-exposed cells transfected with a lipid droplet surface protein construct (pDsRed-ADFP) confirmed this co-localization. Cultured adipocytes exposed to BDS develop blue fluorescence in their large lipid droplets, further supporting the localization of fluorescent PAHs to

lipid stores within cells. Quantitative RT-PCR results revealed a time-dependent upregulation of Phase I biotransformation enzymes (CYP1A1, CYP1B1 and ALDH3A1) secondary to PAH exposure. TCDD-inducible poly(ADP-ribose) polymerase (TIPARP) and plasminogen activator inhibitor 2 (PAI-2 or SERPINB2) also were up-regulated.

356 MONO-FUNCTIONAL MUSTARDS FORM FREE RADICALS WHEN ENZYMATICALLY REDUCED MAKING THEM USEFUL AS MODEL COMPOUNDS FOR MECHANISTIC STUDY AND DRUG SCREENING FOR SULFUR MUSTARD.

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During the past twenty years it has been the prevailing opinion among many who study sulfur mustard (2,2'-bis-chloroethyl sulfide) toxicity that tissue damage arises from the alkylation and cross linking of chromatin in affected tissues driven by sulfonium ion formation. That theory led to the conclusion that monofunctional mustards such as mechlorethamine (HN1), chloroethylthyl sulfide (CEES) and chloroethylmethyl sulfide (CEMS) could not act via the same mechanism as sulfur mustard because they lacked the capacity to cross link DNA and, therefore, could not serve as model compounds for the study of sulfur mustard toxicity. Current work from our laboratory, however, indicates that the toxicity of mustards is a consequence of free radicals arising from enzymatic reduction of the immonium and sulfonium ions which form as intermediates in the hydrolysis of nitrogen and sulfur mustards, respectively. Electron paramagnetic resonance (EPR) spectroscopy using a Bruker ESP 300E instrument can detect these free radicals in an *in vitro* system consisting of 2.4 mM NADPH, up to 4.0 mM sulfur mustard, CEES, or HN1, a 1.03 M concentration of the spin trap α -(4-pyridyl-1-oxide)-N-t-butyl nitron, and 0.016 μ M human NADPH cytochrome P450 reductase in 490 μ L 0.1 M potassium phosphate buffer, pH 7.5 containing 250 mM NaCl in an EPR flat cell with a capacity of 0.5 mL. The work presented here indicates that monofunctional mustards are also activated to free radicals by enzymatic reduction. As such they offer an excellent model to study the toxic mechanism of sulfur mustard and the usefulness of potentially therapeutic and prophylactic compounds without use of the chemical warfare agent itself. Some variance between the monofunctional compounds and the behavior of sulfur mustard may arise from slight differences in solubility.

357 EFFECTS OF DAIDZEIN ON TCDD-INDUCED IMMUNOSUPPRESSION IN MURINE SPLENOCYTES IN VITRO.

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Phytochemicals have various pharmacological properties including antioxidant activity, anticancer, and immunomodulatory effects. In this study, we evaluated the immunomodulatory effects of 13 phytochemicals, such as daidzein, genistein, formononetin, biochanin A, kaempferol, galangin, quercetin, myricetin, chrysin, baicalein, luteolin, naringenin and hesperetin, on 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced immunosuppression in splenocyte cultures from female BALB/c mice *in vitro*. Daidzein, kaempferol, galangin, quercetin, myricetin and luteolin recovered TCDD-induced inhibition of lymphoproliferative response by LPS. In contrast, only daidzein recovered TCDD-induced suppression of proliferative response by Con A. Moreover, the number of intracellular CD4⁺IL-2⁺ cell and IL-2 mRNA expression reduced by TCDD were recovered by daidzein. Taken together, the present results indicated that daidzein might interact with the Ah receptor in TCDD-induced immunosuppression. (Supported by a grant from KOSEF and a BK 21 grant)

358 STUDY ON DOXORUBICIN-INDUCED CARDIOTOXICITY IN RATS USING UPLC-MS-BASED METABONOMICS.

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The use of Doxorubicin (DOX) as anticancer drug is limited by its cardiotoxicity. Until now the precise mechanisms of DOX-induced cardiotoxicity is not entirely clear. Metabonomics, the study of metabolites and their roles in various disease states, is a novel methodology arising from the post-genomics era. The present study investigated the cardiotoxic effects of DOX in male Wistar rats by using

UPLC/MS-based metabolomics of urine, as well as clinical chemistry and heart histopathology. Through single intraperitoneal injection, DOX is administered to the rats at the doses of 5mg/kg, 10mg/kg and 20mg/kg respectively, and the rats in control group are treated with equal volume of saline. Urine samples were collected from 0 to 4 days after treatment. Results of clinical chemistry and histopathology examinations prove the appearance of cardiotoxic effects with a dose-time-dependant manner. After the UPLC analysis, the retention time and m/z data pair for each peak was detected. Partial least squares discriminant analysis (PLS-DA) and coefficient of correlation analysis were used for marker selection and identification. The scatter for different groups is centralized in different places and is related to the administered dose. One day after administration, the changes of the metabolite spectrum of the rats treated with DOX have appear, which are correlative with the rat's weight, changes of clinical chemistry and histopathology. With the dose and time increased, such contents in the urine get augmented as uric acid, tryptophan, phenylalanine and bile acid, at the same time the hippuric acid and glucuronate conjugates are decreased. Based on above results, these metabolites may be involved in DOX-induced cardiotoxicity, but the role of them need more research. Metabonomics proves more sensitive than traditional method for toxicity evaluation, and thus can be applied to the selection of drug toxicity and early diagnoses of disease. Supported by China Natural Science Foundation grant 30572281, 30371705.

359 BEAT-TO-BEAT QT VARIABILITY CORRELATES WITH PRO-ARRHYTHMIC RISK IN CONSCIOUS PRIMATES.

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The appearance of the ICH S7B guideline, requiring the investigation of arrhythmogenic potential of candidate drugs prior to the initiation of clinical studies, has highlighted the need for improved predictors of pro-arrhythmic potential other than QT prolongation. Recent approaches to identify the risk of arrhythmia have been proposed based on parameters to quantify beat-to-beat QT variability (BBQTV) and the correlation of these parameters with the induction of arrhythmia has been examined in anesthetised dog preparations (Thomsen et al, 2004; Van der Linde et al, 2005). In the present study we have evaluated this approach in a conscious telemetered monkey study, using astemizole (AST) as a model pro-arrhythmic drug. Six female cynomolgus monkeys implanted with telemetry transmitters were orally dosed with either astemizole (30 mg/kg) or the vehicle (methylcellulose) and ECG signals monitored from 1h before to 12h after dosing. QTc (Bazett) was estimated from the QT and RR intervals. Estimates of BBQTV were provided by Short Term Instability (STI) Long Term Instability (STI) and Total Instability (STI) (Van der Linde et al, 2005). The frequency and severity of arrhythmia induction was graded for each animal. Treatment with Astemizole resulted in QTc prolongation and the occurrence of large U waves in all animals and arrhythmic events were detected in two animals. Increases in values for STI, LTI and TI correlated with the severity of arrhythmia observed and these parameters returned to the normal range after recovery from the arrhythmic periods. Our results demonstrate a good correlation between BBQTV and pro-arrhythmic potential in monkeys treated with AST. Further studies are warranted with different classes of pro-arrhythmic and pure QT prolonging drugs. Furthermore, data is also required on the sensitivity of these markers at lower dose-levels, where arrhythmias are not observed, in order to evaluate the predictive potential of this approach in safety studies.

360 THE CARDIOVASCULAR HEMODYNAMICS OF THE ACUTE TOXICITY OF RECOMBINANT HUMAN ACID SPHINGOMYELINASE ADMINISTERED TO ACID SPHINGOMYELINASE KNOCKOUT MICE AND THE MODIFICATION OF THIS TOXICITY WITH THE ADMINISTRATION OF L-N6-(IMINOETHYL) LYSINE-5-TETRAZOLE-AMIDE.

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Acute toxicity of recombinant human acid sphingomyelinase (rhASM) in acid sphingomyelinase knock-out (ASMKO) mice occurs following a 20 mg/kg dose of rhASM. Several studies designed to establish cardiovascular biomarkers/ mechanism of toxicity for use in clinical trials, have observed dose responsive changes in heart rate, blood pressure and activity.

We have designed studies to provide supplementary analysis of cardiovascular hemodynamics and have noted significant changes in the P-R and Q-T interval on the electrocardiograph (ECG) and increases in nitric oxide (NO) production, following a 20 mg/kg dose of rhASM, but not a 10 mg/kg dose of rhASM. NO is a key homeostatic biological messenger in the cardiovascular system. Under normal conditions, NO mediates vasodilatation and therefore normal heart rate/blood

pressure parameters. To investigate the possible role of NO, in the observed changes in cardiovascular hemodynamics, L-N6-(Iminoethyl) Lysine-5-Tetrazole-Amide (L-Nil), a selective interstitial nitric oxide synthase (iNOS) inhibitor, was co administered with 20 mg/kg rhASM. Under the conditions of the studies, L-Nil modified observations of toxic lethargy.

Changes in cardiac hemodynamics, specifically blood pressure, are paramount as the mechanism of action is unclear. Further characterization of the role of NO and specifically, iNOS in the modification of toxicity following a 20 mg/kg dose of rhASM, is underway.

361 EMBRYONIC ZEBRAFISH EXPOSED TO 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN EXHIBIT DEFECTS IN CARDIAC VALVE FORMATION AND FUNCTION.

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Zebrafish embryos develop cardiac valves at three sites: the sinus venosus (SV) valve at the inflow to the heart; the atrioventricular (AV) valve between the atrium and the ventricle, and the bulbo-ventricular (BV) valve at the outflow. The valves originate from endocardial cells that migrate to form an endocardial ring and prevent blood regurgitation at presumptive valve locations at approximately 48 hours post fertilization (hpf). These endocardial cells then undergo a process termed epithelial to mesenchymal transformation [60-72 hpf] in which the endothelial cells delaminate into the cardiac jelly to form cardiac cushions [96 hpf]. The cushions are subsequently remodeled to form valve leaflets [120-168 hpf]. High-speed video microscopy showed that zebrafish embryos exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) develop blood cell regurgitation at the nascent valve sites [72 hpf], suggesting that TCDD induces a defect in cardiac valve formation or function. GFP labeled vascular endothelial cells showed that TCDD (1 ppb) disrupts the normal endothelial cell congregation at the presumptive AV valve site. Although TCDD did not prevent the initial formation of the ring of endothelial cells [48 hpf], the ring lost spatial restriction and became progressively diffused in TCDD exposed hearts. Similar results were observed at the BV valve. In-situ hybridization using AV valve-specific markers *bmp4* and *notch1b* indicates that valve specification was not initially prevented by TCDD at early time points [48 hpf]. However, these markers became significantly less restricted in pattern as heart development progressed in TCDD exposed embryos [72 hpf]. Confocal microscopy of TCDD exposed larvae showed no evidence of cushion formation or AV valve leaflets. This is the first report of TCDD affecting cardiac valve formation and function in a vertebrate species. (Supported by NIH RO1 ES012716 and UW Sea Grants R/BT17, R/BT20 and R/BT22).

362 BETA-NAPHTHOFILAVONE INDUCED INHIBITION OF BETA 2-ADRENERGIC RECEPTOR PROMOTER DRIVEN LUCIFERASE ACTIVITY IS NOT MEDIATED VIA PUTATIVE DIOXIN RESPONSE ELEMENTS.

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Beta-adrenergic receptor (β -AR) signaling plays an important role in several cardiovascular system processes that are disrupted by TCDD exposure. We hypothesized that TCDD directly alters β -AR gene expression via putative dioxin response elements (DREs) that are located in the 5' enhancer regions of the β_1 - and β_2 -AR genes. Electromobility shift assays (EMSAs) were performed using [³²P]-labeled hCYP1A1 (positive control) or h β_1 - or h β_2 -AR oligos incubated with *in vitro* translated hAhR and hARNT proteins in the presence or absence of cold competitor oligos, with or without β NF. EMSA data indicate that AhR and ARNT do not bind the five putative DREs of the h β_1 -AR gene, however, strong binding was observed with one of the three putative DREs of the h β_2 -AR gene. Luciferase reporter activities of a positive control vector, hCYP1A1 DRE luciferase construct, pGudLuc6.1 (generous gift from M. Denison, UC-Davis), and of a pGL3-Basic vector (Promega, Madison, WI) containing a 660 nucleotide piece of the h β_2 -AR promoter were determined in transiently transfected HepG2 cells and were normalized to protein concentrations and to renilla transfection control vector expression levels. As expected, exposure to 5 or 10 μ M β NF for 24 hrs increased pGudLuc6.1 vector's luciferase activity 7- or 20-fold over vehicle treatment alone. In contrast, β NF exposure for 8 hrs decreased β_2 -AR promoter driven luciferase activity 30-fold below vehicle treatment levels. Promoter constructs containing a single point mutation in the one putative β_2 -AR DRE that exhibited AhR/ARNT binding in EMSAs; or in all three of the putative β_2 -AR DREs also exhibited similar magnitude decreases. Thus, the β NF-induced inhibition of β_2 -AR promoter driven luciferase activity does not appear to be directly mediated by AhR and ARNT at the

putative DREs of the β_2 -AR gene. The inhibition is, however, specific to β -AR promoter constructs and is not due to overt toxicity. Supported by NIH R15 ES011806 and RR16463-04.

363 MICRO-ELECTRODE ARRAY CHARACTERIZATION OF PHARMACOLOGICAL RESPONSES OF CRYOPRESERVED NEONATAL RAT CARDIOMYOCYTES IN CULTURE: CARDIAC CELL CHIP

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Primary cells in culture are an important tool for investigating physiological mechanisms and responses to pharmaceuticals or toxicants. Cryopreservation of large batches of dissociated embryonic cells has proven to be a valid and convenient substitute for fresh dissections as a source of cells for primary neuronal cultures of cortex, hippocampus, striatum and dorsal root ganglia. We sought to expand on previous reports that benefits of convenience, animals care concerns and economies of scale could be derived from the cryopreservation of neonatal rat cardiomyocytes. Using our method for isolation and cryopreservation of cardiomyocytes we obtained rat cardiomyocytes that could be subsequently thawed and plated in a variety of multi-well formats and in MultiElectrode Array (MEA) chambers. At intervals from 1 to 5 weeks cultures were fixed and immunostained for alpha-actinin and connexin 43 (Cx43). MEA recordings were made at comparable time points. In some experiments cultures were treated with drugs directed toward gap junction coupling. The effect on Cx43 immunofluorescence and the time of coupling events in the MEA were observed. The cardiomyocytes were observed to beat and to show electrical activity 24 to 48 hours after plating. Correlation of activity across individual electrode points of the MEA and Cx43 immunofluorescence indicated formation of an electrical syncytium. Treatment with isoproterenol and carbachol caused predictable alterations in excitation-contraction. These data show that cryopreserved neonatal cardiomyocytes when thawed and cultured, maintain the important characteristics of freshly cultured cardiomyocytes.

364 IN VIVO ASSESSMENT OF TEMPORAL AND TRANSMURAL VARIABILITY OF CARDIAC REPOLARISATION AS A TOOL FOR EVALUATING PRO-ARRHYTHMIC RISK

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Drug-induced prolongation of the QT interval of the ECG, in laboratory animals and humans, is generally considered as indicative of a pro-arrhythmic risk. However there is now evidence that the prolongation of QT interval as such is not an accurate predictor of this risk and that other parameters of cardiac repolarisation are more reliable. In particular instability of action potential duration and increase in transmural heterogeneity of myocardial repolarisation are considered as key parameters. The former can be evaluated as changes in beat-to-beat variability of the QT interval and the latter can be evaluated by changes in T wave morphology. This study was aimed at testing these changes in dogs treated orally with the I_{K_r} blocker astemizole (AST: 3 to 15 mg/kg), the adrenergic agonist isoproterenol (ISO: 2.5 to 10 μ g/kg) or in hypokalemic conditions (hypoK⁺: plasma K⁺ = 3.1 to 3.5 mmol/L) induced by furosemide.

Beat-to-beat variability of QT, was evaluated by 2 formulas: the coefficient of variation of QT as $CVQT = SD_{QT}/mean_{QT}$ and the formula of QT temporal dispersion described in the literature and used in clinical investigations: $QTdt = \log_{10}(CVQT/CVRR)^2$. QTdt formula was found inappropriate for dogs in which the sinus arrhythmia is generally marked and therefore QTdt reflects more the change in RR than in QT variability. In contrast, CVQT showed that AST increased the beat-to-beat variability of QT.

The T wave recorded in CV5RL lead, which is positive and monophasic in untreated healthy animals, was notched after treatment with AST or ISO and in hypoK⁺, which all modify transmural heterogeneity of repolarisation. Notching was characterized by a second peak in the T wave. In hypoK⁺, the changes were most marked, with occasional biphasic or inverted T wave, in addition to various degrees of notching.

In conclusion, we have established a method for in vivo assessment of transmural and beat-to-beat variability of cardiac repolarisation, which may help in evaluating the pro-arrhythmic risk of drugs.

365 SEX DIFFERENCE IN ELECTROPHYSIOLOGICAL PROPERTIES OF CYNOMOLGUS MONKEY PAPILLARY MUSCLES.

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It's reported that women have longer QTc intervals than men and are at greater risk of associated arrhythmia, such as drug-induced torsades de pointes (TdP). Female gender is also a risk factor for the occurrence of syncope and sudden death in congenital long QT syndrome. The purpose of this study was to investigate whether there are sex differences in action potential (AP) repolarization and responses to proarrhythmic drugs in cynomolgus monkey papillary muscles. Ion channel expressions in cynomolgus monkey left ventricles were evaluated to elucidate the mechanisms of sex differences. [Methods] AP in cynomolgus monkey papillary muscles was measured using the microelectrode recording technique. Ion channel protein (KvLQT1, ERG and Cav1.2) expressions were evaluated by Western blot technique. [Results] APD90 in female cynomolgus monkey papillary muscles was approximately 15% longer than that in males. At stimulation of 0.2 Hz, but not at 1.0 Hz, E-4031 at 1 μ M elicited a significantly higher increase in APD90, incidence of early after depolarizations (EADs) and triggered activities (TAs) in papillary muscles from females compared with those from males. In low potassium conditions at stimulation of 0.2 Hz, EADs and TAs were induced at a lower concentration of E-4031 in papillary muscles from females compared with those from males. KvLQT1 and Cav1.2 expressions were higher in female left ventricles than those of males. [Conclusion] In the present study, we provide experimental evidence suggesting sex differences in cynomolgus monkey AP repolarization, and this might be attributable to ion channel protein expression differences. Our electrophysiological findings are consistent with the clinical observations that women have longer QTc intervals and that female gender is a risk factor for drug-induced TdP. Therefore, papillary muscles from the female cynomolgus monkey could be a useful test model for safety pharmacological assessment in evaluating potential proarrhythmic events.

366 CARDIOPULMONARY EFFECTS OF INHALED OZONE AND PARTICULATE MATTER IN TWO MOUSE STRAINS.

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Ozone, a potent oxidant gas with established pulmonary toxicity is a principal air pollutant that coexists with particulate matter (PM) in urban ambient air. While human and animal studies are replete with associations between PM exposure and adverse pulmonary and cardiovascular events, ozone has received little attention outside the realm of pulmonary toxicity. We tested the hypothesis that ozone has a potent cardiopulmonary effect and that variation in interindividual responses occur with specific air pollutants. After implanting radiometers to measure heart rate (HR), we exposed two different mouse strains (i.e. C3H/HeJ, C3 [n=3] and C57Bl/6J, B6 [n=3]) to 3 different daily exposure protocols for 3 consecutive days each. The order of the exposure protocols was as such: 1) 2 hrs of filtered air (FA) followed by 3 hrs of FA; 2) 2 hrs of FA followed by 3 hrs of carbon black (CB, CMD = 0.7 μ m, MMAD = 1.01 μ m); and 3) 2 hrs of ozone (O3, 550 ppb) followed by 3 hrs of CB. We observed predictable strain differences in HR following 2 hrs of FA between C3 and B6 mice (507 $\pm$ 27 vs. 406 $\pm$ 11 bpm). We also observed a notable decline in HR after O3 exposure in both C3 and B6 mice (313 $\pm$ 17 vs. 292 $\pm$ 13 bpm). The O3-induced HR response in C3 mice appeared to be more persistent over time compared with that in B6 mice. Selected heart rate variability (HRV) parameters in the time domain were also altered with O3 exposure. The O3-induced HRV changes are associated with altered parasympathetic activity in regulating HR. Using whole-body plethysmography, we observed striking strain differences in the O3-induced ventilatory response. The C3 mice responded by increasing respiratory rate following ozone exposure while the B6 mice showed no significant changes in respiratory rate when compared to the FA exposure. Further analyses and future studies will focus on the adverse mechanisms of O3 and PM exposure on cardiopulmonary function. Moreover, we aim to elucidate genetic factors that may modify individual responses and susceptibility to these air pollutants.

367 SUBCHRONIC EFFECTS OF ENVIRONMENTAL TOBACCO SMOKE (ETS) EXPOSURE ON CARDIAC FUNCTIONS IN APOE^{-/-} MICE.

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ETS exposure has been associated with increased cardiac morbidity and premature mortality. However, cumulative effects of long-term ETS exposure have yet to be fully explored and systematically monitored. This subchronic study therefore evaluated alterations in cardiac functions in ApoE^{-/-} mice during a 6-mo series of daily 6-hr side stream smoke (SS) exposures.

Thirty-two ApoE^{-/-} mice, fed with either high-fat chow (HFC) or normal chow (NC), were exposed to SS (a surrogate for ETS, avg. 450 µg/m³) or filtered air, 6 hours per day, 5 days per week for 6 months. Electrocardiogram (ECG), Heart Rate (HR), Deep Body Temperature, and Activity Counts data were constantly sampled for 35 seconds/5 minutes throughout the experiment using implanted DSI telemetry transmitters (TA10ETA-F20). Heart Rate Variability (HRV) analyses, and methodologies for assessing cardiac vulnerability and mortality, were conducted using conventional methods (SDNN, standard deviation of RR intervals; RMSSD, root mean square of successive RR-differences; PSD, power spectral analysis.) and a new method that was developed in our laboratory.

The results showed that: (1) HR was significantly reduced in both high-fat and normal chow mice over the 75 days of SS exposure; and (2) the effects of SS exposure varied across different time segments of the day. Although no significant HRV alterations were found in NC-fed mice exposed to SS, the HFC-fed mice exposed to SS had greater acceleration in HRV reduction than those in the NC-fed control group.

Our results showed that subchronic SS exposures at concentrations encountered in human environments, could produce abnormal cardiac functions in ApoE^{-/-} mice. Our results provide additional information for elucidating pathophysiologic pathways between ETS exposure and cardiac diseases.

368 ACUTE CARDIOVASCULAR EFFECTS OF NICKEL AEROSOL IN APOE^{-/-} MICE.

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Although exposure to fine ambient particulate matter (FPM) is known to be associated with excess daily mortality and morbidity, the major component responsible for these effects remain unknown. Our recent study using a mouse model of atherosclerosis (ApoE^{-/-}) exposed to concentrated (10X) ambient air FPM (CAPs) for 6hr/d, 5d/w, for 6 mo showed that Ni concentration was significantly associated with acute changes in heart rate (HR) and HR variability (HRV). The objective of this pilot study was to investigate if a similar pattern of acute changes in HR and HRV could be seen when ApoE^{-/-} mice were exposed specifically to Ni.

Male ApoE^{-/-} mice, 16 weeks-old, were fed with high fat chow for 2 months and implanted with ECG transmitters three weeks before the start of the exposure. They were exposed either to filtered air or 0.2 µm NiSO₄ aerosol at ~300 µg/m³ for 3h/d, 5d/w for 2 weeks. ECG, HR, activity, and body temperature data were sampled for 30 sec every 5 min throughout the experiment.

We found that average HR decreased in 3 out of 4 Ni-exposed mice between midnight and 6 am, an effect consistent with previous findings in mice exposed to CAPs. HRV, expressed as log standard deviation of normal-to-normal beat intervals (SDNN), decreased significantly during the exposure period in both Ni- and sham-exposed mice. However, decreases in HRV persisted in Ni-exposed mice for at least 3 more h after the exposure, while HRV returned to normal shortly after in the sham-exposed animals.

Our results indicated that Ni aerosol could produce changes in HR and HRV in mice susceptible to atherosclerosis and confirmed our earlier observations that Ni could be an important component of ambient FPM in eliciting alterations in cardiac functions. Further study is needed to explain the biological mechanism of this phenomenon, and of chronic effects of Ni exposure at environmentally relevant levels.

369 HIGH DOSE PRENATAL ARSENIC EXPOSURE DECREASES CORONARY ARTERY DENSITY AND INCREASES CARDIAC FIBROSIS IN ADULT MICE.

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Arsenic is a naturally occurring groundwater contaminant found at levels higher than 10 ppb in more than 30% of the private wells in Maine. Epidemiological studies have associated arsenic exposure with cardiovascular disease. The goal of this study was to determine whether prenatal arsenic exposure could adversely impact the adult cardiovascular system. Pregnant mice were exposed to high levels of

sodium arsenite (10, 20, 40, or 80 ppm) via drinking water, *ad libitum*, starting on day 5 of gestation and continuing until the day of birth. Hearts from one male and one female offspring from four different litters were collected in adulthood and processed for histochemical analysis. Coronary arteries and arterioles were visualized with α -smooth muscle actin staining. Arteries and arterioles >5 µm in diameter were counted from a single section at each of the lower, middle and upper areas of the midventricular region. Incidence and severity of cardiac fibrosis were observed by Masson trichrome stain of ten sections evenly distributed from the apex to base of each heart. Only data from male offspring have been analyzed. In males, increasing arsenic exposure decreased artery densities for all areas of the midventricular region with the largest effect observed in the lower midventricular region. Lower midventricular artery densities (mean $\pm$ SE) were 12.5 $\pm$ 2.1, 10.5 $\pm$ 2.5, 9.8 $\pm$ 3.7, 7.9 $\pm$ 3.0, and 8.1 $\pm$ 3.3 no./mm² for the 0, 10, 20, 40 and 80 ppm treatment groups, respectively. Likewise, a trend for increasing fibrosis was observed with increasing arsenic exposure. Mean ($\pm$ SE) total area of fibrosis was 1.58 $\pm$ 0.40, 1.32 $\pm$ 0.42, 3.32 $\pm$ 2.04, 3.62 $\pm$ 2.37, and 4.62 $\pm$ 3.60 1x10⁵ pixels for the 0, 10, 20, 40 and 80 ppm treatment groups, respectively. Cardiomyocyte hypertrophy was observed adjacent to areas of fibrosis. Thus, prenatal exposure to arsenic may increase risk of cardiovascular disease in adulthood.

370 METHODS FOR THE ASSESSMENT OF CARDIOVASCULAR PARAMETERS IN THE MINIPIG.

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The dog and primate are the usual large animal species for evaluating test article effects on the cardiovascular system, however they may not always be suitable for some compounds. As an alternative species, the minipig may be used given their many similarities to humans. A telemetered model was prepared to assess simple parameters such as blood pressure (BP), heart rate (HR) and ECG, in addition to a more complex cardiovascular profile in both anesthetized and conscious animals. In the telemetry study, treatment with dl-Sotalol induced prolongation of PR and QT-intervals of the ECG, with telemetry demonstrating to be an accurate method for long-term collection of cardiovascular data in this species. Additional parameters including pulmonary artery pressure (PAP), left ventricular pressure (LVP) and cardiac output (CO) were also assessed in male minipigs (Yucatan and Gottingen) administered Saline, Diphenhydramine or Isoproterenol. Large volumes of saline resulted in a small transient elevation in BP in anesthetized animals with no effect in the conscious restrained model. Diphenhydramine caused a small transient decrease in HR and elevated systemic BP, which was contrary to results in conscious unrestrained animals. Isoproterenol dosing resulted in acute elevations in HR, LVP, PAP and decreased systemic BP. The minipig is therefore considered an appropriate model for use as an indicator of potential effects on the cardiovascular system.

371 A COMPARISON BETWEEN COMPOUND POTENCY IN AN IN VITRO PHOSPHOLIPIDOSIS ASSAY AND AT THE HERG-ENCODED POTASSIUM CHANNEL.

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Inhibition of the Human Ether-a-go-go Related Gene (hERG) encoded K⁺ channel and phospholipidosis (PLD) are potential safety concerns and liabilities to early compound progression in the drug discovery process. Although PLD has not been causally linked to aberrant cellular function and its clinical implications are not well understood, the association of PLD with frank toxicity within a given tissue is common; and presence of PLD in cells/tissues is an issue to regulatory agencies and for the drug development process. The hERG - encoded K⁺ channel underlies the repolarising I_{Kr} current of the cardiac action potential, inhibition of the I_{Kr} current can lead to potentially fatal cardiac arrhythmias. Cationic, amphiphilic drugs (CADS) are reported to cause local or systemic PLD after chronic exposure in preclinical studies and, in very broad terms, inhibitors of the hERG-encoded K⁺ channel also fall into this chemical category. In view of this and the observation that some CADs that induce PLD *in vivo* have also been reported to prolong the QT interval, it was considered to be worth testing whether a larger list of agents known to prolong QT interval are phospholipogenic and whether there was any correlation between potency of CADS in *in vitro* PLD and hERG-encoded channel assays. Forty-five compounds representing various therapeutic indications were tested in an *in vitro* PLD assay using the mouse splenic I-13.35 cell line and in the IonWorks™ HT hERG assay using the Chinese hamster ovary K1 cell line. Potency values were obtained in both assays for 33 of these, 26 of which are known to cause QT prolongation, with 24 of the 26 causing PLD *in vitro*. Correlations between hERG potency (IC₅₀) and either PLD induction (EC₅₀) (r=0.04) or extent (% PLD maximal response) (r=0.4) were not observed. Lack of a potency correlation indicates that hERG data cannot be used as a quantitative indicator of *in vitro* PLD potency or vice versa.

372 A DOSE RANGE-FINDING STUDY TO DETERMINE THE HEMODYNAMIC EFFECTS OF A SINGLE IV INFUSION AND A 14-DAY CONTINUOUS INTRAVENOUS (CIV) OF KSR-1 AS-214231 (NSC-731442) IN CYNOMOLGUS MONKEYS.

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The objectives of this study were to determine the hemodynamic effects and cardiovascular (CV) toxicity of KSR AS-214231 (KSR) when administered as a single 15-minute IV infusion at 9, 18, or 27 mg/kg and as a 14-day ambulatory continuous intravenous infusion (CIV) at 0, 14, or 28 mg/kg/day in cynomolgus monkeys. This compound is a phosphorothioated antisense oligonucleotide to KSR-1 being developed for use in the treatment of pancreatic cancer. The animals were instrumented with telemetry for blood pressure (BP), heart rate (HR), and electrocardiograms (ECG). For the 15-minute infusion groups (2 to 4/group), there were no BP or HR alterations (9 mg/kg). At 18 mg/kg, three of four animals exhibited variable CV alterations, including BP increases (15 to 25 mmHg) peaking at 2 hours post dosing and HR increases (50 BPM). At 27 mg/kg one of two animals responded with BP increases (50 mmHg) and apparent reflex bradycardia (50 BPM) associated with the end of dosing. There were no ECG alterations attributed to the test article. For the CIV groups of 1 male[M]/1 female[F] were used. At 14 mg/kg/day there were alterations of HR and BP primarily in the F that were not considered test article-related. At 28 mg/kg/day, the M exhibited acute hypotension with apparent reflex tachycardia at dosing start. HR and BP were stable through Day 6 and then progressively declined until the end of the infusion. HR decreased (35 BPM) and BP decreased (25 mmHg) across the dosing period. The F exhibited HR increases (30 BPM) and BP increases (20 mmHg) from Day 7 to Day 14. There were no ECG alterations. The no effect levels were 9 mg/kg (single IV infusion) and 14 mg/kg/day (CIV infusion). All dose levels were potentially tolerable. [Supported by NCI Contract NO1-CM-42200].

373 VALIDATION OF GUINEA PIG LANGENDORFF HEART ASSAY TO EVALUATE CARDIOVASCULAR TOXICITY OF DRUG CANDIDATES.

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Cardiovascular toxicity of pharmaceuticals is a major concern in public healthcare. As such, effective methods must be established to detect unwanted, off-target cardiovascular signals during early drug development. The current study was conducted to validate the sensitivity and specificity of the isolated guinea pig Langendorff heart assay for detection of cardiovascular toxicity of drug candidates. Isolated guinea pig hearts were perfused in Langendorff mode under either sinus rhythm or paced at 200 and 300 beats per minute via the right atrium, and multiple parameters of hemodynamic function (coronary and left ventricle pressure), cardiac electrical function (electrocardiogram and monophasic action potential (MAP)), and indices of arrhythmogenic propensity (MAP triangulation, reverse-use dependence, increased repolarization dispersion and beat-to-beat instability), together with overt arrhythmia (ventricular tachycardia, Torsades de Pointes, etc.) were simultaneously evaluated with 26 reference compounds that possess diverse QT liability/arrhythmogenic potential, inotropy, chronotropy and vasodilatory actions. Ascending concentrations up to either 100-fold the hERG IC50 value or 100 μ M were routinely tested utilizing 4-6 hearts for each reference compound. The validation results revealed that each reference compound exhibited a unique cardiovascular profile; compounds associated with high arrhythmogenic risk in the clinic often exhibited more than two "positive signals" on parameters linked to arrhythmogenesis at concentrations within 30-fold of their maximal therapeutic free plasma concentration. A scoring system was then developed to assist in rank-ordering the cardiovascular toxicity of these cardiotoxicants as well as new drug candidates. The arrhythmogenic potential predicted by this scoring system generally correlated well with that observed in the clinic, suggesting that the isolated Langendorff heart assay in conjunction with the newly developed scoring system may be a useful tool in early cardiac risk assessment.

374 A HESI STUDY OF DOXORUBICIN INDUCED CARDIOTOXICITY IN RATS.

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This study was initiated by the Mechanism-Based Markers of Toxicity Working Group as part of the HESI Committee on the Application of Genomics in Mechanism-Based Risk Assessment. Doxorubicin (Dox), a broad spectrum anthracycline antineoplastic agent, is associated with a cumulative dose-dependent cardiomyopathy in patients. In the current study, the cardiotoxicity potential of Dox was investigated and compared with the effects of etoposide (Etop), a pharmacologically comparable negative control. Sprague Dawley rats received weekly intravenous dosing of Dox (0.5, 1, 2, and 3 mg/kg/week) or Etop (0.5, 1, and 3 mg/kg/week) for 6 consecutive weeks. The highest dose of Dox was associated with low incidence of mortality and dose-dependent decrease in body weight gain was observed in animals that received Dox. Dox treatment caused profound pancytopenia (RBC, WBC and lymphocyte), and prominent changes in various serum chemistry parameters (BUN, CREA, ALKP, CHOL, and ALB). Cardiotoxicity markers, such as creatinine kinase (CK), CKMM and troponin T, were elevated in the animals receiving Dox at doses of 2 and 3 mg/kg. Dexrazoxane (Dex), a protective agent for Dox induced cardiotoxicity, significantly ameliorated Dox's effects on body weight, serum parameters, and cardiotoxicity markers. Histopathological examination of toluidene-blue stained heart sections revealed macrovesicular vacuolation of atrial and ventricular myocytes following high dose Dox. High-density gene expression profiles will be analyzed to study the molecular mechanisms underlying the cardiotoxic effects mediated by Dox and inform for potential safety biomarkers.

375 EFFECT OF β -BLOCKERS ON LEFT VENTRICULAR FUNCTION IN HEALTHY AND DISEASE HEARTS OF NON-HUMAN PRIMATES USING REAL-TIME THREE-DIMENSIONAL ECHOCARDIOGRAPHY.

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β -Blockers remain a cornerstone in the therapy of all stages of ischemic heart disease, albeit the negative inotropic effect of acute β -blockade. The purpose of this study was to examine whether effects of β -blockers on left ventricular (LV) function are different between healthy and diseased hearts in monkeys. Male cynomolgus monkeys (n=25) were used in this study. To induce acute cardiomyopathy as diseased heart, animals received two repetitive epinephrine infusions (10 μ g/kg/min for 3 h) at an interval of 24 hours. LV function was measured using real-time three-dimensional echocardiography (RT3DE, SONOS 7500). End-diastolic and end-systolic volumes (EDV and ESV) significantly ($p<0.01$) increased in monkeys treated with epinephrine. Thereby, LV ejection fraction (EF) was significantly ($p<0.01$) depressed ($<40\%$) compared with pre-value (60%). In healthy hearts, propranolol and metoprolol (0.1 and 0.3 mg/kg/10 min, iv; n=3-4) caused a dose-dependent increase in ESV, but little effect on EDV, resulting in a decrease in EF as an indication of heart contractability. On the other hand, propranolol and metoprolol (each 0.3 mg/kg/10 min, iv; n=4-6) ameliorated a reduction in EF associated with a decrease in ESV in monkeys with LV dysfunction. These results demonstrated that β -blockers might present a different action mode in the diseased heart compared with effect on the healthy heart. Additionally, RT3DE provides the feasibility and accuracy for the detection of the deterioration of LV function.

376 EVIDENCE FOR ISOPRENOID DEPLETION PRIOR TO NECROSIS IN A RAT MODEL OF STATIN-INDUCED MYOPATHY.

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Statins are lipid-lowering agents that inhibit HMG-CoA reductase, the rate-limiting enzyme in the synthesis of isoprenoids and cholesterol. The isoprenoid pyrophosphates of farnesol (FPP) and geranylgeranyl are attached to proteins by three prenyl transferases (with different substrate/protein specificities) and act as membrane anchors. Rare instances of myopathy are associated with statin use, and this may involve depletion of isoprenoids and FPP-derived products such as the respiratory chain intermediary ubiquinone. We have indirectly assessed isoprenoid level in

a rat model of statin-induced myopathy by measuring the appearance of unprenylated Ras, Rap1A and Rab5A (representing substrates for each prenyl transferase) in skeletal muscle cytoplasmic fractions. Female Han-Wistar rats were administered simvastatin (80 mg/kg/day) and skeletal muscle necrosis (defined by histopathology) was induced after 10-14 days in glycolytic fibres (predominant in gastrocnemius muscle) but not in oxidative fibres (predominant in soleus muscle). Unprenylated Ras, Rap1A and Rab5A were increased in gastrocnemius muscle of rats with necrosis but not in matched soleus muscle. Changes in protein prenylation also occurred prior to the induction of necrosis, as unprenylated Rab5A was evident in gastrocnemius muscle after two days of simvastatin administration and continued to increase for the duration of the study. Combined oral administration of simvastatin with farnesol (220 mg/kg/day) or geranylgeranyl (145 mg/kg/day) failed to prevent skeletal muscle damage. Unprenylated Rab5A was comparable in the gastrocnemius muscle of all groups, implying that insufficient exposure of isoprenoids was achieved in skeletal muscle. In conclusion, these data suggest that isoprenoids are decreased in glycolytic skeletal muscle several days prior to the initiation of statin-induced myopathy in the rat. This could be as a prelude to depletion of ubiquinone or more directly implicate protein prenylation in the mechanism.

377 EFFECTS OF MODERATE DIET RESTRICTION AND HIGH FAT DIET-INDUCED OBESITY ON DOXORUBICIN-INDUCED CARDIOMYOPATHY.

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Clinical use of doxorubicin (DOX, Adriamycin[®]) is limited due to its oxyradical-mediated cardiomyopathy. Susceptibility of the obese sensitive population to drug-induced cardiomyopathy has been sparsely investigated. Moderate diet restriction (DR) has a plethora of beneficial health effects. Our objective was to investigate the potentially opposing effects of obesity and DR on DOX-induced cardiomyopathy (DOXIC). Challenge of male SD rats (250-275 g) maintained on DR [35% less food than ad libitum (AL), 42 d], with a LD₁₀₀ dose of DOX (12 mg/kg, ip) on d 43, led to 100% mortality in AL rats between 7 to 13 d, whereas all the DR rats survived. Serum creatine kinase-MB (CK-MB), cardiac troponin-I, cardiac MDA, and histopathology revealed progressive cardiac injury in AL rats in stark contrast to the DR rats. Accumulation of DOX and its toxic metabolite, doxorubicinol, did not differ in the AL and DR rat hearts. Further mechanistic studies revealed that DR rats were protected due to: (1) increased cardiac antioxidants leading to markedly lower oxyradical stress decreasing cardiac uncoupling proteins 2 & 3; (2) lowered cardiac triglycerides leading to higher adiponectin, higher mitochondrial AMPα2 protein kinase, and 2-fold higher cardiac ATP levels; and (3) increased cardiac erythropoietin and decreased SOCS3 resulting in upregulation of cardioprotective JAK/STAT3 pathway. For the obesity studies, male SD rats (250-275 g) were maintained on 40% high fat diet for 42 d. Upon challenge with a normally LD₁₀ dose of DOX (8 mg/kg, ip) on d 43, 10% of the AL rats died by d 19 compared to 80% mortality in obese rats between d 7 to 18. Serum CK-MB and cardiac MDA levels were significantly higher in obese rats compared to AL rats. These results indicate that obesity sensitizes, while DR renders high resiliency to DOXIC. Moreover, the remarkable cardioprotection against DOXIC by DR appears to be due to a combination of lower oxyradical stress and favorable cardioprotective cellular signaling.

378 ENHANCED SUSCEPTIBILITY OF MYOCARDIAL FAILING RABBIT HEART TO DOFETILIDE-INDUCED TORSADES DE POINTES.

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Sponsor: M. Hejtmancik.

Cardiac remodeling after myocardial infarction is associated with increased risk of ventricular arrhythmias and susceptibility to proarrhythmic effects of torsadogenic drugs. The rabbit model of myocardial failing has been shown to be prone to develop torsades de pointes (TdP) when exposed to torsadogens. However, there are little data concerning the cellular basis of arrhythmias in this model. This study was aimed to investigate the dispersion of repolarization and the myocyte action potential duration (APD90) of the normal and myocardial failing rabbit heart in response to dofetilide. Comparison with normal conscious rabbits, rabbits with myocardial failing hearts had a slower heart rate (228±10vs244±10bpm; p=ns), prolongation of QT (p<0.001) and QTc (p<0.001) intervals (154.3±4.5vs133.3±2.4ms and 280.9±6.2vs243.4±6.1ms, respectively). Short-term variability was increased (p<0.05) in myocardial failure group (0.38±0.02ms) when compared with normal conscious group (0.29±0.01ms) whereas long-term variability tended to increase (0.39±0.03vs0.33±0.02ms; p=ns). Myocytes isolated

from border zone of infarcted area could be separated into two groups: non-hypertrophic cells and hypertrophic cells with pathological parameters of EC coupling. APD90 of hypertrophic failing myocytes (912±44ms) was prolonged (p<0.01) compared with action potentials from the same area of normal rabbits (388±19ms). In normal myocytes, dofetilide increased APD90 (p<0.01) at 0.1 μM (from 388±19to921±68ms) with evidence of EADs. In hypertrophic myocytes, dofetilide (1.0 μM) increased APD90 21±8 % from baseline (p<0.05) and EADs were observed both before and after applied dofetilide. It can be concluded that (1) the re-entrant circuit from different APD profiles, (2) EADs triggered activity and (3) increase dispersion of repolarization are responsible for genesis of TdP in the failing rabbit heart.

379 ATTENUATION OF ANGIOTENSIN II-INDUCED EARLY CELL DEATH BY METALLOTHIONEIN RESULTS IN A PREVENTION OF THE LATE DEVELOPMENT OF CARDIOMYOPATHY.

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Angiotensin II (Ang II) plays a critical role in the diabetic cardiovascular diseases including cardiomyopathy. We have demonstrated that cardiomyocytes with high levels of metallothionein (MT) are significantly resistant to diabetes- and Ang II-induced apoptotic cell death mediated by NADPH oxidase (NOX) activation. To investigate the effects of MT on Ang II - induced cardiac injury in vivo, we administered the cardiac-specific MT-overexpressing transgenic (MT-TG) and WT mice with a single injection of Ang II at 1 mg/kg body weight and collected the cardiac tissues 7 hr after the treatment. Ang II caused a significant increase in apoptotic cell death, examined by TUNEL assay and Western blotting for the caspase-3 activation along with an increased 3-NT formation in the heart and an increased expression of NOX isoform p47phox in the membrane proteins, examined by Western blotting. All of these effects of Ang II were significantly prevented in the heart of Ang II-treated MT-TG mice, indicating the protective effect of MT on Ang II-induced apoptosis via suppression of NOX activation. To further evaluate antiapoptotic effect of MT on Ang II, MT-TG and WT mice were chronically infused with Ang II at a subhypertensive dose of 0.5 mg/kg body weight for two weeks and then cardiac tissues were collected at 3 and 6 months. Ang II chronic treatment caused a significant increase in cardiac fibrosis, shown by increased collagen content and up-regulation of connective tissue growth factor, an important profibrotic factor, in the heart of WT, but not in MT-TG, mice. These results suggest that Ang II induces cardiac apoptosis through activation of NOX and subsequent generation of peroxynitrite. MT prevents Ang II-induced cardiac cell death through suppression of NOX activation, peroxynitrite formation and nitrosative damage. The protection of Ang II-induced early cell death by MT leads to a prevention of the late development of cardiomyopathy (Supported in part by ADA, NIH and AHA research grants).

380 METALLOTHIONEIN PREVENTS DIABETES-INDUCED TRIGLYCERIDE AND LIPIDS ACCUMULATION IN THE HEART: EFFECT ON STIMULATING AKT-DEPENDENT GLUCOSE METABOLISM.

Y. Wang. Medicine, University of Louisville, Louisville, KY. Sponsor: L. Cai.

Yuehui Wang, Y. James Kang and Lu Cai, Medicine, University of Louisville, Louisville, KY

Cardiac-specific metallothionein (MT)-overexpressing transgenic (MT-TG) mice were highly resistant to diabetes-induced oxidative damage and the development of diabetic cardiomyopathy. Increasing evidence indicates that diabetic lipotoxicity plays a critical role in initiating the oxidative damage at the early stage, leading to cardiac dysfunction at the late stage. Therefore, the present study was aimed at investigating mechanism by which MT protects the heart from diabetes-induced lipotoxic damage. Both MT-TG and wild-type (WT) mice were given a single dose of streptozotocin to induce hyperglycemia. Two and three weeks after the treatment, cardiac triglyceride and lipid accumulation were significantly increased, along with increases in lipid peroxidation in the heart of the WT diabetic mice, but not in the MT-TG diabetic mice. To explore the underlying mechanisms, Akt abundance and phosphorylation was examined at two weeks and two months after the onset of diabetes. Although Akt abundance was not significantly changed, Akt phosphorylation was significantly decreased in the heart of WT diabetic mice under basal condition or in response to insulin stimulation. The decreased phosphorylation of Akt in the hearts of WT diabetic mice at two weeks and two months after the onset of diabetes was accompanied by decreases in phosphorylation of GSK-3b, a critical kinase related to glucose metabolism. However, neither phosphorylation of Akt nor GSK-3b kinase was decreased in the MT-TG diabetic mice, suggesting the preservation of normal glucose metabolism. Therefore, these results suggest that MT prevents diabetes-induced increases in cardiac triglyceride and

lipids accumulation, likely through preservation of the Akt-dependent glucose metabolism (Supported, in part, by research grants from ADA, Philip Morris USA, Inc. and NIH).

381 ACIZOL EFFICACY IN EXPERIMENTAL CARDIOLOGY.

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Acizol, an antidote to carbon monoxide belonging to the series of zinc organic compounds, is an effective antioxidant. We studied Acizol specific pharmacology activity at intragastric introduction on cardiac ischemia. Two experimental models were used - cardiac infarction and calcium arrhythmia. Acizol dose was 60 mg/kg. Survival rate, electrocardiography and histology indices were registered. Experimental studies showed that Acizol can increase experimental animals survival rate by 20%, decrease heart attack frequency by approximately 2 times, increase cardiac tonus 2 times, and accelerate atrioventricular conductivity by 25% in comparison with the controls. Acizol has antiarrhythmic properties: lengthening of the latent period by 70% and lengthening of the fibrillation time by 80%. Increasing time of life of experimental animals by 40% in comparison with the control was registered. Acizol is a perspective drug for prophylaxis and cardiac ischemia treatment.

382 IDENTIFYING CARDIOTOXIC DRUGS USING A TWO CELL MODEL COMBINED WITH MULTI-PARAMETRIC ANALYSIS: APPLICATIONS FOR *IN VITRO* TOXICITY SCREENING.

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In vitro cell-based models able to predict toxicity specific to the heart would be of considerable value in early drug development. Predicting organ-specific toxicity requires cells from different tissues and a test set of drugs with known organ-specific toxicity. Drugs considered primarily toxic to the heart (anthracyclines) were compared to those considered primarily toxic to the liver (camptothecin and rotenone). Rat cardiomyocytes isolated from 1-2 day old neonates and a rat liver hepatoma (H4IIE) cell line were used as the cell models. Both cell systems were exposed to 1, 5, 10, 20, 50, 100, and 300 μ M of the test drugs for 1, 3, 6, or 24 hr. Cytotoxicity was assessed using markers for general toxicity, oxidative stress, apoptosis, and hypertrophy. Mitochondria were the most sensitive targets in both cell models. However, the TC50 values were 2-to-3 fold lower in the liver cells when compared to the heart cells. The average TC50 value for adriamycin in cardiomyocytes at 24 hr was 5 μ M versus 18 μ M in H4IIE cells. In addition, a reduction in reduced glutathione was observed in cardiomyocytes with a concomitant dose- and time-related increase in ROS as measured by DCFDA. Cardiac hypertrophy markers ANP and BNP were also measured. At 3 hr, adriamycin showed a 2-fold increase in BNP at 50 μ M, and Endothelin-1 at 0.1 μ M showed a 2-fold increase in ANP and a 4-fold increase in BNP. Camptothecin and rotenone were more toxic in the H4IIE cells. A decrease in cell proliferation and reduction in mitochondrial function were observed in H4IIE cells that was much greater than in the cardiomyocytes. The average TC50 value for camptothecin in H4IIE cells at 24 hr was 77 μ M versus 298 μ M in cardiomyocytes. The average rotenone TC50 was 0.3 μ M in H4IIE cells versus 32 μ M in cardiomyocytes. These data suggest that a two cell model may allow *in vitro* screens to predict cardiac versus non-cardiac toxicity. Thus, the incorporation of a two cell model for identifying organ specific toxicity appears to be a useful tool.

383 STRAIN DIFFERENCES IN SERUM CARDIAC TROPONIN-I CONCENTRATION AND MYOCARDIAL NECROSIS AFTER ISOPROTERENOL TREATMENT IN MICE.

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Cardiac troponin-I (cTnI) is a common biomarker of cardiac injury in humans, but satisfactory application of cTnI in toxicologic pathology requires greater characterization of its properties. Increases in the concentration of cTnI in serum are thought to be diagnostic of myocardial cell membrane disruption and breakdown of myofiber. Interpretation of cTnI concentration across different mouse strains has not been described. This study evaluated cTnI in age-matched female CD1 and balb/c mice after beta-adrenergic stimulation. Subcutaneous administration of isoproterenol (10 mg/kg) to CD1 mice produced a peak cTnI concentration of 5.5 ± 2.1 ng/ml 4 hr after dosing and returned to baseline (0.1 ng/ml ± 0.03) after 24 hr. CK activity in serum was increased and returned to normal within 24 hr in these animals. These mice exhibited dose-dependent increases in myocardial necrosis,

graded as minimal to slight, which was restricted to the left ventricle, and characterized by myofiber hyalinization, fragmentation and karyorrhexis. In contrast, balb/c mice treated with 10mg/kg isoproterenol reached a peak cTnI concentration of 27.5 ± 13.1 ng/ml 4 hr post dose and did not return to baseline (0.1 ng/ml ± 0.03) for 72 hr. CK activity in serum was markedly increased 1 hr post-dose, and by 24 hr, some mice still had marked increases in the activity of CK. Myocardial necrosis, graded as minimal to moderate, was characterized by hyalinization and karyorrhexis, and varied in distribution from a few myocytes in the left ventricles to necrosis of a majority of the myocytes in the left ventricle and several in the right ventricle. Collectively these data demonstrate the sensitivity of cTnI as a biomarker of cardiac injury, and suggest that balb/c mice are more susceptible to isoproterenol induced cardiac injury.

384 FABP3 AS A BIOMARKER OF CARDIAC AND SKELETAL MUSCLE INJURY IN THE RAT.

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Striated muscles are subject to compound-mediated toxicity, including injury caused by statins, PPARs, and other lipid-altering drugs. Histopathologic assessment of muscle is the most definitive method to confirm injury but is resource intensive and lesions that occur at low incidence and in specific fiber types can be difficult to detect. Clinical pathology biomarkers of muscle injury, such as CK and AST, are useful, but tend to be less sensitive than is desired. As part of a broader effort to develop additional biomarkers of muscle injury, we investigated the use of fatty acid binding protein 3 (Fabp3) as a biomarker of both cardiac and skeletal muscle injury. Fabp3 is abundant in heart and skeletal muscle and is released into circulation with muscle injury. When quantified in a panel of markers with cardiac troponin I (cTnI) concentration, the cardiac or skeletal muscle source of serological Fabp3 may be inferred. Histologic sections of muscle were examined and serological Fabp3 and cTnI concentrations were quantified in Sprague Dawley and F-344 rats following treatment with compounds that cause cardiac and/or skeletal muscle injury. The kinetics and treatment-dependant increases in Fabp3 concentration were evaluated and compared with histologic assessments and clinical pathology parameters. Those doses producing histologic muscle lesions had corresponding serological Fabp3 concentrations up to 70 times control values. Specifically, nine compound treatments that produced skeletal muscle necrosis in rats at an incidence of 80-100% had Fabp3 concentrations increased 7.5 to 70 times control (mean = 32.2x). Ten compound treatments that produced skeletal muscle necrosis at an incidence of 33-66% had Fabp3 concentrations increased up to 17.3 times control (mean = 6.7x). The correlations between histologic muscle lesions and serological Fabp3 concentrations suggested that Fabp3 is a sensitive and predictive biomarker of cardiac and skeletal muscle injury in the rat in pre-clinical safety studies.

385 EFFECTS OF CALCIUM CYCLING ALTERATION ON THE GENESIS OF TORSADES DE POINTES IN MYOCARDIAL FAILING RABBIT HEART.

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QT interval prolongation may be antiarrhythmic, but also may be associated with increased risk of arrhythmias, in particular torsades de pointes (TdP). Early afterdepolarizations (EADs) and transmural dispersion of repolarization have been known to serve as a physiological substrate and predictor for TdP. It is thought that abnormal calcium cycling is the proximate cause of EADs, and it is known that calcium cycling is abnormal in heart failure. However, the mechanisms for drug-induced TdP in heart failure are not well understood. The purpose of this study was to search for torsadogenic-modifying effects of verapamil, ryanodine and KB-R7943 on ventricular premature depolarizations (VPD) and TdP in the conscious failing rabbit heart, a model known to be prone to develop TdP. Failing rabbit hearts were pretreated with propranolol followed by test articles before continuous infusion of dofetilide, used to induce TdP. In the vehicle control hearts, VPD and TdP were induced in all rabbits (8 of 8) and the onset of VPD and TdP were 3.6 ± 1.3 min and 10.3 ± 1.4 min, respectively. Intravenous infusion of dofetilide was associated with lengthening of RR, QT and QTc interval. Verapamil and ryanodine significantly delayed onset of VPD ($p < 0.05$) and suppressed occurrence of TdP ($p < 0.01$). On the other hand, KB-R7943 accelerated onset of TdP. Blockade of L-type calcium channel and blockade of calcium release channel of the sarcoplasmic reticulum prevent dofetilide-induced TdP, suggesting roles of intracellular calcium overload in drug-induced TdP. Also, our data indicates that blockade of sodium/calcium ex-

change (NCX) may favor the development of TdP in the setting of heart failure, and suggests potential new avenues for development of antiarrhythmics in the presence of heart failure.

386 PHYSIOLOGICALLY-MOTIVATED PHARMACOKINETIC MODELING OF SATURABLE, RENAL RESORPTION OF PERFLUOROALKYLACIDS IN MONKEYS AND RATS.

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Perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) are xenobiotic amphiphilic surfactants that are present in human blood, likely from the wide variety of commercial and product applications that they have been used in or can be derived from. Both are unreactive, essentially unmetabolized, distributed primarily in extracellular space, and largely protein bound in serum; however, both have elimination half-lives in human serum of several years. The combined properties of biopersistence, potential bioaccumulation, and evidence of toxicity have resulted in international scientific and regulatory attention. Human elimination rates are much lower than those determined in monkeys and rodents. Previous pharmacokinetic (PK) analyses and risk characterizations for these compounds have been performed using a one-compartment model; however, PFOA and PFOS in the monkey have kinetic behaviors that are not consistent with a single-compartment model. We developed a physiologically-motivated pharmacokinetic (PK) model for PFOA and PFOS was developed to evaluate quantitative evidence for a role of renal resorption in controlling persistence (*Toxicol* 227, 156-64, 2006). Here we extend this model by adding time-dependent changes in distribution in monkeys and in rats that appear to be due to pharmacodynamic properties of these compounds. Observed changes in kinetics appear to be associated with altered distribution within the body, coupled with reductions in elimination clearance on single doses. Higher, repeated dosing leads to saturation of resorption and more rapid excretion. The physiological basis of this model makes it feasible to develop a human model that can be used to estimate the exposures associated with blood levels of PFOA and PFOS measured in a population, as well as to evaluate the potential role of variability in renal function on potential individual susceptibility.

387 BAYESIAN ANALYSIS OF PARAMETERS FOR PHARMACOKINETIC MODELS.

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Bayesian statistical approaches are increasingly being used to estimate the distributions of parameters for physiologically-based pharmacokinetic (PBPK) models. These models introduce many parameters for which there is often little, if any, knowledge. Bayesian analysis estimates all parameters simultaneously using data sets of various types along with any prior parameter estimates. When we assume that the kinetic parameters for individuals are drawn from the same probability distributions, we may both estimate the population distributions (by estimating their parameters) and use information about the population to refine the individual estimates. This hierarchical approach to the statistics also allows us to distinguish between error and biological variability. We examine the results of a series of experiments [Kemper (2003), DuPont-7473, USEPA AR-226.1499] observing the concentration of perfluorooctanoic acid (PFOA) in adult rats. We jointly analyzed time-courses for plasma concentration in some experiments and fecal and urinary elimination in others to estimate the distributions of PK parameters for PFOA in the overall population of rats. In some subjects, plasma concentration declines in two distinct phases, indicating the need for multi-compartmental analysis. We analyzed one- and two-compartment models with separate urinary and fecal clearances and determined the information gained with the two-compartment model. For example, in male rats using plasma data alone we can only estimate a mean total clearance of 0.7 mL/kg/h. The inclusion of elimination data allowed by our hierarchical analysis found that the mean urinary and fecal clearances could be estimated as 0.65 and 0.08 mL/kg/h respectively. Though we observed large biological variability, the estimated errors are relatively small. By simultaneously using both the plasma and elimination data, we obtained a more complete characterization of the distribution of model parameters. This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy.

388 PHARMACOKINETIC/PHARMACODYNAMIC MODELING OF PERMETHRIN IN THE RAT.

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Pyrethroid insecticides typically represent complex mixtures of isomers, both diastereomeric and enantiomeric in form, some more potent than others in eliciting their neurotoxic effects. Isomer potency is thought to be a function of intrinsic ac-

tivity at the target site as well as susceptibility to detoxication through biotransformation. For example, trans-permethrin is hydrolyzed in vitro approximately 20 times faster by mouse liver microsomal esterases than cis-permethrin. Consequently, it is unclear whether pharmacokinetic models used to predict target tissue dose following exposure must account for the individual isomers to reasonably predict response. A coupled pharmacokinetic/pharmacodynamic (PK/PD) model was used to characterize potential bias in predicted response. A physiologically-based pharmacokinetic (PBPK) model was used to describe the pharmacokinetics of permethrin and calibrated using experimental data on concentration time-course of cis- and trans-permethrin in rat blood and brain tissues following oral administration. A pharmacodynamic model was proposed based on a Hill equation representation of the fractional population of modified sodium channels. Dissociation constants were varied to simulate varying potency. The PK/PD model was employed to test three scenarios: (i) accounting for the isomers individually; (ii) lumping of the isomers as one; (iii) considering only the 'active' form of the isomers. Initial modeling results suggest that both diastereomers, cis- and trans-permethrin, must be accounted for individually to accurately predict response. (Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.)

389 A QUANTITATIVE STRUCTURE-ACTIVITY-BASED PREDICTION OF ACUTE INHALATION TOXICITY FOR NON-REACTIVE VOLATILE ORGANIC COMPOUNDS (VOCs).

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A large majority of the chemicals used in occupational settings are volatile non-reactive compounds for which inhalation concerns mandate an assessment of respiratory toxicity. Unfortunately, comprehensive toxicity testing is not available for all chemicals and convenient, accurate screens are needed. Attempts to develop reliable quantitative structure-activity relationship (QSAR) models for inhalation toxicity that are suitable both within and across species have been largely unsuccessful due to a large variability in the quality of test data and an integrated thermodynamic approach. The objective of this project was to compile a high quality toxicity database from which we can develop QSAR-based models for predicting inhalation toxicity. A comprehensive rodent inhalation toxicity database was developed that includes entries for over 150 chemicals, each of which conform with a stringent standard for quality assurance. From this, a correlation was established wherein the acute toxicity of non-reactive VOCs can be predicted with an accuracy comparable to the reproducibility of the testing methods. In conclusion, it is possible, based on QSAR principles, to accurately estimate the acute toxicity of non-reactive chemicals based strictly on thermodynamic principles that govern dosing metrics. For reactive chemicals, the derived value represents a baseline of minimum toxicity from which points of departure can be identified and further explored. The success of the model demonstrates the potential for deriving rapid, inexpensive and biologically-derived in silico screens for estimating inhalation toxicity for incompletely characterized chemicals.

390 DEVELOPMENT OF QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIP (QSAR) MODELS TO PREDICT CARCINOGENIC POTENCY OF CHEMICALS.

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Two important disadvantages of long-term animal bioassays are that testing involves substantial amounts of time and money, and that high doses are usually used in the testing process. These disadvantages can be circumvented using QSARs. Most carcinogenicity QSAR models relate the carcinogenic potency to measures of carcinogenicity such as mutagenicity, lethal dose (LD50) or the MTD. In one such QSAR, Krewski et al. demonstrated a good correlation ($r = 0.952$, $N = 191$) between tumor dose (TD50) and the MTD. The objective of this study was to predict the carcinogenic potency of a wide variety of chemicals. Initially, this study aimed to duplicate the results of Krewski et al. (1989) using TD50 data published in the Carcinogenic Potency Data Base (CPDB). The MTDs, LD50s and LOAELs of 467 chemicals from the CPDB were estimated using TOPKAT, a QSAR software for toxicity prediction. The correlation between TD50 and MTD for the 467 CPDB chemicals ($N = 467$, $r = 0.538$) was poor compared to the result from the study by Krewski et al. (1989). This study also developed QSAR models using both TD50 and oral slope factor (from USEPA's IRIS database; 95% upper bound on the dose-response slope) as measures of carcinogenic potency. The lowest energy conformers of the chemicals were obtained using CAChe software. Four descriptor-generating programs were used to generate the descriptors for the QSAR model: Dragon,

Molconn-Z, CAChe and CODESSA. Results of this research indicate that sex, species, and mechanism-specific QSARs have a greater predictive ability than generic QSARs that considered all chemicals irrespective of sex, species or mechanism of action. In addition, while the QSARs developed in this study may predict the carcinogenic potency of a wide variety of chemicals, they may not be able to differentiate between carcinogens and non-carcinogens. Hence, separate discriminative QSAR models may need to be developed to differentiate carcinogens from non-carcinogens.

391 A GENERALIZED CONCENTRATION ADDITION MODEL PREDICTS JOINT EFFECTS OF TCDD AND PARTIAL-AGONIST PCBs.

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The Toxic Equivalency Factor (TEF) method used to assess the joint effects of mixtures of dioxin-like chemicals is a special case of the method of concentration addition. However, the TEF method implicitly assumes that the individual agents are full agonists with parallel dose-response curves that differ only by potency. We have derived a generalization of concentration addition (GCA), intended to more accurately describe interactions of combinations of full and partial agonists, and which is supported by kinetic models of simple receptor systems.

The GCA and TEF models were compared to AhR-dependent expression of EGFP by binary combination doses of TCDD and each of two mono-ortho partial agonist PCBs (2,3,3',4,4'-pentachlorobiphenyl, PCB105, and 2,3',4,4',5-pentachlorobiphenyl, PCB118) in the H1G1.1c3 (mouse hepatoma) cell line. The GCA model performed better than TEF at predicting the observed data. Each assay showed a reduced effect at the highest-dose combinations; this reduction is not predicted by the TEF method, but in the GCA model can be seen as the result of competition by the partial agonist for free receptors. While the TEF method overpredicts combination effects at the highest combination doses, it may also underpredict the joint effects of lower (more environmentally relevant) doses, depending on the specific TEF values chosen.

392 QUANTITATIVE MODELING OF THE EFFECTS OF MEMBRANE FLUIDITY ON THE ONSET OF TYPE-2 DIABETES.

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Type-2 diabetes represents one of the highest health care costs based on VA data. Multiple mechanisms leading to this disease have been identified, such as insufficient insulin sensitivity, genetic factors, and oxidative stress from environmental insults. Interventions need to be focused for uncovering specific mechanisms of disease onset and progression. Our working hypothesis is that decoupling between insulin levels and glucose uptake into cells (insulin resistance) is largely the result of reductions in cell membrane fluidity leading to reductions in glucose transporter capacity. Further, that such changes are the result of changes in the fatty-acid (FA) composition of the membrane phospholipids, e.g. as a result of diet and/or oxidative stress and other toxicological processes. Experimental support of this hypothesis includes Read and McElhaney (1975), who demonstrated that uptake of *n*-glucose by the bacterium *A. laidlawii* is dependent on the FA and cholesterol content of the plasma membrane and exhibits a direct dependence on the fluidity of the membrane lipids. Our analysis of their glucose uptake shows that V_{max}/K_m is reduced by a factor of ~4X due to the experimentally induced changes in membrane fluidity. More recent work by Napoli et al. (1995) shows changes in V_{max} in skeletal muscle of streptozotocin diabetic rats decreases about 6X from a baseline of 7.5 to a value of 1.2 nmol/(mg-sec) as diabetes is induced over 10 days. Thus, changes in membrane fluidity are capable of producing the magnitude of change in glucose transport V_{max} observed in an animal model of diabetes. These quantitative relationships can be exploited to develop a biologically-based pharmacokinetic/dynamic (BBPK/PD) model for the disposition of glucose and insulin. The specific benefits of a mechanistic understanding of the processes underlying diabetes incidence include: early identification of insulin resistance, identification of susceptible individuals and potential environmental stressors, identification of potential biomarkers, and development and screening of more effective and/or novel treatments.

393 INTEGRATION OF TOXICOKINETICS AND TOXICODYNAMICS BY MECHANISM-BASED MATHEMATICAL MODELING.

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A mechanism-based mathematical modeling approach was applied to integrate and analyze data from safety studies in rats of an experimental drug for use in diabetes. Based on the postulated mechanism that the pathological finding (i.e., sciatic nerve

degeneration) was associated with glucose-lowering effects of the test article, the model was designed to explore this relationship using toxicokinetic and toxicodynamic data. The toxicokinetic process was depicted by a physiologically-based toxicokinetic model, which also considered reversible hepatic biotransformation between the parent and its major metabolite. A mathematical toxicodynamic model was constructed to mimic the glucose-lowering effect in the plasma compartment, which considered glucose production, elimination and positive and negative feedback mechanisms that are responsible for maintaining euglycemia. In the toxicodynamic model, the extent and duration of glucose-lowering in the treated animals were described as reductions in the glucose AUC as compared to the control animals. The degree of sciatic nerve degeneration was translated into numerical data for each dose level using both the individual incidence and the severity of the findings. A sigmoidal Emax model was used to describe the relationship between reductions in glucose AUC and sciatic nerve degeneration. Simulation outputs showed good agreement with the experimental findings. The modeling efforts demonstrated a clear relationship between glucose-lowering effects and sciatic nerve degeneration. This approach suggests that mechanism-based mathematical modeling could be a useful tool for integration of experimental findings and for quantitative safety assessment in preclinical drug development.

394 BBPK MODEL OF THE HYPOTHALAMIC-PITUITARY-THYROID AXIS IN ADULT MALE RATS.

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Some environmental chemicals affect endocrine function and may alter hormone systems at low doses. The hypothalamic-pituitary-thyroid (HPT) axis controls many physiologic functions, including metabolism, development, and reproduction. Here we present the approach for development of the biologically-based pharmacokinetic model (BBPK) euthyroid HPT axis model, its features, and model simulations. The model for the adult male rat includes sub-models for dietary iodide, thyroid stimulating hormone (TSH), as well as thyroid hormones, thyroxine (T4) and 3,5,3'-triiodothyronine (T3). First, the individual sub-models were developed independently of one another using radiolabeled tracer studies to estimate various model parameters. Then, the models were combined to form one endogenous model that includes (1) feedback of T4 on TSH production, (2) stimulation of T4/T3 production and thyroidal iodide uptake by TSH, and (3) the use of available thyroidal iodide in hormone production. Further model development will include linking the HPT axis model with PBPK models for thyroid active chemicals to predict HPT axis disturbances based on the primary mode of action of the chemicals. Key model features have already been built into the HPT axis model to facilitate this linkage. Hepatic UDPGT conjugation of T4 in the liver was described because HPT axis disturbances may result from an increase T4-glucuronide formation. Active uptake of iodide into the thyroid at the sodium/iodide symporter was included because environmental chemicals may inhibit thyroidal iodide uptake, decreasing the amount of iodide available for hormone production. This mechanistically formulated HPT axis model integrates a variety of physiologic processes that will be used predict complex, non-linear dose responses resulting from exposure to thyroid toxic chemicals, alone and in combination. Funded by ATSDR #U61/ATU472105 and EPA STAR Fellowship #FP916793.

395 DEVELOPMENT OF BBPK RADIOLABELED SUB-MODELS FOR THE HPT AXIS IN PND 14 SD RAT PUPS.

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To assess the developmental neurotoxicity of chemicals that act by disruption of the HPT axis, we are developing a BBPK model for the PND 14 SD rat pup. This model includes integration of feedback mechanisms among thyroxine (T4), triiodothyronine (T3), and thyroid-stimulating hormone (TSH). Growth equations will expand the model to all developmental stages. To begin the model, submodels for iodide, T4 and T3 were created using radiolabeled kinetic data for each material. These models establish kinetic parameters such as metabolic rate constants and distribution behavior. The submodel for iodide included flow-limited compartments for plasma and liver and diffusion-limited compartments for thyroid and GI tract. Active uptake via the sodium-iodide symporter was accounted for in the thyroid and GI tract. The model predictions compared well with experimental data for radiolabeled iodide in the thyroid, GI tract, and plasma. Both the T4 and T3 models include a flow-limited volume of distribution compartment and diffusion-limited brain (cortex) and liver compartments. Active transport and metabolism via glucuronidation and Michaelis-Menton (MM) Type 1 5' deiodination was accounted for in the T4 model liver. 1st order clearance of T4 from the brain was in-

cluded. In the T3 model, active transport and 1st order clearance were included in the liver and MM Type III 5' deiodination was included in the brain. The BBPK model predictions compared well with experimental radiolabeled brain, liver, and plasma data. A submodel for TSH was created to establish kinetic parameters to describe the feedback relationships between serum concentrations of both TSH and T4. The BBPK model predictions were compared to endogenous TSH and T4 data. Pup submodel parameterization allows comparison with kinetic behavior of the axis in adults. Future efforts includes integration of submodels to create an HPT axis BBPK model with homeostatic feedback processes and chemical induced disruptions.(USEPA RD832134)

396 CRITICAL CELL-CYCLE-EVENTS IN CANCER DEVELOPMENT.

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Cancer can result from interactions between genetic elements and genotoxic agents in the ambient environment. Population density of animal populations, and especially human populations, is affected by age specific cancer mortality rates. Decrease in latency from initiation to frank tumor correlates with a decrease in the number of cell-cycle steps or a reduction in control by targeted tumor suppressor genes independently and in combination with expressed macromolecular p53, p27, H2AX, Blm, etc. We have systemically examined the decrease in steps to cancer development based on estimates from survival analysis and Weibull's model of the combinations of these targeted antioncogene-knockout mice. Thus, the multistage and multistep carcinogenic process at the organismic level finds its counterpart at the cellular level. The cause-and-effect relationship between cancer development and the loss of these genetic functions can be explicitly shown in p53-MDM2 demonstrating escape from apoptosis- senescence as the initiation-transformation-immortality process of cancer development. The importance of these initiating events is confirmed by further mathematical modeling with targeted knockouts in Rb-p107-p130, and Arf-myc-p53.

Disclaimer: The opinions and conclusions expressed in this paper are those of the authors and do not necessarily reflect those of the National Center for Environment Assessment, Office of Science and Technology, Office of Water, Office of Research and Development, United States Environment Protection Agency.

397 COMPUTATIONAL MODELING OF NRF2-MEDIATED OXIDATIVE/ELECTROPHILIC STRESS RESPONSE.

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To limit excessive accumulation of reactive oxygen species (ROS) and electrophiles, mammalian cells have a transcription factor Nrf2-mediated gene regulatory network, which is activated in the face of oxidative and electrophilic stressors. In this study, we analyzed the antioxidant gene regulatory network from an engineer's perspective. We decomposed the network into transducer, controller, and plant, which are functional units often seen in a classical negative control system. We then formulated a computational model to study the systems behavior of oxidative/electrophilic stress responses. This model reproduced the response profile of a variety of intracellular species including ROS, electrophiles, Nrf2, GSH, GSSG, and antioxidant/phase II enzymes (e.g. GCL, GPx, GST, etc.), in both normal and impaired conditions such as GSH depletion. The model predicted that prior exposure to small-doses of oxidative stressors would protect cells from damage by a subsequent large-dose exposure. These predictions were confirmed experimentally using hypochlorous acid as the oxidant stressor in RAW 264.7 macrophages. For species such as hydrogen peroxide and lipid peroxide, which are removed by reactions converting GSH into GSSG, detoxification is limited by glutathione reductase and the pentose pathway supplying NADPH. In contrast, for electrophiles which are primarily removed by conjugating with GSH either enzymatically or non-enzymatically, the ability of *de novo* GSH synthesis is the limiting factor. Model simulations indicate that the steady-state dose response between intracellular electrophiles and stressors is likely to follow a superlinear-to-sublinear transition as the dose increases. This study represents one of the first efforts to understand cellular control systems related to a key oxidative stress pathway in a quantitative manner and to use the control module to understand expected dose-response relationships.

398 A HYBRID CFD-PBPK MODEL OF CHLORINE GAS UPTAKE AND TISSUE DOSIMETRY IN THE UPPER RESPIRATORY TRACT (URT) OF F344 RATS.

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Chlorine (Cl₂) is very reactive in water and an irritant. Nasal extraction of Cl₂ is high and resultant lesions in the respiratory tract show a proximal to distal distribution indicating that airflow and exposure concentration play a key role in patho-

genesis. Our hypothesis for the mode of action (MOA) for Cl₂ is that its irritant effects are caused by oxidative stress mediated by hypochlorous acid (HOCl). HOCl would be formed in epithelial tissues by hydrolysis of Cl₂, and to a lesser extent, by downstream biological responses including inflammation. To describe tissue dose metrics associated with different endpoints of this proposed MOA for use in dose response analysis, a recent computational fluid dynamics-physiologically based pharmacokinetic (CFD-PBPK) model of inhaled hydrogen sulfide (Schroeter et al., Toxicol. Sci., 2006, 90(1), 198) was modified to address experimental dosimetry data on Cl₂. These data included gas uptake of Cl₂ delivered unidirectionally at 3 different flow rates and concentrations, measured in situ in the isolated URT of both sexes of F344 rats; and tissue chlorotyrosines (Cl-Tyr; 3-chloro- and 3,5-dichlorotyrosine) in samples obtained from 4 different regions representing respiratory and olfactory tissues in both the septal and lateral airstreams. Cl-Tyr were used as an internal dosimeter. The CFD mesh was segmented to provide estimates of Cl₂ flux in each of the 4 regions. The PBPK model of the tissue in each region describes rates for Cl₂ hydrolysis, reaction of HOCl with proteins, and scavenging of reactive species by soluble anti-oxidants, including glutathione, obtained by both evaluation of the literature and fitting to dosimetry data. This model can be used to estimate flux to the URT, and tissue HOCl or Cl-Tyr as improved dose metrics for risk assessment. Correlation of these dose metrics with lesion incidence and location will provide insights on their utility in characterizing oxidative stress as the MOA for inhaled Cl₂ in the respiratory tract. (This abstract does not reflect Agency policy.)

399 INTER-SPECIES EXTRAPOLATION OF HYDROGEN SULFIDE NASAL DOSIMETRY USING PHARMACOKINETIC-DRIVEN COMPUTATIONAL FLUID DYNAMICS MODELS.

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Human exposure to hydrogen sulfide (H₂S) gas results in cardiovascular, neurological, and respiratory effects that increase in severity with increased exposure level. Animal studies show that the olfactory epithelium is especially sensitive to H₂S inhalation. Subchronic exposure of rats to 30 or 80 ppm H₂S results in olfactory neuron loss and basal cell hyperplasia (with a No-Observed-Adverse-Effect-Level (NOAEL) of 10 ppm). These lesions commonly border main airflow streams, indicating that differential epithelial tissue responses likely result from increased tissue dose. Anatomically accurate computational fluid dynamics (CFD) models of the rat and human nasal passages were developed to predict H₂S tissue dose in the olfactory regions. Air-tissue flux was defined in terms of H₂S solubility, diffusivity, and reaction kinetics in nasal tissue. Kinetic parameters for the rat were estimated from an air-tissue pharmacokinetic model that was fit to experimental nasal extraction data. Using this pharmacokinetic-coupled CFD model, there was a close correlation of predicted air-tissue flux with lesion incidence within olfactory epithelium. Kinetic parameters were allometrically scaled from rats to humans to predict olfactory tissue dose with the human nasal CFD model. Assuming that equivalent H₂S flux values induce similar tissue responses in rats and humans, a NOAEL Human-Equivalent-Concentration was estimated to be 5 ppm. This estimate was based on quantitative interspecies extrapolation of olfactory tissue dose estimates from the CFD models and is approximately 10-fold higher than the current estimate used by the U.S. EPA that relied on default dosimetric assumptions.

400 ESTIMATION OF GAS-PHASE MASS TRANSFER COEFFICIENTS FOR DOSIMETRY CALCULATIONS IN THE RAT NASAL PASSAGES.

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Dosimetry of inhaled gases in the upper respiratory tract depends on nasal geometry and airflow patterns. Recently, hybrid computational fluid dynamics-physiologically based pharmacokinetic (CFD-PBPK) models were developed to estimate nasal tissue dose and systemic transport of inhaled chemicals. In this approach, a CFD model of the rat nose was used to compute gas-phase mass transfer coefficients (MTCs) for implementation in a PBPK model. Specifically, a model developed by Frederick et al. (Toxicol. Appl. Pharmacol. 152:211-231, 1998) divided the rat nose into compartments based on airflow patterns and epithelial type. MTCs were estimated for each compartment. However, the CFD model used in that study contained only the anterior rat nose so some MTCs had to be estimated by other means. In this study, a CFD model of the entire rat nose was used to estimate localized MTCs using a zonal approach (Jarabek et al., Toxicol. Sci. 60(1-S):150, 2001). Airflow simulations were conducted at inhalation flow rates of 100, 300, and 500 ml/min with FIDAP (Fluent, Inc.). The rat nasal passages were partitioned into 16 zones separated by coronal cross-sections including the compartment boundaries defined by Frederick et al. (1998). Gas uptake simulations were

conducted in which anterior zones were progressively made non-absorbing, with all distal regions being completely absorbing. Localized MTCs were then calculated in each zone and used to compute compartmental MTCs. This approach avoids the problem of high extraction efficiency in the rat nose as a whole under maximal uptake conditions, and estimates the filtering efficiency of different regions of the nose. These updated MTCs can be implemented in future CFD-PBPK models for improved dosimetry estimates of inhaled gases. This research was funded by the U.S. EPA (Req. No. 06262W5E48). The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

401 EXPERIMENTAL AND COMPUTATIONAL APPROACHES TO EVALUATE RESPIRATORY TRACT UPTAKE AND DOSIMETRY OF HEXAMETHYLENE DIISOCYANATE IN RATS.

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Hexamethylene diisocyanate (HDI) is a reactive chemical used in the commercial production of polyurethanes. Human exposure to HDI may initiate occupational asthma. Toxic effects in animals exposed to HDI vapor include respiratory tract irritation and degeneration of olfactory epithelium. Olfactory degeneration was observed at 0.025 ppm HDI in a chronic two-year inhalation study, and pathological changes were seen in the alveolar airways at higher exposure concentrations. These observations suggest that inhaled HDI results in dose dependent effects that permeate throughout the respiratory tract. In this study, a dosimetry model was developed to predict HDI tissue dose in the rat respiratory tract. The model consisted of an anatomically accurate computational fluid dynamics (CFD) model of the rat nasal passages that was linked to a lung dosimetry model. Airflow and HDI transport in the nasal passages were simulated using the finite element software FIDAP (Fluent, Inc.). Transport of HDI through lung airways was simulated by convection and axial dispersion and was implemented in MATLAB (Mathworks, Inc.). The nasal and lung components of the dosimetry model included tissue phases where elimination of HDI was governed by diffusion and first-order clearance. HDI nasal extraction was experimentally measured in the surgically isolated upper respiratory tract of anesthetized F344 rats. The CFD model was used to fit a first-order clearance term to the nasal extraction data that was subsequently used throughout the respiratory tract lining. Tissue dose was predicted in the nasal passages and in all generations of the lung. The simulations predicted that the upper respiratory tract and upper bronchial airways received the highest tissue dose, yet there was still penetration of HDI vapor to the pulmonary airways. These results allow more accurate estimation of the relationship of tissue dose with adverse responses from a chronic HDI inhalation study in rats and can be scaled to human inhalation.

402 COMPUTATIONAL FLUID DYNAMICS PREDICTIONS OF HYPER-POLARIZED HELIUM FLOWS.

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Computational fluid dynamic (CFD) simulations of respiratory airflow provide a quantitative basis for optimizing inhaled drug delivery, understanding the risks posed by airborne pollutants, and predicting human health effects. However, to have confidence in the model it must be validated against experimental data. We have previously demonstrated the feasibility of validating CFD predictions with magnetic resonance (MR) imaging of hyperpolarized (HP) ^3He . However, the direct comparison between measured and predicted flows with HP remains challenging, due to the high degree of helium self-diffusion which blurs the predicted velocities. The forward problem of applying a probabilistic blurring to predicted data is possible and accurate but remains computationally expensive even in simple geometries. In the current study, the forward problem is efficiently and accurately solved on arbitrary geometries. The approach is based on physical arguments which show each component of the velocity field is blurred independently via convolution with a diffusion propagator which obeys the diffusion equation and whose solutions can be linearly superposed; thus each component of the blurred velocity field can be solved for independently and simultaneously over the whole domain. To illustrate the approach, comparisons were made between measured and predicted flows in a phantom. Two dimensional maps of HP gas flow at select imaging planes were created using two phase-sensitive images acquired with different bipolar gradient strengths. The phantom geometry was derived from separate ^1H MR images. The steady-state Navier-Stokes equations were solved with a commercial finite-volume solver (Star-CD). Each solution was then evolved with a custom solver to account for statistical blurring over the acquisition window. Results showed good correlation between measured and predicted flows. Larger airways showed a greater level of detail in the velocity profile than smaller airways in keeping with theoretical considerations. Funded by NHLBI RO1 HL073598-01.

403 PARTICLE DEPOSITION IN AN IMAGING-BASED GEOMETRY OF THE SPRAGUE-DAWLEY RAT.

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Many investigators have modeled the physics of particles in order to investigate potential interactions between respiratory diseases and environmental challenges. Most of these early simulations were based on idealized geometries and the approach to particle deposition was likewise idealized. However, the details of 3D geometries are strong determinants of micro-dosimetry, particularly in upper airways. In this study, laminar vs. turbulent flow assumptions were compared with fine and ultra-fine particle deposition in an imaging-based rat lung. Cast imaging was performed using an Avance MRI spectrometer and a 11.7-Tesla magnet. Airway geometry was extracted as an isosurface and subjected to volume-conserving smoothing. A paved, boundary-fitted CFD-compatible tetrahedral mesh was generated directly from the isosurface with a novel scale-invariant meshing algorithm (LaGriT-PNNL) that creates cells orthogonal to the boundary. A transient inhalation/exhalation case was simulated using a commercial finite-volume solver (Star-CD). The inlet pressure was prescribed from plethysmograph data; outlet pressures were prescribed 0.0 kPa. Turbulent simulations were performed with the Reynolds averaged k- ω model. Particles were assumed to have one-way interaction with the pulmonary flow. Particle trajectories and deposition patterns were predicted with the PNNL Lagrangian particle tracking code PT6. Turbulent influence on particle trajectories is based on the eddy-interaction model and accompanied by a near-wall correction. Results from a grid refinement study show our solution has acceptably small discretization error. Deposition efficiencies and patterns were a function of particle size, flow rates, and the addition of turbulence. Capabilities of the particle tracking code include the handling of particle dynamics in moving meshes, and we hope to extend our analysis to moving airways to better predict the in vivo data we are currently acquiring to validate these predictions. Funded by NHLBI RO1 HL073598-01.

404 ADVANCES IN CANCER RISK ASSESSMENT FOR INHALATION EXPOSURES TO ASBESTOS.

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Efforts are underway to develop a new approach to assessing cancer risk associated with inhalation exposures to asbestos at contaminated sites. The draft methodology is based on a meta-analysis of available epidemiological studies that provide quantitative exposure-response information on relative risk of lung cancer or incidence of mesothelioma. The influence of mineral type (amphibole or serpentine) and fiber dimensions are considered by "binning" fibers, and a number of different binning strategies are evaluated. An over-dispersed Poisson model is used to fit the predicted number of deaths to the observed number in each exposure group using maximum likelihood estimation (MLE), rather than fitting individual studies. Probability distribution functions are used to address uncertainty in the data, including cumulative exposure, disease, and estimation of bin-specific concentration values. The output of the model includes uncertainty distributions for each bin-specific potency factor and for any specified mixture of asbestos bins.

405 MULTISCALE CARDIAC MODEL OF NANOPARTICLE DRIVEN ATHEROSCLEROSIS.

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There is growing evidence that exposure to particulates may be involved in lesions of the coronary endothelium that lead to atherosclerosis. It is also well known that deleterious alterations in fluid shear stress and coronary transmural pressure perturb endothelial biochemistry and exacerbate the local inflammation at the root of plaque formation. We have initiated a program to develop a multiscale computational model of the heart to investigate the relationship among atherosclerosis, the presence of nanoparticles and the secondary perturbations of respiratory inflammation. The project aims to link cardiovascular function with respiratory function and biomechanics with biochemistry. A preliminary mouse heart geometry with cellular resolution was reconstructed from serial cryomicrotome images via serial nonlinear co-registration. In addition, a high-contrast, high resolution, whole mouse, contrast-infused magnetic resonance (MR), dataset was acquired. A detailed segmentation of both tissue and blood domains was performed with custom algorithms including fuzzy connectivity, and a network preserving algorithm for extracting the coronary vasculature. Work is currently underway to co-register the cryomicrotome data with the MR data. To create a computational mesh, tissue surfaces, blood surfaces and blood-tissue interface were extracted with a multi-material marching

cubes algorithm. This was followed by application of a novel scale-invariant, multi-material mesh-generation algorithm that produces a quality, nearly orthogonal paving of biological geometries based on the concept of local scale. Finally, we have developed efficient constitutive models for myocardium, papillary muscle, chordae tendinae, cardiac valve tissue and coronary artery. Much work remains to be done. However, we believe that a predictive computational model that links circulation to respiration can become an important tool for predicting regional deposition of ultrafine particulates and site selectivity of atherosclerotic plaque formation. Funded by DOE LDRD DE-AC05-76RL01830

406 *IN VITRO* 2-METHOXYACETALDEHYDE ADDUCT FORMATION USED FOR PROTEOMIC-BASED GLYCOL ETHER BIOMARKER DEVELOPMENT WITH SURFACE ENHANCED LASER DESORPTION IONIZATION (SELDI) ANALYSIS.

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The protein adduct formation of 2-Methoxyacetaldehyde (MALD, CAS 10312-83-1) was tested as a potential biomarker of glycol ether exposure. MALD is a reactive intermediate in the metabolism of 2-methoxyethanol (2ME, CAS 109-86-4), a glycol ether known to produce reproductive effects in laboratory animals and is a potential carcinogen. Bioactivation of 2ME is reported to occur via oxidation by alcohol dehydrogenase to MALD and subsequent aldehyde dehydrogenase to methoxyacetic acid (MAA, CAS 625-45-6). Protein and DNA adducts have been reported for 2ME, and MALD has been suggested as the likely source of macromolecular adduct formation. In the current study MALD, along with 2ME and MAA were tested *in vitro* to evaluate adduct formation using human hemoglobin or albumin. Surface Enhanced Laser Desorption Ionization (SELDI) with Time of Flight mass spectrometry was used to detect adduct formation in 0.1 µg hemoglobin or albumin incubates. The study showed that the reactivity of MALD with albumin was several fold greater than for hemoglobin. The reaction of MALD with the alpha-globin chain was greater than that of the beta-globin portion of hemoglobin. After 3 hours the MALD-adduct levels for albumin > alpha-globin > beta-globin. The MALD adduct levels did not continue to increase further during the 24-hour test period, and no corresponding adduct formation was noted for 2ME or MAA. Tryptic digests of the proteins may be utilized for specific proteomic-based biomarkers of exposure to glycol ethers.

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407 (2-METHOXYETHOXY)ACETIC ACID: A URINARY BIOMARKER OF EXPOSURE TO JP-8 JET FUEL.

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(2-Methoxyethoxy)acetic acid (MEAA), the major urinary metabolite of the deicing agent 2-(2-methoxyethoxy)ethanol (diEGME) added to JP-8 jet fuel, was used to measure exposure to JP-8 in air force personnel at six airbases in the United States. JP-8 is a kerosene based fuel containing over 200 different compounds, including aliphatics, aromatics, benzene, and naphthalene, all of which vary in concentration depending upon the specific batch of JP-8. DiEGME is always present at 0.1 %. Post-shift urine specimens were obtained from personnel who performed fuel tank maintenance and their attendants (high category, n = 98), fuel handlers (moderate category, n = 38) and personnel with no direct JP-8 contact (low category, n = 61). Urine containing a deuterated (2-butoxy)acetic acid internal standard was extracted with ethyl acetate, followed by esterification to the ethyl ester. The ethyl ester was extracted with methylene chloride and concentrated. Quantitation was by a gas chromatograph equipped with a mass spectrometer. The mean urinary MEAA level was significantly greater in the high (6.9, SD = 15.5 µg/ml) category, as compared to the means of the moderate (0.4, SD = 0.9 µg/ml, p = 0.0024) and low (0.1, SD = 0.02 µg/ml, p = 0.0002) categories. The maximum concentrations of urinary MEAA were 110.0 µg/ml, 4.8 µg/ml, and 0.2 µg/ml and the percent of measurements below the limit of detection (0.1 µg/ml) were 6%, 67%, and 97% for the high, moderate, and low categories, respectively. This study demonstrated that urinary MEAA can be used as a biomarker of exposure to JP-8.

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408 URINARY 2-BUTOXYACETIC ACID: DEVELOPMENT OF AN EFFECTIVE GAS CHROMATOGRAPHIC TEST METHOD FOR QUANTIFICATION.

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A simple and effective general test method for 2-butoxyacetic acid (2-BAA) in urine samples was developed. 2-Butoxyacetic acid is a metabolite and biomarker for exposure to 2-butoxyethanol (2-BE), a glycol ether and is of concern because of the general toxicity of this class of compounds. Glycol ethers have been frequently reported to damage the male reproductive system, hematopoietic system, and fetal/embryonic development. Occupational exposure by these widely used glycol ethers is likely, since they are readily absorbed through the skin. Specifically, 2-BE is used as a component in paints, inks and common household cleaning products, and is of interest to this laboratory for exposed populations. For the use of this test on urine, specimens were first spiked with deuterated 2-BAA; the deuterated analog was used as a procedural internal standard. The samples were extracted with ethyl acetate, concentrated, and treated by acid catalyzed esterification to produce the corresponding ethyl esters of 2-BAA. Subsequently, the ethyl ester derivatives were extracted using methylene chloride and concentrated to produce the final solution for gas chromatographic analysis. A mass selective detector (MSD) using a 50-m X 0.20-mm (id) HP-1 capillary column and a temperature program of 60 to 150°C was used for the gas chromatographic measurement. Ion m/z 57 was monitored for the ethyl ester of 2-BAA and ion m/z 66 was monitored for the internal standard. A series of recovery studies using 1, 5, 10 and 20 µg/ml 2-BAA spiked urine samples demonstrated good accuracy and precision; recovery varied between 100-102% of theory. The limit of detection (LOD) was found to range from 0.005 to 0.015 µg/ml for this analysis method.

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409 EXCRETION OF URINARY BIUREA IN WORKERS EXPOSED AZODICARBONAMIDE.

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Azodicarbonamide (ADCA), a causative agent of occupational asthma in plastics and rubber industry workers, is an organic low molecular weight agent used as a blowing agent in the manufacture of expanded foam plastics. ADCA is rapidly converted to biurea and that biurea is then eliminated rapidly from all tissues through urine. The urinary biurea has been used as biomarker of ADCA exposure, yet limited studies have been done. Accordingly, we measured airborne ADCA in workplace, urinary biurea, and total IgE in serum, and analyzed the relationship among indices. The study population contained 14 exposed and 14 non-exposed workers in a ADCA manufacturing plant. Urine samples were obtained at prior to shift (PS) and the end of shift (EOS) on the survey day and blood samples collected on the survey day. Analysis for biurea was achieved by oxidation of biurea to ADCA prior to derivation with triphenylphosphine. The derivative was extracted with dichloromethane, and then evaporated. The dried sample was reconstituted in acetonitrile, and quantified using HPLC. Total IgE in serum was analyzed via immunoassay. The mean of environmental ADCA was 1.553±1.930 TWA mg/m³, and 80 percent among the collected samples were higher than 1 mg/m³, recommend level of Health and Safety Executive. The mean of PS biurea, EOS biurea, and total IgE were 0.19±4.68 mg/g creatinine, 0.18±4.02 mg/g creatinine, and 163.78±4.09 IU/mL, respectively. There was significant correlation between the urinary EOS biurea and the airborne ADCA (r=0.733, p<0.05) and PS biurea (r=0.574, p<0.05) in the exposure group. From the results of simple regression analysis about the urinary concentration of EOS biurea and that of ADCA in the exposed group, coefficient of determination was 0.34 (p<0.05). There was significant correlation between the urinary EOS biurea and the airborne ADCA. The urinary biurea was an effective index as a biomarker of airborne ADCA in workplace, despite the limitation of sample size and variables.

410 GENOTOXIC EFFECTS IN WORKERS EXPOSED TO FUMES AND AEROSOLS OF BITUMEN (FAB).

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Recently, DNA damage in form of increased strand break frequencies could be determined in white blood cells (WBC) of 66 workers exposed to FAB (Marczyński et al., *Cancer Epidemiol. Biomarker & Prev.* 15: 645; 2006). Meanwhile, 202 exposed

workers were studied using a cross-shift design. 1-hydroxypyrene (1-OHP) and the sum of 1-, 2-, 3-, 4- and 9-hydroxyphenanthrenes (OHPhe) in urine as well as 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-OHdG) and DNA strand breaks in WBC were analysed before and after the shift. Personal air sampling during shift was carried out to assess external exposure to FAB. 55 construction workers not exposed to FAB served as reference subjects. Compared to controls, exposed workers showed higher levels of 1-OHP and OHPhe after the shift. Significantly more 8-OHdG and DNA strand breaks were found before and after the shift in exposed workers. Concentrations of 8-OHdG increased ($P < 0.0001$), while DNA strand break frequencies decreased ($P < 0.05$) during shift in both, exposed and control individuals. Airborne concentrations of FAB correlated significantly with urinary concentrations of 1-OHP ($r = 0.25$, $P < 0.001$) and OHPhe ($r = 0.36$, $P < 0.001$) after the shift. In addition, 1-OHP in post-shift urine was weakly, but significantly associated with post-shift values of DNA strand breaks ($r = 0.19$, $P = 0.001$). However, no associations could be found between external exposure to FAB and genotoxic parameters as well as between OHPhe in post-shift urine and genotoxic parameters. Overall, the findings strengthen our previous results that at workplaces with exposure to FAB higher levels of DNA damage are observed. However, because of a weak association between 1-OHP and DNA strand breaks only and because of lacking associations between airborne FAB and genotoxic effects as well as between OHPhe and genotoxic effects the reasons for increased DNA damage in FAB exposed workers remains unclear.

411 PAH EXPOSURE AND BIOMARKERS IN HUMAN POPULATIONS.

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Polycyclic aromatic hydrocarbons (PAH) are released from the incomplete combustion of organic materials such as gas, oil, and wood. PAH contamination occurs as complex mixtures that often contain over one hundred different chemicals. Humans may be exposed to polycyclic aromatic hydrocarbons (PAHs) in the environment from voluntary sources, such as tobacco smoke and diet, and involuntary sources, for example industrial processes. Animal studies have clearly linked exposure to PAHs with a variety of cancers. Human populations may also be at increased risk of cancer if the dose and duration of exposure are adequate. However, there is limited information regarding the absorption and distribution of PAHs from non-occupational exposures. The current study has measured biomarkers in plasma and urine from three generations in a total of 70 families (37 urban, 33 rural) from the country of Azerbaijan. Measurements collected from these populations include the concentration of PAHs in plasma, PAH-DNA adducts in peripheral lymphocytes, and 1-hydroxypyrene in urine. The median serum concentration for total PAHs in the urban population was 148 ng/mL, while a median level of 67 ng/mL total PAHs were detected in the rural population. Total DNA adduct levels were significantly higher in the urban population than in the rural population. Analyses of urine samples from urban and rural populations indicate that 1-hydroxypyrene concentrations are highly variable. The data also indicate that plasma PAH levels may reflect DNA damage in lymphocytes.

412 ASSESSMENT OF CHRONIC EXPOSURE TO PESTICIDES OF POPULATION IN HELLA, PELOPONNESUS AND CRETE, GREECE.

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In the present report we assessed chronic exposure of the population to currently used (endosulfan, dimethoate, diazinon, fenthion, chlorpyrifos, methyl parathion, malathion, methidathion, phosphamidon and methomyl) and two banned organochlorine pesticides (HCHs and DDT). The population was divided to two large groups. One had current occupational exposure to pesticides and one did not. The levels of the aforementioned pesticides were measured in the head hair of the population. The organochlorine pesticides were measured as follows: The samples were analyzed by GC-ECD following distraction of the hair matrix by overnight incubation in 2 M HCl, cleanup with liquid-liquid extraction with 2ml hexane: dichloromethane (4:1) and final purification on a cartridge containing basic alumina, acidified silica and anhydrous sodium sulphate. The rest of the pesticides were measured by GC-MS following a simple methanolic extraction. The limits of detection for the currently used pesticides in the GC-MS analysis ranged from 1-40 ng/g. Currently used pesticides (diazinon, fenrthion, malathion and endosulfan) were detected at trace amounts in a very small percentage of our samples. Mean values of both organochlorine pesticides and their metabolites in the hair of whole

population were as follows: α -HCH: 0.18 ng/g, HCB: 2.34 ng/g, lindane: 0.38 ng/g, op DDE: 1.16 ng/g, pp DDE: 0.33 ng/g, op DDD: 1.6 ng/g, pp DDD+op DDT: 0.90 ng/g, pp-DDT: 0.23 ng/g.) The values observed in the present study are lower than those in one of our earlier studies. The pesticides though are still detected despite the long time that they have been banned. This report is part our ongoing population and environmental study of exposure to pesticides and assessment of its effects on public health.

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413 EXPOSURE FROM ARTISANAL METAL PROCESSING AND MANUFACTURING FISHING TOOLS INCREASES BLOOD LEAD LEVELS IN CHILDREN FROM CARTAGENA, COLOMBIA.

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Lead poisoning is one of the most frequent health concerns in children around the world. Low levels of blood lead (BPb) have been linked to impairment in neurobehavioral and growth development. In order to assess lead exposure in a Latin-American country where this metal has been removed from gasoline, BPb concentrations, morphometric and demographic parameters, as well as several hematological variables were evaluated in 189 randomly chosen children, 5-9 years old, attending primary schools located in different neighborhoods in Cartagena, Colombia. The arithmetic mean $\pm$ standard error BPb level was 5.5 ± 0.2 μ g/dL (range $<1.0 - 21.0$ μ g/dL). Fourteen children (7.4%) had BPb levels above the US Centers for Disease Control and Prevention's threshold of concern (10 μ g Pb/dL). BPb levels were weakly but significantly correlated with red blood cell count, and inversely with child stature, age, mean corpuscular volume, and mean corpuscular hemoglobin. BPb levels did not differ significantly between boys and girls, but significant differences were observed between locations ($P < 0.001$). Among the children with BPb levels greater than 10 μ g/dL, four had microcytic homogeneous and nonregenerative anemia, and an equal number also presented basophilic stippling. These children were visited at their homes, and in all cases, lead source was linked to artisanal metal melting-related processes, and fishing net sinker production by fishermen. An educational program about the health effects of Pb exposure is being implemented to prevent poisoning in children from Colombia. Supported by CICTE, UniCartagena.

414 DEVELOPMENT OF A MAGNETIC IMMUNOSENSOR TO BIOMONITOR FOR THE CHLORPYRIFOS METABOLITE TRICHLOROPYRIDINOL.

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Biological monitoring approaches are being developed using portable analytical systems to quantify dosimetry utilizing readily obtainable body fluids (i.e. blood, urine, and saliva). To assess dosimetry to the insecticide chlorpyrifos (CPF), a fast, simple, and sensitive bioelectrochemical magnetic immunosensing method was developed to measure the metabolite trichloropyridinol (TCP). Under optimal conditions the immunosensor detected a TCP concentration as low as 6 ng L⁻¹, which is 50-fold lower than the reported limit for a commercially available TCP ELISA assay. To establish the utility of the immunosensor, in vivo pharmacokinetic studies were conducted. Rats were given single oral gavage doses (1, 10 or 50 mg/kg) of CPF, and saliva and blood were collected from groups of animals (4/time-point) at 3, 6, and 12 hr post-dosing, and were analyzed for TCP. Trichloropyridinol was detected in both blood and saliva at all doses and the concentration in blood exceeded saliva; although the kinetics in blood and saliva were comparable. The results from these experiments were then used to further develop a physiologically based pharmacokinetic (PBPK) model for CPF that incorporated a compartment model to describe the blood and saliva time-course of TCP. The computational model adequately simulated the results over the dose ranges evaluated. The model was further used to simulate the blood and saliva TCP concentrations for human dietary CPF exposures in the range of the Allowable Daily Intake (ADI) or Reference Dose (RfD) for CPF (0.01-0.003 mg/kg/day). The simulations suggest that the immunoassay has adequate sensitivity to detect and quantify TCP in blood and saliva at these low exposure levels. However, further studies are needed to fully understand the pharmacokinetics of CPF and TCP, particularly at low environmentally relevant doses. These initial findings suggest that the TCP immunosensor repre-

sents a novel approach with broad application for evaluating both occupational and environmental exposures to CPF. (Supported by CDC/NIOSH grant R01 OH008173-01)

415 CHARACTERIZATION OF PROTEIN ADDUCTS OF SULFURYL FLUORIDE WITH ALBUMIN PROTEIN IN THE MALE F344 RAT.

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Inhaled test materials may react with nucleophilic macromolecules (hemoglobin, albumin) following absorption. Quantitation of these adducts can provide useful information to assess exposure to xenobiotic compounds. Sulfuryl fluoride (SO₂F₂) is a structural and post-harvest fumigant used to control a wide variety insect pests. SO₂F₂ is a key alternative to methyl bromide, a stratospheric ozone depleting substance scheduled for phaseout by the Montreal Protocol. The tissue distribution of radioactivity after exposure to ³⁵S-SO₂F₂ has been shown to be highest in lungs and nasal mucosa (Mendrala 2005), suggesting significant uptake of inhaled SO₂F₂ or its metabolites in both nasal and pulmonary tissue. SO₂F₂ has also been shown to be highly reactive with endogenous nucleophiles such as hydroxide and amine moieties of protein (Mielke 1964; Cady 1974). To characterize potential covalent adducts of SO₂F₂ with albumin, a major protein in blood and nasal/lung lavage fluids, *in vitro* experiments were conducted to characterize the extent and site of alkylation to this protein. Treatment of rat albumin with high vapor concentrations of SO₂F₂ (500-50,000 ppm x 4hr) resulted in addition of 1-10 SO₂F moieties to each protein molecule, as measured by ESI-TOF-LS/MS. ESI-LC/MS/MS analysis of tryptic digests of these treated albumin samples and data analysis with MASCOT allowed for the identification of two unique peptides containing a tyrosine residue alkylated with SO₂F. Subsequent analysis of tryptic digests of plasma and lung lavage fluid from male F344 rats exposed to 300 ppm SO₂F₂ (4 hr) afforded identification of one of the alkylated peptides seen in the *in vitro* experiment in both sample matrices. These data indicate that SO₂F₂ has the potential to react with albumin present in the airways of the rat, and possibly peripheral circulation. Future studies are planned to evaluate the site- and dose-dependence of this adduct formation.

416 GLOBIN S-PROPYLCYSTEINE AND URINARY N-ACETYL-S-PROPYLCYSTEINE AS BIOMARKERS OF 1-BROMOPROPANE EXPOSURE.

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1-Bromopropane (1-BP), an alternative to ozone-depleting solvents, is a neurotoxin and a reproductive toxicant in animals and humans. In the study presented here, the dose response of urinary N-acetyl-S-propylcysteine (AcPrCys) and S-propylcysteine (PrCys) adduct formation on globin and neurofilament preparations were evaluated as potential biomarkers of exposure. To evaluate the response of these indices to differing exposure levels, male Wistar rats were exposed to 1-BP by inhalation for two weeks at either 0, 50, 200, or 800 ppm (8 hr/day, 5 days/week). Urinary AcPrCys and PrCys protein adducts all increased with increasing exposure levels as determined by LC/MS/MS. To evaluate the response of AcPrCys and PrCys adducts to different exposure durations male Wistar rats were either exposed to filtered air or 1-BP for 4 weeks at 50 ppm (8 hr/day, 5 days/week). Urine was collected before the first day of exposure and blood and urine collected after the fifth day of exposure each week. After 4 weeks half of the animals were euthanized and blood collected and one week later the remaining rats were euthanized and blood obtained. Urinary AcPrCys and globin PrCys adduct levels were determined using LC/MS/MS and observed to increase with duration of exposure; and a first approximation of elimination kinetics obtained. Blood was also obtained from workers in a 1-BP production facility and analyzed for globin PrCys adducts by LC/MS/MS. A significant increase in globin PrCys adducts was observed in the 1-BP exposed workers relative to nonexposed workers. These results demonstrate the ability of 1-BP to covalently modify proteins within the nervous system as well as peripheral proteins and support urinary AcPrCys and PrCys adducts as potential biomarkers of 1-BP exposure.

417 FIPRONIL URINE BIOMARKERS IN HUMANS FOLLOWING TOPICAL TREATMENT OF DOGS.

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Fipronil (I; 5-Amino-1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-4-(1,R,S)-(trifluoromethyl)sulfinyl)-1H-pyrazole-3-carbonitrile; CAS number 120068-37-3) is an important insecticide in 47 registered products for pest management. Pet owners

often protect dogs and cats from fleas with topical applications of liquid formulations at rates of up to 5 mg fipronil/kg dog. The extent of human exposures resulting from these applications has not been reported. We have used spot urine specimens from persons familiar with our biomonitoring research who have inquired about the extent of their I exposure. Composite samples of two persons, A and B, were created using 30 ml of urine from 15 spot samples from each person to give two 450 ml composites for protocol analysis. Composites were stored at -10C until needed for analysis. Samples were acid hydrolyzed at 24, 40, and 80 C in 0.5N HCl from 0-21 hr and passed through a Bond Elut-Certify II SPE Cartridge (Varian, Harbor City, CA). Samples were eluted with 4 mL ethyl acetate and saturated with sodium sulfate. Elutions were analyzed using HPLC with UV/Vis detection at 275 nm. Acid hydrolysates of urine contain microgram amounts of the corresponding sulfone (II) and sulfide (III). Preliminary urine biomarker indices of exposure (0-1.3 µg/mL urine and 0-2.7 µg/g creatinine) represent potential aggregate exposure following safe use of I products. Biomarkers appear within 1 day of use of I and urine elimination of progressively smaller amounts of II and III continues for several days to 2 weeks depending upon the extent of exposure. Primary contact with treated pets and secondary contact with indoor residues are likely based upon previous studies with chlorpyrifos products. When knowledge of absorption, distribution, metabolism, and elimination are available these data will be useful to estimate absorbed dosage (µg/person) for risk characterization. Alternatively, urine biomarkers may be useful to construct biological exposure indices.

418 A NOVEL APPLICATION OF N-ACETYLCYSTEINE (NAC) IN BIOLOGICAL MONITORING OF METHYLMERCURY (MEHG) EXPOSURE – A PRELIMINARY STUDY.

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Scalp hair is the current media of choice for assessing MeHg exposure in humans. However, the new EPA hair reference level of only 1-2 ppm is close to the background level found in hair, limiting the utility of hair in monitoring relatively low levels of exposure. Cord blood concentration is a better predictor of nervous system deficits determined during postnatal follow-up, but cord blood is not always accessible for analysis, and by the time it is obtained it is too late to reverse any damage that might have been done. The present work was embarked upon to search for a new method of bio-monitoring for MeHg exposure using NAC. We took the advantage of our previous finding that NAC is remarkably effective at enhancing urinary excretion of MeHg in mice and hypothesized that an NAC challenge dose will produce a transient increase in urinary MeHg excretion that is proportional to the MeHg body burden. Unlike other chelating agents including diethiols, NAC does not alter tissue distribution of essential metals, and has minimal side effects. Anesthetized Wistar rats (250-300g) were given different doses of NAC intravenously following intravenous injection of [14C] MeHg and urine samples were collected at 30 min intervals. In addition, a selected NAC challenge dose was applied to graded doses of [14C] MeHg that mimics the body burden of MeHg following a meal of contaminated fish. The results show that the NAC challenge dose consistently produced a transient (<2h) increase in urinary MeHg excretion that was proportional to the body burden. This rapid, reproducible, and dose-dependent response to NAC challenge makes it a promising bio-monitoring agent for MeHg exposure, and thus for predicting the MeHg body burden. Current studies are evaluating different NAC dosing regimens for applications under conditions that more closely mimic environmental MeHg exposure, with the hope that a similar protocol may eventually be tested in humans. (Supported in part by NIH grants ES01247, ES07026, and ES06484)

419 SPECIATION OF ARSENIC IN BIOLOGICAL MATRICES BY AUTOMATED HG-AAS WITH MULTIPLE MICROFLAME QUARTZ TUBE ATOMIZER (MULTI-ATOMIZER).

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Analyses of arsenic (As) species in body fluids and tissues of individuals chronically exposed to inorganic arsenic (iAs) provide essential information about the exposure level and pattern of iAs metabolism. This information facilitates the risk assessment of disorders associated with iAs exposures. We have previously described an oxidation state-specific analysis of As species in biological matrices by hydride-generation atomic absorption spectrometry (HG-AAS), using a cryo-trap for preconcentration and separation of arsines. In order to improve performance and detection limits of

the method, we have recently automated the cryotrapping and HG step and replaced a conventional flame-in-tube atomizer with a newly developed multiatomizer. Arsenines from As(III)-species are generated in a mixture of 0.75M Tris-HCl (pH 6) and 1% sodium borohydride. For generation of arsenines from As(V)-species, samples are pre-treated with 2% L-cysteine. The analytical performance of this system was characterized using cultured cells and tissues of mice exposed to iAs. Detection limits have improved significantly reaching 12 pg for iAs, 17 pg for MAs, and 13 pg for DMAs in cell lysates. Recoveries were between 92-98%. The redesigned system was used for As speciation in exfoliated bladder cells isolated from 21 individuals exposed to iAs in drinking water. All samples contained iAs, MAs and DMAs, however, the amounts and proportions of these arsenicals varied among donors and did not correlate with As levels in urine. The sum of As species in cells ranged from 4 to 18 ng. Analysis of As species in small samples from human tissues targeted by iAs exposure may provide an effective tool for risk analysis of diseases associated with this exposure. (This abstract does not reflect EPA policies.)

420 NOVEL APPROACH TOWARDS IDENTIFICATION OF BIOMARKERS FOR HORMONE EXPOSURE.

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Traditional risk analysis often implies estimations by extrapolating from dose-response relations established in animal experiments. Often, physiological effects in animals can only be induced at high doses. DNA microarray technology, combined with statistical techniques, may offer the possibility to identify biomarkers for effects at lower doses.

To address this issue, we have performed an animal study in which we exposed immature female rats for 3 days to increasing doses of ethinyl estradiol (EE2, 0.03–10 µg/kg/day) and examined uterus weight and several additional parameters, including uterine gene expression profiles. Subsequently, we used flexible spline models to assess how much of the variation in uterus weight was explained by the variation of the dose. Furthermore, spline models were fitted to explain gene regulation from dose, as well as, uterus weight from gene regulation. From the two latter models the explanatory power of each gene as a biomarker for the dose–response relation was estimated and calculated over the total dose range, or restricted to the low doses. Additionally, we aimed to select biomarkers that suffice as a unique intermediate link in the causal relation from dose to response.

A general conclusion is that many genes, including Gjb2, Syk, Gfra2, apelin, calbindin and Gap43, showed high correlations with both dose and response, and also with each other. These genes could therefore be selected as sensitive biomarkers for exposure to and as biomarkers for effects of estrogenic compounds. Future applications could consist of *in vitro* systems expressing these genes in a comparable manner as the *in vivo* system, allowing for high-throughput testing of estrogenic compounds without animal testing. Furthermore, this method of biomarker identification could be applied to any group of chemicals of interest.

421 MASS SPECIFIC DISCOVERY OF DNA ADDUCTS.

R. W. Giese and P. Wang. *Pharmacology Sciences/Barnett, Northeastern University, Boston, MA.* Sponsor: R. Schatz.

Up until the present time, unknown DNA adducts in biosamples generally have been detected with low specificity, impeding their development as biomarkers for cancer, for example to help individualize cancer prevention. We are reporting a new method (IMI-MS) that broadly discovers unknown DNA adducts with high specificity by mass spectrometry. In our method, enriched adducts from a nuclease digest of DNA (50–100 µg) are phosphate-specifically tagged with an isotopic pair of benzoyl-histamines and detected by MALDI-TOF-MS. Examples of adduct data from the method are as follows. Calf thymus DNA exposed in a test tube to cigarette smoke and DNA from smoker lung share the following adducts: 355.067 (etheno-dAMP), 387.093, 401.110 and 441.105. The latter might be N7-phenylfapydGMP (exact mass 441.105) derived from exposure to benzene oxide. Adduct 359.064, that is observed in many DNA samples, may be formyl-dAMP (exact mass 359.064), a previously undetected lesion that might arise from the reported oxidative intermediate formylphosphate on DNA. In a test tube exposure, p-benzoquinone, a metabolite of the ubiquitous carcinogen benzene, is observed for the first time (perhaps with some propensity) to adduct onto 5-methylcytosine in DNA, in addition to forming known C, A and G adducts. Especially if this BQ-MeC lesion, by perturbing local DNA structure, enhances damage in one way or another to a diagonal MeC deoxynucleotide, then exposure to benzene could erase epigenetic sites. The first direct detection by the method of N6-methyladenine in mammalian DNA samples (e.g. human leukocytes) remains to be interpreted as bacterial contamination (uninteresting result), or as further evidence for this compound as the sixth base of DNA (interesting observation). The method is a new tool for studies of both simple and complex (e.g. human) exposures. It will help to determine unknown DNA adduct structures, to make precise adduct connections

between different exposures, and to formulate and test adduct-related hypotheses. (Supported by CA84641, CA106006, and the Environmental Cancer Research Program. Tissue samples were from NDRI.)

422 GCSPRI: A MULTIPLEXED MICROARRAY FOR PATHOGEN, TOXIN AND TOXICANT BIOSENSING.

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Surface plasmon resonance (SPR) is a technology that can measure minute changes in the optical properties of an aqueous medium close to a metal surface, represented by a shift in the SPR angle, allowing for the sensitive detection of changes in mass at that surface that result from the capture of molecules or cells. SPR analysis has been used to detect various pathogens or toxins with great sensitivity, suggesting the potential of the technology as a biosensor, but the traditional Kretschmann SPR configuration is limited in the number of analytes that can be simultaneously detected. Grating-Coupled Surface Plasmon Resonance Imaging (GCSPRI) represents a novel method for detecting a wide range of pathogens, toxins, and toxicants. Our experiments show that GCSPRI is capable of sensitive and quantitative real-time detection of multiple analytes, including viruses, bacteria, protein toxins, and toxicants. In line with the requirements of a robust environmental biosensor, we have demonstrated the capability of a single GCSPRI sensor chip to detect multiple analytes in succession, simultaneously, and from a surrogate environmental samples. By immobilizing an antigen on the chip in order to measure the amount of a cognate antibody in serum samples, it is also possible to monitor pathogen- or toxin-specific seroconversion in order to identify previous exposure to such agents. Our results demonstrate that GCSPRI technology is capable of high-throughput, real-time, label-free, sensitive detection of a broad range of representative pathogens, toxins, and toxicants highlighting the technology's numerous potential applications in the fields of biodefense and environmental monitoring. Supported by N43 ES 2490, DMI-0321646, USDA grant 58-1940-0-007

423 PLUCKED HUMAN ANAGEN HAIR IS UNIQUELY SUITED FOR BIOMARKER DEVELOPMENT; A COMPARATIVE STUDY OF PLUCKED MAMMALIAN HAIR FROM DIFFERENT SPECIES.

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Because the anagen (actively growing) hair follicle is such a metabolically active organ, plucked hair is being used with increasing frequency to define the efficacy of compounds, especially those that influence the cell cycle. The purpose of this study is to further define the utility of plucked hairs for biomarker development. Plucked hair from mice, rats, dogs, minipigs, cynomolgus monkeys and humans were stained with hematoxylin and characterized as being in anagen, telogen or undetermined (for humans). Telogen and anagen hairs were evaluated for morphology and cellularity. In all species, telogen hairs had differing club morphologies. In dogs, minipigs, monkeys and humans, telogen hairs were lined by a small layer of epithelial cells surrounding the club region. In anagen follicles, only human plucked hairs could be extruded intact, defined by the presence of an inner and outer root sheath. Plucked minipig hairs were sometimes surrounded by an extensive outer sheath but no inner sheath. Because plucked human anagen hairs were morphologically intact, confocal microscopy was used to further evaluate their morphology. Confocal microscopy was shown to be an improvement over standard sectioning procedures for evaluation of targets of interest that can be identified with fluorescent labels in plucked hairs. In summary, plucked human anagen hair offers considerable potential to establish tissue biomarkers; however, because only human anagen hairs can be extruded intact, this method may not be highly translatable to other species.

424 AN ASSESSMENT OF OVERALL ESTROGEN EXPOSURE FOR THE GENERAL POPULATION AND SEVERAL SUB-POPULATIONS IN THE UNITED STATES.

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Detections of estrogens in the environment have raised concerns in recent years because of their potential to affect both wildlife and human development. Often little context is provided as to whether potential drinking water exposures in the United States are large or small compared to other sources of exposure (e.g., dietary intake).

This study estimates the combined environmental and dietary exposure on a mass basis to endogenous, synthetic, natural product and plant-derived estrogens; and, quantifies the relative importance of the different types and sources of estrogens. For most of the U.S. population, greater than 99% of the total estrogen exposure comes from estrogens in the diet (most of which is comprised of phytoestrogens). The exception is women taking estrogens for contraception or hormone therapy for whom the prescribed source can be important. Drinking water exposures make a minor contribution. Moreover, estrogens derived from therapeutic use that are predicted to be present in drinking water (from excretion after human-use) represent less than 0.0001% of total exposure to estrogens for all age groups. Using the results of this study to characterize the potential effects of estrogens is challenging and complex. Because estrogens affect many endpoints and relative biological activity varies among endpoints, a detailed risk assessment would require investigating multiple endpoints and adjusting the estimated exposures presented in this study for differences in biological activity for each endpoint being investigated. Nevertheless, comparison of the relative contribution of different sources of estrogens clearly indicates: diet dominates total estrogen exposure; exposure to prescribed estrogens through drinking water is a very small fraction of total exposure; and, the contribution of prescribed estrogens via drinking water to total estrogen exposure is so small that adverse effects to human health from prescribed estrogens in drinking water are not likely to occur in the United States.

425 DEVELOPMENT OF PCDD/F AND DIOXIN-LIKE PCB SERUM CONCENTRATION REFERENCE VALUES FOR THE GENERAL U.S. POPULATION USING THE 2005-WHO TEFS AND THE 2001-2002 NHANES DATA.

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The availability of serum concentration reference values has become essential as biomonitoring data is being used more often to determine if individuals have potentially been exposed to specific chemicals. Serum concentration reference values were recently published for PCDD/Fs and dioxin-like PCBs based on the 2001-2002 NHANES data and the 1998 WHO TEFS. Since that time, however, WHO has revised the TEF values for a number of congeners. In accordance with these changes, it was necessary to determine new reference statistics by applying the 2005-WHO TEFS to the weighted 2001-2002 NHANES serum concentration data. Updated summary statistics were developed for several demographic subgroups including males and females, Hispanics and non-Hispanics, smokers and non-smokers, as well as for several representative age groups. In addition, the contribution of individual congeners to the total TCDD TEQ and the effect that the revised TEFS had on total TEQ serum concentrations for the general U.S. population were also determined. The updated TEFS had little impact on the overall percent contribution of the various congeners to the total TEQ: 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD, 2,3,4,7,8-PeCDF, and PCB 126 still contributed the most to the total TEQ. However, both PCB 156 and PCB 157 were no longer significant contributors, instead being displaced by 1,2,3,4,7,8-HxCDF and PCB 169. The effect of the new TEFS on total TEQ was consistent with the change in magnitude of the TEFS. In general, the decrease in TEFS for the mono-ortho substituted PCBs decreased their contribution to total TEQ appreciably. Using the new TEF scheme, TEQ17-9 was approximately equal to TEQ17-3. Within groups, differences in TEQs using the two schemes were more evident in groups with higher serum levels (e.g. Age 60+) than in groups with lower levels.

426 DEVELOPMENT OF A FRAMEWORK FOR DETERMINING THE APPROPRIATENESS OF A BIOMARKER OF EXPOSURE.

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The ILSI Health and Environmental Science Institute's Biomonitoring Technical Committee is sponsoring work to develop a practical method for determining the appropriateness of a biomarker for specific questions of exposure. The resulting framework is based upon the use of interpretive criteria developed for the International Biomonitoring Workshop co-sponsored by the ILSI Health and Environmental Sciences Institute. The criteria cover a wide range of attributes regarding a biomarker's characteristics; for example, how well the human pharmacokinetics of the biomarker are understood, the strength of association of the exposure to the level of the biomarker, the temporality of the biomarker, and numerous criteria dealing with analytic measurement of a biomarker. A criterion is quantifiable based upon its relationship to the marker being assessed, thus making it possible to compare the results of the criteria for different biomarkers to help the assessor determine the best choice of biomarker. Further, the framework is accessed

through a simple spreadsheet, thus making it easily assessable and user friendly. The overall framework will help assessors make a decision regarding the best choice of biomarker for specific questions of exposure; the possible application/development of a similar framework for biomarkers addressing health risk is discussed.

427 INTERPRETING HUMAN BIOMONITORING DATA IN A RISK ASSESSMENT CONTEXT: BIOMONITORING EQUIVALENTS.

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Advances in analytical methodology have made it possible to detect and quantify trace amounts of synthetic and natural environmental chemicals and their metabolites in various human tissues and biological matrices. However, conventional exposure and risk assessment paradigms are structured in terms of administered dose rather than the absorbed dose or tissue concentrations. Health-based screening criteria are generally presented in terms of administered dose (e.g., reference doses or cancer slope factors) or external media concentrations (reference concentrations in air, for example). Thus, the snapshot of integrated uptake of a chemical provided by a biomonitoring sample cannot be directly interpreted in terms of these traditional risk criteria. We propose here application of pharmacokinetic modeling techniques to derive blood or urinary levels of analytes that are consistent with exposure at existing health-based screening guidelines such as reference doses. These levels are termed "biomonitoring equivalents" or BEs. Approaches for using pharmacokinetic data from a variety of sources including clinical experiments, occupational monitoring data, etc. are described. The BE approach leverages existing regulatory health-based exposure guidelines to provide a screening tool for evaluation of preliminary biomonitoring data, allowing prioritization of chemicals for further, more detailed study using epidemiological techniques or to delineate exposure pathways. BEs are presented for several case study chemicals.

428 ANALYSIS OF 1, 2,3, 4-DIEPOXYBUTANE SPECIFIC PROTEIN ADDUCT IN OCCUPATIONALLY EXPOSED WORKERS.

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Butadiene (BD) is an industrial chemical used in the production of synthetic rubber which is also found in gasoline and cigarette smoke. It is a multisite carcinogen in rodents, with mice being more susceptible than rats. BD is categorized by NTP and US EPA as carcinogenic to humans by inhalation. BD is metabolically activated to several epoxides that differ in their mutagenic potency by up to 200-fold and are known to form DNA and protein adducts. To advance our understanding in BD metabolism and carcinogenesis it is important to have accurate measure for the internal formation of the individual epoxides. Therefore, the internal formation of the promutagenic 1,2,3,4-diepoxybutane (DEB) in vivo was determined indirectly by analysis of the corresponding N,N-(2,3-dihydroxy-1,4-butadiyl)-valine (pyr-Val) adduct. To achieve this, globin samples from BD-styrene polymerization plant workers in the Czech Republic were analyzed for pyr-Val using a recently established method (Boysen et al 2004). Stable isotope internal standard was added to 50 mg globin, followed by hydrolysis with trypsin, purification by immunoaffinity (IA) chromatography and quantification by LC-MS/MS. The LOD and LOQ were 5 fmol and 10 fmol, respectively. Among the analyzed 104 subjects from the Czech study (26 female controls, 23 female BD exposed workers, 25 male controls and 30 male exposed workers) 12 samples had quantifiable amounts of pyr-Val ranging from 0.2 to 0.8 pmol/g globin. Mean 8-h exposure levels to BD were 0.0035 and 0.180 ppm for female controls and exposed, respectively, and 0.0032 and 0.370 ppm for male controls and exposed, respectively. At the time of abstract submission, all samples remain blinded for exposure and gender groups. Following completion of a second independent set of analyses and data submission, codes will be broken for exposure, gender and genotype assignment. These data will be available on the poster.

429 IN UTERO AND LACTATIONAL EXPOSURE TO 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) PERMANENTLY ALTERS CARDIOVASCULAR MORPHOLOGY AND EXTRACELLULAR MATRIX REMODELING.

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Studies have shown that the murine heart is a sensitive target of TCDD during fetal development. Microarray analysis demonstrates that in utero and lactational TCDD exposure significantly induces the expression of cardiac genes involved in

extracellular matrix (ECM) remodeling. We tested the hypothesis that this exposure would permanently disrupt cardiac ECM expression and be associated with cardiac remodeling in adulthood. Time-pregnant C57Bl6 female mice were treated with vehicle control (corn oil) or 1.5, 3.0, or 6.0 μg TCDD/kg on GD 14.5. Dams were sacrificed on GD 17.5 and fetal hearts were assessed for ECM mRNA expression, using quantitative RT-PCR. Additional dams from the 6.0 $\mu\text{g}/\text{kg}$ group were allowed to deliver, and adult offspring were sacrificed at approximately 3 months of age and were assessed for ECM mRNA expression as well as cardiac morphology. Quantitative RT-PCR of fetal hearts revealed a significant induction of CYP1A1, endothelin (ET-1), osteopontin, and matrix metalloproteinase (MMP) 13 mRNA following exposure to 3.0 or 6.0 μg TCDD/kg, while MMP9 was significantly induced at 6.0 μg TCDD/kg, and 18s rRNA was significantly induced at all three doses. While hearts of adult offspring exposed to TCDD no longer showed induction of CYP1A1 or ET-1, they did exhibit induction of fibrosis markers and regulators of ECM, including collagen I, osteopontin, MMP2, MMP9, and MMP13. Further, TCDD-exposed offspring exhibited increased left ventricular mass and induction of cardiac hypertrophy markers, atrial natriuretic peptide and β -myosin heavy chain. These results suggest that early TCDD exposure may predispose animals to cardiac remodeling and hypertrophy with age. Future studies will determine if alterations in fetal gene expression are mediated by the aryl hydrocarbon receptor and whether early exposure increases the susceptibility to cardiovascular disease following a second cardiovascular insult in adulthood. Supported by ES012335 to MKW and ES012335-0281 to ACA.

430 ARYL HYDROCARBON RECEPTOR PARTICIPATES IN POST-TRANSCRIPTIONAL CONTROL OF WILMS' TUMOR SUPPRESSOR GENE AND MURINE NEPHROGENESIS.

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The molecular mechanisms governing ontogenesis are regulated by environmental and somatic factors in utero that activate or repress the expression of numerous genetic elements. The aryl hydrocarbon receptor (Ahr) is an important ligand-activated nuclear transcription factor during embryogenesis and throughout maturity in multiple organisms. Environmental hydrocarbons acting as Ahr ligands deregulate Ahr functions to induce deleterious developmental deficits in several systems, including the kidney. The Wilms' tumor suppressor gene (Wt-1) encodes a Cys2-His2 zinc finger protein that mediates transcriptional repression/activation of target genes involved in the regulation of mesenchymal-to-epithelial (MET) trans-differentiation during renal development. Wt-1 is regulated at the mRNA level via insertion of lysine-threonine-serine residues (KTS) between the third and fourth zinc finger resulting in modification of Wt-1 DNA binding specificity. Using a metanephric kidney organ culture system we have shown that benzo(a)pyrene (BaP), an Ahr ligand, inhibits nephrogenesis via an Ahr dependent mechanism in embryonic day 11.5 (E11.5) C57BL/6J (C57) mouse embryos and alters -KTS/+KTS Wt-1 mRNA ratios after a consecutive four-day exposure. Altered Wt-1 mRNA ratios correlate with a reduction in mRNA for Wt-1 transcriptional targets (syndecan 1, Pax2, Egf-receptor, and RaR α), and renal differentiation markers (Sfrp1, Igf1r, Igf2r, Wnt4, Lhx1, and E-cadherin). Metanephroi from B6.D2N-Ahrd/J (D2N) a co-isogenic mouse strain expressing an Ahr variant with reduced ligand-binding affinity are protected from BaP-induced deficits in renal cell differentiation. Thus, BaP and related Ahr ligands may disrupt nephrogenesis in vivo by altering the ratio of Wt-1 mRNA splice variants and expression of downstream Wt-1 targets. These data establish a link between Wt-1 and Ahr in the regulation of transdifferentiation events for diseases of the kidney such as Wilms' tumor, Denys-Drash syndrome, Frasier syndrome and Beckwith-Wiedman syndrome.

431 DEVELOPMENTAL AND MOLECULAR INTERACTIONS BETWEEN THE HYPOXIA AND ARYL HYDROCARBON RECEPTOR (AHR) PATHWAYS IN ZEBRAFISH.

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Previous zebrafish (*Danio rerio*) studies have shown that exposure to the model PAH β -naphthoflavone (BNF) or the environmentally relevant PAH benzo[a]pyrene (BaP) substantially increases CYP1A expression and activity, while co-exposure to the CYP1A inhibitors α -naphthoflavone (ANF) or fluoranthene (FL) blocks the BNF or BaP-mediated induction of CYP1A. This reduction in CYP1A activity is thought to account for the marked synergistic developmental toxicity (e.g. pericardial edema and jaw deformities) observed in BNF/ANF and BaP/FL coexposed embryos, effects not observed in embryos exposed to these compounds independently. This synergistic embryotoxicity seems to be a generalized response to the coexposure of an AHR agonist and CYP1A inhibitor. A plausible ex-

planation for this interaction is that reduced metabolism leads to prolonged activation of the AHR, which appears to underlie the toxicities of recalcitrant AHR agonists (e.g. TCDD). We hypothesized that a hypoxia-induced reduction in CYP1A activity in BNF or BaP exposed embryos would similarly enhance pericardial effusion and other developmental abnormalities. Various combinations of BNF/ANF or BaP/FL were performed under hypoxia and normoxia. CYP1A activity in the BNF/hypoxia and BaP/hypoxia embryos was reduced by approximately 50% relative to normoxia embryos. Although CYP1A activity was reduced, we did not observe any increase in pericardial edema. However, pericardial edema increased in co-exposed embryos (BNF/ANF or BaP/FL) under hypoxic conditions compared with those under normoxic conditions. These results suggest that reduced CYP1A activity may not underlie the observed developmental toxicity, and that hypoxia may exacerbate the developmental toxicity of some PAH mixtures. This research was partially funded by the NIEHS Duke Superfund Basic Research Center (P42 ES10356) and the NIEHS-supported Integrated Toxicology and Environmental Health Program (T32 ES07031).

432 TCDD IS A POTENT DEVELOPMENTAL TOXIN, BUT FAILS TO AFFECT SPERMATOGENESIS IN OFFSPRING OF CHRONICALLY TREATED CRL:WI(HAN) RATS.

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One of the most potent adverse effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is on the developing male reproductive system, after in utero exposure [1]. We have previously found that a single dose of TCDD causes no decrease in F1 sperm levels [2], and we have now undertaken a study to determine if chronic dosing of TCDD affects the developing rat.

CRL:WI(Han) rats were weaned and exposed to TCDD for 12 weeks before pairing, then during pairing until the end of gestation. Animals were killed for determination of TCDD levels, with ~30 animals per group littering. >90 F1 animals per group (67 for the high dose group) were analysed.

The high dose group had 8 animals with total litter loss (vs. 3 in control), there was an adverse effect on pup viability and a dose-related decrease in pup weight at birth. The F1 males showed a significant, dose-related delay in balano-preputial separation (BPS), and the high dose group were slightly less active in motor activity trials. There were no adverse effects of maternal treatment on seminology data at PND70 or 120, nor any effect on the fertility of the animals. Although there was a decrease in epididymis weight at PND 70 in the high dose group, there were no decreases in the weight of accessory sex organs at PND120; nor were there any significant findings in tissues examined by light microscopy at PND120.

Our data from both an acute and a chronic dosing study show that developmental exposure to TCDD does not decrease sperm counts of F1 animals. However, our chronic dosing data reveal BPS to be an extremely sensitive response to maternal TCDD exposure, and that the dosing regime is a key determinant of response.

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1. Mably, et al (1992) Tox. App. Pharm. 114, 118-126

2. Bell, et al (2005) Toxicologist Abstract 681

433 670NM LIGHT TREATMENT MITIGATES DIOXIN-INDUCED HEPATIC OXIDATIVE STRESS.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is an acutely toxic anthropogenic chemical. Previous studies have shown that treatment with a red to near-infrared (630 – 1000 nm) light emitting diode (LED) attenuates toxicant-induced oxidative stress and energy deficit in rodent neuronal cell culture. For this study, fertile chicken eggs (*Gallus gallus*) were injected once at the start of incubation with sunflower oil vehicle or 200 pg TCDD/g egg (200 parts per trillion), an environmentally relevant dose. Daily LED treatment (670nm; 4J/cm²) from embryonic day 0 – 20 after TCDD exposure significantly reduced embryonic mortality by 47%, and doubled the probit-determined LD₅₀. Exposure to TCDD alone increased the hepatic oxidized-to-reduced glutathione ratio (GSSG/GSH) by 134%, which was accompanied by a 42% decrease in glutathione reductase (GRx) activity, the key enzyme that actively restores GSH from the oxidized state (GSSG). LED treatment of TCDD-exposed eggs completely reversed the glutathione redox imbalance by decreasing GSSG/GSH by 88% and increased GRx by 55%, restoring activity to the control levels. Our study demonstrates that 670 nm phototherapy can mitigate the oxidative stress resulting from developmental exposure to TCDD and restore he-

patric enzyme activities to control levels in TCDD-exposed embryos. The effective attenuation of TCDD-induced embryo toxicity by LED treatment could extend to mitigating the effects of other teratogens that induce oxidative and energy stress.

434 A DEVELOPMENTAL STUDY OF ENERGY AND GLUTATHIONE REDOX BALANCE IN BRAIN AFTER DIOXIN EXPOSURE AND 670NM LIGHT TREATMENT.

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Daily treatment with a red light (670 nm; 4J/cm²) emitting diode (LED) has been shown to effectively mitigate 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) embryo toxicity in the domestic chicken (*Gallus gallus*) model. Work in other organ systems suggests that TCDD decreases available energy (ATP) and suppresses antioxidant enzyme activity prior to and during hatch (embryonic day 20 - 21), as well as that daily LED treatment (embryonic day 0 - 20) mitigates toxicant induced energy deficit and improves both cytochrome c oxidase activity and energy production. The present study quantifies cellular energy production (ATP), cytochrome c oxidase activity, and glutathione in the developing brain of the chicken exposed in ovo to TCDD. In the developing chicken brain, exposure to TCDD alone (200 ppt) decreased ATP content by 5% and cytochrome c oxidase activity by 17%, and significantly increased the ratio of oxidized (GSSG) to reduced (GSH) glutathione by 178%. In TCDD-exposed chicks given LED treatment throughout development, those trends were completely reversed; ATP was significantly increased by 21% and cytochrome c oxidase activity increased by 27%. The GSSG/GSH ratio was significantly reduced by 69%, primarily by blocking the TCDD-induced 154% increase in GSSG. These results support the hypothesis that daily LED therapy *in ovo* decreases toxicant-induced oxidative and energy stress, and these changes may underlie our previous report of the LED therapy-induced decrease in embryo mortality induced by TCDD. We are currently conducting multiple studies to elucidate the mechanism(s) by which LED therapy attenuates toxicant-induced stress in the developing central nervous system.

435 THE EFFECTS OF RED LIGHT THERAPY ON TCDD-INDUCED OXIDATIVE STRESS IN CHICKEN KIDNEY DURING EMBRYOGENESIS.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent developmental toxicant that causes increased embryo mortality and sub-lethal changes in multiple organs, including the liver and the kidney. TCDD has been shown to induce oxidative and energy stress by increasing production of oxygen radicals at the cellular level. In this study, we investigated TCDD induced oxidative stress and the effects of daily red light (670nm at peak; 4 J/cm²) therapy (RLT) in the chicken kidney during development. Eggs were injected once prior to incubation with sunflower oil vehicle control, 2pg/g, 20pg/g, or 200pg/g TCDD; half of the eggs in each dose were then treated with red light once per day through embryonic day 20 (E20). Upon hatching (E21), the kidneys were collected and assayed for glutathione peroxidase [GPx], glutathione reductase [GRx], catalase [CAT], superoxide dismutase [SOD], reduced glutathione [GSH], and ATP. The effectiveness of the red light treatment at the 200 pg/g TCDD dose is evident in GPx activity (RLT:14% vs. Non-RLT: 36% decrease), GRx activity (RLT: 22% vs. Non-RLT: 32% decrease), CAT activity (RLT: 13% vs. Non-RLT: 22% decrease), SOD activity (RLT: 19% vs. Non-RLT: 29% decrease), GSH concentration (RLT: 5% vs. Non-RLT: 36% decrease), and ATP level (RLT: 6% vs. Non-RLT: 25% decrease). Our findings indicate that TCDD induced oxidative and energy stress in chicken kidneys and that daily RLT throughout development attenuated decreased ATP, GSH depletion, and lowered antioxidant enzyme activities. These changes also suggest that RLT therapy could counteract renal damage induced by increased oxygen radicals at cellular levels.

436 EXAMINATION OF METAL INDUCED TOXICOGENOMIC RESPONSE DURING NEURULATION IN RESISTANT AND SENSITIVE MOUSE STRAINS.

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Differential sensitivity to environmentally induced Neural Tube Defects (NTDs) has been observed between inbred mice strains. In particular, the C57BL/6(C57) is more sensitive than the SWV to Cd exposure during neurulation. We hypothesize

that Cd induces NTDs by impacting gene expression in cell cycle and apoptosis pathways and that the observed difference in Cd-sensitivity is due to its differential effect on these pathways. We used the Codelink Uniset 20K mouse I array to evaluate for gene expression alterations in embryos of C57 and SWV mice, 12 and 24 hrs post-injection (GD8.0, ip) of either vehicle or 4mg/kg(BW) Cd. To determine associations between developmental stage, treatment, and strain, we employed MAPPFinder(GenMAPP) to observe categorical and pathway-based changes in gene expression. In both the C57 and SWV, Cd induced up-regulation of genes involved in the cell cycle pathway and kinase activation(12h) as well as down-regulation of genes related to development and neurogenesis(24h). Exclusively, in the C57, Cd induced up-regulation of genes involved in apoptosis(12h). These genes included caspase 8, cyclin g1, and pmaip. In addition, in the C57 we observed down-regulation of genes responsible for cell adhesion, corresponding to dysfunctional adhesion and fusion of the neural tube(24h, GD9.0). Our initial microarray analysis suggests Cd impacts apoptotic and other cell signaling pathways differentially in the C57 compared to the SWV. In both strains, we observed Cd-induced changes in cell cycle genes. Using pathway based assessment approaches, we were able to observe differential impact in specific gene targets. Preliminary western blot analysis of p53, p21, and caspase 3 further supports these specific conclusions. Using a comparative microarray approach, we have correlated Cd-induced alterations in gene expression of these selected signaling pathways with differential neural tube closure sensitivity. EPA:R826886, NIEHS:5P30ES07033, 2P01ES09601, U10ES11387 and NSF:0CE0434087

437 EFFECTS OF PERFLUORINATED ACIDS ON PULMONARY SURFACTANT PROPERTIES *IN VITRO*.

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Dose-dependent reductions in neonatal survival were observed in rodents treated during gestation with perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) but not with perfluorohexane sulfonate (PFHS) or perfluorobutane sulfonate (PFBS). The pathophysiological basis of the neonatal mortality is unknown but clinical signs suggest impaired respiratory function. Studies with PFOS found no evidence of delayed lung maturation or altered surfactant composition or quantity in rats. In the present study, direct effects of these perfluorinated acids (PFA) on surfactant surface properties were evaluated using a captive bubble surfactometer (CBS). The CBS chamber was filled with aqueous media and an air bubble was allowed to float up to the agarose ceiling. A 1µL droplet of bovine lipid extract surfactant (BLES) was spread at the air-liquid interface to form an equilibrium film of 23 mN/m in < 1sec. PFA was added to the media at concentrations of 0.5, 0.1, 10⁻³, 10⁻⁵ and 10⁻⁶% w/w. After 5 min, 20 compression-expansion cycles at 20 cycles/min were conducted. Minimum surface tensions (ST) and the amount of film area compression needed to achieve them were determined. Minimum ST at 0.5% and 10⁻⁶% PFA (means, n=5) were: PFOA 11.9, 1.7; PFOS 16.4, 5.7; PFHS 15.6, 2.9; PFBS 11.1, 2.9 mN/m, respectively, upon film area compressions of 60-80%. The minimum ST for BLES without PFA was 1.0 mN/m (mean, n=5) on film area compressions of <20%. At 0.5% PFA, minimum ST were significantly higher than those at 10⁻⁶% (p<0.01). At 10⁻⁶% PFA, minimum ST approached normal values, but only at film area compressions well above those of normal surfactant films *in vitro* (~20%) or *in situ* in lungs (<30%). Thus, these PFA appear to directly interfere with normal surfactant properties *in vitro* even at relatively low concentrations. Further studies are needed to assess the toxicological significance of these findings. (Supported by a grant from 3M Corporation)

438 EFFECTS OF AMPHETAMINE DRUG CHALLENGE DURING AN INHIBITORY CONTROL TASK IN RATS DEVELOPMENTALLY EXPOSED TO PCBs AND/OR MEHG.

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Developmental PCB exposure has been reported to produce dopamine (DA) hypo-functionality in rats. *In vitro* studies suggest MeHg exposure may exacerbate this effect. To examine this more closely, we conducted a drug challenge study to determine if developmental exposure to an environmental PCB mixture, alone and in combination with MeHg, alters catecholamine function. Female Long-Evans rats were exposed to corn oil (controls), PCBs alone (1 or 3 mg/kg/day in corn oil), MeHg alone (1.5 or 4.5 ppm in drinking water) or a combination of PCBs and MeHg (1 mg/kg PCB + 1.5 ppm MeHg or 3 mg/kg PCBs + 4.5 ppm MeHg) beginning 4 weeks prior to breeding and continuing until litters were weaned on post-natal day 21. One adult male and female per litter were trained to asymptotic performance on a differential reinforcement of low rates (DRL) operant task which has been shown to be prefrontal cortex (PFC) dependent. Rats had to wait 15 s between lever presses to obtain a food reinforcer. Presses occurring too soon reset the

timer and delayed the reinforcer. Testing occurred Monday-Saturday and replicate doses of 0, 0.5 and 1.0 mg/kg amphetamine (AMPH) were given IP 10 minutes before testing on Tuesdays and Fridays via a counterbalanced Latin square design. The 1.0 mg/kg dose of AMPH was less disruptive to responding in male rats receiving 3 mg/kg/day PCBs as well as in males receiving 1.5 ppm MeHg both alone and in combination with PCBs. Paradoxically, rats exposed to 4.5 ppm MeHg, either alone or in combination with PCBs, did not show this effect, and female rats did not differ significantly from controls in their response to AMPH. Interestingly, groups exposed to both PCBs and MeHg did not show a greater effect than groups exposed to PCBs or MeHg alone. Overall, the findings lend support to the hypothesis that either developmental PCB or MeHg exposure may produce DA hypofunctionality in the PFC. (Supported by USEPA R82939001 & NIEHS PO1 ES11263)

439 THE EFFECTS OF INHALED VAPORS OF DIMETHYL DISULFIDE ON EMBRYO/FETAL DEVELOPMENT IN RATS AND RABBITS.

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Dimethyl Disulfide (DMS) is a promising zero-ozone depleting, methyl bromide replacement candidate for soil fumigation applications and a known effective agent used in the sulfiding process of oil refining. These studies were designed to provide information about unique toxicities emanating in prenatal development when DMS was exposed to pregnant rats and rabbits throughout the period of major organogenesis. Four groups of 27 female Crl:CD(SD) rats were exposed to atmospheres of 0, 5, 20, and 80 parts per million (ppm) and four groups of 24 female NZW rabbits were exposed to atmospheres of 0, 15, 45, and 135 ppm. All animals were observed twice daily for morbidity and mortality, and clinical observations, body weight, and food consumption were recorded at appropriate intervals. Laparohysterectomies were performed on rats and rabbits on gestation day 20 and 29, respectively, and the following parameters were examined: Gravid uteri weights, number of early and late resorptions, number of total implantations, number of corpora lutea, number of fetuses, fetal weights, fetal sex, and fetal examination of malformations and variations (external, visceral, and skeletal). From an overall maternal toxicity perspective, the rabbits were not as sensitive as the rats, as reductions in body weight, body weight gain, gravid uterine weight, and food consumption were manifested at 80 ppm in the rats but not at 135 ppm in the rabbits. The high exposure concentration resulted in in utero growth retardation in the rats, but not in the rabbits. Other manifestations of developmental toxicity (prenatal mortality or structural malformations) were not observed in either species at all evaluated concentrations. A NOEL for both maternal and developmental toxicity in rats and rabbits was 20 ppm and 135 ppm DMS, respectively.

440 STRUCTURAL DIFFERENCES BETWEEN THALIDOMIDE AND LENALIDOMIDE CAUSE DIFFERENT DEVELOPMENTAL EFFECTS IN RATS AND RABBITS.

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Lenalidomide (LEN) and thalidomide (THAL) are structurally similar. Developmental toxicity (DT) studies were conducted to compare their effect patterns. LEN (oral gavage) was given to 24 Crl:CD (IGS) BR female rats/group at 0, 100, 300 or 500 mg/kg/day (MKD) on GDs 7-17 (C-section GD 20; standard evaluations). A pulse-dosing and 2 full DT studies (oral, gavage) were conducted in Crl:NZW/Kb BR or Hra:(NZW)SPF rabbits. In the pulse-dosing study (5/group), LEN (100 MKD) or THAL (250 MKD) was given on GDs 8-10. In DT study 1 (20/group), LEN was given at 0, 5, 15 or 25 MKD on GDs 7-17 (THAL: 250 MKD). In DT study 2 (25/group), LEN was given at 0, 0.3, 10, 20 MKD on GDs 7-17 (THAL: 180 MKD). Doses based on range-finding studies (5/group; LEN: 0, 5, 10, 25, 60, 100 MKD; THAL: 0, 10, 20, 60 and 180 MKD). C-section GD 29 with uterine and fetal examinations. LEN maternal and developmental NOELs in rats = 300 MKD (decreased weight gain, feed consumption and fetal weight, increased supernumerary ribs and growth retardations at 500 MKD; postimplantation loss unaffected although the classic effect of THAL in rats). In rabbits, LEN maternal and developmental NOELs = 3 MKD (decreased weight gains, feed consumption and fetal weights and increased postimplantation loss, external, soft tissue and skeletal variations but no malformations at 10 MKD; THAL at 180 MKD selectively reduced fetal weight and increased postimplantation loss, fetal limb abnormalities and other malformations/variations). In the pulse-dosing study, 100 MKD of LEN caused maternal weight loss without affecting conceptuses; at 250 MKD THAL selectively reduced fetal body weight and increased postimplan-

tation loss and limb abnormalities. Thus, despite structural similarity, in two responsive species, LEN and THAL had different developmental effects, confirming observations by Nau (1995) that minor structural differences can remarkably affect developmental outcomes.

441 EVALUATION OF THE DEVELOPMENTAL TOXICITY OF LINALOOL IN RATS.

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The developmental toxicity of linalool, a widely used fragrance ingredient, was evaluated in rats. Linalool or the vehicle, corn oil, were administered orally (via gavage) to pregnant rats (25 rats/group) once daily on days 7 through 17 of gestation at doses of 0 (vehicle), 250, 500 or 1000 mg/kg/day. The rats were observed for viability and examined for clinical signs, abortions, premature deliveries and deaths. Body weights and feed consumption were also recorded. On day 21, all rats were sacrificed and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The uteri were removed and examined for number and distribution of implantation sites, early and late resorptions and fetal mortality. Each fetus was examined for gross external alterations. Approximately one half of each litter was examined for soft tissue alterations and the remaining fetuses were examined for skeletal alterations. No maternal deaths occurred. No clinical signs and no gross changes attributable to linalool were observed at necropsy. During the dosage period, mean relative feed consumption was significantly reduced by 7% and mean body weight gains were reduced by 11% at the highest dose level. During the post-dosage period, feed consumption values at the highest dose level were significantly higher than vehicle control values, which corresponded to the increase in body weight gains during this period. No adverse effects on embryo-fetal development were observed in this study. Caesarean-sectioning or litter parameters, as well as fetal alterations, were not affected by dosages of linalool as high as 1000 mg/kg/day. On the basis of this data, the maternal no observable adverse effect level (NOAEL) of linalool is 500 mg/kg/day and the developmental NOAEL is greater than 1000 mg/kg/day.

442 DEVELOPMENTAL AND PERI-POSTNATAL STUDY IN CYNOMOLGUS MONKEYS WITH BELIMUMAB.

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Belimumab is a fully-human monoclonal antibody specific and antagonistic for the B-lymphocyte stimulator protein (BLyS). BLyS is a soluble protein secreted by monocytes/macrophage that stimulates B lymphocyte maturation and survival. This study assessed the potential in utero and postnatal effects of continuous belimumab exposure in cynomolgus monkeys during pregnancy. Dose levels of 0 (control), 5 and 150 mg/kg were administered intravenously bi-weekly from Gestation Day (GD) 20 to GD150 to pregnant monkeys, in a two-phase study design. In Phase 1, the adult females were evaluated for multiple endpoints of toxicity, immunological response, toxicokinetics (TK) and pharmacodynamics (PD). Fetuses were delivered by C-section on GD150 for teratologic evaluations (external, visceral, skeletal), TK and PD. In Phase 2, the adult females were allowed to deliver and were maintained with their infants for one-year postnatal and evaluated for TK and PD. Infants were evaluated for growth and development, neurobehavior, clinical pathology, immunology, and then necropsied for complete pathologic evaluation. Results showed no test article related toxicologic effects in adult females during gestation or postnatally, and no developmental or toxicologic effects in fetuses or infants. The primary effects observed were related to expected pharmacology, mainly reduced numbers of total (CD20+) and mature (CD20+/CD21+) B lymphocytes in peripheral blood and tissues. These effects were observed in the adult females during dosing, in GD150 fetuses, and in infants, and were reversible upon cessation of exposure. In conclusion, this study demonstrated that belimumab was well tolerated at pharmacologically active dose levels in pregnant cynomolgus monkeys and their infants after exposure throughout pregnancy.

443 DEVELOPMENTAL TOXICITY EVALUATION OF BIS (METHYL SALICYLATE) CARBONATE (BMSC; CAS NO. 82091-12-1) ADMINISTERED BY ORAL GAVAGE TO CD® (SPRAGUE-DAWLEY) RATS ON GESTATIONAL DAYS (GD) 6 THROUGH 19.

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BMSC, a chemical intermediate, was evaluated initially in a 28-day study in adult rats; there were no effects at doses ≤ 1000 mg/kg/day (mkd). Subsequently, time-mated CD® rats (25/group) were gavaged with 0, 25, 250, or 1000 mkd BMSC in

corn oil on gd 6 through 19. Maternal body weights (MBW), feed consumption (FC), and clinical observations (CO) were recorded throughout gestation. On gd 20, gravid uterine (GUW), and liver weights were recorded. Live fetuses were counted, sexed, weighed, and examined externally (E;100%), viscera (V;50%), and skeletally (S;50%). No maternal mortality or treatment-related CO occurred. At 0-250 mkd, no litters were fully resorbed. At 1000 mkd, 20 (83.3%) had fully resorbed litters. MBW and MBW gain were reduced at 1000 mkd on gd 6-19, gd 0-20, GUW on gd 20 and when corrected for GUW. At 250 mkd, reduced MBW was reduced for gd 6-19 only. MFC was reduced at 1000 mkd throughout gestation and gd 6-9 at 250 mkd. Fetal BW/litter was reduced at 250 and 1000 mkd. The % resorptions and affected fetuses/litter was increased for E, V, S, and total malformations and for E, S, and total variations at 1000 mkd. The % affected fetuses/litter was increased at 250 mkd for S and total variations. Malformations at 1000 mkd included craniorachischisis, exencephaly, abnormal forebrain and lateral ventricles, anophthalmia, anasarca, gastroschisis, umbilical hernia, dextrocardia, unossified skull plates, 15 ribs, and abnormal vertebral cartilage/bone. In conclusion, BMSC resulted in maternal and embryofetal toxicity and teratogenicity at 1000 mkd and maternal and fetal toxicity (but not embryotoxicity or teratogenicity) at 250 mkd. The NOAEL for dams and conceptuses was 25 mkd. The proposed mode of action involves monomeric methyl salicylate (from hydrolytic cleavage of BMSC). Salicylates are not teratogenic in humans. Supported by GE Plastics, Pittsfield, MA.

444 TERATOLOGY STUDIES IN NZW RABBITS FOLLOWING IMMUNIZATION WITH BONE MORPHOGENETIC PROTEIN-7 (BMP-7).

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Bone morphogenetic proteins are members of the TGF- β super family of proteins. BMP-7 is currently under marketing approval in trauma and spine orthopaedic indications. The objective of reproductive and developmental studies in NZW rabbits was to evaluate toxicity to dams and offspring from maternal BMP-7 antibody formation. Two separate studies were performed: prenatal and postnatal assessment. Maternal animals were treated with BMP-7 (350 μ g/animal in 0.5mL) in CFA on pre-mating day 1 and boosted with BMP in IFA on pre-mating days 14, 28, and GD12, and GD26 (postnatal only). Maternal examinations were performed in-life and at necropsy on GD 29 (prenatal) and LD 28 (postnatal). Prenatal fetal assessment included external, visceral, and skeletal exams. Postnatal kits were euthanized on LD 28 and examined. ELISA was used to measure maternal, prenatal and postnatal serum IgG and IgM antibody levels. Anti-BMP-7 antibody levels were elevated in the treated dams in both studies. Fetal and kit levels were elevated corresponding to maternal levels. Postnatal antibody to BMP-7 was detected in maternal milk. No mortality or differences in pregnancy status between BMP-7 treated and untreated dams were noted. No treatment effects in body weight, food consumption, uterine or ovarian parameters (prenatal), gestation length (postnatal), dystocia (postnatal), organ weights, and necropsy findings were noted. Fetal and kit survival rates and development were not altered by elevated antibodies in the BMP-7 treated group. In conclusion, large doses of BMP-7 (total dose = 1.4 mg in prenatal and 1.8 mg in postnatal) induced elevated anti-BMP-7 antibodies in maternal animals. These elevated antibody levels cross the placenta and were distributed among the litters. No teratogenic effects were noted in the presence of elevated anti-BMP-7 antibodies.

445 A TERATOLOGY STUDY OF ORAL GAVAGE OF SI-WU-TANG IN RATS.

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Si-Wu-Tang is a traditional Chinese herbal medicine has been used to improve the female reproductive function for hundred of years. However, it has been questioned used Si-Wu-Tang in pregnant women might cause adverse effect on the fetuses. The purpose of this study was to evaluate the effects of Si-Wu-Tang on maternal pregnancy, embryo-fetal developments and the fetuses in rats.

Four groups of mated Sprague-Dawley rats (21-22) were dosed daily during gestation days 6 to 15 via oral gavage with 0, 100, 300 and 500 mg/mL of Si-Wu-Tang solutions with the dosing volume of 10 mL/kg. Rats were observed daily throughout gestation days 6 to 20. The maternal body weights and food consumption were recorded at designated time intervals. At near term G20, the dams were euthanized and Cesarean sectioned. The number of viable or dead fetuses per litter was recorded. External, visceral and skeletal abnormalities of fetuses were examined.

Significant decreases of maternal food consumption during G6-G9, G9-G12 and G6-G15 were found at 5000 mg/kg/day group ($p < 0.05$). There were no other maternal significant differences among control, low, medium and high dose groups in all parameters. Low incidences of spontaneous external, visceral and skeletal abnormalities of fetus were observed in both the treated and/or control groups. The daily recommended human dose is 15-20 g for a 60-kg subject, therefore, the maximal daily recommended dose estimated is 333 mg/kg approximately. Based on the results, rats received dose up to 5000 mg/kg/day of Si-Wu-Tang did not reveal any maternal toxicity and teratogenicity in the maternal, which provided a safety margin of 15 folds over the recommended human dose basing on a conversion of body weight. It is concluded that Si-Wu-Tang is unlikely to induce detrimental embryo-fetal toxicity and teratogenicity in rats.

Key words: Si-Wu-Tang, fetus, teratogenicity, safety margin

446 ETHYLENE GLYCOL: TOXICOKINETIC STUDY IN PREGNANT NEW ZEALAND WHITE RABBITS.

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Large doses of ethylene glycol (EG) are teratogenic in rats, but not rabbits. Therefore, a rabbit toxicokinetic study was conducted to test the hypothesis that the insensitivity of rabbits is due to limited embryonic exposure to the EG metabolite, glycolic acid (GA); the proximate teratogen in rats. NZW rabbits were gavaged with 100 or 1000 mg EG/kg on gestation day (GD) 9 or 1000 mg/kg on GD 15. At various times post-dosing, rabbits were sacrificed and maternal blood and kidney, embryo, extraembryonic fluid (EEF) and visceral yolk sac were collected and analyzed for EG, GA and/or acid-base status. Data were compared to similarly conducted studies in pregnant rats, with an internal bridging control included in the present study which ensured comparability of the analytical values. In GD 9 rabbits given 1000 mg/kg, peak maternal blood EG (891 μ g/g) was almost identical to that reported for pregnant rats similarly dosed (886 μ g/g). However, peak maternal blood GA (146 μ g/g) was less than half that of rats (363 μ g/g). Levels of both EG and GA in embryo and yolk sac were much lower than maternal blood. As a result, peak levels of GA in the rabbit embryo (45.7 μ g/g) were only one-tenth that reported for rat embryos (479 μ g/g). Following a dose of 100 mg/kg on GD 9, GA was barely detectable in maternal blood, and not detected in embryo or yolk sac. GD 15 kinetics were generally similar to GD 9, except that levels of EG and GA in the embryo and yolk sac tended to be much higher on GD 15, most likely due to more efficient transfer via the chorioallantoic placental circulation relative to the yolk sac placenta of GD 9. The 10-fold difference between rabbit and rat embryos in embryonic GA exposure could be an important factor in the species-specific teratogenicity of EG, although intrinsic insensitivity of the rabbit conceptus to GA also needs to be considered as a possible explanation. Sponsored by CEFIC Ethylene Oxide and Derivatives Sector Group, Brussels, Belgium.

447 COMPARISON OF MATERNAL AND EMBRYO-FETAL DEVELOPMENT DATA AMONG AND ACROSS TWO STRAINS OF RABBITS OBTAINED FROM DIFFERENT VENDORS.

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Rabbit colonies at different vendors are susceptible to infection with a variety of bacterial, viral or parasitic agents, as well as subjected to variable housing conditions; therefore, it is important to investigate the impact of using same strain, time-mated rabbits obtained from different vendors on data analysis. This study was designed to compare the maternal and embryo-fetal developmental data from New Zealand White rabbits or Dutch Belted rabbits obtained from two different vendors (Covance and Myrtle's Rabbitry). Forty non-treated, time-mated female rabbits were assigned to one of four groups based on their strain and purchase vendor. Maternal and embryo-fetal development end-points were evaluated. Maternal body weight and food consumption were not statistically different between same strain rabbits obtained from different vendors. All Dutch Belted rabbits were pregnant and 9 of 10 and 8 of 10 New Zealand White rabbits obtained from Covance or Myrtle, respectively, were pregnant. No significant differences in number of corpora lutea, implantations, resorptions (early and/or late), live or dead fetuses, mean litter weights, mean fetal weights (males and/or females) or fetal morphological (external, visceral and skeletal) were noted between same strain rabbits regardless of vendors. In comparing the two different strains obtained from the same vendor, a greater number of resorptions were noted for the New Zealand White rabbits than the Dutch Belted rabbits regardless of vendor. Dutch Belted rabbits had higher pre-implantation loss and higher percentage of viable implants, lower total number of live fetuses, and mean litter weight as compared to the New Zealand White rabbits regardless of vendor. In conclusion, maternal and embryo-fetal development data were similar for the same strain of time-mated rabbits obtained from different vendors and the embryo-fetal development data was different between both strains regardless of vendor.

448 INTEGRATION OF DEVELOPMENTAL TOXICITY DATA INTO RISK ASSESSMENT: A CASE STUDY OF DIMETHOATE.

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Dimethoate is an organophosphate pesticide used to control a wide variety of insects on agricultural crops and ornamentals. To ensure that dimethoate is used safely, it is important to determine exposure levels that protect against adverse effects at all life stages, including the developing fetus, infants and children. Based on an analysis of a developmental neurotoxicity study, a cholinesterase (ChE) sensitivity study, a cross-fostering study, and several single and multi-generational reproductive toxicity studies, two potential critical endpoints for dimethoate were identified: brain ChE inhibition (ChEI) and pup mortality. An initial evaluation concluded that pup mortality was a preferable endpoint, based on an increased number of pup deaths born to dams dosed with > 3 mg/kg dimethoate via oral gavage. Closer examination, however, revealed that the pup deaths were clustered in a low number of litters in which the dams providing postnatal care exhibited maternal care deficits. When the data were instead analyzed using the dam as the unit of statistical significance, the USEPA-recommended practice, a significant increase in the average litter proportion of pup deaths was only observed when dams postnatally dosed with 6 mg/kg dimethoate were raising the pups. Gestational exposure to 6 mg/kg dimethoate had no effect on pup survival. Furthermore, a benchmark dose (BMD) meta-analysis approach revealed that BMDL10 (the lower 95% bound of the dose resulting in a 10% reduction in the parameter of interest) for ChEI was >3-fold lower than the BMDL10 for pup mortality (0.19 and 0.68 mg/kg, respectively; Reiss and Gaylor, Regul. Toxicol. Pharmacol., 2005). Thus, we conclude that brain ChEI is the correct critical endpoint for assessing risk. Overall, this study underscores the importance of using the dam as the unit of statistical significance, and demonstrates the utility of the BMD approach.

449 HISTORICAL CONTROL DATA OF CRL:(WI) BR-WISTAR RATS, IN SEG II STUDY.

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Control data are frequently considered when interpreting teratology results from common laboratory species. The Middle Atlantic Reproduction and Teratology Association (MARTA) database includes data from studies performed using Sprague-Dawley (SD) rats, a frequently used outbred strain in the U.S. In the current study, control data are presented for the Crl:(Wi) BR-Wistar rat (WI), a frequently used outbred strain in Europe. Control groups from rat Segment II studies included 233 litters consisting of 3221 fetuses from the two study sites. Scoring evaluations were normalized by cross-site training. All studies were conducted using criteria for GLP studies and were compliant with ICH Segment II or OECD 414 guidelines. Fetuses were collected from timed-pregnant females by C-section data on GD20 and evaluated for external, visceral, and skeletal parameters. Fetal abnormalities were calculated on a per-litter basis by determining percentage of affected/total for each litter, and then calculating the overall average % for the groups. There were no incidents of an entire litter being resorbed. The mean numbers of implants and live fetuses per litter were 14.9 (range 14.3–15.1) and 13.8 (12.7–13.9), respectively, while mean % postimplantation loss 9.8% (7.6–12.0%). Comparing our results with the MARTA SD database suggests that WI rats have slightly lower mean numbers of implants and live fetuses whereas postimplantation loss was observed at a slightly higher frequency. However, numbers for all parameters are well within observed SD ranges. Morphological examinations of fetuses revealed some differences between the strains in the incidence of spontaneous malformations. For example, abnormal origin of right subclavian artery from aortic arch was found to be 0.4% in WI compared with approximately 0.1% in SD. It is not clear whether these trends will continue as additional fetuses are added to the WI database. Our findings demonstrate that although there may be slight differences between WI and SD teratology parameters, these appear to be minimal.

450 USE OF THE ZEBRAFISH FOR EMBRYOTOXICITY SCREENING.

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Several screening assays have been developed as early indicators for in vivo embryotoxicity/teratogenicity traditionally studied in rats and rabbits, but so far none of them has gained broad acceptance in drug discovery. Embryotoxicity assays in the

zebrafish (*Danio rerio*) provide several advantages compared to other screens such as the EST or the WEC: in the developing fish egg the whole embryofetal period is covered, the assay is easy and quick to perform and requires no use of higher vertebrates.

To evaluate the predictivity of the zebrafish embryotoxicity screen, 13 compounds for which in vivo data were available, were provided as blinded samples by 3 pharmaceutical companies and were screened by Oxford-based VASTox in zebrafish embryos, which were exposed to the test substances at 4 hours post fertilization (sphere stage or midblastula; equivalent to 6 days in rats/rabbits) and scored at 96 hours p.f. (free swimming larva; equivalent to 16/18 days in rats/rabbits). The score consisted of the assessment of at least 22 morphological and functional parameters at each dose tested.

Out of the 10 embryotoxic substances, 8 were classified correctly, whereas none of the 3 non-embryotoxic compounds was correctly predicted as negative. Thus, the sensitivity of the test system was found to be 80% but the specificity of the assay was only 0%. By adding VASTox's bioanalysis to check compound uptake for the 2 false negatives and an acute toxicity test in zebrafish larvae for the 3 false positives to determine embryotoxicity versus general toxicity, the predictivity may be improved considerably.

This evaluation study points towards the zebrafish embryotoxicity test being a promising method for the early screening of lead compounds during early drug discovery. However, a higher number of compounds needs to be tested to further validate and especially improve specificity of the zebrafish model in order to become an accepted early screening system for embryotoxicity/teratogenicity.

451 ROLE OF THE INHIBITOR OF DNA BINDING-6 (ID6) IN NORMAL AND ALL-TRANS RETINOIC ACID EXPOSED ZEBRAFISH EMBRYO DEVELOPMENT.

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Id proteins (Inhibitor of DNA Binding) are a class of helix-loop-helix (HLH) proteins that lack a DNA binding domain and function by forming inactive heterodimers with other HLH proteins. The Id proteins promote G1/S phase transition of the cell cycle and are important regulators of lineage commitment and terminal differentiation. Morpholino knockdown of Id6 resulted in pericardial, yolk sac, and/or brain edemas, as well as notochord herniations indicating that Id6 is necessary for proper development of these structures. In addition, we have found that all-trans retinoic acid (atRA) exposure results in increased expression of zebrafish Id6. atRA is a powerful regulator of cell growth and differentiation and is known to result in abnormal embryo development, including truncation of the heart tube and alterations in the midbrain and hindbrain. Id6 in situ hybridization data demonstrate that an increase in Id6 expression is detectable in the posterior heart of atRA-exposed embryos at 48 and 72hpf. These data suggest that defects in zebrafish heart development following exposure to atRA may result, at least in part, from increased expression of Id6. In human endothelial cells, Ids are involved in vascular endothelial growth factor (VEGF)-induced activation of angiogenesis. Our data show that atRA also induces VEGF expression in the developing zebrafish. Injection of VEGF in zebrafish embryos causes disruption of vessel and heart development, similar to vascular defects we have observed in atRA-exposed embryos, such as pericardial edema and disruption in the formation of the subintestinal vessels and the common cardinal vein. Further evidence that Id6 expression in the developing zebrafish is a target for the atRA signaling pathway is demonstrated by exposure to DEAB, an inhibitor of atRA signaling, which results in reduced Id6 expression. Taken together, these data indicate that Id6 is a key player in atRA signaling in the embryo and that it may be acting through increased expression of VEGF.

452 MATRIX METALLOPROTEINASE-2 AND -9 ARE ESSENTIAL FOR ZEBRAFISH EMBRYOGENESIS AND SERVE AS TARGETS FOR GLUCOCORTICOID EXPOSURE.

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Expression and activity of the matrix metalloproteinases (MMPs) are essential for the extracellular matrix remodeling that occurs during embryonic tissue morphogenesis. The gelatinases MMP-2 and MMP-9 catalyze the degradation of intact type IV collagen and denatured collagens (gelatins). Glucocorticoids (GCs) inhibit MMP gene expression in mammalian cell cultures systems and cause developmental abnormalities in several species. Therefore, we hypothesized that GCs impede normal development through alteration of MMP expression. Microinjection of morpholinos targeting MMP-2 and MMP-9 was utilized to transiently knock down expression and demonstrated the necessity of these genes for normal embryogenesis. Exposure to 100 mg/L dexamethasone (DX) or hydrocortisone (HC) caused an induction of MMP-2 (1.5-1.6 fold over control) and MMP-9 expression (7.6-9.0 fold over control) by 72 hours post fertilization (hpf) as measured by real-time PCR. Further, DX- and HC-treated embryos exhibited a decreased head-

trunk angle, craniofacial abnormalities, truncated body axis, altered somitogenesis, and/or modification of the yolk sac shape and size. *In situ* hybridization (ISH) confirmed GC-induced MMP-2 and MMP-9 expression in treated embryos at 72 hpf. Increased expression was seen in the brain, heart, and anterior kidney. Increased gelatinase activity was observed via *in vitro* zymography in embryos treated with 100 mg/L DX or HC through 72 hpf (3.0-3.1 fold over control). These data confirmed that increased transcript levels detected via real-time PCR and ISH resulted in increased enzyme activity. Through the addition of specific inhibitors, it was determined that this activity was due to the metalloenzymes MMP-2 and MMP-9. Based on these findings, MMP-2 and MMP-9 are critical for normal zebrafish embryogenesis and GCs are capable of modulating their expression, thereby providing insight into how this class of compounds exerts its teratogenic effects.

453 ARYL HYDROCARBON RECEPTOR REGULATED GENE EXPRESSION DURING SYNERGISTIC DEVELOPMENTAL TOXICITY OF POLYCYCLIC AROMATIC HYDROCARBONS IN ZEBRAFISH (DANIO RERIO).

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Polycyclic aromatic hydrocarbons (PAH) are pollutants created by the incomplete combustion of carbon, and are increasing in the environment largely due to burning of fossil fuels. PAH occur as complex mixtures, and some combinations have been shown to cause synergistic developmental toxicity in fish embryos, characterized by pericardial edema and craniofacial malformations. Previous studies have indicated this toxicity is regulated by the aryl hydrocarbon receptor (AHR). In this study, we examined the model PAH and AHR agonist β -naphthoflavone (BNF) with and without the AHR partial agonist/antagonist and CYP1A inhibitor α -naphthoflavone (ANF) to determine 1) whether ANF was acting as an AHR antagonist and 2) what other alterations BNF and ANF both alone and in combination had on mRNA expression AHR regulated genes cytochromes P450 (CYP) 1A, 1B1, and 1C1, and the AHR repressor (AHRR2). Zebrafish embryos were exposed from 24-48 hours post fertilization to either BNF, 1-100 μ g/L, ANF, 1-150 μ g/L, a BNF+ANF co-exposure (1 μ g/L + 100 μ g/L), or a DMSO vehicle control. RNA was extracted and examined by quantitative reverse transcription real time PCR. Both BNF and ANF alone caused a dose dependent increase in mRNA of CYP1A, CYP1B1, CYP1C1, and AHRR2, confirming their activities as AHR agonists. In the BNF+ANF co-exposures, expression of these genes was greater than additive, and expression levels of the AHR regulated genes resembled the higher doses of BNF alone, indicating that ANF is not acting as an AHR antagonist and suggesting that ANF's inhibition of CYP1A may be increasing the half-life of the parent compound and toxicity. This study furthers a mechanistic understanding of PAH synergistic toxicity by and will be important for developing more robust tools for risk assessment. This research was supported by the NIEHS-supported Duke University Superfund Basic Research Center (P42 ES10356), Integrated Toxicology and Environmental Health Program (T32 ES07031), and EPA STAR fellowship to A.T.L.

454 OVER-EXPRESSION OF PGC-1 ALPHA DOES NOT CONFER PROTECTION BUT PROMOTES RECOVERY FROM OXIDANT INDUCED RENAL MITOCHONDRIAL AND CELLULAR INJURY.

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Renal cell death as a result of mitochondrial dysfunction and compromised bioenergetics is a common consequence of ischemia-reperfusion injury and exposure to nephrotoxins. Promoting the biogenesis of mitochondria is therefore an attractive therapeutic strategy to sustain oxidative phosphorylation and maintain ATP-dependent cellular functions. The goal of this study was to determine if increased mitochondrial biogenesis prior to or following oxidant exposure prevents cell death and/or accelerates recovery of renal proximal tubular cells (RPTC) in primary culture. Over-expression of the mitochondrial biogenesis regulator PPAR- γ cofactor-1 alpha (PGC-1 α) in control RPTC resulted in a 52% increase in mitochondrial number. However, the increased mitochondrial number did not protect mitochondrial function after t-butylhydroperoxide (TBHP) (200 μ M for 30 mins.) exposure, but rather potentiated mitochondrial dysfunction and cell death measured by cellular respiration and propidium iodide staining. In contrast, increased mitochondrial biogenesis following TBHP exposure, accelerated recovery of mitochondrial function 48 hours after TBHP injury. Control RPTC exhibited a 50% reduction in the expression of electron transport proteins (ATP-synthase β and ND), ATP, basal, uncoupled, and ouabain-sensitive respiration, whereas all measures of mitochondrial function in injured RPTC over-expressing PGC-1 α after injury returned to control values. We suggest that the up-regulation of mitochondrial biogenesis prior to oxidant exposure promotes renal cell death whereas the up-regulation of mitochondrial biogenesis following injury accelerates the recovery of mitochondrial and cellular functions.

455 LINDANE-INDUCED MODULATION OF GLUTATHIONE STATUS IN MDCK CELLS DOES NOT RESULT IN NECROTIC INJURY.

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Lindane (LIN), a chlorinated hydrocarbon pesticide, is toxic to the liver, kidneys, immune, reproductive, and central nervous systems. Oxidative stress has been suggested as a possible mechanism of toxicity, although the mechanism of renal toxicity has yet to be identified. Our laboratory has shown LIN-induced morphological changes indicative of apoptosis, and a concentration-dependent increase and time-dependent decrease in generation of reactive oxygen species (ROS) in distal tubule cells. To determine whether LIN exposure alters cellular glutathione (GSH) levels, MDCK cells were treated with vehicle (DMSO), a known oxidant-stress inducing compound, H₂O₂ (50 μ M), and LIN (10 & 100 μ M). Total GSH was measured using a DTNB microassay after incubating for 15 and 30 min, 1, 2, and 6 hr time points. GSH levels increased above control values 15 min after LIN exposure at both concentrations. From 30 min onward, a time-dependent decline in GSH was noted. Control, vehicle, and H₂O₂-treated cells exhibited similar GSH levels over time. Cells were pretreated with N-acetyl cysteine (NAC) or buthionine sulfoximine (BSO) to evaluate the role of GSH on LIN-induced oxidative stress. Changes in cellular response to LIN exposure were shown after pretreatment with NAC and BSO. NAC enhanced GSH levels, while BSO caused further depletion of GSH. Necrotic injury was assessed by measuring lactate dehydrogenase (LDH) leakage at 15 and 30 min, 1, 2, 4, 8, 24, and 48 hours after LIN treatment. No LDH leakage was observed for all treatments at all time points. This finding corresponds to our previous findings of LIN-induced apoptosis, as opposed to necrosis. Initial elevation in total GSH corresponds to the spike in ROS generation at 15 minutes. The time-dependent decline in ROS generation correlates to the time-dependent depletion of GSH. This decline may be attributed to quenching of free radicals by GSH, thereby decreasing the cellular stores of this antioxidant. ROS generation and alterations in cellular protective mechanisms did not result in necrotic injury.

456 TOXICOGENOMICS ANALYSIS CORRELATES ATTENUATION OF NRF2 FUNCTION WITH RENAL TOXICITY IN THE RAT.

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Renal toxicity is a significant cause for clinical failure of drug candidates and for post-marketing withdrawal of approved drugs; however, the mechanisms of chemically-induced renal toxicity are not well understood. In order to gain understanding of the mechanisms of chemically-induced renal toxicity, we first curated 4 key biological pathways focused on kidney function and injury from a thorough review of the literature. Genes in these 4 pathways were then used to systematically query and analyze kidney data in DrugMatrix®, a large-scale contextual microarray gene expression database of drug-treated rat tissues. Data mining revealed a tightly-clustered set of experiments, including treatments with compounds of well documented renal toxicity such as 4,4'-methylenedianiline, 6-mercaptopurine, cerivastatin, rifampin and thalidomide. Pathway analysis revealed that genes involved in TGF- β signaling, PI3K/MAP signaling, inflammation, and oxidative stress response are consistently up-regulated by these compounds, potentially leading to hypertrophy, proliferation, fibrosis and cell death, consistent with renal injuries such as renal tubular necrosis and acute interstitial nephritis that have been reported for some of these compounds. Surprisingly, heme-oxygenase-1 (HO-1), a key cytoprotective mediator of oxidative stress response in kidney, was not up-regulated in these experiments, while many transcription factors that activate HO-1 transcription were consistently up-regulated. In-depth analysis revealed that in these experiments, the function of Nrf2, a key transcription factor that regulates the expression of many genes including HO-1, thioredoxin, and peroxiredoxin, involved in the amplification of the mammalian defense system against oxidative stress, was compromised by the induction of Bach1, a transcriptional repressor of Nrf2. These results suggest that attenuating the anti-oxidative response mediated by Nrf2 via Bach1 induction may be an important mechanism for compound-induced renal dysfunction, and illustrates the value of using gene expression to understand mechanisms of toxicity.

457 A METHOD FOR THE EVALUATION OF SITE-SPECIFIC NEPHROTOXIC INJURY IN THE INTACT RAT KIDNEY USING ETHIDIUM HOMODIMER.

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As part of our ongoing research to identify the mechanism by which cadmium produces its nephrotoxic effects, we have developed a novel method to quantify proximal tubule epithelial cell viability that involves perfusing the intact rat kidney with

the cell membrane impermeable fluorochrome, ethidium homodimer. The objective of the present study was to determine if this procedure could be used to characterize toxic injury in other regions of the kidney. Rats were treated with the site-specific nephrotoxicants, gentamicin (proximal tubule; 100 mg/kg i.p. per day for 8 consecutive days) and indomethacin (papillus; 20 mg/kg oral gavage, single dose). At the end of the respective treatment periods, ethidium homodimer (5 μ M) was infused (3 ml/min for 10 min) into the left intact kidney while the animal was anesthetized. The kidney was then perfused with a physiologic saline solution, removed, placed in embedding medium, frozen, cryosectioned at a thickness of 5 μ m and ethidium-labeled cells counted. The gentamicin-treated animals exhibited polyuria and proteinuria with no change in urinary creatinine excretion. Indomethacin-treated animals did not show significant differences in either urine volume or urinary protein, but did show significantly lower urinary creatinine levels. Results of the viability assay indicated that gentamicin treatment resulted in proximal tubule cell death that was localized in the cortex of the kidney. Renal cell death resulting from indomethacin treatment was localized primarily in the papillus. However, the kidneys of the indomethacin-treated animals also showed modest evidence of cell death in the cortex and medulla. These results show that this *in situ* viability assay provides a sensitive and accurate way to identify cell death in different regions of the kidney following exposure to the site-specific nephrotoxicants. Supported by Grant RO1-ES006478 from the NIEHS.

458 METABONOMIC EVALUATION OF RENAL PAPILLARY NECROSIS: COMPARISON OF THREE CHEMICALLY-DIVERSE AGENTS.

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NMR-based metabolomics was used to study the metabolic consequences of renal papillary necrosis (RPN), a major cause of renal failure. The mechanism of RPN is not fully understood and is further hampered by the lack of methods to detect its development and progression. Patterns of metabolite changes elicited by three compounds, 2-bromoethanamine (BEA), 2-chloroethanamine (CEA) and N-phenylanthranilic acid (NPAA) were compared. BEA and CEA cause RPN to develop acutely while NPAA requires several days of dosing to manifest a lesion. Sprague Dawley rats (n=30) were given a single IP dose of 15 mg/kg or 150 mg/kg of BEA, or 100 mg/kg or 1000 mg/kg of CEA. NPAA was orally dosed for three days at 200 mg/kg/day. Urine and serum samples were collected at sequential time points up to 168 hours post dose and metabolic profiles were determined using high-resolution NMR. The multivariate classification method, orthogonal-projection on latent structures was applied to NMR spectral data. Passage of time was included explicitly in the models to improve separation of general and specific toxic effects from recovery effects. BEA and CEA demonstrated similar markers of toxicity but the time-course and extent of the metabolic changes differed. Urinary metabolic changes common to BEA and CEA were increased levels of glutaric acid, choline- and N-acetyl moieties together with reduced levels of 2-oxoglutarate, succinate, hippurate and dimethylglycine. In addition for BEA, increased levels of adipic acid, glucose, lactate, alanine, formate and reduced levels of N-methylnicotinamide, dimethylamine and TMAO were identified. The metabolic changes identified for NPAA were much less extensive; reduced levels of 2-oxoglutarate, hippurate and creatinine were identified. Serum metabolic profiles were also compared. These data support the conclusion that NPAA causes RPN by a mechanism distinct from that of BEA and CEA.

459 TOXICOGENOMIC INVESTIGATION OF MECHANISMS AND MARKERS OF NEPHROTOXICITY BY INCORPORATING LASER CAPTURE MICRO-DISSECTION AND A CONTEXTUAL MICROARRAY DATABASE.

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To investigate mechanisms and markers of renal tubular toxicity, SD rats were administered a single i.p. dose of a renal tubular toxicant, *p*-aminophenol (PAP), at 50 and 150 mg/kg. The major histopathological lesions observed were renal tubular dilation, necrosis, and regeneration. Microarray gene expression data from the whole renal cortex and from proximal tubular regions, isolated by laser capture microdissection (LCM), were obtained at 6, 24, and 48 hours. Applying Iconix's Drug Signatures® gene-based biomarker technology to the PAP data revealed positive Signature matches consistent with histopathological and *in-life* findings, and provided a means of addressing mechanisms of toxicity. The use of LCM was more informative than the whole cortex in providing Signature-based evidence of renal injury at low dose. Analysis of genes most consistently perturbed at low dose in LCM but not in cortex revealed prominent repression of mitochondrial oxidative phosphorylation and energy production pathways, likely due to enrichment of affected

cells by LCM. These pathways could reflect events that precede the occurrence of minimal tubular necrosis at low dose. Leveraging the contextual richness of DrugMatrix®, a large-scale reference database of rat microarray experiments, hierarchical clustering was performed on genes commonly perturbed in >60% of experiments with renal tubular injury findings, selected from all kidney experiments in the database (n=395). The majority of PAP experiments fall in a tight cluster enriched with heavy metal toxicants. Oxidative stress and mitochondrial injury are some ways heavy metals can cause renal tubular injury, and may provide additional insight into the mechanisms of PAP toxicity. Taken together, this study demonstrates the utility of genomics and LCM in unraveling mechanisms of drug-induced toxicities, and of using a contextual toxicogenomic database to enhance the depth and breadth of analysis.

460 NITRIC OXIDE-MEDIATED NEPHROTOXICITY IN RATS AFTER ACUTE EXPOSURE TO GENTAMICIN, MERCURY OR CHROMIUM.

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The present study was undertaken to morphologically characterize acute kidney injury in gentamicin (Gen)-, mercury (Hg)-, or chromium (Cr)-treated rats and explore the role of nitric oxide (NO) in renal pathogenesis. Sprague-Dawley rats were injected with Gen (100 mg/kg, sc, daily for 3 days), HgCl₂ (0.25 mg Hg/kg, iv), K₂Cr₂O₇ (5 mg Cr/kg, sc), or vehicle (control). At 24 or 72 hr after the last dose, kidneys were collected and formalin-fixed renal sections were used for histopathologic evaluation, TUNEL assay and immunoperoxidase staining with monoclonal antibodies of inducible nitric oxide synthase (iNOS), endothelial nitric oxide synthase (eNOS), and nitrotyrosine. The severity of renal lesions was assessed using a semiquantitative scale of 0 to 5. Histopathology revealed that Gen-, Hg-, or Cr-induced renal lesions were characterized by epithelial necrosis, degeneration, and regeneration of the proximal convoluted tubules (PCT), tubular dilatation, protein casts, glomerular vacuolization with or without capillary thrombosis, and interstitial lymphocytic infiltration. Renal lesion scores indicated greater severity at 72 hr compared to 24 hr (3.3 vs 1.5, 3.8 vs 3.0, and 4.8 vs 2.8 in Gen-, Hg-, or Cr-treated rats, respectively). TUNEL analysis demonstrated that epithelial apoptosis of the PCT in Gen-, Hg-, or Cr-treated rats was more frequent at 72 hr than at 24 hr. Rats treated with Gen, Hg, or Cr exhibited increased expression of iNOS and nitrotyrosine at the necrotic and apoptotic areas of the PCT, the degree of which correlated with the severity of tubular necrosis and apoptosis and increased with time. The increased expression of eNOS was identified in the endothelial cells of glomerular capillaries. These findings suggest that the cytotoxic effects of Gen, Hg, or Cr on PCT are attributable to the free radical NO formed by iNOS that induces epithelial cell necrosis and apoptosis, whereas the cytotoxic effects of these agents on the glomeruli are attributable to NO from eNOS that causes endothelial cell injury.

461 NITRIC OXIDE-ASSOCIATED NEPHRO-TOXICITY IN JUVENILE RATS TREATED WITH GENTAMICIN.

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Nonclinical studies suggest that gentamicin (Gen) induces renal tubular apoptosis in rats via several pathways (caspase-3, cytochrome C, nuclear endonuclease and Bax) and that reactive oxygen species may be responsible for Gen-induced nephrotoxicity. To explore the role of nitric oxide, different age groups of SD rats (25, 40, 80 days old) were treated with saline, 50 or 100 mg/kg/daily (ip) for 6 or 14 days. Gen-induced renal injury was evaluated using a semiquantitative scale (0-5). Histopathological evaluation showed that Gen induced degeneration, necrosis, and regeneration in the proximal and distal convoluted tubules, tubular dilatation and protein casts, glomerular vacuolization, and interstitial lymphocytic inflammation and that Gen-induced renal injury is age-, time- and dose-dependent. The immunoreactivity of apoptosis, caspase-3, cytochrome C, iNOS (inducible nitric oxide synthase), and nitrotyrosine was assessed immunohistochemically in the kidneys. The expression of caspase-3, cytochrome C, iNOS, and nitrotyrosine increased with age, time and dose and the levels of the increased immunoreactivity correlated with degree of tubular apoptosis. These findings suggest that nitric oxide may play an important role in Gen-induced nephrotoxicity.

462 KIDNEY INJURY MOLECULE-1 (KIM-1) EXPRESSION DURING ACUTE KIDNEY INJURY INDUCED BY GENTAMICIN, MERCURY, OR CHROMIUM.

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We previously reported that increases in urinary Kim-1 (U-Kim-1) occurred at lower doses of the nephrotoxicants gentamicin and mercury compared to other routinely-used markers: urinary NAG, BUN and serum creatinine. The objectives

of this study were to determine 1) the temporal relationship between the appearance of U-Kim-1 with routinely-used biomarkers of nephrotoxicity; 2) the correlation of U-Kim-1 and nephropathology; and 3) whether appearance of Kim-1 in urine is specific for renal injury. Sprague-Dawley male rats were injected with a nephrotoxicant (gentamicin, 100 mg/kg, sc, daily for 3 days; HgCl₂, 0.25 mg Hg/kg, iv; or K₂Cr₂O₇, 5 mg Cr/kg, sc), or vehicle (control) and urine collected from each rat prior to and at 0-4, 4-8, 8-24, 24-48, 48-72 h after treatment for assay of Kim-1, NAG, and creatinine. At necropsy (72 h), blood (BUN, creatinine) and kidneys (histopathology) were collected. Results showed that U-Kim-1 was elevated within 24 h after exposure to each nephrotoxicant and remained elevated through 72 h. NAG responses were nephrotoxicant-dependent with elevations occurring early (gentamicin), late (Cr), or no change (Hg). At 72 h after treatment, histopathology indicated injury to ~50% tubules; however, only U-Kim-1 was significantly increased while BUN, serum creatinine and NAG were not different from controls. In rats treated with the hepatotoxicant galactosamine (1.1 mg/kg, ip), serum ALT was increased at 12 and 24 hr, but no increase in urinary Kim-1 was observed. Thus, at nephrotoxicant doses used in this time-course study, elevations in U-Kim-1 occurred within 24 h and were sustained following exposure compared to other biomarkers, correlated with the severity of renal lesions, and were specific for renal injury and not liver injury. U-Kim-1 appears to be a sensitive and tissue-specific biomarker that will improve detection of early acute kidney injury following exposure to nephrotoxic chemicals and drugs.

463 COMPARISON OF URINARY BIOMARKERS TO DETECT METAL-INDUCED NEPHROTOXIC EFFECTS IN GENTAMICIN-PRETREATED RATS.

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Patients with subclinical renal injury (SRI) represent a subpopulation that is potentially more susceptible than healthy subjects to further kidney damage from nephrotoxic compounds. We have developed and validated a rat model of SRI using doses of gentamicin (Gen) that do not produce overt renal failure. Administration of mercury (Hg) or chromium (Cr) to SRI rats results in the development of acute renal failure at doses that do not produce overt effects following administration of these metal doses to healthy control animals. The objective of this study was to determine which urinary biomarkers (NAG, Kim-1, α GST, GST-Yb1 and RPA-1) are best able to detect the effects of either Hg or Cr in Gen-pretreated rats. To induce SRI, male Sprague-Dawley rats were dosed (sc) daily for 3 d with 100 or 150 mg/kg Gen; 24 h after the 3rd injection, rats were injected with HgCl₂ (0.1 and 0.25 mg Hg/kg iv) or K₂Cr₂O₇ (2.5 and 5 mg Cr/kg sc). No interactive effects were seen following administration of 100 mg/kg Gen followed by the lowest dose of either Hg or Cr. In contrast, injection of 150 mg/kg Gen followed by the lower dose of Hg resulted in a marked increase in urinary levels of α GST, GST-Yb1, and NAG. No increases in urinary levels of Kim-1 or RPA-1 were observed, although these levels were increased after exposure to Gen alone. The potentiation of Hg-induced nephrotoxicity in SRI rats would not have been observed if only traditional markers of nephrotoxicity had been used in the study (blood creatinine and BUN). A similar interactive effect was observed at the higher doses of Cr (α GST, GST-Yb1, NAG) and Hg (α GST, GST-Yb1) in rats pretreated with 150 mg/kg Gen. These results suggest that the ability to detect a potentiation of metal-induced nephrotoxic effects in animals with SRI, compared to healthy control animals, depends on the biomarker selected, the agent producing the nephrotoxicity, and the dose of the nephrotoxic agent.

464 ALPHA-GST, GST-YB1, RENAL PAPILLARY ANTIGEN-1 (RPA-1) AND KIDNEY INJURY MOLECULE-1 (KIM-1) ARE SENSITIVE BIOMARKERS OF SUB-CLINICAL RENAL INJURY IN GENTAMICIN-TREATED RATS.

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Elevated serum creatinine and BUN are typically used as indices of acute renal failure; however, greater than 50% of the GFR could be lost before elevations of these markers are detected. Since limitations exist with the use of these markers of kidney damage, and since early detection of even subtle renal injury is important for the implementation of renal protective strategies, there is a need to identify more sensitive biomarkers of altered renal function. The objective of this study was to compare the ability of routinely used biomarkers of renal damage (blood: creatinine and BUN; urine: NAG) and a suite of relatively new urinary biomarkers, notably, α GST, GST-Yb1, renal papillary antigen-1 (RPA-1), and kidney injury molecule-1

(Kim-1) to detect renal injury in rats injected with gentamicin (Gen). Male Sprague-Dawley rats were injected with Gen at 0, 50, 100, 150, 200 or 300 mg Gen/kg, sc, daily for 3 d. Rats were placed in metabolism cages after the 3rd injection and urine collected for 24 h, after which blood and kidneys were removed. Dose-dependent increases in the severity of histopathologic lesions were observed using a semiquantitative scale of 0 to 5. Scores were controls, 0; 50-150 mg/kg, 1.5-2.5; 200-300 mg/kg, 4-5. No increases in BUN, blood creatinine, and urinary NAG were observed except at the highest dose. In contrast, dose-dependent increases in urinary α GST, GST-Yb1, RPA-1, and Kim-1 were observed at doses $\geq$ 100 mg/kg. Increases in urinary markers following injection of 100 or 150 mg/kg gentamicin (no elevation of BUN and creatinine) were 3- to 5-fold for α GST, GST-Yb1, and RPA-1, and 10- to 20-fold for Kim-1. Since elevated levels of α GST, GST-Yb1, RPA-1 and Kim-1 were detected in urine of rats after injection of Gen doses that do not produce overt renal failure, these endpoints have potential to serve as sensitive biomarkers of renal injury.

465 LOW DOSE OF DICHROMATE ALTERS PARACELLULAR AND TRANS-CELLULAR TRANSPORT ALONG THE RAT NEPHRON.

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Potassium dichromate (PD) at mM dose induces renal transport disorders. However, there is scant information of the effects of this metal on whole kidney function at μ M dose. This study investigates the effect of PD on the transport of mono and divalent cations, glucose, total proteins and phosphate along the nephron, in rats i.v. infused. A tracheotomy was performed on anesthetized adult Wistar female rats. One catheter was inserted into the left jugular vein for infusion (inulin-FITC, with or without PD), other into the femoral artery for blood sampling and arterial blood pressure recording, and another into left ureter for urine collection. PD was infused during 1 h to attain in plasma 5 μ M. Glomerular filtration rate (GFR) was estimated from inulin-FITC clearance (fluorometric analysis), the renal solute transport was evaluated through fractional excretions (FE) of Na⁺, K⁺, Ca²⁺, Mg²⁺ (AAS), phosphate (colorimetric method), glucose (hexokinase method), proteins and urinary and serum osmolarities (vapor pressure). Experiments were performed in three stages in the same animal: 1) control (inulin-FITC), 2) exposed (PD with inulin-FITC) and 3) recovery (inulin-FITC). Infusion of PD induced a decrease in the inulin U/P ratio and GFR. Renal transport of cations was also affected: FE of Na⁺ and K⁺ increased, whereas those of Ca²⁺ and Mg²⁺ decreased. FE of glucose augmented, proteins and phosphate decreased. Reabsorption of Na⁺, glucose and phosphate were transiently decreased and transport of Ca²⁺ and Mg²⁺ were increased by PD infusion. Interestingly in the recovery period all parameters return to the control values except the FE of Ca²⁺ and Mg²⁺. These results show that the infusion of PD to low doses caused deleterious effects on the transcellular and paracellular transport along the nephron. Further studies are required to evaluate the effects of PD into the distal transport of cations across the paracellular pathway.

466 REVERSIBLE BINDING OF 14C-HEXAMETHYLDISILOXANE (14C-HMDS)/METABOLITES TO ALPHA 2U-GLOBULIN IN MALE F344 RATS.

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Studies with Fischer 344 female and male rats exposed to HMDS have demonstrated the following in male kidney; increased protein droplets, the presence of alpha 2u-globulin (α 2u) by immunohistochemical staining, increased tubular regeneration, hyaline and granular casts, tubular and papillary mineralization, kidney lesions and renal tumors. To investigate the reversible binding of HMDS/metabolites to α 2u, 14C-HMDS was administered to rats in corn oil by oral gavage at 100 mg/kg bw. Kidney cytosol was prepared using an improved procedure to minimize loss of volatile/lipophilic siloxanes. Established direct and indirect approaches that demonstrate a chemical- α 2u interaction such as gel filtration, DEAE anion exchange chromatography and headspace gas chromatography (GC) were unsuccessful in characterizing binding between HMDS/metabolites with α 2u most likely due to the extreme hydrophobic and volatile nature of HMDS. A new approach using Hydrophobic Interaction Chromatography (HIC) was developed to isolate the α 2u specifically found in the kidneys of male rats but not female rats. A dot blot using polyclonal antibodies raised against purified rat α 2u-urinary globulin was used to positively identify the presence of α 2u in the fraction isolated by HIC. Radioactivity co-eluting with the α 2u peak was monitored to determine the binding of HMDS/metabolite to the protein. D-limonene oxide, a chemical with a high

affinity for $\alpha 2u$, displaced/reversed the binding of HMDS/metabolites to $\alpha 2u$ in samples of male rat kidney cytosol. Reversed phase-high pressure liquid chromatography and GC-mass spectrometry analysis of solvent extracts of HMDS/metabolites from kidney cytosol provided additional evidence that HMDS/metabolites were indeed reversibly bound to $\alpha 2u$ in male rats. Reversible binding coupled with the observed nephropathy and renal tumors and the observation of $\alpha 2u$ in kidneys by immunohistochemical staining support the criteria of EPA and IARC for an $\alpha 2u$ mechanism of male rat kidney tumors induced by HMDS.

467 AMINOACYLASE III MEDIATED CYTOTOXICITY OF THE TRICHLOROETHYLENE-DERIVED MERCAPTURIC ACIDS IN MOUSE PROXIMAL TUBULE CELLS.

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Trichloroethylene (TCE) and other halogenated alkenes are known environmental contaminants with cytotoxic and nephrotoxic effects, and potential carcinogens. Their metabolism via the mercapturate metabolic pathway was shown to lead to their detoxification. The final products of this pathway, mercapturic acids or N-acetyl-L-cysteine S-conjugates are secreted in the lumen in the renal proximal tubule. The proximal tubule may also deacetylate mercapturic acids, and the resulting cysteine S-conjugates are transformed by cysteine S-conjugate β -lyases to nephrotoxic reactive thiols. The specificity and rate of mercapturic acid deacetylation may determine the toxicity of certain mercapturic acids whereas enzymology of this process is not known in details. In the present study we characterized kinetics of the recently cloned and purified to homogeneity mouse aminoacylase III (AAIII) towards wide specter of halogenated mercapturic acids and N-acetylated amino acids. In general, V_{max} of AAIII was significantly larger with chlorinated and brominated mercapturic acids whereas fluorination significantly decreased V_{max} . The enzyme deacetylated the mercapturic acids derived from the TCE metabolism, N-acetyl-S-(1,2-dichlorovinyl)-L-cysteine (NA-1,2-DCVC) and at higher rate N-acetyl-S-(2,2-dichlorovinyl)-L-cysteine (NA-2,2-DCVC). Both mercapturic acids induced cytotoxicity in the mouse proximal tubule derived mPCT cells, which exogenously expressed AAIII. An inhibitor of β -lyase, aminooxyacetate, significantly decreased the cytotoxicity. The toxic effect of NA-2,2-DCVC was smaller than NA-1,2-DCVC indicating that factors other than the AAIII intracellular activity may be involved in mediating the cytotoxicity of these mercapturic acids. Our results indicate that in the proximal tubule cells AAIII plays an important role in deacetylating NA-1,2-DCVC and 2,2-DCVC and this process may be involved in their cyto- and nephrotoxicity.

468 STRUCTURE OF DIMERIC AMINOACYLASE III.

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Aminoacylase III (AAIII) plays an important role in deacetylation of acetylated amino acids and N-acetylated S-cysteine conjugates (mercapturates) of halogenated alkenes and alkanes in kidney. AAIII was recently cloned from mouse kidney and partially characterized. *In vitro* mouse AAIII (35 kDa) is a mixture of tetramers and dimers. In the present work the AAIII dimer was shown to be enzymatically active and was studied using electron microscopy, three-dimensional (3D) reconstruction and limited trypsinolysis. The 3D reconstruction of the micrographs of negatively stained AAIII dimer particles was performed using EMAN software from Steven Ludtke (Baylor College of Medicine). In the resulting 12.5 Å resolution model, the AAIII monomer was subdivided into two unequal domains. Only proximal ends of the domains participated in the dimer formation. Protein protrusions from both monomers surrounded the central cavity in the dimer, creating a "cage", which might be a substrate-binding site. In agreement with the model, limited trypsinolysis identified a ~9 and ~25 kDa fragments. By Western blotting the smaller domain was localized N-terminally and the larger domain C-terminally. These domains did not demonstrate the AAIII activity separately or together indicating that a specific structure is necessary for the catalysis.

469 MITOCHONDRIAL CALPAIN 10 REGULATES ATP SYNTHESIS IN RENAL PROXIMAL TUBULE CELLS.

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Calpains are a family of Ca^{2+} -activated cysteine proteases that have been implicated in numerous physiological and disease processes. Recently our laboratory has determined that calpain 10 is located in the mitochondria and is active in the mitochondrial matrix. We have developed an adenoviral-delivered calpain 10 shRNA to

knockdown calpain 10. To determine the physiological role of mitochondrial calpain 10, we infected renal proximal tubular cells (RPTC) with calpain 10 shRNA and examined mitochondrial function. After 3 days of infection, mitochondrial calpain 10 decreased to undetectable levels. While cytosolic calpain 10 levels did not decrease until 5 days after infection, measurement of mitochondrial membrane potential and oxygen consumption revealed no differences in control, virus-scramble, or calpain 10 shRNA infected cells. However, cellular ATP levels increased 25% four days and 50% five days after knockdown of calpain 10. Three days after calpain 10 shRNA infection there was an increase in ATP synthase β subunit of the F1 complex in the mitochondria that was not seen in the control or scramble shRNA treated cells. In addition, the loss of mitochondrial calpain 10 was correlated with increased protein expression and phosphorylation of AMP-activated protein kinase (AMPK), which is known to mediate transcription of respiratory subunits and components of the F1/F0 ATPase. In summary, we suggest that mitochondrial calpain 10 regulates cellular ATP levels under basal conditions.

470 CHLOROFORM RENAL TOXICITY DOES NOT REQUIRE HEPATIC CYTOCHROME P450-DEPENDENT CHLOROFORM METABOLISM IN MICE.

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Chloroform, which can be generated by chlorination of drinking water, can cause hepatic and renal toxicity in a number of species. Chloroform is believed to be metabolically activated by P450 to form phosgene, which targets cellular macromolecules to initiate toxicity. It has been proposed that nephrotoxic chloroform metabolites are produced by P450 enzymes within the kidney, leading to damages in the proximal tubule, although direct *in vivo* evidence for the contribution of renal P450s in chloroform-induced nephrotoxicity has not been reported. The aim of this study was to determine whether chloroform renal toxicity persists in a conditional knockout mouse model (the liver-Cpr-null mouse), in which there is a liver-specific deletion of the P450 reductase gene. We compared the extent of chloroform-induced renal toxicity in wild-type and the liver-Cpr-null mice. The levels of blood urea nitrogen (BUN), and histopathological changes in the kidney, were examined at 24 hr following an oral dose of chloroform at 150 or 300 mg/kg. Mice in the vehicle control groups received corn oil only. At the 150 mg/kg dose, the levels of BUN were three times higher in the exposed group than in the vehicle-treated group for the liver-Cpr-null mice, but were not different between exposed and vehicle-treated groups for the wild-type mice. Furthermore, lesions were found in the proximal tubules of the liver-Cpr-null, but not the wild-type, mice. At the chloroform dose of 300 mg/kg, kidney toxicities were observed in both mouse strains, but the severity of toxicity, as well as the extent of elevation of BUN levels, were greater in the liver-Cpr-null than in the wild-type mice. These findings indicate that the loss of hepatic P450-dependent chloroform metabolism does not protect the kidney from chloroform-induced renal toxicity, suggesting that renal P450-dependent metabolism plays an important role in chloroform toxicity in the kidney. (Supported in part by NIH grant ES07462).

471 SERINE PROTEASE INHIBITOR TREATMENT AMELIORATES ANTISENSE OLIGONUCLEOTIDES (ASO)-INDUCED RAT NEPHROPATHY.

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Glomerular proteinuria is associated with ISIS104838 treatment in rats. It does not appear to be related to ASO induced off-target effect, proinflammatory effect, immune deposition or complement activation. Inhibitors of several classes of protease were examined as part of the mechanistic studies. Rats were given single dose of ISIS104838 at 500 mg/kg on Day 1. Serine protease inhibitors (PIs) (AEBSF and Aprotinin), cysteine PI (E-64), or metallo PI (doxycycline) were given daily on week 1, and 3-times a week on weeks 2-3. Urine protein/creatinine (P/Cr) was monitored weekly. Glomerular morphology (H&E), α -dystroglycan (α -DG) staining (IHC), urine and plasma total protease activities were determined. A single dose of ISIS104838 at 500 mg/kg induced glomerular proteinuria with P/Cr>40 after 3 weeks. IHC revealed decreased staining of α -DG, prior to glomerular changes. Both plasma and urine total protease activities were elevated concurrently with urine protein increases. Non-toxic ASO ISIS141923 does not affect protease activities in either plasma or urine. Serine PI dose-response study showed a complete inhibition of glomerular proteinuria in high-dose serine PIs treated group and 60% reduction of urine protein in low-dose group after 3 weeks of treatment. Part of the high-dose group were kept for continuous monitoring urine protein changes without additional serine PI treatment. These rats briefly developed proteinuria (P/Cr=15) after 2 weeks but were able to recover eventually with P/Cr=3. In another study, daily treatment of serine PIs for the first week of the study also showed

benefit, suggesting the serine protease activation is the initiating event for ISIS104838-induced glomerular injuries. In both studies, serine PI treatment partially preserved glomerular morphology and α -DG staining, whereas cysteine or metallo PIs showed no effect. Current data suggest the causative role of serine protease in ISIS104838-induced kidney injury, most likely due to the systemic or local proteolytic events that are unique to the rats.

472 UNIQUE EFFECTS OF ANTISENSE OLIGONUCLEOTIDE (ASO) TREATMENT IN THE RAT KIDNEY.

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The effects of ASO on kidney in rat are distinct relative to mouse, monkey and human. Renal toxicity in the rat has been investigated using ISIS 104838, a 2nd-generation ASO targeted against human TNF α , and control ASO ISIS 141923 at 30, 60 and 150 mg/kg/wk. Kidney effects were assessed by urine chemistry and histopathology. Both ASOs induced tubular proteinuria within the first week of treatment [protein to creatinine (P/C) ratio <10]. Tubular proteinuria was independent of sequence and reversible upon cessation of treatment. ASO uptake by proximal tubule epithelial cell is an endocytic process, the same process and cell responsible for low MW protein reabsorption in kidney. ASO-induced tubular proteinuria is probably due to a competition for uptake or interference of receptor-protein interaction. Tubular proteinuria is not attributed to tubular degeneration/regeneration in these rats. A small subset of ASOs also induces glomerular proteinuria in rat at doses ≥ 30 mg/kg/wk after 4-6 weeks [P/C ratio >10]. In contrast to the tubular proteinuria, the high grade proteinuria is highly sequence dependent. The high grade proteinuria is associated with thickening of the glomerular basement membrane (GBM) and focal glomerular sclerosis. Ultrastructural changes revealed podocyte degeneration and fusion of foot processes. Decreased staining of α -dystroglycan (DG), a podocyte associated protein, has been found prior to glomerular damage. These renal effects have not been observed in mice, monkeys or man. In monkey, no increase of P/Cr ratio, no change in glomerular morphology and no decrease in α DG staining was observed after 13 weeks treatment of ISIS104838 at 35 mg/kg/wk or 4 weeks treatment at 140 mg/kg/wk. Rats in general are more sensitive to glomerular damage with aging rat nephropathy a commonly encountered lesion in laboratory rats. The present data clearly suggest the ASO-induced the kidney effects are highly sequence specific and unique to rats.

473 PROTEOMIC ANALYSIS OF THE EFFECTS OF S-(1, 2-DICHLOROVINYL)-L-CYSTEINE-INDUCED ACUTE RENAL FAILURE.

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It has been shown that renal tissue repair stimulated by a low dose of S-(1, 2-dichlorovinyl)-L-cysteine (15 mg DCVC/kg, ip) 72 h prior administration of a normally lethal dose (75 mg/kg, ip), protects mice from acute renal failure (ARF) and death (autoprotection). Objective of the present study was to identify the proteins indicative of DCVC-induced nephrotoxic mechanisms of autoprotection. Male mice (25-29 g) received distilled water (10 ml DW/kg), low, high, and high dose of DCVC after priming. Renal dysfunction and injury were assessed at 36 h after DCVC in low dose, high dose, and autoprotected mice (36 h after high dose of DCVC), by assessing plasma creatinine and renal histopathology, respectively. Proteins from the whole kidney homogenates at 36 h in DW-treated, low dose, high dose, and autoprotected mice were separated by two dimensional electrophoresis and quantified. Proteins significantly altered by DCVC ($p \leq 0.01$) in low dose, high dose, and autoprotected mice when compared to DW controls, were quantified. Expression of 18 common proteins was maximally changed (≥ 10 fold) in all the groups compared to controls. These proteins were conclusively identified by LC-MS/MS of peptide digests and by gel matching. These 18 proteins are involved in several biochemical processes, such as stress response, fatty acid transport, protein repair, protein turnover, and energy metabolism. ATP levels were significantly reduced after the high dose of DCVC when compared to low dose alone and autoprotected mice. These findings suggest that proteomic studies of experimental animal models like DCVC-induced ARF and nephro-autoprotection may be useful in understanding molecular mechanisms of injury and recovery following ARF-bound injury (Supported by DK 61650).

474 NEPHROTOXICITY INDUCED BY N-(3, 5-DICHLOROPHENYL)-3-HYDROXYSUCCINAMIC ACID: EFFECT OF DIMETHYL SULFOXIDE IN THE VEHICLE.

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The agricultural fungicide N-(3,5-dichlorophenyl)succinimide (NDPS) induces selective nephrotoxicity in male rats at intraperitoneal (ip) doses of 0.4 mmol/kg or greater. NDPS nephrotoxicity is mediated by metabolites arising from oxidation of the succinimide ring. One NDPS metabolite, N-(3,5-dichlorophenyl)-3-hydroxy-succinimide (3-NDHSA), was a nephrotoxicant at doses lower than NDPS, but heating of the sesame oil vehicle was required to achieve solution. The purpose of this study was to determine the effect of the use a combination of dimethyl sulfoxide (DMSO) in sesame oil as a vehicle that did not require heating to provide 3-NDHSA in solution prior to administration. Male Fischer 344 rats (4 rats per group) were administered 3-NDHSA (0.1, 0.2 or 0.4 mmol/kg, ip) or vehicle (25% DMSO in sesame oil; 2.5 ml/kg) and renal function monitored for 48 hr. No evidence of nephrotoxicity was observed in the 0.1 mmol/kg treatment group. In the 0.2 mmol/kg treatment group, transient diuresis and proteinuria were observed. In the 0.4 mmol/kg treatment group, marked nephrotoxicity was observed characterized by diuresis, proteinuria, increased kidney weight and blood urea nitrogen (BUN) concentration, and proximal tubular necrosis. These results demonstrate that use of a DMSO/sesame oil vehicle reduces 3-NDHSA nephrotoxicity. Supported by NIH Grant DK31210.

475 NEPHROTOXICITY OF EXTRACT FROM FUNGI DERMOCYBE SANGUINEA CONTAINING ANTHRAQUINONES.

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The toxic action of orellanine and several cortinarines from *Cortinarius speciosissimus* is very well known. Until recently the nephrotoxicity of *Dermocybe sanguinea* was at issue. The chemical composition of *D. sanguinea* presents 5.3% water soluble anthraquinones, especially emodin and dermocybin in O-glycosyl forms. Acute effects of *D. sanguinea* extract 1:1 (DSE) were evaluated on rats. Rats received a dose of DSE (10 ml/kg, p.o.) on day. At 24, 48, 96 h after the administration with DSE, urine, blood and kidney samples were collected. Normal Wistar-rat kidney, urine and blood samples were examined prior to the study. Creatinine and urea levels in urine and plasma were determined. Active tubular secretion (ATS) was determined by Rowntree-Geraity method. The kidneys were examined histologically. Exposure with DSE caused a decrease of ATS at 58 and 46% after 24 and 96 h, respectively. Plasma creatinine and urea nitrogen levels were increased after 48 h. Diuresis and urine creatinine and urea nitrogen levels were decreased after 96 h. In this dose group degenerative lesions were localized in the large segment of the proximal tubules and include lymphohistiocytic infiltrates in the interstitial area. The results of this study implicate that the extract of *Dermocybe sanguinea* was altered of rat's kidneys. The authors conclude that the acute intoxication of *Dermocybe sanguinea* may be associated with nephrotoxicity of natural anthraquinones.

476 MECHANISTIC TOXICANT PATHWAYS FOR ROTENONE AND 7-BUTYL HYDROPEROXIDE IN RAT RENAL CORTICAL SLICE CULTURES.

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The precision-cut renal cortical slice culture model is a unique in vitro tool that maintains the 3-dimensional cell/matrix interactions found in the kidney in vivo. The purpose of this study was to characterize biochemical response of this culture model to two diverse toxicant classes, namely a mitochondrial respiratory chain inhibitor (rotenone) and a pro-oxidant (*p*-butyl hydroperoxide or tBHP). A dose response was performed in precision-cut renal cortical slices prepared from male Sprague-Dawley rats and cultured on screens in a roller incubator for 24 hours at seven concentrations between 0.1-300 μ M for rotenone and 1-10,000 μ M for tBHP. Two hours before harvesting slices, radiolabeled ³H-leucine and ¹⁴C-tetraethylammonium chloride (¹⁴C-TEA) were added to the culture media. After the incubation period, media was collected and assayed for lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activities (indices of cytotoxicity) and nitrate products [nitric oxide (NO) index]. The slices were harvested and content of ATP, GSH, protein, thiobarbituric acid-reactive substances [lipid peroxidation (LPO) index], organic cation transport (NMeN⁺-sensitive ¹⁴C-TEA uptake) and protein synthesis (acid-insoluble ³H-leucine incorporation) were determined. The LDH release response generally mimicked the ALP release response. Maximal ALP and LDH release occurred at 1 μ M rotenone and 1 mM tBHP. NO production was moderately increased by tBHP treatment (2.5 fold), but unchanged by rotenone treatment. Similarly, LPO was markedly increased (7 fold) at cytolytic concentrations of tBHP, and only moderately (2 fold) increased in rotenone treated samples. Profound declines in ATP, organic cation transport, protein synthesis, and GSH

were observed at non-cytolytic (< 20% maximal LDH release) doses (0.1 μ M) of rotenone; whereas only cytolytic concentrations (> 1 mM) of tBHP elicited these declines. This data suggests these two diverse toxicants exert effects on discrete mechanistic pathways.

477 EPIDERMAL GROWTH FACTOR RECEPTOR REGULATES DE-DIFFERENTIATION/RE-DIFFERENTIATION IN REGENERATING RENAL PROXIMAL TUBULAR CELLS FOLLOWING OXIDANT INJURY.

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Repair of the injured renal epithelium is thought to be mediated by the surviving renal proximal tubule cells (RPTC) which must dedifferentiate to allow the proliferation and migration necessary for epithelial regeneration. RPTC then redifferentiate to restore tubular function. Current models suggest epidermal growth factor receptor (EGFR) activation, subsequent to p38 mitogen activated protein kinase (p38), or transforming growth factor- β 1 (TGF- β 1) is required for dedifferentiation characterized by vimentin expression and the spindle morphology. However, the mechanisms of the redifferentiation have not been investigated; therefore, a model of redifferentiation using rabbit RPTC in primary cultures was developed. The dedifferentiated phenotype was induced by treatment with 1 mM H₂O₂ for 5 hours and persisted > 48 hr. Redifferentiation occurs from 72 to 120 hours as indicated by a decrease in vimentin expression and the return of control epithelial morphology. Inhibition of EGFR by AG1478 during dedifferentiation (24 hr post H₂O₂) restored epithelial morphology and decreased vimentin expression to control levels at 48 hours. Conversely, EGF increased vimentin expression and produced the spindle morphology at 48 hours. The p38 and TGF- β 1 receptor inhibitors (SB 203580 and SB431542, respectively) had no effect on redifferentiation following H₂O₂ injury. These experiments represent a new model for the investigation of RPTC redifferentiation following acute injury and reveal that the dedifferentiated phenotype is maintained following injury by EGFR activation independent of p38 and the TGF- β 1 receptor.

478 COMPOUND-INDUCED CRYSTALLURIA DOES NOT PREDICT OCCURRENCE OF OBSTRUCTION NEPHROPATHY IN FEMALE RATS.

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Oral administration of compound A induced crystalluria in rats and dogs. Compound A contains a sulfonamide moiety, has poor, pH-dependent aqueous solubility and is renally eliminated. Urinary calculi and associated obstruction nephropathy was noted only in female rats. Administration of the compound to Beagle dogs resulted in crystalluria without renal lesions. Follow-up studies in rats were conducted to understand the association between crystalluria and calculi formation, determine the chemical nature of the urinary crystals, and identify putative biomarkers of precipitate/calculi-induced urinary tract lesions. In rats, doses $\geq$ 100 mg/kg/day resulted in marked crystalluria in all animals. Crystals were identified as compound A by confocal dispersive Raman microscopy. At high doses, lesions in the urinary tract consistent with calculus formation and obstruction nephropathy were observed in female rats after 7 days of treatment. Gross lesions included calculi and/or white precipitates in kidney, ureters and urinary bladder, renal pelvic and ureteral dilatation, and mucosal hemorrhages in ureters and bladder. Correlating microscopic renal lesions included tubular dilatation with epithelial degeneration, mucosal hemorrhages, pelvic dilatation and urothelial hyperplasia. Crystalline precipitates within the renal tubules, often sufficient enough to occlude the papillary ducts, were noted in kidney sections and visualized under polarized light in frozen sections. Urine and serum chemistry and additional renal injury biomarker panels reflected acute renal failure in affected animals; however no predictive parameters were identified as judged by absence of these markers in animals that had crystalluria but no histopathological changes. Conclusion: Precipitation of crystals and formation of calculi in the kidney, ureters, and urinary bladder in rats was due to the poor solubility of compound A at physiological pH and high concentrations of the compound achieved in the rat urine.

479 MECHANISMS OF BROMIDE- AND BROMATE-INDUCED KIDNEY AND LIVER CELL DEATH.

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The mechanisms of bromide (Br⁻) and bromate (BrO₃⁻)-induced death in Normal Rat Kidney (NRK) and liver cell (CRL1469) lines was investigated. Cell death was assessed based on the staining of 3-(4, dimethylthiazolyl)-2, 5-diphenyltetra-

zolium bromide (MTT), cell morphology, annexin V and propidium iodide (PI) staining and cell cycle analysis. Both BrO₃⁻, (added as KBrO₃) and Br⁻ (added as NaBr) induced concentration-dependent decreases in MTT staining after 48 hr in kidney and liver cells. However, BrO₃⁻ decreased MTT staining at lower concentrations (0.1 to 1.6 mg/mL) than Br⁻ (1.6 to 25.6 mg/mL). Further, BrO₃⁻, but not Br⁻, induced greater decreases in MTT staining in kidney cells compared to liver cells. These data were confirmed by analysis of cell morphology, which demonstrated that concentrations of BrO₃⁻ as low as 0.2 mg/mL induced alterations in kidney cell morphology, compared to 0.8 mg/mL in liver cells. The role of the sodium iodide symporter (NIS) in cell death was investigated by exposure of cells to the NIS inhibitor NaClO₄ (20 mM). Treatment with NaClO₄ prior to exposure to either BrO₃⁻ or Br⁻ increased MTT staining in kidney cells, but not liver cells. To study the mechanisms of cell death, the effect of BrO₃⁻ on annexin and PI staining and cell cycle was assessed in kidney cells. BrO₃⁻ exposure increased both annexin V and PI staining in tandem, suggesting that cell death was primarily mediated by necrosis as opposed to apoptosis. Cell cycle analysis demonstrated that BrO₃⁻ exposure doubled the percentage of cells in G2-M, which was not inhibited by NaClO₄. These data demonstrate that BrO₃⁻ and Br⁻ cause concentration-dependent death in kidney and liver cells, that BrO₃⁻ is a more potent toxicant than Br⁻, that kidney cells are more susceptible to BrO₃⁻-induced cell death than liver cells, and suggests that the mechanisms of BrO₃⁻-induced cell death involves both a G2-M arrest and NIS. This work was partially supported by the Awwa RF and the Georgia Cancer Coalition.

480 RESPIRATORY MEASUREMENT VALIDATION STUDY IN CYNOMOLGUS MONKEYS USING A HEAD PLETHYSMOGRAPH: EFFECTS OF MORPHINE (IV) AND HISTAMINE.

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The objective of this project was to validate procedures for inhalation exposures and respiratory measurements in nonhuman primates. In addition to inhalation exposures, the methods will be used for safety pharmacology studies and inhaled drug dosimetry estimates. A combination head-only exposure helmet/head plethysmograph system was used in conjunction with the Data Sciences International (DSI) PONEMAH® Physiology Platform. Prior to test agent treatments, the 2-3 year old male Cynomolgus monkeys were acclimated to chair restraint and the helmet system over a 6-week period. To evaluate the performance of the exposure helmet/plethysmograph and data collection system, tidal volume, breathing rate and minute volume were measured prior to, during and following intravenous dosing with morphine and inhalation of histamine. Respective mean baseline values prior to morphine and histamine treatments were 28 and 23 mL for tidal volume, 45 and 50 breaths/minute for breathing rate and 1248 and 1145 mL/minute for minute volume. Treatment with IV-injected morphine at dose levels of 100 and 1000 μ g/kg resulted in mean maximum decreases of 3% and 28% in tidal volume, 10% and 27% in breathing rate and 13% and 43% in minute volume, respectively, when compared to baseline levels. The approximate time to peak effect on respiratory parameters was 15-20 minutes post-dose for the 100 μ g/kg level and 5 minutes post-dose for the 1000 μ g/kg level. Exposure of monkeys to inhaled histamine aerosols generated from 0.02% to 0.2% saline solutions resulted in a mean maximum decrease of 42% in tidal volume and a mean maximum increase of 63% in breathing rate when compared to baseline levels. Peak effect on respiratory parameters was noted approximately 2 minutes after inhalation of histamine. These results provide a bio-phase validation for use of the exposure helmet/plethysmograph and the DSI PONEMAH® system with nonhuman primates.

481 WHOLE BODY PLETHYSMOGRAPHY IN RATS AS AN APPROPRIATE METHOD TO TEST RESPIRATORY FUNCTION ACCORDING TO THE ICH S7A GUIDELINE.

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Whole body plethysmography as established in our lab is a state-of-the-art technique for quantitative routine measurements of pulmonary parameters which is considered to facilitate the detection of potential unwanted effects of new drug candidates to the respiratory system prior to first dose in man and to fulfill the requirements of the ICH guideline S7A.

A pressure signal is detected by a transducer from the animal placed into a chamber, from which respiratory parameters, i.e. respiration rate, tidal volume and minute volume, are derived using pre-determined conversion factors. The ultimate pressure signal measured results from the animal removing air from the chamber and the expansion of the thorax due to inspiration. The latter creates a signal greater than that

originating from removing air from the chamber since warming and humidifying of the inspired air are additional factors to this process. A new, internally developed study design allows the routine evaluation of respiratory parameters in combination with a functional observation battery (FOB) for neurological effects in one study without extra-use of animals. The rat was selected as an appropriate model for this type of experiments.

Baseline data confirmed the absence of any statistically significant difference in respiratory parameters between individual animals, sexes and time of recording with little evidence of habituation to repeated and/or long time exposure. Positive controls were employed (amphetamine, theophylline, baclofen) to test for adverse effects. Compared to control animals, the test articles clearly induced alterations of the respiratory function.

Our results demonstrated that changes in pulmonary parameters can reliably be detected. The experimental conditions and the equipment in use provide a basic understanding of the adverse effects on the respiratory system as a fundamental part of the safety assessment of drug candidates.

482 DEVELOPMENT OF A LCMS METHOD FOR QUANTIFYING OXIDATIVE MODIFICATIONS OF LIPIDS IN BIOLOGICAL SAMPLES.

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Oxidized phospholipids have been shown to play an important pathological role in both acute and chronic vascular disease. We hypothesize that inhalation of certain toxic agents and environmental pollutants can induce oxidative modifications to endogenous phospholipids. To address this, we have developed a method to measure the specific phospholipid, 1-palmitoyl-2-arachidonoyl-sn-glycero-phosphocholine (PAPC) and its oxidized by-products in tissues and plasma. Phospholipids were eluted with an isocratic mobile phase consisting of methanol and water (90:10) with a flow rate of 1 mL/min. With reversed-phase chromatography, PAPC elutes at approximately 14 minutes. Auto-oxidation of PAPC (oxPAPC), over a 3 day period, results in the formation of a complex array of compounds eluting at approximately 6 minutes. Mass spectrometry (MS) was performed on an atmospheric pressure ionization (API) triple-quadrupole biomolecular mass analyzer (Applied Biosystems API 4000) equipped with an electrospray ionization source. Phospholipids were introduced into the electrospray MS by liquid chromatography. oxPAPC produced several signals that are both higher in molecular weight (from oxidation) and lower in molecular weight (from oxidative decomposition). PAPC was defined by the presence of a signal at m/z 782.7. The presence of PAPC could be detected as low as 100ng/mL. oxPAPC was defined by the presence of 3 previously identified molecular ions (m/z 594.3, 610.2, 828.6). These methods will enable the detection of oxidative modifications of endogenous phospholipids in inhalation toxicology studies.

483 MECHANISMS OF MECHANICAL VENTILATION-INDUCED INFLAMMATORY AND OXIDANT RESPONSES.

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Rationale: Mechanical ventilation (MV) is an unavoidable consequence in a variety of respiratory diseases. Mechanical strain associated with ventilation contributes to inflammation and ventilator-induced lung injury (VILI). Implicated in this injury are inflammatory mediators and reactive oxygen species (ROS) signaling. Tumor necrosis factor- α single and double receptor KO mice (TNF α R1-/- and TNF α R1/2-/-) and interleukin-1 β (IL-1 β) KO mice were used to investigate inflammatory and ROS signaling pathways in VILI. Methods: Wild type (WT), TNF α R1-/-, TNF α R1/2-/-, and IL-1 β -/- mice were mechanically ventilated with a 10cc/kg tidal volume, 150BPM for 6 hours. Analysis included pulmonary function testing, histological analysis, and proinflammatory cytokine and chemokine mRNA measurements by RNase protection assay. Immunohistochemistry for heme oxygenase-1 (HO-1) and 8-oxo guanine were performed to determine antioxidant and oxidant stress responses. Results: Decreased compliance and increased peak pressure were seen after 6hrs of MV, indicating a change in lung function. Ventilated mice showed no significant change in lung morphology as compared to unventilated mice. Ventilation increased alveolar expression of HO-1 protein in WT, TNF α R1/2-/-, and IL-1 β -/- mice. MV decreased 8-oxo guanine staining in all groups. mRNA message for MIP-2, IL-1 β , KC and IL-1 receptor II increased after 6hrs of MV. TNF α and IL-1 message increased in WT and KO mice. Conclusions: MV for 6hrs does not significantly alter lung morphology in all groups. Increased HO-1 protein may be contributing to decreased oxida-

tive DNA damage as seen by decreased 8-oxo guanine staining with MV. The development of ventilator-induced inflammation in TNF α R-/- and IL-1 β -/- mice demonstrates that TNF α and IL-1 β are each not solely necessary for a ventilator-induced inflammatory response.

484 COMPARATIVE EVALUATION OF RISK FACTORS FOR CARDIOVASCULAR DISEASE (CVD) IN GENETICALLY PREDISPOSED RATS.

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Rodent CVD models are increasingly used for understanding individual differences in susceptibility to environmental stressors such as air pollution. We characterized pathologies and a number of known human risk factors of CVD in genetically predisposed, male young adult Spontaneously Hypertensive (SH), Stroke-Prone SH (SHSP), Heart Failure SH (SHHF) and Diabetic (JCR) rats. These were compared to healthy Sprague Dawley (SD) and parental Wistar Kyoto (WKY) rats. Baseline differences in pathology of the heart, lung and kidney, antioxidants and gene expression, along with levels of systemic inflammation and coagulation markers were analyzed to understand distinct disease-related phenotypes. There were various degrees of myopathy in all strains; JCR appeared to be affected the most. No major pathological lesions were apparent in the lungs of any strain. However, lavage fluid neutrophils, marker for lung inflammation, were increased in SH and SHSP relative to others. This was associated with higher baseline levels of lung glutathione but lower ascorbate. Lung ascorbate levels were highest in SD and JCR. Although having high hematocrit values, coagulation parameters in JCR were comparable to WKY. These were most affected in SHSP relative to WKY. JCR and SD rats had the highest number of circulating white blood cells and lymphocytes relative to all other strains. Further, JCR and SHHF, both with obese phenotype, demonstrated remarkable kidney lesions. Thus, each model demonstrates distinct disease- and tissue-dependent anomalies that are likely to be associated with the degree of compensatory response. Understanding the pathobiologic manifestations of the disease in the chosen animal model is essential for accurate interpretation of susceptibility data. (This abstract does not reflect US EPA policy. Supported in part by UNC/EPA CT829471 and NRC Research Associateship Award at NHEERL, EPA).

485 ACUTE CARBON MONOXIDE POISONING: TREATMENT WITH NORMOBARIC OR HYPERBARIC OXYGEN AND ITS CORRELATION WITH MITOCHONDRIAL COMPLEX IV ACTIVITY FROM PERIPHERAL BLOOD LYMPHOCYTES.

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Background: Symptoms of acute carbon monoxide (CO) poisoning (ACOP) are generally attributed to hypoxia due to carboxihemoglobin (COHb), although there is also an effect on cells due to the inhibition of the mitochondrial complex IV (mCIV). Objective: To establish 1) the activity of mCIV in patients suffering from ACOP; 2) the level of its recovering depending on the treatment (normobaric oxygen -NBO- vs hyperbaric oxygen -HBO-), and 3) the correlation between mCIV activities and the severity of intoxication. Patients: 26 subjects with ACOP were distributed as: a) severe (COHb > 20%) (n:13), randomly treated with one (n:8) or two (n:5) sessions of HBO (SHBO1 and SHBO2), and b) moderate (COHb: 10-20%) (n:13), randomly treated with NBO (MNBO) (n:8) or one session of HBO (MHBO1) (n:5). Method: 1) Register of clinical manifestations, 2) COHb determination and 3) mCIV activity (spectrophotometry) in lymphocytes from peripheral blood (Ficoll technique), before and immediately after the treatment, as well as at 24 h, 1 month and 3 months later. Analysis of results: 1) analysis for intention-to-treat principle depending on the assignment of groups; 2) comparison of MNBO-MHBO1, SHBO1-SHBO2 and MHBO1-SHBO1; 3) analysis of variance for repeated measures; 4) comparison of COHb and mCIV (nonparametric test for independent data). Results: mCIV activities were decreased in severe and moderate ACOP compared to controls ($p = 0.014$ and 0.027 , respectively), but were not different to immediately after-treatment values. We did not find differences in after-treatment mCIV activities between MNBO and MHBO1, nor between SHBO1 and SHBO2. Recovery of mCIV activity along time was significant in SHBO1, SHBO2 and MHBO1 ($p = 0.036$, 0.048 and 0.016 , respectively), but not in MNBO. Conclusions: In severe ACOP one single session of HBO is enough effective in restoring mCIV activity, and it is also more effective than NBO in moderate ACOP. (FIS PI041239).

486 POSSIBLE ROLE OF ANGIOTENSIN II (ANG II) INDUCED CONNECTIVE TISSUE GROWTH FACTOR (CTGF) MEDIATED ELEVATION IN ELASTIN AND COLLAGEN LEVELS IN RAT PLEURAL MESOTHELIAL CELLS (RPMCS, CCL 216).

U. B. Doshi and J. M. Cerreta. *PHS, St. John's University College of Pharmacy and Allied Health Professions, Jamaica, NY.* Sponsor: *L. Trombetta.*

Perturbation in extracellular matrix homeostasis is the hallmark of tissue fibrosis. While many mechanisms are involved in regulation of matrix alteration, studies carried out with antagonists of Renin Angiotensin System (RAS) have implicated a role for Ang II, an effector peptide of RAS, in several fibrotic disorders. Ang II mediated increase in collagen production has been shown to occur via a peptide, CTGF, in renal and cardiac fibrosis. However, in the lung a direct link between Ang II and CTGF expression has not been established with respect to extracellular matrix perturbation. The current study in RPMC was undertaken to examine a possible link between Ang II mediated increase in elastin and collagen levels and CTGF. Cells were grown in supplemented F-12-Nutrient Mix (Ham)-media containing 50µg/ml ascorbic acid or without ascorbic acid and 5% CO₂. Cell viability was assessed by MTT assay. Sirius-red assay, ELISA and western blot analysis were carried out to measure collagen, elastin and CTGF levels respectively. Cultures were challenged with various concentrations of Ang II (10nM-1µM) for 24 hrs, following which the cells were either used for MTT assay or allowed to synthesize matrix for 7 days and then used for estimation of protein levels. At concentrations used Ang II did not show any toxicity. Ang II induced a 40 % increase in elastin and 47.1 % increase in collagen (p<0.01) levels at a concentration of 1µM by day 7 as compared to control. In western blot analysis, Ang II treatment at 1µM concentration caused an increase of CTGF as measured at day 7. These data indicate that a treatment of RPMC with 1µM concentration of Ang II causes CTGF increase between 0-7 days of treatment. Further, due to such increases the levels of collagen and elastin are elevated by day 7. These data suggest a positive correlation between Ang II, CTGF and matrix proteins synthesis.

487 LUNG PROTEIN EXPRESSION FOLLOWING ACUTE FORMALIN EXPOSURE – EFFECT OF THE SUBSTANCE P DERIVATIVE SAR⁹, MET(O₂)¹¹-SUBSTANCE P.

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Previous studies suggest that Sar⁹,Met(O₂)¹¹-Substance P (SPa) may attenuate lung injury by blocking the action of endogenous substance P at the neurokinin-1 receptor (NK1r) on pulmonary alveolar macrophages and other potential lung cell receptor sites. We sought to determine if SPa pretreatment would attenuate protein alterations associated with formalin-induced acute lung injury. F344 male rats [control; formalin-only (F); or SPa/formalin] were exposed to 67 mg/m³ (67 ppm) aerosolized formalin or air for 1.5 min while others were administered 35 ppm aerosolized 10 µM SPa over a 10 minute period just before formalin exposure. At ~42 minutes post-formalin or air exposure, rat lungs were removed for protein analysis. Replicate rats were administered pulmonary function and lung permeability tests. The F rats demonstrated a 33-fold increase in inspiratory dynamic lung compliance compared to the SPa rats. Increased compliance was matched by a 6-fold increase in F rat lung permeability versus the SPa group. Two-dimensional gel electrophoresis revealed altered abundance of 71 proteins whose formalin-mediated changes were attenuated in the SPa rats. Given the brief exposure period, the low abundance of the detected proteins (potential charge variants), and their identities, we hypothesize that this formalin effect relates to altered protein posttranslational modification and not protein synthetic/catabolic mechanisms. Conversely, immunoblotting suggests a limited set of proteins may be up- or down-regulated. Though the exact nature of the observed expression changes remains debatable, this study clearly demonstrates the protective effect of SPa against some aspects of acute formalin toxicity. Supported by AFOSR Grants FA9550-06-1-0083 & FA9550-05-1-0216 to FAW and FA9550-04-1-0231 to MLW.

488 MOUSE MODELS OF BERYLLIUM-INDUCED SENSITIZATION AND GRANULOMATOUS LUNG DISEASE.

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Exposure to beryllium (Be) is an occupational hazard that can cause chronic beryllium disease (CBD), an irreversible, debilitating granulomatous lung disease, in as many as 3-5% of exposed workers. CBD begins as an MHC Class II-restricted,

Th1 hypersensitivity. CBD can be viewed as a biphasic disease: first peripheral sensitization occurs, and then, in some, there is progression to pulmonary granuloma formation. In order to look at both aspects of the disease, we performed a mouse ear-swelling test (MEST) to correlate with the sensitization process and a 5-month oropharyngeal aspiration study to look at granuloma development in mice. In the MEST, 21 different inbred mouse strains were utilized to see if they would exhibit varying hypersensitivity responses to Be. In two separate experiments, mice were placed into either group: C/C group (sensitized with vehicle, challenged with vehicle) or Be/Be group (sensitized with beryllium sulfate, challenged with beryllium sulfate). The SJL/J strain appeared to exhibit one of the greatest hypersensitivity responses with a 37.7% increase over the baseline ear thickness in the Be/Be group compared with a 2.6% increase in the control group. The FVB/N strain, in contrast, had statistically insignificant, increases in ear thickness. The results for the hypersensitivity MEST response were as follows: SJL/J, AKR, DBA/2J>>PL/J, LG/J, MRL/MpJ>> FVB/N, 129/SvImJ, SWR/J. The data have been analyzed by haplotype mapping to uncover genes associated with sensitization to beryllium. In the aspiration study, seven inbred strains aspirated either 20µg, 35µg, or 50µg of beryllium metal powder or water vehicle monthly. Clear dose and strain differences in beryllium-induced lymphogranulomatous nodules were observed. Uncovering the genes responsible for the hypersensitive phenotype and granuloma formation in mice may prove useful in learning more about the mechanisms involved in CBD.

489 BINDING OF THE MACROCYCLIC TRICHOETECENE SATRATOXIN G TO RIBOSOMES IN RAW 264.7 MACROPHAGES AND PC-12 NEURONAL CELLS.

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Satratoxin G (SG), a macrocyclic trichothecene produced by the black indoor air mold *Stachybotrys chartarum*, causes both inflammation and neurotoxicity in the murine intranasal lavage model suggesting its potential to adversely affect human health. The molecular mode of action for SG and other trichothecenes is believed to involve ribosomal binding and intracellular kinase activation followed by inflammatory and apoptotic gene expression. The purpose of this research was to characterize both concentration-response and kinetics of SG binding to ribosomes in RAW 264.7 macrophage and PC-12 neuronal cell models. Following exposure of the cells to SG at various concentrations over different time intervals, ribosomal fractions were separated from cytoplasmic proteins using a sucrose gradient system. Each fraction was then measured by a competitive ELISA using anti-SG antibodies. SG was preferentially found in the monosome fraction with smaller and negligible concentrations found in the polysome and cytoplasmic fractions, respectively. When SG was incubated at 0, 2.5, 5, 10, 50, 100 ng/ml with the cells for 30 minutes, SG binding to ribosomal fractions increased in a concentration-dependent fashion up to 10 ng/ml with saturation of a single ribosomal binding site. SG binding was largely unaffected by boiling and isopropanol precipitation of the ribosomal fractions. Furthermore, exhaustive RNase treatment for removal of ribosomal RNA, failed to reduce SG content in the ribosomal protein fraction. Taken together, SG has the capacity to rapidly and tightly bind to macrophage and neuronal ribosomal proteins. Both the concentration-dependence and kinetics of this interaction were consistent with previously observed kinetics of SG-induced intracellular kinase cascade activation as well as downstream inflammatory and apoptotic sequelae.

490 ACTIVATION OF RAT ALVEOLAR MACROPHAGES AND TYPE II EPITHELIAL CELLS FOLLOWING OZONE INHALATION.

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Ozone (O₃) inhalation is associated with an inflammatory response, characterized by macrophage (AM) and neutrophil accumulation in the alveolar regions of the lung. These cells release mediators important in the pathophysiology of O₃-induced injury. We speculated that Type II alveolar epithelial cells also participate in inflammatory responses to O₃ and this was investigated. Female Wistar rats (200-225 g; 8-10 weeks) were exposed to air or O₃ (2 ppm) for 3 h. Animals were sacrificed 3-72 h later. Following O₃ inhalation, greater numbers of inflammatory leukocytes were recovered in BAL fluid 6-12 h post-exposure. This was correlated with an increase in BAL protein demonstrating that O₃ induces alveolar epithelial injury. Exposure of animals to O₃ also resulted in an increase in mRNA for the inflammatory cytokine, TNF-α, in AM and Type II cells which was evident after 3 h. Expression of COX-2, the enzyme mediating prostaglandin production, was also increased after O₃. Whereas a rapid and persistent induction of COX-2 was noted in Type II cells, increases in expression of this enzyme were delayed for 72 h in AM. Antioxidant production in the lung is critical in limiting oxidant-induced damage.

AM and Type II cells constitutively expressed SOD and HO-1 mRNA. Although SOD mRNA levels were not significantly altered by O₃, HO-1 mRNA decreased over time in AM, but not in Type II cells. Similar results were observed with SOD and HO-1 protein expression. PPAR- γ , a nuclear hormone ligand activated transcription factors, has been reported to reduce lung injury associated with inflammation. Both AM and Type II cells constitutively expressed PPAR- γ mRNA. O₃ caused a transient decrease in PPAR- γ mRNA in AM, but a rapid increase in Type II cells. Taken together, our data demonstrate that O₃ inhalation results in augmentation of genes mediating the inflammatory response. Moreover, both AM and Type II cells produce mediators that help to limit the inflammatory response and maintain normal lung homeostasis. Support: ES004738, ES005022, ALA-NJ.

491 EXPOSURE TO GASOLINE ENGINE EXHAUST CAUSES OXIDATIVE STRESS IN RATS.

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Oxidative stress is a possible mechanism for some of the adverse health effects attributed to air pollution exposure. Some effects have been reported to be greater in populations living adjacent to heavily traveled roads, consistent with the hypothesis that fresh vehicle emissions may contribute disproportionately to the hazard. Diesel engine exhaust has been widely studied, but gasoline engine exhaust (GEE) has received much less attention. We used in situ chemiluminescence to assess the effect of inhalation of GEE in rats. This technique measures the decay to ground state of singlet O₂, a highly reactive form of O₂. Rats were exposed sequentially to exhaust from two 1996 General Motors 4.3 L gasoline engines, each run for 3h (total 6h) on a cycle simulating the Unified Driving Cycle 6. This protocol thus included two "cold starts." Rats were exposed to diluted GEE containing 60 $\mu\text{g}/\text{m}^3$ (average) of total particulate matter (TPM), to similarly diluted emissions passed through a HEPA filter to remove the particles, or to filtered air as a control. TPM, NO_x, CO, and total hydrocarbons were measured. Following exposure, the rats were immediately removed from the exposure chambers, deeply anesthetized, and placed on a ventilator. The lungs, heart, and liver were exposed and covered with saline-saturated gauze and an opaque drape with a 1 cm diameter hole. The hole was aligned sequentially over the lungs, heart, and liver, and photon counting from each organ was performed using a high sensitivity photomultiplier tube and data acquisition system. The results showed a significantly higher signal in the organs (Lung > heart $\approx$ liver) of the rats that had been exposed to whole GEE, compared with those exposed to filtered air, but little effect of the filtered GEE. These results are consistent with the presence of increased reactive oxygen species due to inhalation of the particle phase of fresh GEE. Supported by the Electric Power Research Institute and the National Environmental Respiratory Center.

492 GASOLINE EMISSIONS AFFECT CLEARANCE OF INTRA-TRACHEALLY INSTILLED *PSEUDOMONAS AERUGINOSA*.

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Gasoline emissions (GE) are contributors to ambient and occupational air pollution. Of the health outcomes associated with ambient pollutant exposures, respiratory infection is a major contributor to morbidity and mortality. As part of a larger health assessment of multiple anthropogenic source emissions, mice were exposed to GE and instilled with an inoculum of a common respiratory pathogen, *Pseudomonas aeruginosa*. GE was generated from two mid-mileage 1996 4.3 L GM engines operated on a simulated version of the Unified Driving Cycle 6 and fueled by national average unleaded gasoline. Groups of young C57BL/6 mice were exposed to clean air, whole GE diluted to 18 (High), 12 (Mid), and 2 (Low) ppm oxides of nitrogen (NO_x), and a particulate matter (PM)-filtered level (18 ppm NO_x). PM concentrations were ~ 60 , 30 and $7\mu\text{g}/\text{m}^3$ at the High through Low levels respectively. Exposures were conducted for 6h/d for 7 consecutive days. Mice were instilled with bacteria subsequent to the last exposure and sacrificed after 18h in clean air. Pulmonary inflammatory histopathology and colony counts of serially diluted lung homogenate were assessed. GE had no discernable effect on exacerbation of pulmonary pathology. Similar to observations with diesel emissions, GE decreased bacterial clearance in an exposure-dependent manner. PM filtration partially attenuated the response. These results indicate that resistance to respiratory bacterial infection may be modulated by multiple on-road sources.

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493 CYTOGLOBIN IMMUNOLocalISATION IN LUNG PARENCHYMA IN SEVERE COPD.

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Cytoglobin is a novel member of the globin family that has a putative role as a tissue oxygen sensor. It is expressed in fibroblasts and has been shown to be an inducer of fibrosis, raising the possibility of a role in the pathogenesis of fibroproliferative toxicity and disease. In this study, we profiled cytoglobin expression in a cohort of patients with severe COPD undergoing lung volume reduction surgery, in which alveolar fibrosis and loss of vascularity are a feature. Ethically acquired COPD lung tissue was classified according to the fibroproliferative phenotype: pneumonitis (P), diffuse fibrosis (DF), marked alveolar fibrosis without loss of septation (AF) and organising fibrosis with loss of normal lung parenchyma (OF). In lung areas presenting with P, there was marked cytoglobin staining of macrophages, predominately a vesicular staining pattern suggestive of protein phagocytosis. There was little signal in adjacent areas. In DF areas, there was a diffuse signal within the interstitium, within fibroblasts and occasional mononuclear cells. As fibrosis increased from AF to OF, there was a progressive loss of cytoglobin signal from the interstitium, an increase in signal within the pulmonary vasculature and an increase in signal within pan-cytokeratin positive cells bordering areas of consolidated matrix – although typical epithelium within the conducting airways showed only a weak signal or negative signal. Additionally, a marked cytoglobin signal seen in the pulmonary vasculature was associated with vessels surrounded with fibrosis. In conclusion, cytoglobin expression in a disease cohort showing fibroproliferation and loss of vascularity shows a progressive loss of signal as the lesion matures to a more acellular phase, or where vascular structures are lost or surrounded by fibrosis. The histologically significant finding of cytoglobin expression in the peri-fibrotic cytotkeratin positive cell colony raises the possibility that this expression may be an attempt by normal lung to limit the spread of hypoxic stimuli.

494 THE USE OF A FLOW CYTOMETRY-BASED CYTOKINE ANALYSIS FOR BRONCHOALVEOLAR LAVAGE WITH CONFIRMATION BY REAL-TIME RT-PCR IN A MOUSE MODEL OF PULMONARY INFLAMMATION.

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Bronchoalveolar lavage (BAL) provides a valuable tool for insight into the lung inflammatory state following a toxic or antigenic exposure. Toxicological injury evaluation routinely uses a concentrated first fraction BAL (<600 μl) to accurately measure various lung inflammatory parameters in mice. However, 100-250 μl of this BAL fraction is typically used in most conventional assays limiting the researcher to only a few measurements. Recently, flow-cytometry based cytometric bead arrays (CBA) have been developed to simultaneously determine concentrations of multiple analytes in a single 25-50 μl sample saving both cost and time. In this confirmatory study, we used a mouse inflammation CBA kit to analyze IL-6, IL-10, IL-12p70, MCP-1, IFN- γ , and TNF- α protein in BAL fluid obtained from A/J or C57BL/6J mice exposed to welding fume. Since inflammatory mediators are usually accompanied by both an increase in gene expression and protein, real-time RT-PCR was done on isolated RNA from whole lung homogenates to validate the findings. It was found that a limitation of the CBA kit was sensitivity. In particular, samples with low concentrations of an analyte were often reported as extrapolated below sensitivity or zero thus limiting statistical analysis. These findings made validation by some other method necessary. Real-time RT-PCR strongly supported the findings of the CBA kit by displaying similar trends in the mouse lung inflammatory response caused by welding fume exposure. Therefore, the mouse inflammation CBA kit was an acceptable method for measuring cytokines in studies involving limited sample size and represents a cost efficient screening method for pulmonary inflammation. Also, it is recommended that another method such as RT-PCR or ELISA be performed if increased sensitivity is required.

495 COMPARISON OF LUNG INJURY AND INFLAMMATION AFTER REPEATED TREATMENT WITH WELDING FUMES COLLECTED FROM DIFFERENT WELDING PROCESSES.

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Welding fumes are a complex mixture of different metals. Depending on the welding process, some welders may be exposed to fumes that contain vastly different metal profiles. The goal was to compare the lung response in rats after multiple

treatments with welding fumes that are vastly different both chemically and physically. Welding fumes were collected from three different processes: gas metal arc-mild steel welding (GMA-MS); manual metal arc-hardsurfacing welding (MMA-HS); flux-cored arc-hardsurfacing welding (FCA-HS). Male Sprague-Dawley rats were treated by intratracheal instillation 1/wk x 7 wk with 0.5 mg/rat of the fume samples. Controls were treated with saline. Bronchoalveolar lavage was performed 4 days after the final treatment, and parameters of lung injury (lactate dehydrogenase and albumin) and inflammation (neutrophil influx) were assessed. Metal analysis indicated that the GMA-MS fume was primarily composed of Fe (1.08 µg Fe/gm total metal) and Mn (0.32 µg Mn/gm total metal), whereas the Mn content of the FCA-HS (2.0 µg Mn/gm total metal) and MMA-HS (1.8 µg Mn/gm total metal) fumes was ~6x higher than in the GMA-MS fume. The FCA-HS (0.07 µg Cr/gm total metal) and MMA-HS (0.304 µg Cr/gm total metal) fumes contained Cr which was present in only trace amounts in the GMA-MS fume. The FCA-HS and MMA-HS fumes were found to be more water-soluble than the GMA-MS fume. Significant elevations in lactate dehydrogenase, albumin, and the number of lung neutrophils were observed for the MMA-HS and FCA-HS groups compared to the GMA-MS and saline groups. No significant differences in lung injury and inflammation were observed between the GMA-MS and saline group. The greater lung response caused by the FCA-HS and MMA-HS fumes is likely due to the presence of Cr and higher levels of Mn. Results from this study indicate that welders who are exposed to fumes generated from specific processes may be at a greater risk for adverse pulmonary effects.

496 TOXICOKINETICS OF SURFACTANT PROTEIN AND INFLAMMATORY GENE EXPRESSION IN PRIMARY RAT LUNG ATII CELLS AND FIBROBLASTS EXPOSED TO PENICILLIUM CHRYSOGENUM AND STACHYBOTRYS CHARTARUM TOXINS.

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Not a great deal is known about the effects and modes of action of the spores and toxins of the majority of molds encountered in the indoor environment on lung biology. In vivo models of lung disease indicate that fungal spore and toxin exposures lead to a variety of species and toxin-specific inflammatory responses. However, it is unclear whether all the lung cells participate in these inflammatory changes. In this study we used gene expression profiling using custom made RT-PCR based microarrays to compare surfactant protein and inflammatory gene responses in primary fetal rat lung ATII cells and fibroblasts to atranones A&C from *Stachybotrys chartarum* (Sc) and melagrins and roquefortine C from *Penicillium chrysogenum* (Pc). In Pc-toxin exposed ATII cells, inflammatory genes were transiently up-regulated, generally in dose and time dependent ways, within 2 to 4 hr and de-expressed by 12 hr post exposure (PE), especially in melagrins exposed cells. SP-A, B & C genes were down regulated at 4 hr PE. SP-D expression was unaffected by the Pc toxin exposures. Inflammatory genes were generally up-regulated in atranone A but down regulated in atranone C exposed ATII cells from 2 to 24 hr PE, and in a dose dependent fashion. SP-A & C genes were down regulated in both atranone A & C exposed cells, while SP-B & D expression was unaffected. In fibroblasts, gene expression was generally limited to SP-D up-regulation, while MIP-3α was down regulated, from 2 to 4 hr PE. The results indicate that toxins from two mold species common on damp materials in buildings provoke compound-specific toxic responses with different toxicokinetics. They also provide further insight into molecular mechanisms of spore and toxin induced lung disease onset and development, and may be useful in the identification of fungal specific molecular biomarkers of disease.

497 REPEATED EXPOSURE TO 1→3-β-GLUCAN SUPPRESSES LUNG DEFENSE RESPONSES AND SLOWS THE PULMONARY CLEARANCE OF LISTERIA MONOCYTOGENES IN RATS.

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Chronic exposure to low levels of mold has been reported to increase susceptibility to respiratory infections. Currently, the mechanism is not well understood. This study investigated lung defense effects after repeated low-dose zymosan (a 1→3-β-glucan from yeast cell wall) exposure on bacterial infection (*Listeria monocytogenes*) in male Sprague-Dawley rats. On days 0, 3, 7 and 10, rats received one dose of zymosan A (0.6 mg/kg body weight each dose) via intratracheal instillation or vehicle control (saline). On day 17 (one week later), rats were intratracheally inoculated with 5x10⁵ bacteria. Rats were euthanized on days 20, 22, and 24. Bacterial clearance was determined by measuring colony-forming units cultured from the left lungs. Bronchoalveolar lavage (BAL) was performed on the right lungs.

Inflammation and lung injury were assessed by measuring (1) neutrophil (PMN) infiltration and (2) albumin and lactate dehydrogenase levels in BAL fluid. Multiple zymosan treatments induced greater lung injury and inflammation as indicated by elevations in PMN, LDH, and albumin at days 22 and 24 compared to control. In addition, repeated low-dose exposure to zymosan slowed the clearance of the bacteria at day 20 and 22 compared to controls, suggesting a possible suppression in lung immune responses. In contrast, previous results have shown that a single acute dose of zymosan at a concentration (2.5 mg/kg body-weight) equivalent to the total of the four repeated concentrations used in the current study enhanced lung immune responses and increased the rate of bacterial clearance from the lung. This study demonstrated the importance of treatment concentration and dosing regimen in understanding lung immune effects associated with 1→3-β-glucan exposure.

498 90 DAY INHALATION TOXICITY STUDY WITH DISK-SHAPED POTASSIUM OCTATITANATE PARTICLES (POT; TERRACESS TF) IN RATS.

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Fibrous particles such as asbestos and some man-made fibers have been known to produce carcinogenic or fibrogenic effects upon inhalation. Non-fibrous, disk-shaped potassium octatitanate particles (POT; TerraceSS TF) have been manufactured to avoid potential fiber toxicity. Four groups of 20 male and 15 female rats each were exposed to POT aerosol at concentrations of 0, 2, 10, or 50 mg/m³ for a 90 day period followed by a 15 week recovery period. The mass median aerodynamic equivalent diameter of the aerosols ranged from 2.5 to 2.9 µm. Lung burdens of POT were determined in 5 males/group at the end of the exposure period and after 3 and 15 weeks recovery. The clearance 1/2 times for POT were estimated to be in the order of 2 to 3 months for the 2 and 10 mg/m³ groups and 6 to 9 months for the 50 mg/m³ group. There were no POT related adverse effects in clinical observations, body weights, clinical pathology, or neurobehavioral measurements. At the end of the 90 day exposure, a slight increase in lung-to-body weight ratios was observed at 50 mg/m³ in males; this effect was not seen during the recovery periods. Female lung weights were normal. Microscopically, inhaled POT particles were mostly phagocytized by alveolar macrophages (AMs) in the alveoli and alveolar walls maintained their normal structure at 2 and 10 mg/m³. At 50 mg/m³, some alveoli were distended and filled with aggregates of particle laden AMs. The alveolar walls showed slight Type II pneumocyte hyperplasia but neither active inflammation nor alveolar fibrosis was present. The lung responses and lung clearance rates of POT were comparable to those of "nuisance" type dusts at these concentrations. The slower clearance rates at 50 mg/m³ and the presence of aggregated particle-laden AMs in alveoli at this level were considered signs of alveolar "overloading" and 50 mg/m³ was considered an effect level; the NOAEL was 10 mg/m³.

499 ESTABLISHMENT OF A BIOASSAY FOR DETECTION OF LUNG TOXICITY DUE TO FINE PARTICLE FROM BASED ON RESULTS OF DOSE RESPONSE STUDY.

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In order to establish an appropriate bioassay for detection of lung damage after fine particle exposure, sequential histopathological changes were examined after intratracheal instillation to quartz, as a typical lung toxic agent, into F344 male rats.

Experiment 1: A total of 60, 10-week-old male F344 rats, were randomly separated into 6 groups and each group was consisted 10 rats. Groups 1-4 were exposed to 4mg, 2mg, 1mg and 0mg (control) quartz (DQ-12) suspended in saline (0.2ml) using an aerolizer and subgroups were sacrificed 5 rats on Days 1 and 28 thereafter. Groups 5 and 6 were exposed to 4mg and 0mg (air only) quartz powder without suspension using special aerolizer and sacrificed on the same days. All groups underwent assessment of lung histopathology.

Experiment 2: A total of 72, 10-week-old male F344 rats, were randomly separated into 6 groups. 60 rats (5 groups) were exposed by intratracheal instillation to quartz, titanium dioxide, hydrotalcite and β-cyclodextrin, 2mg/rat, suspended in 0.2ml saline (established by Experiment 1) and subgroups of 6 rats were sacrificed on Days 1 and 28. 12 rats were exposed by intratracheal instillation to 0.2ml saline as control groups, remaining 12 rats were not exposed as untreated group and subgroups of 6 rats were similarly sacrificed on the same days. All groups underwent assessment of lung histopathology and immunohistochemical demonstration of BrdU and iNOS as end-point markers.

From Experiment 1, 2mg quartz suspended in 0.2ml saline were suggested to be most appropriate for sensitive detection of inflammatory changes. By histopathology and immunohistochemical assessment, the level of toxicity were considered

quartz > hydrotalcite > β -cyclodextrin > titanium dioxide from Experiment 2. This instillation model can be used for more sensitive detection of acute or subchronic lung toxicity due to fine particles.

500 PARTICLE SIZE DISTRIBUTION OF NICOTINE IN MAINSTREAM SMOKE OF 2R4F CIGARETTES UNDER DIFFERENT PUFFING REGIMEN.

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Nicotine's dose and rate of delivery to the brain play an important role in its addiction and cardiovascular effects. Nicotine is mainly present in the particulate phase of cigarette smoke and since particle size distribution controls the deposition behavior of particles in the respiratory tract, changes in the particle size distribution can affect its regional and total dose to the lung. Variation in the regional and total dose can also change its absorption rate and delivery to the brain. The particle size distribution of mainstream smoke (MS) can be varied by changes in puffing regimen, cigarette design and composition. In this study, we measured nicotine in different particle size fractions of MS generated from 2R4F cigarettes using three puffing regimens: (1) FTC – 35 ml over 2 sec.; (2) short puff – 50 ml over 2 sec.; and (3) long puff – 100 ml over 10 sec. MS was generated in a chamber at 37°C and > 95% RH to simulate respiratory tract conditions. Size-separated particle fractions from the first puff were collected using two RJR cascade impactors. Particle size distribution of MS was determined by spectrophotometric analysis of size-segregated fractions. These particle fractions were analyzed for nicotine using gas chromatography and mass spectrometry. Quantifiable nicotine was present only on two of the impactor stages (0.4-1.1 μ m and 1.1-1.9 μ m). Although the MMAD and GSD for each puffing regimen were similar for total particulate matter (MMAD 0.9-1.1 μ m, GSD 1.6), nicotine's distribution exhibited significant differences. Using the FTC puffing regimen, nicotine was almost equally distributed between 0.4-1.1 μ m (mean $\pm$ SD; 55% $\pm$ 5) and 1.1-1.9 μ m (45% $\pm$ 5) size fractions. However, using the short puffing regimen, nicotine increased in 0.4-1.1 μ m size fraction (72% $\pm$ 0.2) and using the long puffing regimen, nicotine increased in 1.1-1.9 μ m size fraction (60% $\pm$ 5). The changes in nicotine's particle size distributions compared to total particulate matter were statistically significant (P=0.05).

501 STRAIN AND GENDER DIFFERENCES IN TOBACCO SMOKE-INDUCED PULMONARY AIRSPACE ENLARGEMENT AND MUCUS HYPER-SECRETION.

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The correlation between tobacco use and its harmful effects has been well studied; however, factors resulting in differential sensitivity to tobacco smoke are poorly understood. The objective of this study was to examine strain and gender differences in pulmonary susceptibility to tobacco smoke-induced airspace enlargement and mucus hypersecretion. Age-matched male normotensive Wistar Kyoto (WKY) rats, male spontaneously hypertensive (SH) rats, and female SH rats were exposed either to filtered air or to tobacco smoke for 6 hours/day, 3 days/week for 12 weeks at an average total particulate concentration of 90 mg/m³. The left lung lobes were then fixed with 4% paraformaldehyde and examined by hematoxylin and eosin (H&E) staining for morphometric analysis of mean linear intercept (MLI) and alcian blue/periodic acid-Schiff (AB/PAS) staining for mucus glycoconjugate measurement. The MLI of male SH rats exposed to tobacco smoke (118 microns) was significantly increased compared with their filtered air controls (93 microns), whereas no significant changes were observed in WKY rats and female SH rats when compared with their corresponding controls. In addition, the difference in MLI was significantly greater in male SH rats compared with WKY rats and female SH rats. In the airway epithelium, male SH rats also demonstrated significant elevation in positive epithelial cell AB/PAS staining (10%) following 12 weeks of smoke exposure compared with filtered air controls (0.3%). In contrast, no significant changes were found in WKY rats and female SH rats when compared with the control animals. These results not only suggest the male SH rat to be a good model for tobacco smoke-induced airspace enlargement and mucus hypersecretion, but also show strong gender differences in the classical features of tobacco smoke-induced lung disease.

502 CYTOTOXIC AND INFLAMMATORY EFFECTS OF HARDWOOD COMBUSTION PARTICLES ON MOUSE MACROPHAGE CELL LINE.

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Particles from incomplete wood combustion are a major health concern worldwide. We studied wood smoke particles from good and poor combustion of birch (*Betula spp.*) wood logs in one masonry heater. The smoke was diluted in about 1:200 with

filtered laboratory air and cooled in a dilution tunnel. The size-segregated particulate samples (PM_{10-2.5}, PM_{2.5-1}, PM_{1-0.2} and PM_{0.2}) were collected with a high volume cascade impactor. Particulate material was extracted from the sampling substrates with methanol that was subsequently evaporated. Mouse RAW 264.7 macrophages were exposed for 24 hrs to four doses of particulate samples (15, 50, 150 and 300 μ g/ml) and corresponding volumes of filter blanks. TNF α and IL-6 productions were measured immunochemically (ELISA) and cytotoxicity was measured by using the MTT test. Particulate emissions from poor combustion were 2-10 fold compared to good combustion in different size ranges. The largest emissions were measured in PM_{0.2} with good combustion and in PM_{1-0.2} with poor combustion. None of the particulate samples induced significant IL-6 production, but TNF α production was increased. Particles from poor combustion induced in all the size ranges somewhat larger TNF α responses than particles from good combustion. The PM_{2.5-1} samples caused the largest inflammatory responses in both types of combustion experiments. The PM_{1-0.2} and PM_{0.2} samples from poor combustion had higher cytotoxic activity in the macrophage cell line per unit of particulate mass than the samples in other size ranges. In good combustion, the PM_{10-2.5} samples had the highest cytotoxic activity, whereas the PM_{0.2} samples were least cytotoxic. Thus, we managed to show that combustion conditions affect not only the mass but also the toxic activity of emitted particles in different size ranges.

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503 PULMONARY AND SYSTEMIC EFFECTS OF FOUR-WEEK DIESEL INHALATION IN HEALTHY AND HYPERTENSIVE RATS.

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Diesel exhaust (DE) comprises a significant fraction of near road ambient particulate matter (PM). Exacerbated cardiac and pulmonary complications are noted in individuals residing near roadways. We examined pulmonary and systemic effects of diesel exhaust (DE) in healthy and hypertensive rats to investigate potential mechanisms and susceptibility. Male Wistar Kyoto (WKY) and Spontaneously Hypertensive (SH) rats (12-14 weeks age) were exposed whole body to air or freshly generated DE (gas plus particles) at 0.5 and 2.0 mg/m³ x 6h/d x 5d/wk x 4 wks. Lung injury and inflammation were assessed by histopathology and bronchoalveolar lavage fluid (BALF) analysis while systemic effects were assessed by plasma cytokines and hematological measurements. DE caused concentration-dependent increases in BALF neutrophils in both strains but no significant net accumulation of macrophages, despite the presence of particle-laden macrophages. Pulmonary protein leakage was modest but the activity of a cell injury marker, γ -glutamyl transferase was significantly increased in both strains. Lung pathology was characterized by thickening of alveolar septa, presence of alveolar pigmented histiocytes, interstitial inflammation and focal mast cell aggregation. Plasma cytokines, fibrinogen, and hematological parameters were not altered except for modest increases in white blood cells of SH and WKY and hematocrit values of SH rats exposed to DE. Noted inherent strain differences were likely due to presence of cardiovascular disease in SH rats; however, no striking exposure-related differences were apparent in any of the measured markers. These data demonstrate that 4-wk inhalation of rats to DE causes pulmonary inflammation and pathology but only modest systemic effects in healthy and genetically hypertensive rats. (This abstract does not reflect US EPA policy. Supported in part by UNC/EPA CT829471 and NRC Research Associateship Award at NHEERL, EPA).

504 DIESEL PARTICULATE-INDUCED NEUTRAL ENDOPEPTIDASE DOWNREGULATION IS ASSOCIATED WITH CELL PROLIFERATION.

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Neutral endopeptidase (NEP) is a key cell surface peptidase which efficiently inactivates many peptide substrates and plays a central/regulatory role in the maintenance of homeostasis or in control of many physiopathological processes including inflammatory response and carcinogenesis. Therefore, we examined the role of NEP on diesel particulate-induced cell proliferation in human airway epithelial BEAS-2B cells by using RNAi technique. NEP siRNA (small interfering RNA) transfectant cells grew in vitro slower than control siRNA transfectant cells after diesel particulates exposure. Data show that the cell proliferation of NEP siRNA-infected BEAS-2B cells was significantly inhibited at 2 days of transfection with increased DEP concentrations (P < 0.01) and the net proliferation inhibition

rates at 5, 10, 20, and 40 µg/ml DEP were 1.4%, 7.6%, 10%, and 14.4 %, respectively, when compared with their mock controls. This finding indicates that NEP per se involves in cell proliferation in the presence of DEP stimulation. It suggests that down-regulation of NEP expression genetically may be an important mechanism responsible for many pulmonary pathogenesis of diesel particulate exposure. Decrease or loss of NEP expression may indicate a greater susceptibility to the risk of lung disorders including cancer (Supported by HEI).

505 THE EFFECTS OF DIFFERENT DIESEL EXHAUST PREPARATIONS ON THE HOST RESPONSE TO RESPIRATORY VIRAL INFECTION.

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We have previously shown that exposure of human respiratory epithelial cells to an aqueous-trapped solution of diesel exhaust (DE_{as}) enhances the innate inflammatory and anti-viral responses to influenza infections through an increase in toll-like receptor (TLR) 3 expression and signaling. The focus of the current study was to compare and contrast the effects of different DE preparations derived from different sources with differing chemical compositions. The preparations used were: Standard reference material (SRM) 2975, which is derived from a forklift and has a low organic content; DE obtained from a compressor engine (DEP), having an intermediate organic content; extracted organic material (EOM) from both SRM 2975 and DEP; and a preparation of volcanic dust from the Mt. St. Helen's eruption (MSHD), which was used as a particle control. These preparations were compared with DE_{as}, which was derived from a diesel generator. Differentiated human bronchial epithelial cells and A549 cells were exposed to DE or the respective EOM for 2 hours, subsequently infected with Influenza A or challenged with poly(I:C), a synthetic form of dsRNA, and analyzed for the expression of the viral RNA recognition receptors, TLR3, TLR7, RIG-I, and PKR, the pro-inflammatory cytokines, IL-6, IL-8, and RANTES, as well as the anti-viral cytokines IFN-α and IFN-β. When cells were exposed to these preparations prior to infection with influenza or stimulation with poly(I:C), SRM 2975 and its EOM significantly increased levels of IL-6, DEP significantly increased levels of PKR and RIG-I, while its EOM did not, and MSHD significantly increased levels of TLR3, PKR, and RIG-I. Taken together, these data suggest that DE derived from different sources significantly vary in their effects on respiratory anti-viral responses and that the amount and type of organic material as well as particulate matter within preparations may be the cause for the observed differences.

506 INHALED OXIDATION CATALYSIS TREATED DIESEL ENGINE EMISSIONS INDUCE A MARKED SYSTEMIC OXIDANT STRESS. IS NO2 MODULATION THE MAIN TRIGGER?

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Due to the onset of oxidation catalysis (oxycat) as a Diesel emission after-treatment strategy, the oxidant potential of Diesel emissions increased which can be probed by the NO₂/NO_x ratio which correlates with the amount of reactive oxygen species to be generated from the atmosphere to aqueous media containing CPH as a spin probe and assayed by electron spin resonance (ESR). In this study, we assess the impact of 3 consecutive daily inhalations for 3 hours of diluted untreated (UDE) (low oxidant) and Oxycat treated (ODE) (high oxidant) Euro3 Diesel engine emissions (dilution 1:50, PM=0.5mg/m³, NO₂ 0.4ppmUDE and 4ppm ODE) and air and UDE doped with NO₂ at similar concentrations to those found in ODE (4ppm). Plasma TNFα, and lung and heart SOD and GPx activity levels were assayed in healthy (H) and chronic myocardial infarction (MI). ESR measurements of ROS ranked as: AIR<UDE<NO₂=UDE+NO₂<ODE showing a significant contribution of NO₂ as a source of CPH spinprobe oxidation. NO₂ alone induced systemic oxidant stress : lung and heart GPx activities in both H and MI rats while no impact was seen on SOD. ODE had little impact on GPx activity but for increased levels in MI rat heart, while it provoked increased activities of SOD in both H and MI rat lungs and heart. A moderate impact of UDE+NO₂ was seen on SOD activities in MI lungs and hearts, while no impact was seen in H rats. In both UDE+NO₂ and ODE, impacts on lung GPx appear to be less marked than for NO₂, suggesting potential quenching of NO₂ impact by the co-presence of DE acting on SOD activity. Concerning TNFα, increased serum levels were similar in MI rats after NO₂, UDE+NO₂ and ODE, while more disparate records were found in H rats with again a net tendency to increased levels. Diesel emission treatment with oxycat provokes a marked systemic oxidant stress and elevation of serum TNFα, which cannot be totally assigned to the sole modulation of NO₂ concentrations, other ROS might be involved. MI rats appear to be more sensitive than healthy rats

507 REALISTIC ATMOSPHERIC AGING ENHANCES RESPIRATORY TOXICITY OF DIESEL EMISSIONS.

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Objective: Determine if photochemical aging of hazardous air pollutants (HAPS) increases toxicity of air pollution mixtures to the respiratory system.

Methods: Diesel emission particles (DEP) from a Mercedes-Benz were collected on filters as fresh DEP (FRD) or light aged DEP (AGD) after photochemical transformation with an EPA/UNC synthetic urban mixture in an outdoor smog chamber. DEP were then instilled into adult C57 male mice in 3 groups: Control (C)(PBS), FRD & AGD treated mice. DEP (30ug) or PBS (50ul) was instilled on days 1, 4, & 7 & animals were examined on day 8 for pulmonary function changes (n=4/group) or euthanized to collect bronchoalveolar fluid (BAL)(n=6/group). The photochemical aging showed a dramatic change in composition of carbonyl containing compounds (like aldehydes & ketones).

Results: DEP tx led to airway & tissue inflammation by day 8 shown by increased BAL cellularity(37,500 cells/ml in C vs 263,000 cells/ml for AGD) & an influx of neutrophils (PMNs)(<500 PMN/ml in C vs 200,000 PMN/ml for AGD). FRD also elevated cellularity & PMN in BAL(e.g. 37,000 cells/ml & 50,000 PMN/ml) but with less inflammation. Pulmonary function, indexed as total lung resistance(Rt) to methacholine (Mch) challenge (forced oscillation technique) was increased by AGD; for example, in C group the average Rt increased above baseline by 76%(0.68 to 1.20 cmH₂O/ml/s) in response to the highest dose (100 mg/ml), whereas AGD induced increases in Rt to 300% of baseline. FRD also led to an increase in sensitivity to Mch, but to a much lesser degree, increasing Rt by 122%. Additionally, more mice (n=10) were treated with dark aged DEP (non-photochemically transformed) that induced BAL cellularity and functional changes comparable to the effects with FRD & significantly less toxic to respiratory tissues than the AGD DEP.

Conclusion: Realistic atmospheric aging enhances respiratory toxicity of vehicular DEP leading to an inflammatory response (BAL cellularity & influx of PMNs) & impairment of pulmonary function. Supported by NIH: ES12496 & ES011961.

508 COMPARING THE TOXICITY OF FRESH AND AGED DIESEL EXHAUST USING A NEWLY DEVELOPED *IN VITRO* EXPOSURE SYSTEM.

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There is an increasing interest in examining complex urban air pollution mixtures containing both particulate and gaseous components. Our previous studies have shown that atmospheric aging of gaseous pollutants modifies the chemical composition of air pollution mixtures, ultimately affecting its toxicological potential. The goal of this current project is to determine whether aging of complex gaseous and particulate mixtures, in the presence or absence of photochemistry, alters the toxicological potential of that mixture. Specifically, we wanted to determine whether aging of diesel engine emissions (DE) would alter toxicological responses compared to fresh DE emissions. A second goal was to examine the differences between multiple diesel sources; a 1980 Mercedes and a 2005 Volkswagen Passat TDI station wagon. Each freshly emitted DE sample was injected into a 120 m³ outdoor smog chamber and aged utilizing natural sunlight and humidity. Our electrostatic aerosol sampler (EAS) system has the capability to expose human respiratory epithelial cells directly to particulate matter in its natural state across an air-liquid interface. We have compared the toxicity of unreacted "fresh" DE, dark-aged DE (reacting with itself in the absence of sunlight), and photochemically-aged DE. Cells were exposed to the various DE's for 1 hour and analyzed for cytotoxicity (LDH) and inflammatory cytokine production (IL-8) 12 hours post exposure. Both fresh and aged DE from both DE sources increased LDH and IL-8 levels as compared to the clean air control, with the Mercedes engine generating a greater response. In addition, our results suggest that aging significantly enhances the responses compared to fresh DE emissions. Taken together, these results further support the importance of considering engine type and atmospheric aging when studying the toxicity of vehicle emissions.

509 ULTRA-STRUCTURAL EVALUATION OF CYTOTOXIC EFFECTS OF COARSE AND FINE PM ON MACROPHAGES AND ALVEOLAR EPITHELIUM.

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Persistent exposure to high levels of airborne particles (PM) has been associated with the increase of respiratory disorders. Alveolar epithelium and macrophages resident in the airway system are some of the first cell types to be in contact with tox-

ics present in PM. We evaluated the ultrastructural cytotoxicity effects by transmission electron microscopy (TEM) on J774A.1 macrophages (MQ) and A549 alveolar epithelium (AEC) cells exposed for 24 h to 50 µg/cm² of chemically characterized coarse and fine PM collected in Mexico City. Macrophages avidly phagocytized PM. Coarse PM were observed as dense fibrous bodies inside phagolysosomes; cytoplasmic organelle lysis, increase in filopodia and chromatin condensation were also evident. The exposure to fine PM showed agglomerates of particles in cytoplasmic lysosomes and vacuoles, mitochondria swelling and crest destruction, and condensed chromatin and membrane blebbing. AEC also internalized PM. Coarse particles were observed as solid dense aggregates within lysosomes, perturbation of cytoplasmic architecture, chromatin condensation and darkness of lamellar and dense bodies were also apparent. Exposure of AEC to fine PM showed aggregates of particles in lysosomes, presence of filamentous structures in the cytoplasm, mitochondrial swelling and chromatin condensation. TEM revealed a more dramatic organelle lysis and cytoplasmic perturbation from exposure to coarse than to fine PM, yet exposure to fine PM induced mitochondrial damage. Our results indicate that ultrastructural cytotoxic effects in MQ and AEC varied according to PM physicochemical properties despite of the cell type. These observations suggest that coarse and fine PM have a direct effect on organelle structure, particularly mitochondria and cytoskeleton, thus affecting metabolic energy generation and also promoting cell death, events that could contribute to adverse health effects.

510 PULMONARY HAZARD STUDY IN RATS WITH THREE FORMS OF ULTRA-FINE-TiO₂ PARTICLE-TYPES: DIFFERENTIAL RESPONSES RELATED TO SURFACE PROPERTIES.

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The aim of this study was to assess lung toxicity in rats of newly developed, well characterized, ultrafine-TiO₂ particles and compare them to TiO₂ samples in two different size ranges. Groups of rats were intratracheally instilled with doses of 1 or 5 mg/kg of either two ultrafine rutile TiO₂ particle-types (uf-1 or uf-2); rutile R-100 fine-TiO₂ (F-1); 80/20 anatase/rutile P25 ultrafine-TiO₂ (uf-3); or quartz particles. Phosphate-buffered saline (PBS) solution instilled rats served as vehicle controls. Following exposures, the lungs of PBS and particle-exposed rats were evaluated for bronchoalveolar lavage (BAL) fluid inflammatory markers, cell proliferation, and by histopathology at post-instillation time points of 24 hrs, 1 week, 1 month, and 3 months.

The range of lung inflammation/cytotoxicity/cell proliferation and histopathological responses was quartz > uf-3 > F-1 = uf-1 = uf-2. Exposures to quartz and to a lesser degree, uf-3 anatase/rutile TiO₂ particles produced pulmonary inflammation, cytotoxicity and adverse lung tissue effects. In contrast, exposures to F-1 fine-TiO₂ particles or to uf-1 /uf-2 ultrafine-TiO₂ particle-types produced transient inflammation. We conclude that differences in responses to anatase/rutile uf-3 TiO₂ particles vs. the rutile uf-1 and uf-2 TiO₂ particles could be related to crystal structure, inherent pH of the particles, or chemical reactivity. Thus, based on these results, inhaled rutile ultrafine-TiO₂ particles are expected to have a low risk potential for producing adverse pulmonary health effects. Finally, the results demonstrate that exposures to ultrafine-TiO₂ particle-types can produce differential pulmonary effects, based upon their composition, and crystal structure. Thus, the lung toxicity of anatase/rutile uf-3 should not be viewed as representative for all ultrafine-TiO₂ particle-types.

511 EFFECTS OF CONCENTRATED AMBIENT ULTRA-FINE/FINE PARTICLES IN INSULIN-RESISTANT, OBESE RATS.

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We hypothesize that inhaled ambient ultrafine (UF) particles can induce oxidative stress-related injury of target cells in the cardiovascular system, thus providing a plausible mechanism for adverse effects observed in sensitive populations. We measured pulmonary and cardiovascular endpoints related to inflammation and oxidative stress in a diabetic rat model after exposure to concentrated UF/fine particle-containing aerosols. The Harvard ultrafine concentrated ambient particle system (HUCAPS) was used to generate the aerosols (particle number concentration ~0.05-1.3 x 10⁶/cm³; count median diameter ~75 nm) for acute exposures (4 hrs; 3 x 6 hrs). Obese, insulin-resistant homozygous male JCR:LA-cp rats (cp/cp; 13-15

months) as well as non-diabetic, lean heterozygous males (+/?; 5-13 months) were exposed to the HUCAP aerosols; controls were exposed to particle-filtered air. We collected blood, bronchoalveolar lavage fluid, and several tissues (lungs, heart, carotid arteries, aorta, liver, pancreas, spleen, kidneys, brain) after exposure. JCR cp/cp rats had slightly higher total lung lavage cell numbers, more blood leukocytes and leukocyte aggregates, and less glial fibrillary acidic protein in brain. These parameters were not consistently affected by HUCAP exposure. Although lavage neutrophils remained unchanged, there was a trend towards elevated protein, lactate dehydrogenase, and β-glucuronidase in HUCAP-exposed rats that was independent of genotype or exposure duration. After 3 days of exposure, the amplification of aortic mitochondrial DNA (12 kb) was significantly decreased in HUCAP-exposed cp/cp rats as compared to +/? rats and air-exposed controls. These results show that small oxidant stress-related changes occur after acute exposure to real-world particles in an animal model relevant to the study of UF particle effects in humans.

512 INFLUENCE OF ENDOTOXIN AND GLUCAN ON *IN VITRO* OXIDATIVE AND INFLAMMATORY POTENTIALS OF FINE AND COARSE PM FROM PAVED ROADS.

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Biological materials are known to contribute to the composition and toxicity of ambient particulate material (PM), but the nature and extent of contribution are largely unknown. Similarly, PM resuspended from paved road surfaces contributes to exposures, but the nature and toxicity of road dust is poorly documented. Dust was collected from active traffic surfaces at 55 sites in 6 U.S. regions using a non-contact vacuum method. Samples were sieved and aerosolized, and the PM_{2.5} and PM_{10-2.5} fractions were collected. Samples were analyzed chemically and for endotoxin, a lipopolysaccharide marker for gram negative bacteria (limulus amoebocyte assay), and 1,3-β-D-glucan, a carbohydrate marker for fungi and some bacteria (glucatelect lysate assay). Samples were tested in vitro for oxidative potential (oxidation products from incubation with linoleic acid) and for inflammatory potential (cytokines produced by incubation with human blood). Relationships between in vitro potency ranking and endotoxin and glucan contents were examined. Mean values for both endotoxin and glucan were similar for the two PM size fractions, but values varied considerably among individual samples. Overall, neither endotoxin nor glucan were strongly correlated with either oxidative or inflammatory potential, indicating that other chemical components drove those effects. However, both size fractions of dust collected at the perimeters of cattle feedlots in Kansas and Texas were among those having the greatest inflammatory potential, and also had by far the highest endotoxin contents (4x mean value for PM_{2.5}, and 8x mean value for PM_{10-2.5}).

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513 ASSOCIATIONS BETWEEN CHEMICAL COMPOSITION OF FINE PARTICULATE MATTER FROM SIX EUROPEAN CITIES AND INFLAMMATORY RESPONSES IN THE MOUSE LUNG.

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Inflammation is regarded as an important mechanism in associations between urban air fine particulate concentrations and mortality and morbidity among cardiorespiratory patients. We investigated the association of fine particulate (PM_{2.5-0.2}) chemical composition with inflammatory responses in the mouse lung. The PM_{2.5-0.2} samples were collected using an optimized high volume cascade impactor during selected seasons in six European cities. Ions (IC), water-soluble elements (ICP-MS) and PAH-compounds (GCMS-SIM) were analyzed from these samples. Healthy C57BL/6J mice were intratracheally instilled a single dose (10 mg/kg) of the PM_{2.5-0.2} samples. At 4, 12 and 24 hours after the exposure, the lungs were lavaged and the bronchoalveolar lavage fluid (BALF) was assayed for indicators of inflammation and tissue damage: cell number, total protein and cytokines (TNF-α, IL-6 and KC). The responses at the most feasible time-point for each parameter were analyzed for Spearman (ρ) correlation with the measured chemical constituents. All the measured inflammatory responses correlated strongly with each other. Significant positive correlations (ρ=0.83-1.0) of the inflammatory responses were measured with dicarboxylic acids, Ca²⁺, Ni and V. Significant negative correlations (ρ=-0.83 to -1.0) were found with NO₃⁻, NH₄⁺, K⁺, As, and total and genotoxic PAH compounds. In conclusion, the present results suggest that fine particles originating from different sources may have differential effects on the respiratory tract.

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514 SYSTEMIC TRANSLOCATION OF PARTICULATE MATTER (PM)-ASSOCIATED METALS FOLLOWING A SINGLE INTRA-TRACHEAL (IT) INSTILLATION IN WKY RATS.

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Ambient PM contains transition metals with differing water solubilities. Epidemiological studies show a link between PM exposure and an increased risk of cardiovascular disease. Direct translocation of PM-associated metals from the lung into systemic circulation may be partly responsible for this. We measured levels of various metals in lung, plasma, heart and liver of healthy male WKY rats (12-15 wks old) 4 and 24 h after a single IT instillation of saline or oil combustion PM (8.33 mg/kg). IT instillation was used to control for pulmonary deposited dose and nasal absorption. The selected PM contains moderate levels of metals including zinc, vanadium, nickel, aluminum, silica, iron, copper, manganese and lead, each with differing water and acid solubility. Tissues were digested in acid and analyzed for elemental content using Inductively Coupled Plasma-Atomic Emission Spectroscopy. Most PM-associated metals were detected in the lung at both time points, with lower concentrations at 24 h than at 4 h. Both essential and nonessential metals with high water solubility were translocated into extrapulmonary organs. Detection of PM-associated vanadium and nickel in plasma, heart and liver at both time points demonstrates soluble metal translocation following pulmonary exposure. Increased levels of zinc and manganese were also observed in plasma and other organs. Metals with low water solubility were not detected in extrapulmonary tissues despite their decreased level in the lung, suggesting mucociliary clearance. We have demonstrated that PM-associated water soluble metals are rapidly translocated to systemic circulation and extrapulmonary organs following an acute IT instillation, likely via vascular absorption. This process is dependent on the relative water solubility of metals. Inhalation of metal rich PM may elicit a direct effect on pulmonary and extrapulmonary organs. (Abstract does not represent USEPA policy. This research was supported in part by UNC/EPA CT829471.)

515 A NOVEL TECHNIQUE FOR QUANTITATIVE ESTIMATION OF UPTAKE OF DIESEL EXHAUST PARTICLES BY LUNG CELLS.

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While airborne particulates like diesel exhaust particulates (DEP) exert significant toxicological effects on lungs, quantitative estimation of accumulation of DEP inside lung cells has not been reported due to a lack of an accurate and quantitative technique for this purpose. In the present study, we developed a sensitive and reliable new method for quantitative estimation of uptake of DEP by lung epithelial cells and macrophages in culture. The principle of the method is to first isolate the DEP exposed cells by trypsinization and to separate them from free extracellular diesel exhaust particles by Ficoll density gradient centrifugation. Ficoll purified cells were dissolved in 1% sodium dodecyl sulfate solution and insoluble ingested DEP isolated by centrifugation. DEP pellets were subjected to an analysis on a carbon analyzer that could accurately measure the amount of elemental carbon in the sample. Since the DEP preparation used in our studies is about 90% elemental carbon, and normal cells do not have any elemental carbon, the amount of DEP taken up by the cells could be accurately estimated by this method. Using this technique, we studied the time course and dose dependence of uptake of DEP by lung epithelial cells and alveolar macrophages. Qualitative differences were found in the uptake of DEP and ultra fine carbon black (UFCB) by LA4 epithelial cells and MHS alveolar macrophages. Macrophages could ingest DEP as well as UFCB with equal efficiency, whereas epithelial cells were significantly more efficient in taking up DEP as compared to UFCB. This technique can now be used to estimate the deposition and clearance of inhaled DEP and provide correlations between particle load and toxicity. This abstract does not reflect EPA policy.

516 CADMIUM AND ELEVATED TEMPERATURE STRESS SYNERGISTICALLY AFFECT ENERGY METABOLISM IN OYSTER, CRASSOSTREA VIRGINICA.

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Combined effects of temperature and a toxic metal, cadmium, on energy metabolism were studied in a model marine bivalve, the eastern oyster *Crassostrea virginica*, acclimated at 20, 24 and 28°C and exposed to 50 µg/L of cadmium. Based

on the concept of oxygen-limited thermal tolerance in aquatic ectotherms, we show that a persistent pollutant, cadmium, can have detrimental effects on oyster populations by reducing thermal tolerance due to impaired energy metabolism and elevated costs of basal maintenance. At 20 and 24°C but not at 28°C standard metabolic rate of Cd-exposed oysters increased compared to respective controls. Furthermore, highest respiratory time activity was observed in Cd-exposed oysters at 28°C. The results point at a mismatch between oxygen demand and delivery supported by preliminary results that show a decrease in hemolymph oxygen partial pressure during an acute temperature rise (4°C/10h) in Cd-exposed but not control animals (20°C acclimated animals). At 28°C but not at lower acclimation temperatures glycogen content in mantle tissue of Cd-exposed oysters decreased significantly (12.2±4.2mg/g to 5.4±1.3mg/g over 14 days cadmium exposure) suggesting that the combined stressors may increase dependence on glycogen to support maintenance metabolism. Glycogen supplies energy for reproduction as well as for anaerobic metabolism. In line with the continuously decrease in condition and the nearly 50% mortality observed after 14 days of cadmium exposure in oysters acclimated to 28°C we conclude that coupled with the additional stress of cadmium, oysters are less tolerant to cope with increasing energy expenditure with increasing temperature resulting in onset of critical temperature thresholds at lower temperatures. This work was supported by the National Science Foundation IBN-0347238 and Alexander von Humboldt Foundation.

517 MITOCHONDRIAL ACONITASE IS SENSITIVE TO CADMIUM-INDUCED OXIDATIVE STRESS BUT NOT PROTECTED BY UNCOUPLING PROTEINS IN OYSTERS.

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Combined effects of temperature (12, 20 and 28-30°C) and Cd stress on production of reactive oxygen species (ROS) and oxidative stress was studied in a marine poikilotherm *Crassostrea virginica* (the eastern oyster) using mitochondrial aconitase as a sensitive biomarker of oxidative damage, and potential involvement of mitochondrial uncoupling proteins (UCPs) in antioxidant protection was assessed using purine nucleotides (GDP, ATP and ADP) as specific inhibitors and free fatty acids as stimulators of UCPs. Cd exposure in vitro resulted in elevated ROS production and oxidative damage to aconitase which was particularly pronounced at elevated temperature. At higher Cd levels, oxidatively damaged aconitase became resistant to reactivation by Fe²⁺ and thiols suggesting irreversible damage and "point of no return" for this enzyme. Exposure to Cd in vivo resulted in elevated levels of aconitase expression which may indicate a compensatory response to Cd-induced aconitase inactivation. Our study also showed expression of at least three UCP isoforms in *C. virginica* gill tissues but provided no indication that UCPs protect mitochondrial aconitase from oxidative inactivation in oysters. Unexpectedly, oyster mitochondrial aconitase was inhibited by physiologically relevant levels of ATP (IC₅₀ = 1.93 and 3.04 mM at 20 and 30°C, respectively) suggesting that allosteric regulation of aconitase by ATP may be involved in regulation of the tricarboxylic acid flux in oysters. Overall, the results of this study indicate that temperature stress exaggerates toxicity of Cd leading to elevated oxidative stress in mitochondria, which may have important implications for survival of poikilotherms in polluted environments during seasonal warming and/or global climate change, and suggest a novel temperature-dependent mechanism of allosteric regulation of TCA flux in oyster mitochondria. Supported by National Science Foundation IBN-0347238 and the American Physiological Society.

518 COMBINED EFFECTS OF TEMPERATURE AND CADMIUM EXPOSURE ON HEMOCYTE APOPTOSIS AND CADMIUM ACCUMULATION IN THE EASTERN OYSTER CRASSOSTREA VIRGINICA (GMELIN).

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Combined effects of acclimation temperature (12, 20 and 28°C) and exposure to a toxic metal cadmium (Cd, 50 µg/L) on hemolymph parameters related to immune defense and metal transport were studied in a model marine bivalve, *Crassostrea virginica*. Acclimation to elevated temperatures resulted in higher plasma protein concentrations and increased Cd levels in oyster hemolymph plasma and hemocytes. Cd accumulation in hemocytes was linear over the 45 days of Cd exposure and accumulation rates were 0.10, 0.53 and 0.56 µg Cd g⁻¹ dry mass at 12, 20 and 28°C, respectively. Percentage of blood Cd burden associated with hemocytes increased with increasing temperatures from 13-20% at 12°C to 26-47% at 20 and 28°C suggesting a higher role for cellular Cd transport at elevated temperatures. Cd

levels in gills and hepatopancreas were positively correlated with Cd concentration in hemocytes, but accumulation rates were considerably faster, so that after 45 days of exposure Cd levels in gills and hepatopancreas were >10-20 times higher than in hemocytes. As a result of slow Cd accumulation possibly reflecting fast hemocyte turnover rates and/or exocytosis of Cd-containing granules, hemocytes in Cd-exposed oysters did not reach threshold Cd burdens required to trigger apoptosis. This suggests that hemocyte viability is not likely to contribute to immunosuppression in the environmentally relevant Cd range. In contrast, elevated temperature (28 °C) resulted in a significant increase in the percentage of apoptotic hemocytes compared to 12°C or 20°C supporting the notion that 28 °C is physiologically stressful for *C. virginica*. Overall, our study demonstrates that effects of environmental temperature on hemocyte viability and other important blood parameters such as plasma protein content and metal transport capability override potential Cd effects and further emphasizes a key role of temperature in oyster physiology.

519 EFFECTS OF DILTIAZEM AND LANTHANUM ON COPPER INHIBITION OF O₂ CONSUMPTION IN GILL OF THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA*.

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Our oyster rehabilitation studies showed that *Crassostrea virginica* transplanted to Jamaica Bay, NY accumulated copper. Mitochondria are sensitive to oxidative stress caused by metal toxicity. We studied effects of copper on O₂ utilization in *C. virginica* using a YSI Micro-Biological Oxygen Monitor with continuous flow or micro-batch chambers. 5 or 50 mg of CuSO₄ decreased respiratory rates 12 and 32%, respectively. Oysters grown in Jamaica Bay or exposed to copper pretreatments were more sensitive to copper additions. 5 mg CuSO₄ decreased O₂ utilization in excess of 34% and 50 mg caused complete inhibition. We now sought to determine if channel blockers would affect copper's ability to depress mitochondrial respiration. Using micro-batch chambers we measured O₂ consumption in the mitochondrial fraction from gill tissue. Copper reduced respiratory rates in them as previously shown. Pretreating the mitochondria with diltiazem blocked the copper inhibition, while pretreating with lanthanum did not. Lanthanum had a slight inhibiting effect of its own. The study demonstrates that the diltiazem is effective in blocking copper's inhibitory effects on mitochondria and may be useful in blocking copper transport into tissues of other animals as well. The toxic effects of copper on gill mitochondrial could be of physiological significance to the growth and long-term health of oysters and other marine animals living in a copper polluted environment and a copper uptake blocker could be of benefit for preventing diseases caused by high serum copper levels in humans. This work was supported by grants 1R25GM62003 of NIGMS, 0516061071 of NYSDOE and 66288-0036 of PSC-CUNY. We thank Flower & Sons, Inc., Oyster Bay, NY for supplying oysters.

520 PRELIMINARY STUDIES ON THE ACUTE TOXICITY OF SODIUM DICHROMATE TO GOLD FISH, *CARRASSIUS AURATUS*.

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Chromium is a common contaminant in ground water and surface water. It also enters into the environment as effluent from several industrial operations such as tanneries, ceramic, mining, dying, electroplating, metal finishing, printing and pharmaceutical industries. Chromium exists in the environment in divalent, trivalent and hexavalent forms, but the hexavalent form is more toxic than the other forms. Release of chromium-containing-effluents from the industries into the aquatic environment poses a major threat to the growth and survival of ichthyofauna. In this study we evaluated the dose-response and time-response relationships of chromium (sodium dichromate) toxicity to goldfish. The study was conducted using 96 hour static renewal bioassay, according to the USEPA protocol. Study results indicated that the exposure mortality rates of gold fish were increases with increasing concentration of chromium and exposure time periods. Mortality rates of 0 %, 33.32 %, 83.33 %, and 100 % were recorded at the concentration of 0 mg/L, 250 mg/L, 500 mg/L and 750 mg/L of sodium dichromate respectively. In the time response studies, the mortality rates at 0 mg/L were recorded 0 % at all the time periods whereas at 250 mg/L the mortality rates were recorded as 16.66 % for 24 hours, 33.32 % for 48 hours, 33.32 % for 72 hours and 33.32 % for 96 hours. At 500 mg/L concentration, the mortality rates were recorded as 33.32 % for 24 hours, 50 % for 48 hours, 66.66% for 72 hours and 83.33 % for 96 hours. At 750 mg/L concentration, the mortality rates were recorded as 83.33% for 24 hours, 100

% for 48 hours, 100% for 72 hours and 100 % for 96 hours. In summary, these preliminary data indicate that the toxicity of sodium dichromate to *Carrassius auratus* fish is both concentration and time dependent.

521 AN EVALUATION OF TOXICITY OF TRIBUTYL TIN OXIDE AND ZINC PYRITHIONE IN *SEPIA OFFICINALIS* (COMMON CUTTLEFISH).

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Since the ban of tributyltin based antifouling marine paints, new alternatives such as zinc pyrithione have been introduced to control the growth of fouling organisms on the hulls of ships. To evaluate the risk posed to non-target organisms, the toxicity and accumulation of tributyltin oxide (TBTO) and zinc pyrithione (ZPT) were examined in *Sepia officinalis* following acute exposure. *S. officinalis* is native to the coastal waters of the U.K. and Indonesia, where persistence of TBTO and more recently elevated ZPT concentrations, have been measured. This species is harvested commercially by the seafood industry in these areas, which may play a role in introducing these compounds into the human diet. Tank raised *Sepia officinalis* were exposed to environmentally relevant doses of either 50ng/L TBTO or 50ug/L ZPT for 24 hrs. A third group receiving no treatment served as a control population. Following exposure, gill tissue was examined using light, scanning and transmission electron microscopy, graphite furnace atomic absorption spectrophotometry (GFAAS), and western blot analysis. Results indicate that *S. officinalis* is sensitive to TBTO and ZPT exposure. Gill tissue from TBTO and ZPT treatment animals showed necrosis and exfoliation of epithelium as a result of membrane degradation; however damage was greater in TBTO exposed groups as compared to ZPT exposure. GFAAS results showed a significant increase in tin and zinc concentrations in respective treatment groups as compared to control animals. Western blot analysis showed an increase in 4-hydroxynonenal adduct formation in treatment groups as compared to controls, suggesting an increase in lipid peroxidation following exposure to either biocide.

522 THE TOXIC EFFECTS OF MARINE BIOCIDES COPPER PYRITHIONE (COPPER 2-PYRIDINETHIOL-1-OXIDE) ON JUVENILE BROOK TROUT, *SALVELINUS FONTINALIS*.

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Copper pyrithione (CuPT)—a booster biocide developed to replace globally-banned tributyltin (TBT)—acts by disrupting cell membranes, disrupting pH-gradients, and inhibiting ATP-synthesis in target species. However, research concerning the effects of CuPT exposure on non-target species is lacking. This investigation studied the acute effects of copper pyrithione exposure on juvenile brook trout, *Salvelinus fontinalis*. Morphologic changes, as well as copper bioaccumulation in several organ systems were studied. Juvenile brook trout were housed in a 950L aquarium and acclimated to laboratory conditions for 1 month. Trout were divided into groups of 10 and exposed to CuPT (dissolved in DMSO) in separate aquaria for a duration of 2 hours. Concentrations of CuPT ranged from 1ppm to 64ppm. Following exposure, trout were sacrificed and dissected. From each fish, brain and the 2nd and 3rd gill arches were removed and either frozen in liquid N₂ or fixed in 10.0% Sorensen's buffered (pH 7.4) formalin. For light microscopy, gills were embedded in paraffin and sectioned for histological analysis. Frozen tissue was digested in concentrated nitric acid and subjected to graphite-furnace atomic absorption spectroscopy (GFAAS). Results from histological analysis showed the formation of club shaped lamella, edema, epithelial lifting and exfoliation and the fusion of secondary lamella at CuPT concentrations of 16, 32 and 64ppm. GFAAS analysis demonstrated significant increased levels of copper in the brain ($p < 0.005$) and gill arches ($p < 0.001$) of trout as compared to matched controls. These data indicate that the use of CuPT as an alternate biocide could potentially harm non-target aquatic organisms.

523 EFFECTS OF ZINC PYRITHIONE EXPOSURE ON *BUNODOSOMA CAVERNATA* (WARTY SEA ANEMONE).

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Zinc Pyrithione (Zinc Omadine® or Zinc 2-pyridinethiol-1-oxide) is a registered antifouling agent for incorporation into boat paints to control the growth of marine fouling organisms. There is limited documentation on the effects of zinc pyrithione

(ZPT) on anthozoans such as *Bunodosoma cavernata*. To better understand the effects of acute environmentally relevant exposure to this invertebrate, collected specimens were exposed to varying concentrations (5, 50, 100 µg/L) of ZPT for 24 hrs. An additional group served as a control population. Following exposure, anemone tissues were studied using light, scanning and transmission electron microscopy, graphite furnace atomic absorption spectrophotometry (GFAAS), and western blot analysis. Microscopy results showed that anemones exposed to ZPT had exfoliation of epithelial tissues, degradation of musculo-epithelial cells, and loss of mesoglia in a dose dependant manner. Western blot analysis showed an increase in 4-hydroxynonenal protein adducts in tissues of treatment animals as compared to controls, evidence of increased lipid peroxidation. GFAAS results confirm a significant increase in zinc concentration in tissues from treatment animals as compared to controls, indicating bioaccumulation.

524 RELEASE OF THE LIPID PEROXIDATION MARKER 8-EPI-PGF2 α FROM GILL EPITHELIAL CELLS.

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The aim of this study was to compare the capacities of ferric and divalent lead ions to promote lipid peroxidation in gill epithelial cells using the release of the isoprostane marker 8-epi-PGF2 α . Cell suspensions were prepared using fresh gill tissue from fathead minnows. After collagenase treatment and passage of cells through nylon sieves, epithelial cells were isolated on a 51% Percoll gradient using a method previously applied to rainbow trout gills. After centrifugation, gill pavement cells formed a band in the gradient between 1.052 and 1.074 g/mL. Cell viability was estimated as 95%. For morphological studies, paraformaldehyde-fixed cell suspensions were centrifuged and the pellet examined by light microscopy. Pellets were stained for alkaline phosphatase (ALP), because ALP specifically stains pillar cells and vascular elements. Contamination by vascular elements was estimated as < 3%. Basal levels of immunoreactive (ir) 8-epi-PGF2 α were measured by incubating pavement cells in oxygenated modified Cortland's saline and enzyme immunoassay of the supernatant after centrifugation. After 60 min, levels of free ir 8-epi-PGF2 α in medium from control cells ranged from 2.75 - 11.03 ng. The amount of ir 8-epi-PGF2 α in the medium of cells exposed to 5 and 500 µM FeCl₃ for 60 min increased to 14.1 ± 5.2 and 16.1 ± 6.6 ng (% change relative to co-incubated control cells = 149 and 185%, respectively). The enhancement of isoprostane release was statistically significant at 500 µM FeCl₃ (p<.05). By contrast, the release of ir 8-epi-PGF2 α from cells exposed to lead II nitrate (0.6 - 120 µM) did not differ from control cells. In summary, ferric ions but not divalent lead ions are potent in causing oxidant stress to gill epithelial cells as determined by the release of free ir 8-epi-PGF2 α into incubation medium.

525 GENE EXPRESSION PROFILES IN FATHEAD MINNOW EXPOSED TO 2, 4-DNT: CORRELATION WITH TOXICITY IN MAMMALS.

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Toxicogenomics, the genome-wide analysis of gene expression to study the effect of toxicants, has great potential for use in environmental toxicology. Applied to standard test organisms, it has possible applications in aquatic toxicology as a sensitive monitoring tool to detect the presence of contaminants while providing information on the mechanisms of action of these pollutants. We describe the use of a cDNA microarray of the fathead minnow (*Pimephales promelas*) a standard sentinel organism in aquatic toxicology, to better understand the mechanisms of toxicity of 2,4-dinitrotoluene (2,4-DNT) which is released in the environment through military and industrial use. We have constructed a fathead minnow microarray containing 5,000 randomly picked anonymous cDNAs from a whole fish cDNA library. Expression profiles were analyzed in fish exposed to 2,4-DNT for 10 days at three concentrations (11, 22 and 44 µM respectively) below the measured LC50 (58 µM). Sequence analysis of cDNAs corresponding to differentially expressed genes affected by exposure revealed that lipid metabolism and oxygen transport genes were prominently affected in a dose specific manner. We measured liver lipids and demonstrate that lipid metabolism is indeed perturbed following exposure. These observations correlate well with available toxicological data on 2,4-DNT. We

present possible modes of action of 2,4-DNT toxicity and suggest that fathead minnow cDNA microarrays can be useful to identify mechanisms of toxicity in fish and as a predictive tool for toxicity in mammals

526 ASSESSMENT OF THE ESTROGEN ACTIVITY OF PERFLUOROOCTANOIC ACID (PFOA), PERFLUOROOCTANE SULFONATE (PFOS) AND OTHER STRUCTURALLY DIVERSE PERFLUORINATED CHEMICALS IN RAINBOW TROUT.

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PFOA and PFOS, as well as other perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonates (PFSs), are by-products formed during the synthesis of perfluorinated chemicals (PFCs) or intermediates in the manufacture of nonstick and stain-resistant coatings. A recent toxicogenomics study suggests that PFOA acts via an estrogenic mechanism to promote hepatocellular carcinoma in rainbow trout. PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA) and perfluoroundecanoic acid (PFUnDA) have also been shown to be estrogenic *in vivo* using the vitellogenin induction bioassay. However, it is not known whether any PFC directly binds the estrogen receptor (ER). Perfluoroheptanoic acid (PFHpA), PFOA, PFNA, PFDA, PFUnDA, perfluorododecanoic acid (PFDDA), PFOS, perfluorodecane sulfonate, 8:2 fluorotelomer alcohol (8:2 FTOH), 10:2 fluorotelomer alcohol (10:2 FTOH), 8:2 fluorotelomer acrylate (8:2 FTOAc) and N-methyl perfluorooctane sulfonamido ethyl acrylate (MeFOSEA) were tested for their ability to bind the rainbow trout hepatic ER using a standard steroid receptor competitive binding assay. All PFCAs and PFSs tested displaced [³H]-estradiol by more than 75%. Relative binding affinities (RBAs) of these ligands ranged from 0.0003 to 0.003% with PFDA, PFOS and PFDS having the highest affinities for the hepatic ER. These results are similar to those obtained for two weak xenoestrogens, indole-3-carbinol and 4-nonylphenol. Alternatively, 8:2 FTOH, 10:2 FTOH, 8:2 FTOAc and MeFOSEA did not bind the hepatic ER. These results show that multiple PFCs found in the environment are likely weak xenoestrogens. However, variations in PFC chemical structures, namely the specific end group, may influence their bioavailability *in vivo*. Supported NIH grants ES07060 and ES03850 and a DuPont gift.

527 DECREASED SURVIVAL OF RAINBOW TROUT EMBRYOS FERTILIZED WITH SEMEN FROM 17ALPHA-ETHYNYLESTRADIOL EXPOSED MALE TROUT.

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17 α -ethynylestradiol (EE2) is a potent synthetic estrogen that is found in many aquatic environments due to domestic sewage discharges. We previously demonstrated that EE2 exposure to sexually maturing male trout decreases fertility, which was not associated with changes in sperm numbers or motility. In this study, we specifically investigated the effect of EE2 exposure on early embryonic developmental success. Male rainbow trout entering spermatogenesis were exposed for 50 days to waterborne concentrations of 0, 0.8, 8.3, or 65 ng EE2/L (mean values of measured concentration). Semen was then collected from 10 randomly selected individuals in each exposure, the number of sperm quantified, and *in vitro* fertilization trials performed. All fertility trials used eggs obtained from a single unexposed female. Each semen sample was used to fertilize 150 eggs with a sperm:egg ratio of 300,000:1. All treatments were evaluated at four developmental time-points: 0.5 days post fertilization (dpf), 2.5 dpf, 7 dpf, and 14 dpf, representing first cleavage, raised blastula, embryonic keel, and eyed embryo stages, respectively. All EE2 treatments significantly decreased the number of eyed embryos relative to controls (71.8, 84.5, 77.6 % of control values for the 65, 8.3 and 0.8 ng/L treatments respectively). The highest exposure level (65 ng/L) also decreased the number of embryos at all earlier time-points of development (60 % of control). These results clearly demonstrate that trans-generational effects of EE2 are the most sensitive reproductive endpoint in trout. A parallel experiment, which exposed juvenile trout (6 months of age at time of exposure) to 100 ng/L EE2, did not cause subsequent changes in embryonic development when semen from these fish were used in fertility trials. Thus, sexually maturing trout undergoing spermatogenesis appear to be the most vulnerable life stage to EE2 exposure. Supported by NIEHS Grant 5 R01ES012446-03.

528 EFFECTS OF ALKYLANILINES AND ALKYLPHENOLS ON ESTROGEN RECEPTOR BINDING AND GENE EXPRESSION IN RAINBOW TROUT AND HUMANS *IN VITRO* MODELS.

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Estrogen receptor (ER) mediated binding and gene expression assays for both rainbow trout (rt) and human (h) were used to evaluate estrogenic potential of 16 alkylphenols and alkylanilines. This work was part of a larger project to determine which chemicals of EPA concern can bind ER and be ER mediated endocrine disruptors. All 16 alkylphenols and alkylanilines tested in the cytosol rtER competitive binding and rt liver slice/VTG mRNA expression assays competitively bound to rtER and induced VTG expression. Additionally a subset of chemicals tested in the recombinant hER competitive binding assay and two hER mediated gene expression assays, expression of the endogenous estrogen-regulated pS2 gene in the human mammary carcinoma MCF-7 cell line and expression of the estrogen-responsive element luciferase reporter gene construct in the T47D-KBluc cell line, competitively bound to hER and all except high LogP p-n-octylaniline induced gene expression in both hER gene expression assays. For most alkylphenols and alkylanilines gene induction occurred at concentrations greater than or equal to those which resulted in displacement of estradiol in binding assays. The exceptions were high LogP chemicals p-n-octylphenol (5.8) and p-n-octylaniline (5.1) which were not as potent in the rt liver slice model as predicted from rtER binding results. These results suggest chemical uptake and availability are limiting the effect of these chemicals. Furthermore, the efficacy of the rt and h gene expression response for some chemicals appeared to be limited by chemically induced toxicity. Therefore, in addition to binding affinity factors such as chemical uptake, toxicity and metabolism ultimately determined estrogenic potency in rt liver slices and human cell lines.

Disclaimer: This abstract does not necessarily reflect the views of the Agency.

529 REPRODUCTIVE TOXICITY OF VINCLOZOLIN AND KETOCONAZOLE IN FATHEAD MINNOW: ADVERSE EFFECTS AND COMPENSATORY RESPONSES.

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The objective of this study was to characterize responses of fish reproductive systems to dicarboximide (vinclozolin [VZ]) and conazole (ketoconazole [KTC]) fungicides. Although these chemicals have been shown to alter endocrine function in vitro, either by direct interactions with androgen receptors (ARs) (VZ) or by inhibiting activities of cytochrome P450s (CYPs) that mediate steroidogenesis (KTC), little is known concerning their in vivo effects on processes mediated by hypothalamic-pituitary-gonadal axis in fish. We exposed fathead minnows, a model small fish species, for 21-d to waterborne VZ or KTC and evaluated responses at multiple levels of biological organization including: expression of AR and several CYP genes involved in steroid biosynthesis, ex vivo steroidogenic activity, plasma concentrations of sex steroids, gonad histopathology, and reproductive (spawning) success. Exposure to KTC reduced egg production, increased gonadosomatic index in both sexes, and in males caused proliferation of Leydig cells. Also, mRNA transcripts for two key steroidogenic enzymes (CYP side-chain cleavage and CYP17 α hydroxylase /17,20 lyase) were elevated. These changes are indicative of potential adaptive or compensatory responses to impaired steroidogenic capacity. Exposure to VZ resulted in reduced expression of secondary sex characteristics and decreased egg production. VZ also induced compensatory responses – it caused up-regulation of AR, 11 β -hydroxysteroid dehydrogenase mRNA transcripts, and ex vivo gonadal testosterone production. Overall, our data suggest that both VZ and KTC adversely affect endocrine function of fish, but the animals can effectively compensate for these effects to a certain degree. These compensatory responses have important implications for interpretation of data from tests with endocrine-active chemicals.

530 EFFECTS OF TRENBOLONE ON EXPRESSION OF ESTROGEN-RESPONSIVE PLASMA PROTEINS IN ADULT SHEEPSHEAD MINNOW (CYPRINODON VARIEGATUS).

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Protein profiling can be used for detection of biomarkers that can be applied diagnostically to screen chemicals for endocrine modifying activity. In previous studies using sheepshead minnows (SHM), mass spectral analysis detected four peptides

(2950.5, 2972.5, 3003.4, 3025.5 m/z) in the plasma of estrogen-treated male fish and gravid females as opposed to unexposed males. The 3025.5 m/z peptide was positively identified as SHM zona radiata protein fragment 2, an egg membrane protein, further supporting the four peptides as suitable estrogen-responsive biomarkers. In this study, a 21-day aqueous exposure of adult male and female SHM was conducted to investigate possible dose-related effects of the synthetic androgen, 17 β -trenbolone on expression of the four estrogen-responsive peptides and on expression of plasma vitellogenin (VTG). Fish were continuously exposed to 0.005, 0.05 and 5.0 μ g trenbolone/L, a vehicle control (triethylene glycol, TEG), and a seawater (SW) control using an intermittent flow-through dosing system. Plasma was analyzed for presence of the four peptide biomarkers by MALDI-TOF-MS and VTG protein by quantitative ELISA. Male fish from the trenbolone treatments and controls showed no measurable levels of VTG and absence of the four peptide biomarkers. The estrogen-responsive peptides were present in females from the SW, TEG, 0.005, and 0.05 μ g/L exposures, but all four peptide biomarkers were significantly reduced in the highest treatment of 5.0 μ g/L ($p < 0.0002$ to $p < 0.005$). Mean plasma VTG ranged from 11.8 to 4.5 mg/ml in the SW control and 5.0 μ g/L treatment, respectively. No significant difference in VTG was found between the trenbolone and control treatments ($\alpha = 0.05$) which can be explained by VTG's high accumulation and slow clearance from the bloodstream. These results demonstrate trenbolone can inhibit expression of estrogen-responsive proteins and may be capable of causing adverse reproductive effects.

531 MODULATION OF AROMATASE ACTIVITY AS A MODE OF ACTION FOR ENDOCRINE-DISRUPTING CHEMICALS IN A MARINE FISH.

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The steroidogenic enzyme aromatase catalyzes the conversion of androgens (androstenedione and testosterone) to estrogens (estrone and estradiol). In this study, we examine the effects that five endocrine-disrupting chemicals (EDCs) have on reproduction and aromatase activity in brains and gonads from the marine fish cunner (*Tautoglabrus adspersus*). Our goal is to investigate the hypotheses that: 1) modulation of aromatase activity is an indicator of exposure to EDCs that work through diverse modes of action; 2) activity of aromatase isozymes in fish brain and gonad are differently affected by exposures to different EDCs; and 3) modulation of aromatase activity in brains or gonads of spawning fish is linked with reproductive impairment. Spawning cunner were treated with octylphenol, atrazine, estradiol, ethynylestradiol or the aromatase inhibitor androstatrienedione (ATD) using a slow-release implant. Reproductive endpoints (egg production, fertility and viability) were monitored for 14 days, then fish were sacrificed. Brains and gonads were flash-frozen for analysis of microsomal aromatase activity. GSI, HSI and plasma vitellogenin were determined. Results indicate that male brain aromatase activity was significantly changed by exposure to all five EDCs, although not at all tested concentrations, suggesting that such changes are indicative of EDC exposure. Results also show impacts on aromatase activity are tissue-specific. Gonad aromatase activity was significantly changed only in ovaries from fish treated with estradiol or ethynylestradiol. In contrast, male brain aromatase activity was altered by all EDCs. Lastly, results suggest that significant modulation of aromatase activity in the brains of spawning males is consistently linked with reproductive impairment: a significant decrease in any reproductive endpoint was always associated with altered male brain aromatase activity.

532 VITELLOGENESIS INDUCTION IN MALE FISH EXPOSED TO WASTE WATER EFFLUENT ESTROGENS.

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Municipal wastewater has been widely-documented to contain compounds which are introduced to aquatic systems and can significantly impact biota. We investigated environmental endocrine disruption (EED) resulting from exposure to pharmaceutical estrogens (eg, birth control, hormone therapies) from the Boone waste water treatment plant (WWTP) effluent in the S. Fork of the New River, Boone, NC. The effluent release comprises 25-40% of the downstream flow. Environmental estrogen exposure is indicated by measuring a common biomarker, the egg yolk protein vitellogenin (Vtg), in male fish. Fish and water samples (1L) were collected from two sites: the WWTP effluent release and upstream at the ASU greenway (reference). Estrogens were separated from water samples by C18 solid phase extraction columns (to concentrate samples 1000 fold) and analyzed via GC-MS. The optimized protocol resulted in a lower detection limit of 250 ppb. Fish

blood protein samples were characterized using gel electrophoresis and analyzed for Vtg presence by Western blotting, using antibodies specific to fish Vtg. Fish blood samples collected in April 2006 (university in session) indicated sixty percent of male hog suckers, *Hypentelium nigricans*, were producing Vtg (n=5). As expected, Western blots of female plasma exhibited Vtg-specific bands while males demonstrated even denser Vtg bands (likely due to the lack of oocyte uptake of the protein). Analysis of water samples collected in July (university not in session) indicated that estrogen concentrations were below detection limits. September samples (university in session) of water and several fish populations are currently being analyzed. Future sampling will include collection of monthly and quarterly water and fish samples to determine if student estrogens cause temporally increased Vtg production in exposed male fish. Occurrence of EED in fishes with various life history strategies and habitat preferences, as well as spring versus fall spawning fish populations, will be monitored.

533 ESTROGEN RESPONSIVE PLASMA PROTEIN BIOMARKERS IN FOUR FISH SPECIES.

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We previously reported on the use of mass spectrometry to examine plasma from sheepshead minnows (SHM) exposed to estrogen agonists for changes in estrogen responsive protein biomarkers. In this study male fish of four species representative of three orders were exposed to 17 β -estradiol (E2) and examined for the presence of putative estrogen-responsive plasma protein biomarkers. Species and orders evaluated include: Order Cyprinodontiformes (SHM, *Cyprinodon variegatus*, and Gulf Killifish, *Fundulus grandis*), Order Perciformes (Striped Bass, *Morone saxatilis*) and Order Salmoniformes (Rainbow Trout, *Oncorhynchus mykiss*). Blood plasma was sampled from SHM and killifish after 7 d aqueous exposure, striped bass at 3 d post injection and rainbow trout after 14 d dietary administration. Plasma was diluted with acidified aqueous acetonitrile, combined with a sinapinic acid solution and then analyzed by MALDI-TOF-MS without further cleanup. A number of plasma proteins were upregulated in all four species in response to E2 treatment. However, the specific upregulated proteins varied among the species tested. Proteins in the mass range of 22-31 kDa were upregulated in treated compared to control SHM, killifish and striped bass. In contrast, treated rainbow trout exhibited no increased expression of proteins in the 22-31 kDa range but did show increased expression in the 32-35 kDa range. These results indicate the protein biomarker based estrogen effects screening technique developed for SHM should be readily transferable to other fish species and can be used to understand differences in species sensitivity to estrogen agonists. This approach will also permit the development of a less species-dependent technique than ELISA for environmental monitoring of estrogenic effects in multiple fish species.

534 IMPACT OF IMIDACLOPRID ON THE ACTIVITIES OF SERUM ENZYMES IN FRESH WATER FISH, CHANNA PUNCTATUS.

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On the treatment with sublethal concentration of Imidacloprid (organo chloride) the serum enzymes of fish, Channa Punctatus are altered during the toxic exposure periods at different time intervals. The activities of different serum enzymes such as Aldolase, Lactate dehydrogenase (LDH), Iso-citrate dehydrogenase (ICDH), Pyruvate kinase, SGPT, SGOT, Alkaline Phosphatases, Acid Phosphatases and Creatine Kinase (CK) are altered under the impact of imidacloprid. The high activities of Transaminases indicate that Imidacloprid could induce malignance and hepatobiliary disease. Enhancement in Creatine Kinase leads to impairment of central nervous system (CNS) under toxic stress. The activities of enhanced Phosphatases might be due to lysosomal mobilization. The enhanced serum Dehydrogenases reveal the damage of kidney and liver of exposed fishes.

535 CRYOPRESERVED FISH HEPATOCYTES AS A MODEL FOR BIO-ACCUMULATION AND CYTOTOXICITY EVALUATION OF AQUATIC POLLUTANTS.

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The objective of this study was to develop an in vitro model to examine the bioaccumulation and cytotoxic effects of aquatic and wastewater related pollutants. In support of this objective, we developed a method for the isolation of rainbow trout

(*Oncorhynchus mykiss*) hepatocytes in which viability and metabolic activities are maintained. Using a two-step perfusion technique, we successfully isolated hepatocytes with viabilities ranging from 80-95% as assessed by trypan blue exclusion and flow cytometry. Hepatocytes were cryopreserved in an automated, controlled rate freezer with very little loss in viability. On thawing, these cells were >80% viable as assessed by trypan blue exclusion with vial-to-vial variation of only 6% as assessed by flow cytometry. The biotransforming ability of trout hepatocytes was assessed using the following nine specific substrates: dextromethorphan, tolbutamide, phenacetin, testosterone, mephentoin, chlorzoxazone, coumarin, 7-ethoxycoumarin and 7-hydroxycoumarin. While the specific activities of several CYP enzymes were detected, significant CYP3A-like phase I activity was observed during the conversion of testosterone to 6 β - and 16 β -hydroxytestosterone. Similarly, significant activity of the phase II enzyme, glucuronosyltransferase, was evident by the glucuronidation of 7-hydroxycoumarin. Using the above mentioned panel of substrates we also demonstrated the maintenance of enzyme activities following cryopreservation. For these studies, cryopreserved cells were compared to freshly isolated cell suspensions. Taken together, these results demonstrate that cryopreserved trout hepatocytes are viable and exhibit xenobiotic metabolizing enzyme activities. Hence, they represent a suitable model for screening large numbers of hazardous chemicals for their cytotoxicity and ability to be either metabolized or accumulated.

536 EFFECTS OF ETHYNYLESTRADIOL EXPOSURE DURING LARVAL DEVELOPMENT OF THE AMPHIBIAN, XENOPUS TROPICALIS.

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17 α -Ethinylestradiol is a pharmaceutical chemical that primarily enters into the aquatic environment via wastewater from urban centers. This potent estrogen has been reported to impact the endocrine systems of fish species at concentrations in the low ng/l range. In order to evaluate the toxicity of this chemical on an amphibian species, we exposed larval West African clawed frogs, *Xenopus tropicalis*, to ethinylestradiol from 1 day post-fertilization (NF stage 15-20) to metamorphosis (NF stage 66). Exposures were carried out in a continuous flow-through system at concentrations ranging from 10 to 640 ng/l. At metamorphosis, half of the froglets were weighed, blood was collected from the heart, and gonads were excised for quantitative PCR analysis. The froglets not assessed at metamorphosis were placed in clean media and raised until 6 weeks post-metamorphosis at which time they were similarly assessed. Exposure to all concentrations resulted in significantly reduced times to metamorphosis. Ethinylestradiol had no effect on mortality or body mass. The gonads of froglets exposed to ≥ 40 ng/l were all morphologically female based on pigmentation. Additionally levels of vitellogenin, a biomarker of estrogenic exposure, were induced in the plasma at concentrations of ethinylestradiol ≥ 10 ng/l. Quantitative PCR methods for assessing the genes DMRT1 and SOX3 were developed. These genes are expressed predominantly in one sex and are being assessed as biomarkers for intersexuality in amphibians. Results from this study indicate that ethinylestradiol is capable of impacting the endocrine system of amphibians at environmentally relevant concentrations. This abstract does not necessarily reflect EPA policy.

537 HISTOLOGICAL OBSERVATIONS IN NATIVE NEW JERSEY FROGS CAPTURED FROM AGRICULTURAL PONDS CONTAINING ATRAZINE AND OTHER CONTAMINANTS.

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Atrazine is one of the most commonly used herbicides in the United States. This pesticide is routinely found in surface waters where amphibians are non-target species in a unique position for exposure. The literature suggests that low environmentally relevant doses of atrazine have the ability to alter reproductive processes, causing retarded gonadal development (gonadal dysgenesis) and testicular oogenesis (hermaphroditism) in amphibians. To determine whether atrazine poses a risk to frog populations in New Jersey, 391 adult and juvenile green frogs (*Rana clamitans melanota*) and bullfrogs (*Rana catesbeiana*) were collected across nine pond sites during the 2003, 2004 and 2005 breeding seasons in Burlington County, NJ. These sites were evaluated for water levels of atrazine and related compounds. Several pesticide residues were detected in pond waters in addition to atrazine. Resident frogs were assessed histologically and both testicular dysgenesis and oogenesis were observed in captures. The reproductive systems of the two species responded differently to the contaminants in their environment. Retarded gonadal

development was only seen in bullfrogs, while testicular oogenesis was found solely in green frogs. The highest percent incidence of testicular oogenesis was found within the green frog population at the site with the highest atrazine impact. Additionally, parasitic incidence, including brain metacercariae and hepatic protozoan cysts, were compared to the geographic distribution of atrazine use. Further histological observations presented include tissue specific inflammation, tubular degeneration and sperm aggregation within the kidney. This work was supported by the NIEHS training grant in Toxicology ES07148 and NJ DEP contracts SR02-95, SR04-59 and SR05-046.

538 INHIBITION OF TSH-STIMULATED THYROID HORMONE RELEASE FROM THYROID GLAND EXPLANT CULTURES BY THREE CHEMICALS: METHIMAZOLE, PROPYLTHIOURACIL, AND PERCHLORATE.

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As part of an ongoing effort to develop a systems model approach for characterization and prediction of thyroid toxicity, we have utilized *Xenopus laevis* thyroid gland explant cultures for investigating the response of the thyroid gland to stimuli independent of the hypothalamic-pituitary-thyroid (HPT) axis. In this study, methimazole and propylthiouracil, both inhibitors of thyroid peroxidase activity, and perchlorate, an inhibitor of iodide uptake, were used to inhibit T4 release by thyroid explant cultures. Thyroid glands from pro-metamorphic tadpoles were cultured at 21°C in L-15 media supplemented with 0.1% BSA and 1 µM KI. Inhibitory potency of methimazole was tested by culturing glands in control media, media with bovine TSH at a concentration to produce maximal T4 release, or media with TSH and graded concentrations of methimazole. Glands were cultured for 6-8d in test media with regular media exchanges. T4 released into media during the last 48h in culture was determined by radioimmunoassay. Inhibition of T4 release was dose related and occurred at 1 or 5 µg methimazole/ml in glands from NF stage 55 or 59 tadpoles, respectively. These methimazole concentrations are slightly lower than the 12 µg/ml previously reported to alter thyroid histology in tadpoles exposed *in vivo*. Histology of the cultured glands after 8d revealed no evidence of necrosis, and glands treated with TSH alone or TSH with methimazole exhibited follicular cell hypertrophy. Perchlorate and propylthiouracil also inhibited T4 production by cultured thyroid glands at d6-8, and as previously shown with methimazole, the extent of inhibition was related to time and concentration of TSH. This *in vitro* assay system is a useful tool to evaluate the potency of chemicals for inhibiting TSH-stimulated T4 release and can help interpret the effects of chemicals on the HPT axis *in vivo*. This abstract does not necessarily reflect EPA policy.

539 DEVELOPMENT OF GENE EXPRESSION MARKERS OF COMPENSATORY AND DIRECT EFFECTS OF TPO AND NIS INHIBITION IN THE AMPHIBIAN THYROID GLAND.

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An amphibian-based hypothalamic-pituitary-thyroid (HPT) axis model is being developed in a systems biology framework for understanding thyroid toxicity. In developing this model it is important to understand which changes are due to compensatory responses of the animal and which are direct effects of the chemical. Using *Xenopus laevis* as the model organism, responses in the whole organism and thyroid gland culture *in vitro* are being compared to separate compensatory mechanisms from direct effects. The two chemicals used in this study, methimazole and perchlorate, inhibit T4 synthesis by two different mechanisms, TPO inhibition and NIS inhibition, respectively, but each results in decreased circulating T4. The compensatory response of the tadpole to decreasing T4 is to increase circulating TSH to stimulate more T4 production. NF stage 54 tadpoles were treated with methimazole, perchlorate, or dilution water for 6 days. Thyroid glands were removed at 24, 48, 96, or 144 hours. Thyroid glands were processed for histopathology or gene array analysis using Affymetric gene chips. For *in vitro* experiments thyroid glands were dissected from stage 55 tadpoles, cultured in L-15 media with 0.1% BSA at 21°C. Five pairs of glands per treatment were cultured for 24h in media alone (controls) or 500 ng TSH/ml (0.9 mU activity/ml), removed from culture and processed for gene array analysis. Treatment with methimazole and perchlorate resulted in time dependent changes in thyroid gland morphology. Additionally, three patterns of gene expression changes were observed; those characteristic of elevated TSH (based on comparison to the *in vitro* response), those unique to methimazole, and those unique to perchlorate. From these experiments we identified markers within the thyroid gland for thyroid axis inhibition/TSH elevation. We have also established markers for TPO and NIS inhibition, although additional testing with more chemicals is necessary to establish the utility of these markers. This abstract does not necessarily reflect EPA policy.

540 TOXICOLOGICAL EVALUATION OF AN INHALATION EXPOSURE TO BENZENE AND TOLUENE IN A RAPTORIAL BIRD, THE AMERICAN KESTREL (FALCO SPARVERIUS).

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Volatile organic carbon (VOC) compounds are by-products from oil and gas production. The two most common VOCs, benzene and toluene have shown numerous toxic effects in rodents after inhalation exposure. Research here is focused on the exposure of a small raptor, the American kestrel (*Falco sparverius*) to benzene and toluene in a lab atmosphere. The exposure situation is comparable to environmental releases as well as rodent derived LOAELs.

The first year of this project included design and construction of the inhalation chamber and the capturing and dosing of wild American kestrels. The second season utilized a well established American kestrel breeding colony for dosing purposes. In year one a high dose situation was tested (benzene 10 ppm, toluene 80 ppm). In year 2 a low group (benzene 0.1 ppm, toluene 0.8 ppm) was added and high was repeated.

The same endpoints were analyzed in years 1 and 2. *In vivo* and laboratory assays were conducted on collected plasma and tissue samples to evaluate the following toxic responses; immunological (Delayed type hypersensitivity (DTH), Enzyme linked immunoassay (ELISA), bone marrow smear differentials), endocrinological (retinol, retinol palmitate, thyroxine (T4), and triiodothyronine (T3) levels), enzyme activities, and behavioural.

Year 1 concluded that DTH was decreased in exposed birds ($p = 0.01$) as was total leukocyte count ($p = 0.002$). Behavioural variations between exposed and control groups include; activity ($p = 0.013$), ability to focus ($p = 0.007$), and defensive behaviours ($p = 0.037$). Year 2 has shown the same decreased DTH response ($p = 0.001$).

Preliminary results show toxicity in American kestrels exposed to benzene and toluene. The end result of years 1 and 2 will identify any toxicological effect environmental levels of benzene and toluene will have on wild raptors. These data and techniques can then be used for *in vivo* testing of wild birds for biological health monitoring purposes.

541 MECHANISTIC EVALUATION OF THE DEVELOPMENTAL TOXICITY OF NITROGEN OXIDES.

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Nitrates have become global contaminants of most aquatic systems due to their extensive use as fertilizers in agriculture. Recent studies have raised concern that nitrates and nitrites (NO_x) might be reduced to nitric oxide (NO) in the body during mitochondrial respiration. NO is a potent cellular signaling molecule that regulates various processes including steroid synthesis and reproduction. We used *Daphnia magna* as a model to mechanistically investigate the effects of NO_x on reproduction and development. The hypothesis was tested that excess NO, derived from NO_x , can cause abnormal embryo development by interfering with ecdysteroid signaling. We further hypothesized that this effect could be elicited by either NO-dependent suppression of ecdysteroid synthesis or through NO binding to NR1D3 (also called E75), a putative NO sensitive regulator of ecdysteroid signaling. Female daphnids were exposed to concentrations of sodium nitroprusside (SNP, an NO donor), sodium nitrite, and sodium nitrate. Effects on ecdysteroid-dependent development parameters associated with embryos were evaluated. All compounds elicited developmental toxicity consistent with anti-ecdysteroid activity with relative potencies of $\text{SNP} > \text{NO}_2 > \text{NO}_3$. These results were consistent with the hypothesis that NO_x elicits toxicity through its conversion to NO. We have ruled out direct reduction in ecdysteroid levels as a mechanism for this toxicity, based on RIA measurements indicating no decrease in levels from embryos obtained from SNP or NO_x treated maternal daphnids. NR1D3 has been identified and cloned in *D. magna*. The NR1D3 recombinant protein is being expressed and evaluated with respect to sensitivity to NO. Results from this study will advance our understanding of the potential threat posed by NO_x pollution to reproductive success.

542 HAZARD REDUCTION OF THE INCINERATED ASH BY USING WASTE WASHING SYSTEM.

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Since securing new landfill sites has been still demanding in Japan, recycling, renewals, and stabilization of the existing landfills by various treatments are necessary. Ash incinerated from municipal solid wastes (MSW) can contain hazardous contaminants such as heavy metals, organic contaminants, etc. Thus, the leachate from

the landfills filled with the incinerated ash might pose its hazard to the surrounding environments. Semi-full scale model facility (capacity: 1 tons/hr) - Waste Washing System (WWS)- was installed at the Eco-town in Kitakyushu, Japan, and the incinerated ash from MSW was fractionated and washed by this system. Four lysimeters were prepared and each lysimeters were filled with A) the ash before washing (< 25 mm), B) ash after washing (0.065 mm – 10 mm), C) 35% to 65% of mixed ash, and D) 65% to 35% of mixed ash with the ratio of the ashes before washing to after washing. The lysimeters were sprayed with de-chlorinated water every week and the toxicities of the leachate from the lysimeters were monitored every two weeks using Microtox™, ToxScreen-II, and DaphTox tests. It was found that the toxicities of the leachate from the lysimeters B and C were reduced faster than those from A and D and TOC, COD, BOD, T-N, NH₄-N, Cl, Cu, Pb, and As concentrations in the leachate from B and C were also lower than those in the leachate from A and D. Therefore, it was concluded that landfilling with the incinerated ash after passing through the WWS or with the mixed ash with higher contents of washed ash can reduce the toxicity of the leachate from the landfill, suggesting that WWS can be used as a pre-treatment method for hazard reduction.

543 IMPACT OF SODIUM TUNGSTATE ON THE VIABILITY OF SELECTED MICROORGANISMS WITH DIFFERING CAPACITIES FOR BIOREMEDIATION.

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The use of tungsten munitions has the potential to introduce a new contaminant to sites already contaminated with materials such as perchlorate, heavy metals and other organic contaminants and may impact naturally occurring microbial processes such as bioremediation. To address this possibility, microorganisms with different capacities for bioremediation were evaluated for growth inhibition in response to sodium tungstate. The strains included *Shewanella oneidensis*, *Dechlorosoma suillum* and *Pseudomonas putida*. *S. oneidensis* can remediate metals such as chromium and uranium, while *D. suillum* can convert perchlorate to chloride and *P. putida* can degrade a wide range of aromatic hydrocarbons. The initial phase of the study was designed to evaluate different concentrations of tungsten for toxicity prior to evaluating for effects on bioremediation processes. The strains were cultured in 250 ml flasks containing 50 ml nutrient broth and increasing concentrations of sodium tungstate at 30°C for *P. putida* and *S. oneidensis* and 35°C for *D. suillum*. Growth was determined by measuring optical density of the cultures at hourly intervals. Cultures of *D. suillum* and *P. putida* were significantly inhibited by 50 mM sodium tungstate and completely inhibited by 100 mM sodium tungstate. Conversely, *S. oneidensis* exhibited greater resistance, with complete inhibition occurring at 250 mM. Initially *S. oneidensis* was completely inhibited at all concentrations of sodium tungstate at 7 hours post inoculation, but at 24 hours significant growth had occurred, suggesting an inducible resistance mechanism. Taken together, these results indicate that the presence of tungsten in contaminated environments may have detrimental effects on certain bioremediation processes occurring within that environment.

544 ENVIRONMENTAL FATE AND EFFECTS OF NICOTINE RELEASED DURING CIGARETTE PRODUCTION.

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A variety of test methods were utilized to study the biodegradation, bioaccumulation and toxicity of nicotine including determination of the octanol/water partition coefficient, conversion of nicotine to CO₂ in soil and activated sludge; and evaluation of the effects of nicotine on microbiological inhibition, algal toxicity, plant germination and root elongation. The partitioning of nicotine between octanol and water indicated that nicotine will not bioaccumulate regardless of the pH of the medium. The aqueous and soil-based biodegradation studies indicated that nicotine is readily biodegradable in both types of media. The aquatic and terrestrial toxicity tests indicated nicotine has low toxicity to typical environmental microflora. The EPA Persistence, Bioaccumulation and Toxicity Profiler model, based on the structure of nicotine and predictive rates of hydroxyl radical and ozone reactions, estimated an atmospheric half-life of less than 5.0 hours. Using this value in the Canadian Environmental Modeling Centre Level III Model, it was estimated that the half-life of nicotine in water is 3.0 days and the half-life in soil is 0.5 days. This model also estimated that after equilibration of nicotine into the environment, nicotine would be expected to be found predominantly in water at 93% with 4% in the soil, 3% in air, and 0.4% in sediment. Evaluation of all ecological fate and effects test data suggest that nicotine is readily biodegradable in the environment and non-toxic to the aquatic and terrestrial species evaluated under the conditions of these studies.

545 POLYCYCLIC AROMATIC HYDROCARBONS CONTAMINATION IN SOIL AND AIR FROM THE MEXICAN CARBONIFEROUS REGION OF COAHUILA.

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The release of Polycyclic Aromatic Hydrocarbons (PAHs) into the environment during coal pyrolysis to produce coke represents a risk for the populations living in the industry's neighborhood. The level of PAHs contamination is thus a necessary prerequisite to perform environmental quality and risk assessments. Therefore, we measured PAHs concentrations in soil and air particulates from the vicinity of coke oven factories located in the Carboniferous Region in Coahuila, Mexico. We compared PAHs concentrations in soil and air particulate samples collected from Nueva Rosita and Barroterán, these communities have functional coke ovens, and Juárez, a community with no coal industry. PAHs were determined by CG/MS, using the EPA standard recommendations. The results showed that PAHs levels in soil were as follows: Nueva Rosita > Barroterán > Juárez (1708, 1690 and 2.06. mg/kg of soil dry weight, respectively). Soil PAHs content in Nueva Rosita samples exceeds the level considered as major risk health by EPA (1140 mg/ kg dry weight). The most abundant compounds identified in these soils were chrysene, methyl phenanthrene and methyl naphthalene. Based on the soil results, high volume air samples were placed close to the coke chimneys in Nueva Rosita and Barroterán. The air particles contained the following carcinogenic PAHs: benzo(a)pyrene, benzo(a)anthracene, dibenzo(a,h)anthracene and benzo(b)fluoranthene. These results indicate that PAHs are present in the area and should be considered for remediation (Supported by Conacyt-Semarnat project 2004-01-293).

546 TOXICITY OF CHLORINATED POLYCYCLIC AROMATIC HYDROCARBONES.

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Polycyclic aromatic hydrocarbons (PAHs) are discharged from various emission source in aqueous environment. In the case of raw water for drinking water source, these compounds are exposed chlorine in the process for the disinfection in drinking water treatment plant and then are reacted oxidation and/or chlorine displacement. However, there is not much information on the reaction products by chlorination. Additionally, it is afraid the reaction products effect on human health. After Fluoranthene (FL), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo[a] pyrene (B[a]P), benzo[ghi]perylene (B[ghi]P), indeno[1,2,3-cd]pyrene (IP), were exposed with 1mg/L free chlorine in the pure water fortified with 10 mM phosphate buffer (pH 7.0), PAHs and their chlorinated compounds were concentrated by solid phase extraction (SPE) method at the reaction time of 1, 2, 4, 6 and 24 hours, respectively. Under this condition, non-chlorinated PAHs dissolved in water were recovered from 80 % to 120 %. The GC/MS system in selected ion monitoring mode was used for the analysis of PAHs and chlorinated PAHs. PAHs decreased with time-dependent in the case of all six kinds of PAH. The reactivity for the chlorine was sensitive in order of B[a]P > B[k]F > B[b]F = IP > B[ghi]P > FL. Mono-chlorinated PAHs were formed in the case of all six kinds of PAHs. Di-chlorinated PAHs were detected in the case of B[k]F and B[a]P. However, tri-chlorinated forms were not detected in this condition. The extracted compounds from the chlorine solution dissolved PAHs were exposed in HepG2 cells for 24hrs. Mono- and di-chlorinated B[a]Ps showed significant cytotoxicity by lactose-dehydrogenase activity assay, but the chlorinated compounds derived from the other five PAHs hardly showed the cytotoxicity comparing the original compounds. These results indicate B[a]P should be considered most in the chlorination treatment process among six kinds of PAHs.

547 INDOOR AIR QUALITY STUDY-VAPOR INTRUSION OF VOLATILE ORGANIC COMPOUNDS.

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Growing response to residential exposure to Volatile Organic Compounds (VOCs) and Semi-Volatile Organic Compounds (SVOCs) have prompted concern by the EPA to promulgate regulatory limits for VOCs in residential homes. A major source of these fugitive VOCs are dry cleaners, service stations and leaking underground storage tanks. An immediate threat can be realized from the vapors that migrate into residences. This threat can pose chronic health risk for residents if the de-

tectability level is low. The objective of this project is to validate the Johnson-Ettinger (JEM) model and other 2-dimensional and 3-dimensional models using the Brookhaven National Laboratory Air Infiltration Measurement System (BNL/AIMS) technique to determine the subsurface contribution of VOCs intrusion in buildings. Four per fluorocarbon tracers (PFTs) sources were deployed in building 830. Sources were left for approximately 48 hours in six zones. Passive samplers were used to trace the infiltration and exfiltration of PFT sources. Analysis of the samplers in the laboratory showed that the flow rate in three of the six zones were quite comparable. The hallway (Zone 5) had a relatively high concentration rate upwards of 6m³/h. However, the crawl space had low source rates hence there was little detection of PFT sources. This miniature source and sampling tracer kit can measure infiltration rates on the order of 0.2 to 5 changes per hour over time-averaged periods of 1 day to several months or years.

548 UPTAKE OF MERCURY FROM CONTAMINATED SOIL BY DECIDUOUS TREES.

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Mercury (Hg) contaminated landfill material has been used to landscape a city park in Oxford, AL. An ongoing investigation of Hg occurrence in this watershed has found that Hg from the fill material has been redistributing across the fluvial deposition system. The Hg has also been bioaccumulating in various trophic levels of the freshwater ecosystem. This investigation extends the study to include the measurement of Hg in green ash (*Fraxinus pennsylvanica*), a deciduous tree growing in the contaminated flood plain. Soil samples from this plain had an average Hg concentration of 1.195 mg/kg. Root tissue collected from three trees growing in this flood plain had an average Hg concentration of 0.007 mg/kg (dry wt). Root tissue collected from a green ash growing in an area with the local background level of Hg in the soil (0.066 mg/kg) had no detectable Hg. Samples were analyzed using a CETAC Quick Trace Mercury Analyzer M-1600 cold vapor atomic absorption Hg analyzer with an ASX-400 Autosampler. To determine if the trees were degassing Hg through the leaves, contiguous branches with leaves were sequestered in a 1 gal plastic bag along with a passive Hg sampler (SKC passive sampler for Hg, 520 series). After a 24 h sampling period, the sampler contained 0.040 ug of Hg. A second sampler similarly positioned on the same tree for 24 h and open to the atmosphere had no detectable Hg. This would indicate that Hg from the soil is being released into the atmosphere of the park through tree leaves.

549 GENE EXPRESSION PROFILES IN RAINBOW TROUT, ONORHYNCHUS MYKISS, EXPOSED TO A CHEMICAL MIXTURE.

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One of the proposed uses for microarrays in environmental toxicology is the identification of key contributors to toxicity within a complex mixture. The transcriptomic profile of a complex mixture has not been established, and whether the gene expression profiles resulting from exposure to a complex mixture have patterns of altered gene expression with identifiable contributions from each toxicant component of the mixture remains uncertain. We exposed isogenic (cloned) rainbow trout *Onorhynchus mykiss*, to sublethal levels of individual ethynylestradiol (xeno-estrogen), 2,2,4,4 tetrabromodiphenyl ether (BDE-47, thyroid active), and chromium VI or to a mixture of all three toxicants. Following exposure, fish were euthanized, livers harvested and RNA extracted. Fluorescently labeled cDNA were generated and hybridized against a commercially available Salmonid array (GRASP, University of Victoria) spotted with 16,000 cDNAs. Microarrays were scanned to measure abundance of a given transcript in each sample relative to controls. Data were analyzed via Genespring (Silicon Genetics) to identify a list of up and down regulated genes, and to determine gene clustering patterns that can be used as "expression signatures". The gene ontology terms associated with significantly altered genes were also used to identify functional groups that were associated with toxicant exposure. Our analysis indicates that the gene expression profiles resulting from the mixture exposure resemble those of the individual contaminant exposures, but are not a simple additive list. However, patterns of altered genes representative of each component of the mixture are clearly discernible, and the functional classes of genes altered represent the individual components of the mixture. These findings indicate that the use of microarrays to identify gene expression profiles may aid in the identification of key stressors within a complex mixture, ultimately improving environmental assessment.

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FUMONISIN PRODUCTION IS NECESSARY FOR DEVELOPMENT OF THE FULL SPECTRUM OF SYMPTOMS INDICATIVE OF *FUSARIUM VERTICILLIOIDES* MAIZE-SEEDLING DISEASE AND EVIDENCE FOR DISRUPTION OF SPHINGOLIPID METABOLISM AS THE PROXIMATE CAUSE.

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The fungus *Fusarium verticillioides* infects maize and produces fumonisins, inhibitors of ceramide synthase. To determine the role of fumonisins in maize seedling disease, seeds were inoculated with fumonisin producing or non-producing strains of *F. verticillioides*. Seedlings grown from seeds inoculated with the producing strains had detectable fumonisins in roots and soils, and sphingoid bases and sphingoid base 1-phosphates were elevated in roots. Leaf lesions and abnormal leaf developmental were only observed with producing strains and while non-producing strains of *F. verticillioides* caused reduced root and stalk growth the effect on growth was greatest with producing strains. Leaf lesion incidence and severity of effects on root and stalk growth were significantly correlated with fumonisin in roots and the extent of disruption of sphingolipid metabolism in roots. In a subsequent study with the fumonisin producing strain MRC826, seedlings grown from inoculated seeds were harvested on days 7, 14 and 21. Reduced growth of aerial plant parts and roots were seen in seedling as early as day 7, however, leaf lesions were not apparent until day 14. Fumonisins were detected in the roots of seedlings and was maximal on day 14. There were significant increases in free sphingoid bases on day 7 which were maximal on day 14. There were also significant increases in free sphingoid base 1-phosphates on day 7. However, the level of free sphinganine 1-phosphate decreased significantly after day 7. These results suggest a time-dependent adaptive response to disrupted sphingolipid metabolism after prolonged fumonisin exposure, and also support the hypothesis that fumonisin is necessary and sufficient to produce the full spectrum of symptoms indicative of *F. verticillioides*-induced maize seedling disease.

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TCDD DECREASES RETINOID SIGNALING AND VENTRAL PROSTATIC BUD FORMATION IN THE MOUSE UROGENITAL SINUS.

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) interferes with mouse ventral prostate development by blocking initiation of ventral buds from the fetal urogenital sinus (UGS). Retinoic acid (RA) is implicated in many aspects of development but its role in prostate development is unclear. This study tested the hypothesis that decreased RA signaling is partially responsible for inhibiting ventral prostatic bud formation following TCDD exposure of C57BL/6J mouse dams (5 µg/kg, po) on embryonic day (E)15. On E16, TCDD activated the aryl hydrocarbon receptor (AHR), reduced expression of the RA synthesis gene *Raldh2*, and elevated expression of RA metabolizing genes (*Cyp1b1* and *Cyp2c39*) in UGS mesenchyme. These changes resulted in a net decrease of retinoid signaling in the fetal UGS mesenchyme, evidenced by diminished expression of β-galactosidase activity in RARE-*LacZ* retinoid-responsive transgenic mice and diminished expression of the RA target gene *Hoxa13*. TCDD also decreased androgen receptor (AR) protein expression and partially caused a nuclear-to-cytoplasmic redistribution of AR in the peri-urethral layer of UGS mesenchyme cells that also displayed activated AHR. The *Hoxa13* knockout mouse was used to show that loss of *Hoxa13* partially phenocopied TCDD by decreasing expression of AR protein in the UGS mesenchyme and preventing ventral prostatic budding. Together, these results indicate that interference with retinoid signaling in the ventral UGS region of the male mouse fetus may contribute to the UGS region-specific prostatic budding inhibition by TCDD. (Supported by NIH grants F32 ES014284 and R37 ES01332).

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STUDIES ON WHY VENTRAL PROSTATIC BUDDING IN MOUSE UROGENITAL SINUS (UGS) IS FAR MORE VULNERABLE TO 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) THAN DORSAL, LATERAL, OR ANTERIOR BUDDING.

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Experiments were conducted to investigate why *in utero* TCDD exposure can prevent the UGS from forming any ventral prostatic buds while having far less effect on dorsal, lateral, and anterior budding. Pregnant C57BL/6J mice were given 0 or

5 µg TCDD/kg on gestation day (GD) 15 and UGSs were examined on GDs 15, 16 and 17, the critical window for TCDD-induced ventral bud agenesis. Previous results from mice with an aryl hydrocarbon receptor (AHR)-driven *LacZ* reporter gene indicated that the AHR signaling pathway through which TCDD acts is located almost exclusively (in the UGS) in the periurethral mesenchyme. Similar results have now been obtained for the distribution of EROD activity, a reaction catalyzed predominantly by the TCDD-inducible enzyme CYP1A1. In contrast, distribution of mRNA for another TCDD-inducible enzyme, CYP1B1, was far more widespread, and substantial *Cyp1b1* mRNA was seen in vehicle-exposed UGSs. Regional specificity of AHR signaling in the UGS is due largely to localization of the AHR, whose mRNA distribution pattern closely matched results from the *LacZ* reporter mice and the EROD assays, but not to distribution of the AHR dimerization partner ARNT, which was nearly ubiquitous. Smooth muscle is a barrier to the inductive signals by which UGS mesenchyme stimulates prostatic epithelial budding, but smooth muscle actin distribution was not detectably altered by TCDD. Preliminary evidence suggests that TCDD may inhibit ventral budding, in part, by reducing the size of the ventral mesenchymal pad and/or reducing its expression of fibroblast growth factor 10. In summary, the region-specific inhibition of prostatic budding by TCDD does not appear to result from regional differences in the AHR signaling pathway or from enhanced smooth muscle development. Instead, region specificity appears to be due to intrinsic differences in the regulation of prostatic budding by the different UGS regions. (Supported by NIH ES 01332)

553 SENSITIVITY OF FETAL RAT TESTICULAR STEROIDOGENESIS TO MATERNAL PROCHLORAZ EXPOSURE AND THE UNDERLYING MECHANISM OF INHIBITION.

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The sensitivity of fetal testicular steroidogenesis to the fungicide prochloraz (PCZ) was investigated to test the hypothesis that the reported morphological effects from maternal exposure were due to reduced testosterone (T). Pregnant Sprague Dawley rats were dosed by gavage with 0, 7.8, 15.6, 31.3, 62.5, 125 mg/kg/day (n = 8) of PCZ from gestational day (GD) 14 to 18. On GD 18 the effects of PCZ on fetal steroidogenesis were assessed by measuring hormone production from *ex vivo* fetal testes after three hour incubation. PCZ levels in amniotic fluid and maternal serum were measured by HPLC/MS and correlated to the inhibition of steroidogenesis. Fetal progesterone and 17 α -hydroxyprogesterone production levels were increased significantly at every PCZ dose, whereas T levels were significantly decreased only at the two high doses. These results suggest that PCZ inhibits the conversion of progesterone to T through the inhibition of CYP17. To test this hypothesis, the effects of PCZ on CYP17 gene expression and CYP17 hydroxylase activity were evaluated. PCZ had no effect on testicular CYP17 mRNA levels as measured by qRT-PCR. However, microsomal CYP17 hydroxylase activity was significantly inhibited by the fungicide (K_i = 865 nM). Fetal T production was reduced when PCZ levels in amniotic fluid reached ~500 ppb which compares favorably with the K_i for PCZ towards CYP17 hydroxylase activity. These results demonstrate that PCZ lowers testicular T synthesis by inhibiting CYP17 activity which likely contributes to the PCZ induced malformations in androgen-dependant tissues. *This abstract does not necessarily reflect US EPA views or policy.*

554 THE LACK OF A TRANSGENERATIONAL EFFECT OF VINCLOZOLIN FOLLOWING ORAL PRENATAL EXPOSURE TO 100 MG/KG BW.

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This study was performed to investigate a reported transgenerational effect of Vinclozolin on the integrity and performance of the male reproductive system. Pregnant Wistar rats (F0 generation) were treated with doses of 0, 4 and 100 mg/kg bw/day by oral gavage from days 6-15 p.c. All subsequent generations (F1 - F3) were not exposed to the test compound. 50 male pups per group were selected to become F1 parental male animals to generate F2 pups with untreated female rats. Subsequent breeding was continued in the same manner for 2 generations. F1 and F2 generation male rats were observed for mating behavior and success as well as ability to generate healthy offspring. F1, F2 and F3 generation male gonads were examined on postnatal day 130 (sperm evaluation, detailed histopathology of testes, epididymides and accessory sexual glands, as well as quantification of apoptotic cells in testes). Administration of 4 or 100 mg/kg bw to F0 gestating mothers did not result in any anti-androgenic effects. This is considered due to the fact that

the administration period was only until day 15 p.c. Thus, the findings reported by other authors^{1,2} following intraperitoneal application of Vinclozolin at 100 mg/kg bw were not confirmed in this oral study. F1 and F2 generations of male offspring, derived from Vinclozolin-exposed dams, developed normally to sexual maturity and were able to mate and to generate healthy progeny. Neither histopathology nor quantification of apoptotic cells, examined in the testes of F1 generation males, revealed any adverse effect of the test compound. Spermatid/Sperm count, sperm morphology and sperm motility were not affected in F1 and F2 generation male offspring. At present, after the investigation of F1 and F2 pups, the study gives no indication of a transgenerational effect of Vinclozolin on the integrity and performance of the male reproductive system, in particular spermatogenic capacity and sperm viability.

¹ MATTHEW D. et al., *Science* Vol 308: 1466-1469 (2005)

² *ibid*, *Science Supporting Online Material*, doi: 10.1126/science.1108190

555 THE GESTATIONAL EXPOSURE TO THE PESTICIDE METHOXYCHLOR (M) INHIBITS TESTOSTERONE (T) FORMATION BY NEONATAL RAT LEYDIG CELLS (LC).

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M is a pesticide that has been used as a replacement for DDT because it is more rapidly cleared from the body and does not bioaccumulate. M has been reported to exhibit both weak estrogenic and antiandrogenic activities. Recent animal studies suggest that M or its dihydroxylated metabolite, HPTE, inhibits the ability of immature or adult rats to synthesize T. It is not known whether fetal LC present during the last third of pregnancy and for 1-2 weeks after birth in rodents, also are sensitive to M following in vivo exposure. The current studies evaluated whether the daily exposure by gavage of 0, 40, 100 or 200 mg/kg body weight of M dissolved in corn oil to pregnant Sprague-Dawley rats during days 14-22 of gestation, altered the capacity of neonatal (fetal) LC to produce T. Male pups were sacrificed on day 1 after birth, and the serum or testes from all male pups of each litter were pooled into a single sample. The pooled testes were digested with collagenase, and the dispersed LC were cultured in DMEM/F12 medium. Both basal and 10 mIU/ml hCG-stimulated T released into the medium was determined after culture for 4 h. There was a minimum of 11 determinations for each treatment group. Serum T level of animals exposed to 40 or 100 mg/kg M was no different than control; however, the T level declined to ~ 56 % of control in animals exposed to 200 mg/kg M. The basal T level of LC exposed in vivo to 40, 100 or 200 mg/kg M was no different than control. The hCG-stimulated T of LC from control animals was ~ 10.6 fold higher than the basal T from control animals, and the hCG-stimulated T of LC exposed to 200 mg/kg M declined to ~ 37 % of LC from control animals. These studies demonstrate that fetal LC exposed to a higher dose on M during the later third of gestation have reduced capacity to synthesize T.

The findings and conclusions in this abstract have not been formally disseminated by NIOSH and should not be construed to represent any agency determination or policy.

556 A HIGH DOSE OF GENISTEIN INDUCES SIGNIFICANT APOPTOTIC CELL DEATH IN NORMAL HUMAN MYOMETRIAL CELLS (UTSMC), BUT NOT IN HUMAN UTERINE LEIOMYOMA (UTLM) CELLS.

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Uterine leiomyomas (fibroids) are benign tumors of smooth muscle origin and the primary cause of hysterectomy in reproductive-aged women in the United States. Although the etiology of these tumors remains unknown, the overall lifetime exposure of women to estrogen is a known risk factor. The phytoestrogen, genistein, an isoflavone found in soy products, shares structural similarities to the potent estrogen, 17 β -estradiol. In previous studies, using estrogen-responsive UtLM cells and UtSMC, we found that low doses of genistein stimulate proliferation of UtLM cells, but not in UtSMC, and high doses of genistein results in inhibition of cell proliferation and death in both cell types. In this study we were interested in determining if apoptosis was a mechanism of genistein-induced cell death in UtLM cells and UtSMC. A fluorometric assay for caspase-3 and western blot analysis for bcl-2, bax and bak proteins were done in both cell lines after treatment with a high dose of genistein (50 µg/ml) at 24, 72 and 168 h. We found that genistein caused significant increases in caspase-3 activity at all time points in the UtSMC, but only at 168 h in UtLM cells. Furthermore, caspase-3 activity in the UtSMC was 1.8 fold that observed in UtLM cells at 168 h. Bcl-2:bak and bcl-2:bax ratios were analyzed and the bcl-2:bax ratio was increased by approximately 1.6 fold in the UtLM cells at 72 and 168 h; however, no fold changes in proteins were observed in the UtSMC. Using a Hoechst stain, we found that after treatment of both cell types

with genistein at 168 h, apoptotic bodies were more evident in the UtSMC compared to UtLM cells. In conclusion, both cell types are susceptible to the inhibitory/death effects of a high dose of genistein, however, it appears that inhibition/death in the UtSMC is through increased apoptosis, but not in the UtLM cells. Additionally, protection of the UtLM cells from undergoing apoptosis may occur as a result of increased bcl-2 protein expression.

557 DOWN-REGULATION OF TGF- β PATHWAY GENES, ACTIVIN A AND SMAD3, IN HUMAN UTERINE LEIOMYOMA CELLS TREATED WITH A HIGH DOSE OF GENISTEIN.

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Previously, we found that genistein at high doses has an inhibitory effect on uterine leiomyoma (UtLM) cell proliferation. In an attempt to identify the molecular mechanisms of this inhibitory effect microarray studies were performed. Five-hundred and forty-one genes in UtLM cells treated with 50 μ g/ml of genistein with average differences of $> \text{or} = 1.5$ -fold were significantly expressed and regulated ($p < \text{or} = 0.001$). Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com) revealed that significant alterations in gene expression occurred in several functions including cell cycle, cell death, cellular growth and proliferation and in a number of signaling pathways involving integrin, VEGF, purine metabolism, TGF- β and fatty acid biosynthesis (Path 2). Activin A, activin B, Smad3 and TGF- β 2 gene expression levels were all down-regulated in the TGF- β signaling pathway. In addition, there was downregulation of genes related to cell cycle regulation, such as CDK6, A-myb, and Cyclin B2, while P15, a CDK inhibitor was upregulated. Expression data for the above genes were identified and validated by real-time reverse transcriptase-polymerase chain reaction studies. Gene and mRNA expression data for activin A and Smad3 were further confirmed by western blot analysis of lysates of genistein treated UtLM cells. In agreement with decreased gene, mRNA and protein expression of activin A in genistein treated UtLM cells, we found that the growth of UtLM cells was stimulated under activin A treatment and the inhibitory effect of genistein was partially abrogated. The elevated expression of activin A and Smad3 in human uterine leiomyoma tissue samples in comparison with autologous myometrium further supported the contribution of activin A and Smad3 in promoting the growth of UtLM cells. Thus, the inhibitory effect of genistein on UtLM cells may be through cell cycle dysregulation and partially via down-regulation of activin A and Smad3 through the TGF- β signaling pathway.

558 DISRUPTION OF THE FEMALE REPRODUCTIVE SYSTEM BY THE PHYTOESTROGEN GENISTEIN: SUBCUTANEOUS INJECTION VERSUS ORAL EXPOSURE.

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We have previously shown that neonatal exposure to the phytoestrogen genistein (0.5 – 50 mg/kg) by subcutaneous injection (sc) caused adverse effects on the developing female reproductive system including altered ovarian differentiation. Further, ovarian function was disrupted later in life with increased ovulatory capacity in lower genistein doses and reduced ovulatory capacity at higher doses. Prolonged estrous cycles were seen in genistein treated mice with increased severity over time. Reduced fertility was seen in the 0.5, 5, or 25 mg/kg dose groups and infertility was observed at 50 mg/kg. In addition, females generated from the genistein 25 mg/kg group bred to control males have altered ovarian differentiation suggesting that genistein effects can be transmitted to subsequent generations. Thus, neonatal treatment with genistein at environmentally relevant doses caused adverse consequences later in life. Since infants are primarily exposed in the diet to the glycosylated form of genistein, genistin, we directly compared the estrogenic activity of orally administered genistin with genistein administered sc. Female CD-1 mice were treated on days 1-5 with genistein sc in corn oil (0, 12.5, 20 and 25 mg/kg) or by feeding genistin (0, 6.5, 12.5, 25 and 37.5 mg/kg). Body weights and uterine wet weights were determined at 5 days of age. A significant increase in uterine wet weight followed genistein sc at 20 and 25 mg/kg and oral exposure to genistin at 25 mg/kg; the response of oral exposure to genistin 25 mg/kg was comparable to genistein 20 mg/kg sc. Since the majority of the sc genistein enters the circulation, our data suggests that approximately 80% of the oral dose is absorbed into the circulation causing a similar biological response. Further, oral genistin caused similar developmental alterations as those observed with genistein sc. This study demonstrates that data obtained from a genistein exposure by sc route can be extrapolated to oral exposures to genistin and that both can have long term consequences.

559 EXPOSURE TO 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) DECREASES MAMMARY EPITHELIAL CELL PROLIFERATION IN PREGNANT C57BL/6 MICE.

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Development of the mammary gland during pregnancy is a dynamic, orchestrated process involving the proliferation of mammary epithelial cells and their differentiation into cells capable of synthesizing milk components. Alteration in proliferation, differentiation or an early apoptosis of the epithelial cells can lead to a failed lactogenesis. Exposing mice to the aryl hydrocarbon receptor (AhR) agonist TCDD during pregnancy caused the death of pups within 24 hours of birth, but pups that were nursed by control animals survived. Furthermore, pregnant females exposed to TCDD had a poor lobulo-alveolar differentiation and suppressed expression of milk proteins. The present study was conducted to test the hypothesis that exposure to TCDD impairs glandular differentiation by decreasing the proliferation of mammary epithelial cells. Impregnated C57BL/6 mice were treated with vehicle or 5 μ g TCDD/kg on day 0 of pregnancy for animals sacrificed on day 6 of pregnancy, or on days 0 and 7 for animals sacrificed on days 9 or 12 of pregnancy. Three hours before euthanasia, mice were treated with 50 mg/kg bromo-deoxyuridine (BrdU). Left abdominal glands were fixed and stained in carmine-alum to monitor morphological development while the right abdominal glands were paraffin-embedded, sliced and stained with anti-BrdU antibodies to assess cell proliferation. At all points in time examined, mammary glands from TCDD-treated animals had 50% fewer BrdU+ cells than control animals ($p < 0.01$). The effect of TCDD in the number of BrdU+ cells was noted as early as day 6 of pregnancy, when the analysis of the glands showed a 59% decrease when compared with control animals. The results suggest that one of the mechanisms by which AhR activation impairs lactogenesis is through a decrease in epithelial cell proliferation.

560 A BROMINATED FLAME RETARDANT MIXTURE, DE-71, AFFECTS MAMMARY TISSUE OF RAT OFFSPRING FOLLOWING DEVELOPMENTAL EXPOSURE.

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The brominated flame retardant mixture, DE-71, is made up of multiple congeners, primarily penta- and tetra-brominated diphenyl ethers. These compounds are known to be lipophilic and have been detected in house dust, human milk, and are reported to be on the rise in North American residents. To determine whether or not the commercial mixture acts on the developing mammary tissue of the neonate, time pregnant Long Evans rats (N=8-15 dams/group) were dosed by oral gavage with 0, 1.7, 10.2, or 30.6 mg DE-71/kg body weight (BW) on gestation day 6 through postnatal day (PND) 21. BW of the pups was observed on PND 4, 7, 11, 14, 18, and 21. There was no significant difference in BW across dose at any time examined. Mammary glands were removed from female pups on PNDs 4 and 21, fixed and stained with carmine, and evaluated for age-dependent development on a scale of 1-4. There was no significant treatment effect on PND4 gland development. However, there was a dose-dependent decrease in mammary developmental scores at PND21 ($P < 0.001$). The mammary effects of DE-71 are similar to those previously seen following developmental exposure to dioxin. The epithelial portion of glands of female pups exposed to 10.2 and 30.6 mg DE-71/kg/d exhibited a lack of outgrowth, fewer lateral branches and limited terminal end bud development. These data suggest that lactational transfer may be required for this mixture of brominated flame retardants to affect the developing mammary gland early in life. Further studies designed to examine specific exposure parameters necessary for these effects and to determine the long-term effects of these notable changes in mammary gland development will be helpful in evaluating the health risk of these compounds. (This abstract does not necessarily reflect EPA policy).

561 RESPECTIVE CONTRIBUTIONS OF PRENATAL AND LACTATIONAL PFOA EXPOSURES TO ALTERED MOUSE MAMMARY GLAND DEVELOPMENT.

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Perfluorooctanoic acid (PFOA) is a ubiquitous and persistent synthetic compound, which has been detected in human and wildlife sera. Previous studies in mice linked prenatal PFOA exposure to decreased neonatal weight gain and survival, as well as altered mammary gland (MG) development, independent of body weight. To determine the respective contributions of prenatal and lactational PFOA-exposures to

altered MG development in offspring, timed pregnant CD-1 mice (N=28-48 dams/group) received 3 or 5 mg/kg PFOA or control vehicle via oral gavage on gestation days (GD) 1-17. On GD18-19 dams were monitored at intervals until parturition, and litters of similar ages were cross-fostered to yield seven exposure groups: control (CC), lactational exposure from a 3 or 5 mg/kg PFOA-treated dam (3L or 5L), intrauterine exposure from a 3 or 5 mg/kg PFOA-treated dam (3U or 5U), or both lactational and intrauterine exposure from a 3 or 5 mg/kg PFOA-treated dam (3U+L or 5U+L). MG were removed from necropsied female offspring at 3, 6, and 9 weeks of age and scored for age-appropriate development on a 1-4 scale. Excepting 3L at 3 weeks and 3U+L at 6 weeks, MG development was significantly diminished ($p<0.05$; body weight-independent) among all PFOA-exposed offspring, compared to CC at respective time points. MG scores did not differ between prenatal and lactational exposures, within respective doses and time points, except at 3 weeks, when 3L displayed better development than 3U ($p<0.05$). Offspring serum PFOA concentrations at necropsy were dependent on exposure conditions and time, and inversely associated with MG development. Taken together, these findings suggest that serum PFOA concentration is more highly associated with altered MG development than are exposure conditions alone. (This abstract does not necessarily reflect EPA policy; S.S.W. funded by EPA T829472, EPA CR833237, NIH T32 ES007126.)

562 DETECTION OF ATRAZINE AND ITS METABOLITES IN BODY FLUIDS OF PREGNANT AND LACTATING LONG EVANS RATS FOLLOWING A LATE GESTATIONAL EXPOSURE.

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Atrazine (ATR) administration to pregnant Long-Evans rats results in abnormal mammary gland development and delayed vaginal opening in their female offspring and in control neonates that nurse from ATR-treated dams. We hypothesized that residual ATR metabolites are transferred to pups both transplacentally and via milk days after exposure ceases (ATR half-life is 5-17 hr in adult non-pregnant rats). Timed-pregnant dams were gavaged dosed on gestation days (GD)13-19 with vehicle (1% methylcellulose), 100 mg ATR/kg, or 8.7 mg/kg of an ATR metabolite mixture (AMM) containing ATR and 4 common metabolites. Maternal urine, serum, amniotic fluid, mammary tissue, and milk samples were collected on GD19, 20, 21, postnatal day (PND)1, 4, and/or 11, when possible. Isotope dilution high performance liquid chromatography tandem mass spectrometry was used to analyze the samples for the presence of ATR and 10 different forms of its hydroxylated, chlorinated, or mercapturic metabolites. Following ATR exposure, metabolites were detected at high levels in maternal urine at least 6 days post-exposure. Exposure to ATR vs. AMM resulted in differing profiles of free metabolites detected in serum on PND4 (hydroxylated in ATR dams and chlorinated in AMM dams). Diaminochlorotriazine was detected in amniotic fluid on GD20 and 21, following either ATR or AMM exposures. Metabolites were detected in mammary tissue extracts from GD20 to PND4, and ATR itself was present in expressed milk as late as PND4. ATR and its metabolites were below the level of detection in control samples. These results suggest that ATR and its metabolites are available to the developing offspring several days following late gestational oral exposure and that the half-life of these compounds is longer in pregnant and lactating dams than that previously reported in adult non-pregnant rats. This abstract does not necessarily reflect EPA or CDC policy.

563 GESTATIONAL EXPOSURE TO THE ATRAZINE METABOLITES DIAMINOCHLOROTRIAZINE AND HYDROXYATRAZINE ALTERS MAMMARY GLAND DEVELOPMENT IN LONG EVANS RAT.

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Prenatal exposure to a mixture of atrazine (ATR) and its metabolites hydroxyatrazine (HA), diaminochlorotriazine (DACT), deethylatrazine (DEA), and deisopropylatrazine (DIA) has been associated with persistent alterations in postnatal female offspring mammary gland (MG) development in rats. ATR, DEA, and DIA are catabolized to form DACT, an end product of ATR metabolism, while HA, found in ground and surface water, is formed by the hydroxylation of ATR. Both DACT and HA have been detected in the urine and milk of lactating dams six days after gestational exposure to AMM had ended. We examined whether these metabolites, either alone or together, could mimic the effects of the atrazine metabolite mixture (AMM). Using concentrations derived from a previous study involving an 8.7 mg AMM/kg body weight (BW)/d oral exposure, pregnant Long Evans rats (N≥10/treatment) were gavaged with 7.4 mg DACT/kg BW/d, 1.4 mg

HA/kg BW/d, both DACT and HA at those doses, or 8.7 mg AMM/kg BW/d as a positive control on gestation days (GD) 14-18. Mammary glands collected from female offspring on postnatal day (PND) 4 and 40 exhibited delayed development in all prenatally-exposed pups ($P<0.05$). No significant differences were observed in offspring body weight between groups regardless of age. Serum LH levels in PND4 offspring and the day of and weight at vaginal opening were similar across treatment groups. These results are in agreement with previous findings using AMM alone. Mammary glands of DACT, HA, and DACT and HA treated animals exhibited more marked alterations than did AMM treated animals when compared to controls, suggesting that these metabolites, which are presumed to be transfer to the pup, are actively involved in abnormal mammary gland development. (This abstract does not necessarily reflect EPA policy).

564 A NON-INVASIVE MODEL TO EVALUATE THE EFFECTS OF XENOESTROGENS ON PRE-PUBERTAL FEMALE SWINE.

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There is increased interest in both the effects of xenoestrogens, especially those of fungal and plant origin, in non-rodent mammalian species and the ability to detect those xenoestrogenic effects in a non-invasive manner. Prepubertal female swine were used to develop a non-invasive model to quantitatively evaluate the effects of a known xenoestrogenic mycotoxin, zearalenone (ZEA), in this species, and it was hypothesized that antemortem vulvar measurements in ZEA-treated gilts would correlate well with traditional postmortem biomarkers for xenoestrogenic exposure. Twelve weanling gilts (6 per treatment) were kept in individual pens and fed for three weeks with either a nutritionally balanced control diet or the same diet containing 2 mg of ZEA per kg of feed. On days 0, 4, 7, 14 and 21, the width and length of each gilt's vulva were measured by a single individual blinded with respect to animal treatment group assignment. Animals were euthanized on day 21, and their reproductive tracts were removed and weighed, with selected portions fixed in formalin for histopathologic evaluation. Vulvar width was greater ($P<0.05$) in gilts fed ZEA than in controls from day 4 through day 21, and the length of the vulva was similarly greater ($P<0.05$) in the ZEA-treated gilts from day 7 through day 21. As expected with pre-pubertal xenoestrogenic exposure, reproductive tract weights (total reproductive tract, tubular genitalia and vagina alone) were significantly greater ($P<0.01$) in the gilts fed ZEA, as compared to the gilts fed the control diet, and epithelial hyperplasia and squamous metaplasia were consistently observed in the uterus and vagina, respectively, of Zea-treated gilts. Vulvar width correlated significantly ($P<0.01$), with total reproductive tract weight ($r = 0.91$), the weight of the tubular genitalia ($r = 0.84$) and vaginal weight alone ($r = 0.93$). Data indicate that vulvar measurements can be used as quantitative, non-invasive response variables to evaluate the effects of xenoestrogens on pre-pubertal female swine.

565 EFFECT OF ARSENIC AND DIELDRIN MIXTURES IN PREGNANT AND LACTATING RATS.

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The objective of this study is to identify potential toxic effects of sodium arsenite (As) and dieldrin (Ded) mixtures in pregnant rats. After acclimation, plug positive rats were randomly assigned to groups (Gp) (16/each) and orally gavaged on (gestation day) GD4 with As-Ded-mixtures as follows: Gp 1 [0-0], Gp 2 [0.50-0.34], Gp 3 [1.0-0.69], Gp 4 [2.0-1.38], Gp 5 [4.0-2.75] mg/kg/day throughout 21 days of lactation. Biweekly body weights, weekly feed consumption, clinical observations, hematology, clinical chemistry, absolute and relative organ weights and hormones were documented. Randomly selected liver samples were evaluated for biochemical endpoints. Mortalities (2/16) occurred only in Gp 5 (GD 10 & 16). Hyperactivity (10/16) and abnormal behavior (2/16) were noted in Gp 5. Food consumption was lower in Gp5 after the 3rd week (wk) of gestation and the 2nd & 3rd week of lactation. Also the food consumption was lower in Gp 3 during the 2nd wk. There was a trend toward lower body weight throughout the exposure period. Body weight was lower in Gp 5 (from GD10-21, Lactation day (LD) 3, and LD10-21. Body weight was significantly lower in Gp 4 on GD14 and LD10. Increased relative brain to body weight ratio and liver to body weight in Gp 5. Fertility index was 87.5, 87.5, 75, 75, and 75 for Gps 1-5, respectively. The total number of pups were 154, 167, 147, 142, and 139 respectively. These findings suggest that As-Ded may adversely affect body weight (4-2.75), clinical behavior, mortality, relative brain and liver weights of pregnant and lactating dams. (Supported by MHPF/ATSDR Cooperative Agreement # U50/ATU473408-01).

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IN UTERO AND LACTATIONAL EXPOSURE TO 3, 3', 4, 4', 5-PENRACHLOROBIPHENYL MODULATES OVARIAN FOLLICLE DEVELOPMENT AND STEROIDOGENESIS IN IMMATURE RATS.

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To investigate effects of *in utero* and lactational (IUL) exposure to 3,3',4,4',5-penrachlorobiphenyl (PCB126) on follicle development and steroidogenesis during the immature period of the Sprague-Dawley rats, the ovaries and sera were collected on postnatal days (PNDs) 10, 15, 21 and 24 from the offspring of dams, which were orally administered PCB126 at dose levels of 0 (corn oil), 30, or 100 µg/kg on gestational day(GD)15. When the number of follicles in the 100 µg/kg-exposed group was compared with that in age-matched control on PNDs 15 and 24, those of secondary and graafian follicles were decreased on PNDs 15 and 24, and on PND 24, respectively. While serum estradiol-17β (E2) and immunoreactive inhibin levels on PND15 were reduced in the PCB126-exposed groups, their serum FSH levels were rather higher than control. Reduction of E2 synthesis in the ovaries of exposed groups was confirmed by quantification of P450 aromatase mRNA level by real-time RT-PCR. Furthermore, expression of mRNAs encoding P450_{scc}, 3βHSD and 17βHSD, which were enzymes synthesizing progesterone and testosterone, was reduced on PNDs 10 and/or 15. Additionally performed experiment, in which 50 µg/kg PCB126 was given to the same strain of dams on GD 15, revealed increased and decreased serum levels of progesterone and testosterone in the female offspring on PND15, respectively. From these results, it is indicated that IUL exposure to PCB126 affected follicle development during the immature period without reducing the circulating FSH level, and ovarian synthesis of testosterone and E2 is likely a one of toxicological target of IUL exposure to PCB126 in rats.

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MEASUREMENT OF ENVIRONMENTAL PHENOL LEVELS IN THE URINE OF LACTATING WOMEN.

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This study provides data on urinary levels and types of environmental phenolic compounds to which a cohort of North Carolina mothers were naturally exposed. The US EPA conducted a study entitled Methods Advancement in Milk Analysis (MAMA) in which 34 North Carolina lactating mothers age 18-38 donated urine at 2-7 weeks post-partum and 3-4 months post-partum. HPLC-tandem mass spectrometry was used to determine levels of six halogenated phenols including 2,4-dichlorophenol (2,4-DCP), 2,5-dichlorophenol (2,5-DCP), 2,4,5-trichlorophenol (2,4,6-TCP), 2,4,6-trichlorophenol (2,4,6-TCP), triclosan (TRCS) and ortho-phenylphenol (o-PP) found in pesticides, two alkyl phenols including bisphenol A (BPA) and 4-tert-octylphenol (4-t-OP) with estrogenic capacity, and benzophenone-3 (BP-3), a UV blocking agent, also having estrogenic activity. All of these compounds were detected in greater than 50% of donors except for 2,4,6-TCP, o-PP and 4-t-OP, which were found in 21, 18, and 10% of samples, respectively. Visit one and visit two mean concentrations were not significantly different for any of the measured phenols. Mean +/- SEM levels (ppb) of these compounds were 260.9 +/- 80.9 BP-3, 34.43 +/- 13.8 TRCS, 19.5 +/- 12.4 2,5-DCP, 3.4 +/- 0.8 BPA, 1.1 +/- 0.3 2,4-DCP, 0.5 +/- 0.06 2,4,6-TCP, 0.1 +/- 0.02 o-PP, 0.2 +/- 0.02 2,4,5-TCP, and 0.5 +/- 0.4 4-t-OP. BPA levels in this study were similar to the 90th percentile BPA levels in females age 20-59 in the NHANES III call back cohort. The genesis of exposure to these compounds is unknown for this cohort. However, these chemicals are widespread in the environment and are found in drinking water or sewage, in antibacterial products, in sunscreen and light diffracting plastics, in polycarbonate plastics and epoxy resins used in the manufacture of baby bottles, in dental sealants and food can linings, and in pesticide formulations. (This abstract does not necessarily reflect EPA or CDC policy.)

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EXPRESSION OF TRANSPORTER PROTEINS IN RAT PLACENTA.

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ATP-binding cassette (ABC) transporters are a large super family of transmembrane proteins, which mediate trafficking of a wide variety of substrates in an ATP dependent manner across membranes and are fundamental to protecting the foetus from xenobiotic exposure. We investigated the expression profiles of 3 ABC transporters; p-Glycoprotein (p-Gp), Multidrug resistance associated protein 2 (MRP2) and Breast cancer resistance protein (BCRP) in rat placenta using immunohistochemistry, immunoblotting and genomics. Placental tissue from time mated

Han Wistar rats of foetal gestational days 6 -21 were investigated. p-Gp protein and mRNA were found to be expressed in the rat placenta from day 13. MRP2 and BCRP proteins were found to be expressed from day 14 and day 12 respectively. For all proteins examined expression levels increased up to day 20. In contrast, human placental expression of p-Gp is reported to be greatest during the first trimester (the sensitive period of organogenesis) and BCRP is reported to remain constant throughout pregnancy. These temporal differences in expression may lead to differences in foetal sensitivity in the rat, when compared to man, to xenobiotics transported by this superfamily of transporters.

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GENDER SUSCEPTIBILITY IS A FACTOR INFLUENCING OFFSPRING TUMOR RISK AND RESPONSE TO PRENATAL CIGARETTE SMOKE EXPOSURE: A ROLE FOR TESTOSTERONE.

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Gender susceptibility is a poorly studied risk factor. Previous data from this laboratory have shown that 5-wk-old male offspring of pregnant B6C3F1 mice exposed prenatally to mainstream cigarette smoke (MCS) (5 d/wk, 4 hr/d from gestational day 4 to parturition) had a significantly higher tumor incidence in response to EL4 tumor cell injection than age-/exposure-matched female counterparts (64% vs 36%). As males appeared more vulnerable to the tumor-modulating effects of prenatal MCS exposure, we hypothesized that testosterone (T) played a significant role in the observed gender differences. Thus, young adult female mice (prenatally exposed to either MCS [CO=25ppm, TSP=16mg/m³] or filtered air) were injected sc with either T-cypionate (1 mg in 0.1 ml sesame oil) or vehicle 48 h prior to tumor cell injection; serum T levels, determined 7 and 14 d following T injection, were similar to those reported for "normal" male mice (~5 ng/ml). EL4-induced tumor incidence in the T-treated females of both exposure groups (i.e., air and MCS) was significantly greater than the vehicle-injected female counterparts with values approaching those observed previously in exposure-matched males. Air-exposed, T-treated females were more vulnerable to tumor cell challenge than vehicle-injected females, suggesting a role for T in overall tumor susceptibility. Prenatal MCS exposure significantly enhanced tumor susceptibility in the T-injected females compared to their air-exposed counterparts. Results of this study demonstrate that T-induced masculinization of female mice diminishes gender differences in transplanted tumor cell susceptibility, and CS-induced effects on offspring tumor resistance. These findings suggest that sex hormones may be an important contributing factor, that acts synergistically with *in utero* CS exposure, to increase tumor risk in male offspring. Supported by Philip Morris Foundation Inc.



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NON-CLASSICAL MECHANISMS OF RECEPTOR-MEDIATED TRANSCRIPTION.

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Transcription factors/nuclear receptors (TF/NR) are generally thought to mediate changes in transcription through binding to their cognate response elements. Additionally, TF/NR can also influence gene transcription through the sequestration of factor(s) from promoter regions or by tethering to another TF on a promoter. Perhaps the best example of the latter mechanism is the ability of the glucocorticoid receptor (GR) to bind to AP-1 and NFκB resulting in attenuation of inflammatory signaling. Studies in mice that express a DNA binding/dimerization mutant of GR exhibit many of the therapeutic properties observed through GR activation. In a similar vein, the estrogen receptor (ER) can also attenuate AP-1 activity, however until recently this mechanism could not be used for therapeutic purposes due to other physiological effects of ER activation. These examples underscore the importance of TF/NR tethering in modulating inflammatory signaling, and thus reveals many possibilities in drug design yielding unique physiological outputs. The goal of this symposium will be to explore various mechanisms utilized by NR (e.g. GR, ER, AhR, PXR) to modulate transcription and explore how non-response element driven responses can be selectively modulated by receptor ligands.



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PHYSIOLOGIC ROLES OF THE NONCLASSICAL ESTROGEN RECEPTOR ALPHA SIGNALING PATHWAY.

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Estrogen receptor alpha (ERα) can affect gene transcription by classical and non-classical pathways. Classical signaling occurs through ERα binding to estrogen response element (ERE) in DNA, whereas in nonclassical signaling, ERα participates

in protein-protein interactions to alter the activity of transcription factors at non-ERE sites (ie. NF- κ B, AP-1, or Sp1). Previously, our lab generated a Nonclassical Estrogen Receptor Knock In (NERKI) mouse model using a mutant ER α that is unable to bind to ERE but retains intact nonclassical ER α activity. Adding the NERKI allele in the context of the ER α KO genetic background, it is possible to selectively restore nonclassical aspects of ER α signaling. In this report, we describe nonclassical estrogen actions in 2 different physiologic models: 1) Estrogen negative feedback regulation of luteinizing hormone (LH); 2) Estrogen stimulation of uterine responses. We find that selectively restoring nonclassical ER α signaling to the ER α KO significantly decreased LH (0.70 ± 0.18 ng/ml), indicating that the nonclassical pathway mediates the estrogen negative feedback regulation of LH. In the uterine model, the nonclassical pathway was not sufficient to mediate an acute uterotrophic response, characterized by water imbibition after a single injection of E2. However, the uterine epithelial proliferative response to estradiol (E2) and 4-hydroxytamoxifen (4HT) was retained in mice expressing only the nonclassical allele. These results indicate that nonclassical ER α signaling is sufficient to restore luminal epithelial proliferation but not other estrogen-responsive events such as fluid accumulation. We conclude that nonclassical pathway signaling via ER α plays a critical physiologic role in estrogen mediated negative feedback of the hypothalamic pituitary axis and in the uterine response to estrogen.



572 COMBINATORIAL CONTROL OF GENE EXPRESSION BY DIVERSE CLASSES OF AROMATIC HYDROCARBONS AND ESTROGENIC COMPOUNDS.

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Tethering of agonist activated estrogen receptor (ER) to the aryl hydrocarbon receptor (AHR) at the latter's cognate DNA response element results in profound repression of dioxin-inducible AHR target gene transcription. More recently we have obtained evidence that estradiol (E2) and the selective estrogen receptor modulator (SERM), tamoxifen (TAM) dose dependently repress halogenated aromatic hydrocarbon (HAH)-responsive CYP1A1 and CYP1B1 transcription. Paradoxically, E2 has no effect on polycyclic aromatic hydrocarbon (PAH) induction of either CYP1A1 or 1B1, but TAM significantly enhances their transcription in the human breast cancer cell line MCF-7 and the human endometrial cancer cell line, ECC-1. These ER-dependent alterations in AHR-mediated CYP1A1 transcription result in concomitantly altered levels of CYP1A1 protein. Therefore, there is a ligand selective switch from co-repressor to co-activator function in response to ER tethering. This response is dictated by conformational changes induced by AHR ligand activation in response to different classes of activators. To our knowledge, this is the first demonstration of combinatorial control of transcription in response to exposure to different classes of ligands. Furthermore, TAM-mediated co-activation of CYP1A1 transcription and increased CYP1A1 protein levels in response to exposure to the PAH, benzo[a]pyrene, results in significantly higher levels of stable 8-deoxy-guanosine-benzo[a]pyrene-diol-epoxide adduct formation in MCF-7 cells. Therefore, estrogens play an important role in an organism's response to environmental toxicants. These results highlight the importance of the non-canonical role played by activated ER and represent a paradigm shift in our understanding of transcription factor function in general. This work was supported by the National Institutes of Health Grant RO1 ES04869 and ES11834 (to G.H.P.)



573 THE GLUCOCORTICOID RECEPTOR: ONE GENE, MANY PROTEINS, EXTENSIVE POST TRANSLATIONAL MODIFICATIONS: PROVIDE NEW MECHANISMS FOR TISSUE SPECIFIC ANTI-INFLAMMATORY ACTIONS OF GLUCOCORTICOIDS IN HEALTH AND DISEASE.

J. A. Cidlowski, N. Z. Lu, C. M. Jewell, L. J. Lewis-Tuffin, K. Smoak, J. Zhou and P. Housley. *Laboratory of Signal Transduction, NIH/NIEHS, Research Triangle Park, NC.* Sponsor: J. Cidlowski.

Glucocorticoids are an important class of steroid hormones that regulate numerous homeostatic functions in mammals and lower species. Examples of the processes influenced by glucocorticoids include: glucose homeostasis, protein catabolism, skeletal growth, respiratory function, inflammation, development, behavior and apoptosis. Our understanding of how one hormone regulates all of these diverse processes is limited although most of these actions are thought to be mediated via the glucocorticoid receptor, which is a product of a single gene. However, recent studies in our laboratory have shown that multiple glucocorticoid receptor isoforms are produced from combinations of alternative mRNA splicing and alternative translation initiation. In addition these glucocorticoid receptor isoforms are subject to several

post-translational modifications including ubiquitination, phosphorylation and sumoylation. In this lecture, I will show that the receptor isoforms regulate specific subsets of genes and that post translational modification of the glucocorticoid receptor alters the ability of the receptor to select and use specific hormone response elements. We also will show that the behavior (mobility, degradation, nuclear targeting) of glucocorticoid receptors in living cells is controlled by the chemical nature of the ligand to which they bind. Finally, we will describe new studies on the human glucocorticoid receptor β protein whose expression is associated with various states of glucocorticoid resistance in human disease. Specifically we show for the first time that hGR β is a ligand independent transcription factor.



574 AHR TRANSCRIPTIONAL REGULATION AND ITS IMPACT ON MAMMARY TUMOR BEHAVIOR.

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The aryl hydrocarbon receptor (AhR) has been studied primarily for its control of biologic responses to toxic and/or carcinogenic environmental ligands. However, it is becoming clear that the AhR influences a number of important biologic functions including cell growth, cell death, and possibly cell migration in the absence of exogenous ligands. Ironically, much of the research revealing the role for the AhR in normal cell physiology involves AhR activity in neoplastic cells in which the AhR is frequently hyper-expressed and constitutively active. Constitutively active AhR in tumors often behaves differently than environmental chemical-activated AhR, particularly with regard to transcriptional regulation of target genes. These results suggest context-specific interaction of the AhR with co-regulators and/or with other transcription factors. To begin to address this issue, we have evaluated differential AhR regulation of four genes important in mammary gland tumorigenesis: c-myc, Bax, CYP1B1, and AhRR (AhR repressor). While all of these genes have multiple AhR binding sites in their regulatory regions, only CYP1B1 is up-regulated by constitutively active AhR while transcription of the other three genes is constitutively repressed. AhR activation with environmental ligands yields a different set of outcomes. Some possible transcriptional mechanisms responsible for these differential gene effects and for the differences observed between constitutively active AhR and environmental chemical-activated AhR are presented. Particular emphasis is placed on possible interactions between the AhR, other transcription factors, and transcriptional co-regulators. Finally, the influence of the AhR and the cofactors it recruits on mammary tumor cell growth and progression will be discussed.



575 ROLE OF NF- κ B IN REGULATION OF PXR AND AHR-MEDIATED GENE EXPRESSIONS: MECHANISMS FOR THE SUPPRESSION OF CYTOCHROMES P450 BY PROINFLAMMATORY AGENTS.

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The metabolism and disposition of the xenobiotics and/or endobiotics are regulated at transcriptional level by pregnane X receptor, constitutive androstane receptor (CAR) and aryl hydrocarbon receptor (AhR) which are ligand-dependent transcription factors regulating enormous phase I, II enzymes as well as transporters. It has been known that inflammatory responses and infections decrease metabolisms of xenobiotics and endobiotics in human and experimental animals, resulting in pathological conditions such as the cholestatic diseases. NF- κ B is pleiotropic transcriptional factor that regulates the immune and inflammatory processes. We and others have found that there are interactions between the pathways regulated by PXR, AhR and NF- κ B. These interactions converge at the levels of transcription and affecting several critical stages of the transcriptional processes such as the initiation and elongation. We have found that NF- κ B RelA interacts directly with the receptors and these direct interactions could result in transrepression of the receptor-regulated gene expressions. There are several possible modes of action for the NF- κ B regulated transrepressions: (1) squelching mechanism where NF- κ B activation competes for common transcriptional co-regulators; (2) tethering of the NF- κ B to the regulatory regions by receptor-NF- κ B interactions which may result in further recruitment of repressor complex; and (3) direct disruption of receptor binding to the regulatory regions. The interactions between NF- κ B and nuclear receptors is part of the molecular basis for pathway "cross-talk" and also may be the therapeutic target for treatment of diseases such as the cholestasis (funded in part by NIEHS, National Institutes of Health Grants ES 09859 and ES 09106 and American Heart Association Grant 0355131Y).

576 UNDERSTANDING THE LINKAGES BETWEEN ENVIRONMENTAL TOXICITY AND HUMAN DISEASE: FROM OBSERVATION TO MECHANISTIC INSIGHT AND FROM INSIGHT TO THERAPEUTICS.

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Chemical, physical and biological agents in the environment are believed to play a role in the onset and progression of acute and chronic human illness. Such linkages have been established with some degree of certainty in clinical cases of Parkinsonism caused by recreational exposures to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, the occurrence of vaginal cancers in women exposed in utero to diethylstilbestrol, or the rise in sudden cardiac death and emergency room admissions in areas of the world with considerable air pollution, to name a few. In general, however, little progress has been realized in translating basic observations from the research laboratory to the clinical or regulatory setting. Significant impediments to progress are arguably complex and range from the inherent challenges associated with experimental studies in human populations, to deficiencies in coordinated efforts to "bridge the gap" between scientists interested in the study of fundamental mechanisms of toxicity and clinicians interested in the management and prevention of disease. This symposium will highlight recent advances in our understanding of the role of environmental factors in cardiovascular, neurological and oncological health outcomes with the potential for clinical translation. Examples will be presented that identify ways in which cells compensate for toxicant-induced biological deficits, describe potential therapies born out of efforts to elucidate pathogenesis, and the emerging links between therapy and diagnostics. Another goal will be to provide a forum for discussion of novel research paradigms that bridge the gap between mechanistic insight and clinical applications and that exemplify the strength of trans-disciplinary approaches to study of environmental toxicity in human disease.

577 EXAMPLES OF GENE-ENVIRONMENT INTERACTIONS IN PARKINSON'S DISEASE.

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Sponsor: K. Ramos.

The heritability of Parkinson's disease (PD) is low and it does not segregate in families in a simple Mendelian fashion [Ann Neurol 2004;56:495-502; Ann Neurol 2006;59:788-795]. Therefore, it is widely considered that PD is a complex disease and that the environment plays a major role in its cause. While there is epidemiological evidence that pesticide exposures may confer susceptibility, and while there is experimental evidence that toxins can model PD, there is little evidence that specific pesticide exposures cause PD [Environ Health Perspect 2006;114:156-164; Mov Disord, DOI: 10.1002/mds.21009]. Furthermore, there are only rare examples of specific gene-environment interactions in PD [Ann Neurol 2004;55:430-434]. The most convincing evidence that genetic factors also contribute to PD has been derived from linkage analyses of multiplex kindreds. While causal mutations are rare, common variants in some of these genes may confer risk to PD across populations worldwide [Ann Neurol 2004;55:512-521; JAMA 2006;296:661-670]. For one of these linkage-derived susceptibility genes, alpha-synuclein, there is experimental evidence of interactions with pesticides [Neurotoxicology 2002;23:527-536]. In this presentation, we will present preliminary data from a study of the molecular epidemiology of Parkinson's disease (ES10751) regarding possible interactions between the alpha-synuclein gene and pesticides and also provide an overview of similar epidemiological studies of gene-environment interactions in PD. The demonstration of specific gene-environment interactions in PD may provide mechanistic insights that will guide the development of primary and secondary prevention strategies.

578 CHRONIC EXPOSURE TO ACROLEIN, A UBIQUITOUS POLLUTANT, INDUCES DILATED CARDIOMYOPATHY.

S. D. Prabhu, A. Bhatnagar, J. Luo, Y. Gu, R. Ortines, S. Srivastava and B. Chandrasekar. *Molecular Cardiology, University of Louisville, Louisville, KY.*
Sponsor: K. Ramos.

Although the volatile α,β -unsaturated aldehyde acrolein (ACR) is classified as a high priority air and water toxic, the cardiac effects of ACR exposure are unknown. As ACR is a potent electrophile, we hypothesized that chronic ACR exposure would induce oxidative stress and inflammation in the heart that contributes to cardiomyopathy. Adult C57BL/6 mice ($n = 15$ -18/group) were gavaged either 1 mg/kg/d ACR, a dose approximating human daily oral aldehyde consumption, or water (Control) for 48 d. Oxidative stress was increased in ACR vs. Control hearts, evidenced by a 4-fold increase in membrane bound protein-hydroxy-2-nonenal adducts by immunoblotting ($p < 0.05$). Echocardiography revealed left ventricular

(LV) dilatation and systolic dysfunction in ACR mice ($p < 0.05$). Myocyte cross-sectional area (CSA) on tissue sections was increased 2.5-fold in ACR hearts over Control ($p < 0.001$), indicating myofiber hypertrophy. Pressure-volume analysis revealed significant ($p < 0.05$) depression of both systolic function (30% decrease in dP/dtmax) and relaxation (30% increase in tau). ACR hearts further demonstrated a 6-fold increased ($p < 0.001$) activation of the redox sensitive transcription factor and inflammatory mediator nuclear factor (NF- κ B), evaluated by both DNA binding studies and immunostaining for the activated p65 subunit of NF- κ B. Gene expression of tumor necrosis factor- α , an NF- κ B responsive inflammatory cytokine considered to have an important role in pathologic remodeling, was increased 4-fold in ACR hearts ($p < 0.001$). ACR withdrawal studies after chronic exposure revealed only mild reversibility of the LV functional and structural changes. We conclude that chronic environmental exposure to ACR induces myocardial inflammation and oxidative stress, together with myocyte hypertrophy, LV dilatation and dysfunction that is only partially reversible upon removal of exposure. Similar environmental exposures in humans may contribute to the pathogenesis of dilated cardiomyopathy of unclear etiology.

579 CHILDHOOD CANCER AS MODEL FOR DEVELOPMENTAL DISORDERS.

A. F. Olshan. *Department of Epidemiology, University of North Carolina, Chapel Hill, NC.* Sponsor: K. Ramos.

The investigation of childhood cancer has provided some valuable knowledge on the mechanisms of carcinogenesis. Moreover, there is significant potential for childhood cancers to provide unique insight into the role of preconceptional and *in utero* exposures on developmental dysregulation and disease occurrence not only in childhood, but also into adulthood. Early studies of molecular genetic alterations in retinoblastoma, Wilms tumor and neuroblastoma have provided critical data for the delineation of the two-stage model of cancer initiation and progression. Advances in molecular biology have permitted the development of new tools and models, including animal systems, to investigate the etiology of childhood cancer. In parallel, more than a decade of epidemiologic studies on the etiology of childhood cancer has been conducted. Both the experimental animal and epidemiologic studies have provided valuable clues on relevant etiologic agents and mechanisms. Further, the work has identified important challenges in this line of research that apply more broadly to the study of developmental disorders. This presentation will provide an overview and perspective on the rationale, current evidence, and future of research on the etiology of childhood cancer. This presentation will emphasize the continuing need for closer collaboration between toxicological and epidemiologic investigators.

580 HORMONES, THE ENVIRONMENT AND CANCER.

C. Walker. *University of Texas, Smithville, TX.*

The development of hormone-dependent cancers of the breast and female reproductive tract can be modulated by environmental exposures. Both the man-made and natural environment contain compounds such as pesticides and phytoestrogens that can mimic normal ovarian hormones. Exposure to these "environmental estrogens" or xenoestrogens can increase or decrease cancer risk, depending on the exposure and the target tissue. It is now appreciated that the timing of exposure is also a critical determinant of the toxic response to environmental estrogens. The human experience with DES is the best appreciated example of how an environmental exposure to a xenoestrogen during a critical developmental period can cause an adverse health outcome. Women exposed *in utero* to DES during development of the reproductive tract have congenital reproductive tract abnormalities, and are at greatly increased risk for development of otherwise rare cancers such as vaginal clear cell carcinoma. In the breast, xenoestrogen exposure can have opposing effects on breast cancer risk depending on the timing of exposure. Exposures that occur prenatally can increase susceptibility to the induction of breast cancer, while later post-natal exposures can be protective, due in part to the induction of a more differentiated phenotype in the developing mammary gland. Recently, environmental estrogens have been shown to signal via non-genomic mechanisms, and these rapid responses mediated by cell signaling pathways such as MAPK and PI3K may also contribute to toxic response(s) that occur following exposure to these compounds. Finally, we and others have shown that early life exposures to xenoestrogens during critical developmental windows can cause epigenetic alterations that "reprogram" the genome in such a way as to alter patterns of gene expression in the adult and increase the risk of developing hormone-dependent cancer. In some cases these early life exposures have been shown to alter patterns of promoter DNA methylation, but other epigenetic alterations, such as histone modifications, are also candidate mediators for the reprogramming that occurs following developmental exposure to environmental estrogens.

**581** ADVANCES AND CHALLENGES REGARDING MODE-OF-ACTION IN HUMAN HEALTH RISK ASSESSMENT.

N. Keshava² and R. Sams¹. ¹Office of Research & Development, National Center for Environmental Assessment, U.S. EPA, Research Triangle Park, NC and ²Office of Research & Development, National Center for Environmental Assessment, U.S. EPA, Washington, DC.

Mode of action (MOA) analysis is one of the key processes utilized to assess acceptable levels of risk for humans. Central to MOA analysis is the identification of empirically measured key events (i.e. necessary or rate limiting steps) in the development of chemical-induced toxicity. Several recently published guidance documents (USEPA Cancer Guidelines, 2005; IPCS Framework for Analyzing the Relevance of a Cancer MOA for Humans, 2005 Draft for Public Comment) represent a general scientific consensus on the preferred approach for MOA analysis. As the use of MOA information advances, several types of risk assessment issues are emerging. These issues include the weight of evidence (WOE) necessary to establish a MOA for a given compound, harmonization of cancer and non-cancer endpoints through MOA analysis, determining the approach for MOA that supports low dose extrapolation, and how these frameworks should be used for chemical mixtures risk assessment. An effective avenue to overcome these identified issues is to consider risk assessment case studies; since no two chemicals have the same amount or type of MOA information. These case studies should be evaluated based upon the supporting WOE, consistency, and generally through agreement within the scientific community. Additionally, the framework for MOA analysis can be revised based upon recurring issues that are identified through the use of case studies. Throughout this evolving process, approaches that are determined adequate for individual chemicals should be considered by the scientific community as to how the methodology can be applied to "real-world" or comprehensive risk assessments (i.e. chemical mixtures). (Disclaimer: The views expressed in this abstract are those of the authors and do not represent the policy of the U.S. Environmental Protection Agency).

**582** INTERPRETATION OF CONFLICTING GENOTOXICITY DATA TO IDENTIFY AN INDIRECTLY MUTAGENIC MODE OF ACTION: A CASE STUDY INVOLVING CARBON TETRACHLORIDE.

D. A. Eastmond. *Environmental Toxicology Program, University of California, Riverside, CA.*

In the new EPA cancer risk assessment guidelines, the classification of whether a chemical is mutagenic or not plays an important role in determining whether a linear or non-linear approach should be used to extrapolate into the low dose region. However, frequently both positive and negative results are seen in genotoxicity studies. In this presentation, discrepant genotoxicity data on carbon tetrachloride will be discussed and shown to be consistent with an indirect mode of action involving lipid peroxidation and cellular toxicity. Carbon tetrachloride is quite unique in that it is essentially negative in inducing gene mutations in *Salmonella* bacteria but is consistently positive in inducing deletion mutations and aneuploidy in fungi. Studies in mammalian cells in vitro have been largely negative. However, positive results have occasionally been seen. Similarly, a large number of in vivo studies have been performed following treatment of rodents with carbon tetrachloride with mixed, largely negative, results. However, DNA adducts, primarily those derived from oxidation and lipid-peroxidation-derived products, have been repeatedly seen in the liver as have DNA double-strand breaks and micronucleated cells. Mechanistic studies are consistent with a role for lipid peroxidation and toxicity as DNA double-strand breaks can be formed from lipid peroxidation products or during nuclease activation during cell toxicity. Consistent with this proposed mode of action, the positive genotoxicity/mutagenicity results that have been seen in fungi and mammalian systems almost always occur concurrent with or during recovery from extensive cellular toxicity. The implications of this mode of action for extrapolation into the low dose region will also be discussed.

**583** EPIGENETIC PROCESSES AS KEY EVENTS IN THE MODE OF ACTION FOR TUMOR FORMATION.

R. J. Preston. *U.S. Environmental Protection Agency, Research Triangle Park, NC.*
Sponsor: N. Keshava.

The U.S. EPA's Guidelines for Carcinogen Risk Assessment provides a framework for the development of a cancer risk assessment for exposures to environmental chemicals. A major component of this framework for any particular chemical is the assignment of a mode of action for the induction of tumors and a description of the key events that define this mode of action. The concentration for key events has been on the induction of mutations in critical genes for tumor formation and the role of these mutations on enhanced cell proliferation or in parallel the role of cell proliferation in enhancing the production of mutations in critical genes. It is be-

coming increasingly apparent that there is an additional class of cellular alterations that can have a significant impact upon tumor development and susceptibility to tumor induction. These alterations are considered under the general term of epigenetic effects. Epigenetic effects can be heritable changes to DNA or protein codes. These effects include DNA methylation changes that can in turn lead to changes in gene imprinting, chromatin remodeling that can have an impact on all cell house-keeping processes, and the formation of genomic instability. In addition, it has been proposed that there can be developmental reprogramming at early life stages that can change developmental patterns throughout life and indeed appear also to be heritable. The induction of such reprogramming by chemical exposures of individuals during early life stages could potentially lead to an enhanced tumor production at much later life stages. It is necessary to consider these epigenetic changes along with other key events in the tumor process and also what might be their impact upon overall cancer risk assessment. (This abstract does not necessarily reflect EPA policy)

**584** DISRUPTION OF BRAIN CELL REPLICATION AND NEUROTRANSMITTER SYSTEMS DURING DEVELOPMENT LEADING TO COGNITIVE DYSFUNCTION: DEVELOPMENTAL NEUROTOXICITY OF NICOTINE.

W. Slikker¹, Z. Xu², E. D. Levin³ and T. A. Slotkin³. ¹National Center for Toxicological Research, Jefferson, AR, ²Genelogic, Washington, DC and ³Duke University Medical Center, Durham, NC.

Developmental exposure to nicotine in rats results in neurobehavioral effects such as reduced locomotor and cognitive function. Key events in the animal MOA include binding to the nicotinic cholinergic receptor during prenatal and/or early postnatal development. This leads to premature onset of cell differentiation at the expense of cell replication, which leads to brain cell death or structural alterations in regional brain areas. Other events include an initial increase followed by a decrease in adenylyl cyclase activity, as well as effects on the noradrenergic, dopaminergic and serotonergic neurotransmitter systems. Because the nicotine receptor is also present in the developing human brain and the underlying biology for DNA synthesis and cell signaling is comparable, this MOA is likely to be relevant for humans. Although the effects of nicotine exposure in developing humans is not well documented, nicotine exposure as a result of cigarette smoking during pregnancy is associated with several physiological and behavioral outcomes that are reminiscent of the effects of nicotine alone in animal models. As data come available with the advent of the use of the nicotine patch in pregnant humans, the question as to the relative importance of smoking per se vs. nicotine alone may be determined.

**585** APPLICATION OF MODE OF ACTION DATA IN A RISK ASSESSMENT FOR METHYL IODIDE.

M. Gargas¹ and J. H. Kinzell². ¹The Sapphire Group, Dayton, OH and ²Arysta LifeScience North America Corporation, San Francisco, CA.

Recent studies have indicated that acute exposures to methyl iodide (MeI) produce a number of effects in laboratory animals, including fetal toxicity, neurotoxicity, and degeneration of the nasal epithelium. An understanding of the mode of action (MOA) by which these effects are produced by MeI is necessary in guiding critical decisions used in the risk assessment. These include the selection of the appropriate internal dose measure(s) calculated using physiologically based pharmacokinetic (PBPK) modeling, and evaluating the relevance of the observations in animals to human health. Several potential MOAs were evaluated and compared for each endpoint using the modified Hill Criteria. These evaluations and comparisons indicated that: 1) fetal toxicity in rabbits is most likely a result of modulation of thyroid hormones due to elevated iodide concentrations in the fetus resulting from metabolism of MeI; 2) neurotoxicity observed in rats is most likely the result of a generalized inhibition of ion currents in the membranes of nerve cells due to high concentrations of parent chemical; and 3) degeneration of the nasal epithelium of rats is dependent on the sustained depletion of GSH below critical levels due to the metabolism of MeI in the nasal tissues.

**586** APPLICATION OF MODE OF ACTION AND DOSE-RESPONSE INFORMATION IN A CHEMICAL MIXTURES RISK ASSESSMENT.

J. C. Lipscomb. *National Center for Environmental Assessment, U.S. EPA, Office of Research and Development, Cincinnati, OH.*

Humans are virtually continuously exposed to multiple chemicals through dietary, occupational, recreational and interaction with the indoor and outdoor environment. Chemical mixture risk assessment differs from single chemical mixture risk

assessment in several ways, perhaps most importantly in the amount of toxicity data available upon which to base predictions. Absent data on the mixture, or a toxicologically similar (surrogate) mixture, a component-based approach should be undertaken; approaches of this type require decisions about similarity of mode of action to properly group chemicals. Unless data are available to indicate an interaction of the risks from component chemicals, the approach will be based on the assumption of additivity of risks. In that case, similarity or independence of mode of action will determine whether dose addition (i.e., relative potency factor approach) or response addition should be employed, respectively. Most toxicity data are from single-chemical studies conducted at moderate to high dose. In these cases, uncertainty is introduced at two points; 1) whether information on the mode of action warrants application of dose or response addition, and 2) extrapolation of the dose response relationship to lower levels of exposure (those to which humans may be exposed). The success and acceptance of a mixtures risk assessment depends heavily on interpretation of mode of action and dose-response data. This presentation will synopsise mixtures risk assessment approaches and highlight the application of mode of action information.



587 ALTERNATIVE AND INTEGRATED APPROACHES TO SKIN SENSITIZATION TESTINGS.

I. Kimber² and G. Gerberick¹. ¹Procter & Gamble, Cincinnati, OH and ²Central Toxicology Laboratory, Syngenta, Macclesfield, United Kingdom.

Skin sensitization resulting in allergic contact dermatitis is an important occupational and environmental health issue, and there is a continuing need for effective hazard identification and risk assessment. In the past two decades there has been considerable progress in the toxicological evaluation of skin sensitizing chemicals, but there remains a need to identify alternative approaches that will reduce, refine, and eventually eliminate, the need for experimental animals in safety assessment. To address this need, and in response to new and emerging legislation, there has been a substantial and increasing investment in the design and development of alternative approaches to evaluation of skin sensitization. Among the new initiatives are: the further exploitation of structure-activity relationships, measurement of the ability of sensitizing chemicals to form stable associations with proteins and peptides, examination of the stimulation by chemical allergens of changes in the phenotype and function of dendritic cells (DC) and DC-like cells, and the use of integrated testing systems. Progress in each of these areas, and their likely future contributions to hazard identification, determination of relative potency and risk assessment will be discussed.



588 SKIN SENSITIZATION HAZARD IDENTIFICATION AND HAZARD CHARACTERIZATION: WHAT ARE THE ISSUES?

I. Kimber. Central Toxicology Laboratory, Syngenta, Cheshire, United Kingdom.

Skin sensitization resulting in allergic contact dermatitis is an important occupational health issue. Many hundreds of chemicals have been implicated as skin sensitizers, and allergic contact dermatitis is the commonest manifestation of immunotoxicity in humans. There are currently available guinea pig tests and the mouse local lymph node assay for the identification of skin sensitizing chemicals. There is, however, an increasing requirement to design and develop alternative approaches that refine or reduce the use of animals in skin sensitization safety assessment, or that eliminate altogether the requirement for animals. Both in vitro approaches and prediction models based on structure-activity relationships (SAR) are being explored. However, there are difficult challenges to be addressed in developing alternative methods that reflect sufficiently the complexities of immune responses to chemical allergens, and that will provide the required levels of sensitivity and specificity. Moreover, effective risk assessment requires information on relative skin sensitization potency and such data will not normally be available from in vitro assays or SAR analyses. It may prove necessary to consider the application of testing platforms that integrate information from a variety of independent sources to inform judgements on skin sensitization hazards. Progress is being made in each of these approaches and will be described here.



589 THE BENEFITS AND LIMITATIONS OF (QUANTITATIVE) STRUCTURE-ACTIVITY RELATIONSHIPS ((Q)SARS).

G. Patlewicz¹, A. O. Aptula² and D. W. Roberts³. ¹European Chemicals Bureau (ECB), European Commission, Ispra, VA, Italy, ²SEAC, Unilever Research, Sharnbrook, Beds, United Kingdom and ³School of Pharmacy and Chemistry, Liverpool John Moores University, Liverpool, United Kingdom. Sponsor: E. Gerberick.

There is currently considerable interest in exploiting the information derived from (quantitative) structure-activity relationships ((Q)SARS) and expert systems for use in hazard identification and risk assessment. Much momentum has arisen from

preparations for the forthcoming EU Legislation REACH (Registration Evaluation Authorisation of Chemicals), which calls for more widespread use of (Q)SARS to assess existing chemicals. The 7th Amendment to the Cosmetic Directive has also prompted interest in (Q)SARS since a ban on animal testing for cosmetic ingredients will come into effect from 2009 onwards.

QSARS are statistically established correlations relating a quantitative parameter(s) derived from chemical structure to a quantitative measure of biological activity. SARs are (qualitative) associations between a chemical substructure and the potential of a chemical containing that substructure to exhibit a certain biological effect. SARs and QSARS have been developed for skin sensitization. Both these types of models can be found in expert systems, usually commercial programs which comprise a collection of SARs encoding existing knowledge (e.g. DEREK, Hazard Expert), statistically derived QSARS (e.g. TOPKAT, MCASE) or a hybrid of both (e.g. TIMES). Many of the (Q)SARS to date have been developed on the basis of reaction chemistry where the chemical behaves as an electrophile and the skin protein as a nucleophile. Some of the most common electrophile-nucleophile reactions include Michael-type, SN2, SNAr, acylation and Schiff-base formation.

Current work at the ECB is aiming to characterize a number of available (Q)SARS and expert systems for skin sensitization to determine their scope (by using reaction chemistry), benefits and limitations. We will illustrate some examples and discuss the realistic prospects for their use in future safety assessments.



590 PROTEIN AND PEPTIDE BINDING: AN EMERGING STRATEGY.

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Allergic contact dermatitis resulting from skin sensitization is a common occupational and environmental health problem. In order for a chemical to function as a sensitizer it must penetrate the skin, react with protein and be recognized as antigenic by immune cells. Protein reactivity correlates with skin sensitization potential and this relationship is attributed to the fact that chemical allergens, either directly or following biotransformation have electrophilic properties that enable them to form covalent bonds with nucleophilic amino acids, like cysteine and lysine. This relationship was used as the premise for the development of an in vitro assay to measure the reactivity of chemical allergens with peptides containing nucleophilic amino acids (e.g. cysteine). Specifically, in vitro assays using peptides containing cysteine or lysine, or glutathione as surrogate nucleophiles have been evaluated. Findings to date suggest that a strong correlation exists between sensitization potency and the ability of a chemical to react with protein. Analysis of more than 80 chemicals, including sensitizers and non-sensitizers, confirmed the correlation between reactivity and sensitization potential and has supported further evaluation of peptide reactivity testing in interlaboratory studies as a screening approach for skin sensitization testing. These peptide reactivity data were used to develop prediction models based on classification tree methodology. Collectively, data generated support the use of a peptide reactivity assay a potential method to screen the sensitization potential of new chemicals. This approach along with other approaches may lead to a reduction or replacement of animal use in skin sensitization testing.



591 CELLULAR ASSAY SYSTEMS: DENDRITIC CELLS AND BEYOND.

C. Ryan. Procter & Gamble Company, Cincinnati, OH.

The mechanistic understanding of allergic contact dermatitis (ACD) has increased substantially in recent years; the challenge is to apply this knowledge to the design of a predictive cell-based in vitro alternative test. Langerhans cells (LC), considered the principal antigen presenting cell (APC) in the skin, play a key role in the development of allergic contact sensitization. A number of changes are known to occur in LC as a result of exposure to chemical allergens including the internalization of surface Major Histocompatibility Complex (MHC) class II molecules via endocytosis, the induction of tyrosine phosphorylation, the modulation of cell surface markers and cytokine expression. Given the importance of LC in the initiation of skin sensitization, it seems appropriate to explore whether there are opportunities to develop alternative approaches to hazard identification based upon chemical-induced changes in phenotype or function of these cells. While the availability of sufficient numbers of LC isolated from human or murine sources has been a limiting factor, some investigators have focused on events which occur in epidermal LC following their exposure to chemical haptens as the basis for predictive in vitro methods for skin sensitization. However, more work has been conducted using LC-like dendritic cells (DC) derived from bone marrow or cord blood CD34+ precursors or peripheral blood mononuclear cells as greater quantities of these LC-like APCs can be obtained which allow for the characterization of the effects of allergen exposure and permit investigation of their utility as an in vitro model system for the development of predictive tests. More recently, commercially-available human cell lines

such as the KG-1, U-937, THP-1, and MUTZ-3 cell lines are being explored as potential surrogates for DC. Conceptually, a cell-based in vitro test would be integrated into a battery of test methods that together address the key steps involved in the initiation of ACD and could be used in a weight-of-evidence approach for the identification of skin sensitization hazard.

592 INTEGRATED SYSTEMS AND A MODIFIED LOCAL LYMPH NODE ASSAY.

D. Basketter. *Unilever, Sharnbrook, United Kingdom.*

The existing (in vivo) approaches to the predictive identification and characterisation of skin sensitisation hazard have proven very successful. With care, virtually all chemicals which possess intrinsic skin sensitisation hazard for man can be predicted; risk assessment and management can then be applied, particularly through use of relative potency estimation in the local lymph node assay (LLNA). However, the need to reduce, and ultimately replace, animal testing conflicts with the pressure to conduct retrospective assessment of thousands of existing chemicals. A modified LLNA, which uses only the single high dose group, offers the chance to significantly reduce animal use whilst maintaining an accurate identification of skin sensitisation hazard. Unfortunately, it also brings the consequence that data on relative potency are not generated, so compromising risk assessment/management. Strategies for total animal replacement focus on the main elements of the induction of skin sensitisation: bioavailability, electrophilicity, stimulation of cytokine release from keratinocytes and the activation of dendritic cells. Here, the challenge is not only how to use the output from assays based on these elements to determine the presence of skin sensitisation hazard, but also how to integrate them into a holistic view of the relative potency of an identified skin sensitising chemical. One potential paradigm for this will be discussed, particularly in relation to the now extensive published LLNA potency database.

593 ALTERNATIVE AND INTEGRATED APPROACHES TO SKIN SENSITIZATION: RECENT PROGRESS AND THE WAY FORWARD.

W. S. Stokes. *NICEATM, NIEHS, Research Triangle Park, NC.*

Recent significant progress has been made in reducing and refining the use of animals for assessing skin sensitization potential of chemicals. Progress is also being made in developing methods and strategies that may eventually provide for partial or full replacement of animal use. The validation and international regulatory acceptance of the murine local lymph node assay (LLNA) now allows it to be used in place of standard guinea pig based assays in most testing situations and fully eliminates the potential pain and distress that could previously occur with this testing. New approaches such as use of a LLNA limit dose test will further reduce the numbers of animals needed for this procedure. New models and approaches are also being developed that may eventually allow for decisions about skin sensitization potential of a substance to be made without animals. These include advances in structure-activity relationship models, assessment of protein and peptide bonding potential, and the evaluation of responses in mechanism based cellular systems. Integration of the responses from a battery of test models such as these is also expected to provide improved predictions of sensitization potential. Adding knowledge about physical and chemical attributes of substances associated with sensitivity or non-sensitivity may further strengthen such predictions. Evaluation of these integrated approaches will require adequate validation of each individual model as well as the entire test battery with the same set of reference substances for which there is high quality data from the accepted reference test method. High quality data from human studies for the same reference substances are also desirable, where this can be ethically accomplished. Regulatory acceptance of a proposed integrated testing approach will require demonstration that the approach will provide for equivalent or improved prediction of sensitization potential. Further development, validation, and integrated use of mechanism-based sensitization testing models can be expected to further reduce animal use while providing for continued protection of human health.

594 THYROID HORMONE DISRUPTION: FROM KINETICS TO DYNAMICS.

K. M. Crofton¹ and J. W. Fisher². ¹*Neurotoxicology Division, U.S. EPA, Research Triangle Park, NC* and ²*Department Environ Health Sciences, University Georgia, Athens, GA.*

A wide range of chemicals with diverse structures act as thyroid disrupting chemicals (TDCs). Broadly defined, TDCs are chemicals that alter the structure or function of the thyroid gland, alter regulatory enzymes associated with thyroid hor-

mones (THs), or change circulating or tissue concentrations of THs. TDCs represent a group of structurally diverse chemicals that act through a variety of mechanisms. While serum concentrations of THs are useful bio-indicators of TDCs, the relationship between alterations in serum concentration of THs and developmental toxicities is uncertain, particularly at low doses. This symposium will focus on recent collaborative research aimed at reducing the uncertainties by delineating the relationship between developmental neurotoxicity of the brain and perturbation in the maternal/fetal and neonatal hypothalamic-pituitary-thyroid (HPT) axis. A brief introduction of this session will be given explaining how the speakers presentations will fit together and what we expect to accomplish in this workshop.

595 A PRIMER OF COMPARATIVE THYROIDOLOGY: RELEVANCE TO METHODS AND MODELS.

D. C. Ferguson. *Department Physiol Pharmacology University Georgia, Athens, GA.*
Sponsor: K. Crofton.

While the basic principles of thyroid physiology are common in most vertebrate species, there are key regulatory differences between species such as serum thyroid hormone binding proteins, and hormone and iodide turnover rates. Extrapolating results from one species to another (e.g. rat to man), requires careful consideration of the similarities and differences not only circulating hormones, but also differences in tissue responses of deiodinase and conjugative enzymes involved with hormone clearance. Accurate measurement of free T4 by dialysis is a technique which, if applied appropriately, provides an accurate measurement in serum from all species. Free T4, under most circumstances, predicts the steady-state biological response to thyroid hormone including the feedback response of serum TSH concentrations. However, it is now well accepted that changes in thyroid status or development of non-thyroidal disease exert tissue- or cell type-specific effects. To investigate these tissue changes, it is critical to evaluate tissue concentrations of T3 and T4 and the responses of proteins whose expression and/or activity is regulated by thyroid hormone: Type 1 and Type 2 5'-deiodinase enzymes (D1 and D2), membrane thyroid hormone transport proteins such as organic acid transport proteins (e.g. OATP1C1) and monocarboxylate transport proteins (e.g. MCT8). Accurate measurement of serum thyroid hormone and TSH concentrations in model species, while using assay principles applicable to all species, requires recognition of differences in serum binding protein types and quantity, and differences in TSH structure. Beyond this, it is critical to recognize the species-specific developmental stages of hypothalamic-pituitary-thyroid axis regulation. Examination of in vivo and in vitro studies in model species allows the recognition of conserved regulatory motifs, as well as key differences relevant to the interpretation of perturbations induced by environmental xenobiotics.

596 DEVELOPMENTAL TARGETS AND MECHANISMS OF XENOBIOTICS THAT ALTER THYROID HORMONE HOMEOSTASIS.

M. J. DeVito. *U.S. EPA, Research Triangle Park, NC.*

There are multiple targets for environmental chemicals that disrupt thyroid hormone homeostasis in rats. This includes chemicals that affect iodine uptake, hormone synthesis, transport and catabolism. A number of species differences in mechanisms of thyroid disruption create uncertainties and difficulties in extrapolating rodent data to man. In particular, there are species differences in the role of nuclear receptors in thyroid hormone homeostasis and the importance of the different thyroid hormone transport proteins. A more complete understanding of species differences in these processes will improve species extrapolation of thyroid hormone disruptors.

597 LOW-DOSE THYROID INSUFFICIENCY: IMPACT ON NEURODEVELOPMENT.

M. E. Gilbert. *U.S. EPA, Research Triangle Park, NC.*

Thyroid hormones are critical for normal growth and development of the brain. It is well established that severe reductions in thyroid hormone lead to mental retardation in children. Recent evidence indicates that modest hormone reductions in pregnant women result in subtle changes in cognition and IQ deficits in children. Similarly, environmental insults produce modest perturbations in the thyroid axis, and little is known of their potential hazards to normal brain development. Traditional rodent models of hypothyroidism have been limited to severe depletions, with little information available on potential adverse outcomes of milder forms of developmental thyroid insult. New research using rodent models is exploring sensitive biochemical, physiological and morphological biomarkers of adverse neurological outcomes.

598 PBPK AND BIOLOGICALLY BASED COMPUTATIONAL MODELS FOR THYROID ACTIVE CHEMICALS AND THE HYPOTHALAMIC-PITUITARY-THYROID AXIS TO EVALUATE DEVELOPMENTAL NEUROTOXICITY.

J. W. Fisher. *Department Environ Health Sciences, University Georgia, Athens, IL.*

Computational models of developmental thyroid disruption require an understanding of the kinetics of thyroid active chemicals in laboratory animals of various reproductive states. These models require unique reproductive state-specific biological determinants that control dosimetry and mode-of-action. New research is using state-of-the-science computational models to address indirect acting agents that exert their effects on the developing brain via the hypothalamic-pituitary-thyroid axis. Future research is needed to develop computational models of the adverse outcomes on neurodevelopment caused by thyroid disruption.

599 LOW-DOSE THYROID TOXICANTS AND NEURODEVELOPMENT IN RAT: IS IT RELEVANT TO HUMANS?

R. Zoeller. *Department Biology, University Massachusetts, Amherst, MA.*

The original concept of the critical period of thyroid hormone (TH) action on brain development was proposed to identify the postnatal period during which TH supplement must be provided to a child with congenital hypothyroidism to prevent mental retardation. As neuropsychological tools have become more sensitive, it has become apparent that even mild TH insufficiency in humans can produce measurable deficits in very specific neuropsychological functions, and that the specific consequences of TH deficiency depends on the precise developmental timing of the deficiency. Models of maternal hypothyroidism, hypothyroxinaemia and congenital hyperthyroidism have provided these insights. Experimental literature lags behind clinical studies in providing a mechanistic explanation for each of these observations. Recent studies confirm that the specific action of TH depends upon developmental timing. Molecular studies of TH action in the developing brain are generating hypotheses concerning possible mechanisms to account for these pleiotropic actions. New research with rodent models of TH insufficiency is improving our understanding of effects of environmental xenobiotics in humans. Finally, new research directions are required to advance the state of the science for assessing human health and thyroid active chemicals.

600 ANIMAL RIGHTS EXTREMISM AND THE RESPONSIBLE USE OF ANIMALS IN RESEARCH: THE PAST, PRESENT, AND FUTURE.

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The necessary use of animals in biomedical and toxicological research and to fulfill regulatory safety requirements must be acknowledged, with recognition of and respect for the important contribution of animals in the scientific progress. Animals must be humanely treated in accordance with applicable animal welfare laws/regulations and full consideration of the 3Rs - replacement, reduction, and refinement. The Session will call attention to the distinction between animal welfare and animal rights extremism and will explore ways to think about the development of replacement alternatives. How different stakeholders (e.g., the scientific community vs. the animal rights community) look at the 3Rs will be presented. Examples of the actions of animal rights extremists and a new brand of internet-based extremism will be presented and effective and appropriate responses addressed. Measures taken by the scientific community and others in coordinating an effective response to this extremism will be discussed and several "next steps" required to consolidate recent gains highlighted. The processes and practices presented here will help us move forward as humane scientists without sacrificing scientific quality due to illegal tactics and intimidation.

601 WHAT TO DO WHEN YOUR RESEARCH IS UNDER ATTACK.

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When 13 beagle dogs were stolen from the Air Pollution Health Effects Laboratory, replacing the animals was not the most serious problem. The media rushed in, threats were received, staff and their families became fearful, the author's home was picketed, speaking requests arrived, and security systems had to be put in place. Yet, in the midst of the turmoil, opportunities to educate local and national communities were presented. Initially, our efforts were directed by local and national newspaper, magazine, and radio and television reporters. We had the opportunity to explain how and why we did our studies, and why the charges against us were false. As a result, we were usu-

ally given equal, or greater, weight in the media than those who were making inaccurate charges. Good preparation and honest communications helped win the hearts of reporters and the public, and the respect of many of the activists.

If an investigator's home is going to be picketed, they must deal with the family's fears, and let the neighbors know what to expect. During the picketing, it is good to leave your home, leaving your pets in a safe location. Try to arrange with your institution to have security present, and spokespeople to meet the press. Also, inform the local police.

We had numerous interactions with law enforcement. Institutional security focused on improving surveillance and security. Anti-terrorist agencies were interested in finding the animals and the abductors. We were told that the stolen dogs were photographed for fundraising purposes, and then killed. Sadly, we had to identify their charred skeletal remains.

The major lesson that we learned was that it is important to earn the respect and support of the press and the public. That is achieved by open and honest communication.

602 THE SCIENCE OF ALTERNATIVES.

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The word alternatives, often thought of as only replacement, has a deeper and more profound implication when used in its proper context of the 3Rs - replacement, reduction and refinement. There are those in both the scientific community and in the animal rights community that purposefully only focus on replacement. It provides for a more self serving perspective. The 3Rs as used by Bill Russell and Rex Burch in their book "The Principles of Humane Experimental Technique" did not use the word alternative. It was Smyth, several years later, that equated the 3Rs with Alternatives. In meeting after meeting of stakeholders and in the reaffirmation of the 3Rs in a publication in the mid 90's it was decided that the best way to approach this issues was to always use the word alternative with one of the Rs, thus refinement alternative. Practicing the 3Rs is the way to achieve the most humane science. Bill Russell and Rex Burch hypothesized that humane science is the best science. It has taken almost 40 years to be able to prove that this hypothesis is correct. The Science of Alternatives will explore a way to think about the development of replacement alternatives and examine how different stakeholders look at the 3Rs.

603 FACE-OFF AGAINST 21ST CENTURY EXTREMISM.

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Huntingdon Life Sciences, a contract research organization with laboratories located in the US and UK has been the focus of a concerted effort by animal rights extremists to put it out of business by targeting its employees, business associates, financial backers and suppliers. By proactively engaging industry, law enforcement, the courts, and governments on both sides of the Atlantic, HLS has been a successful participant in engineering an outcome that has resulted in high-profile convictions, significantly stronger laws in the UK, similar positive legislative momentum in Washington DC and greatly improved integration of efforts among the various stakeholders in mitigating the threat to research posed by animal rights extremists. This presentation will review measures taken by HLS and others in coordinating an effective response to this new brand of internet-based extremism. It will assess the current environment, and will propose several "next steps" required to consolidate recent gains.

604 THE PHARMACEUTICAL INDUSTRY AND ANIMAL EXTREMISM: ACTION, REACTION OR INACTION?

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The words and activities of animal rights extremists can be truly shocking. However it is the response that those targeted make that actually determines their impact. What is an effective and appropriate response, and how is it implemented?

605 PROTECTIVE MEASURES.

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This presentation will provide proven protective measures that can be implemented to mitigate potential animal extremists from causing harm. While not intended as an "all-inclusive" checklist, these strategies can keep a person out of harms way, take them out of harms way, and effectively deal with extremists tactics using legally

sound, crime prevention-type principles. Areas to be covered include: demonstrations, harassing of neighbors, protection of family and friends, as well as precautions to take at home, the office, and in your vehicle.

606 MODULATION OF DIBENZO[A, L]PYRENE TRANSPLACENTAL CARCINOGENESIS BY GREEN TEA.

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The fetus is a sensitive target for chemically induced toxicity including carcinogenesis. However, few studies have examined the efficacy of chemopreventative agents in the maternal diet. Previous studies showed that a single dose of 15 mg/Kg b.w. of dibenzo[a,l]pyrene (DBP) on gestation day 17 significantly increased mortality in offspring beginning at 12 weeks of age due to an aggressive T-cell lymphoma. All mice surviving to 10 months, exposed *in utero* to DBP, exhibited lung tumors with high multiplicity. In that study, maternal aryl hydrocarbon receptor (AhR) responsiveness was found to decrease offspring mortality ($p = 0.006$). However, AhR-responsive offspring born to these dams had increased mortality compared to their non-responsive litter mates ($p < 0.05$). Interestingly, administration of indole-3-carbinol at gestation day 9 through weaning significantly decreased mortality independent of AhR genotype. Therefore, the goal of the current study was to examine the dietary impact of green tea polyphenols on DBP transplacental carcinogenesis. Pregnant mice were administered DBP as previously described and some were also given caffeinated green tea or decaffeinated green tea from gestation day 0 through weaning at levels reaching 1.5% w/v. Caffeine and epigallocatechin-3-gallate (EGCG) equivalents were also included as separate groups to examine the role of these major constituents found in green teas. The modulation of lymphoma-dependent mortality caused by maternal DBP exposure was significant only in dams receiving caffeinated green tea or caffeine equivalents, suggesting a potential chemoprotective role of caffeine. Current efforts are examining the roles of the AhR and its downstream transcriptional target cytochrome P450 1B1 in mediating DBP-initiated carcinogenesis. Supported by PHS grants CA90890, ES07060, ES03850, and ES00210

607 INHIBITORY EFFECTS OF THE SAPONINS DERIVED FROM ROOTS OF PLATYCODON GRANDIFLORUM ON TUMOR INVASION AND METASTASIS.

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Platycodon grandiflorum is a traditional oriental herbal medicine that is known for its anticancer effects. This study examined the antimetastatic activities of the saponins that were isolated from the roots of Platycodon grandiflorum A. DC (Campanulaceae). Changkil saponins (CKS) were examined using *in vitro* and *in vivo* invasion and metastasis assay. CKS inhibited the invasion of B16-F10 melanoma cells through a reconstituted basement membrane (Matrigel)-coated filter. Zymographic analysis showed that CKS suppressed TPA-induced MMP-9 activity and expression in a dose-dependent manner. We further demonstrated that CKS reduced the transcriptional activity of NF- κ B, an important nuclear transcription factors involved in MMP-9 expression. CKS also inhibited an experimentally-induced lung cancer and increased the survival time *in vivo*. Taken together, these results suggest that CKS can reduce the extent of a lung metastasis of B16-F10 melanoma cells by inhibiting the tumor invasion of the basement membrane possibly by preventing the suppression of MMP-9 expression through inhibiting NF- κ B signaling pathways.

608 EUGENOL PROTECTS AGAINST 7, 12-DIMETHYLBENZ[A]ANTHRACENE-INDUCED DNA DAMAGE IN MCF-7 CELL THROUGH ACTIVATION OF PHASE II ENZYMES.

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Phytochemicals derived from such fruits and vegetables, referred to as chemopreventive agents. Eugenol (2-methoxy-4-allylphenol) is the basic constituent of the clover oil, to which are attributed the chemopreventive and antioxidant properties. Chemopreventive agents induce phase II detoxifying enzymes. NF-E2 re-

lated factor2 (Nrf2) is a transcription factor involved in the cellular protection against oxidative stress through antioxidant response element (ARE)-directed induction of several phase II detoxifying and antioxidant enzymes. This study, examined the chemopreventive effect of eugenol on 7,12-dimethylbenz[a]anthracene (DMBA)-induced DNA damage in MCF-7 cells. Eugenol inhibited the formation of the DMBA-DNA adduct in a dose dependent manner. Furthermore, eugenol increased the expression and enzymatic activity of the phase II enzymes, such as glutathione-S-transferase (GST) and NAD(P)H:quinone oxidoreductase (QR), which detoxify carcinogenic metabolites. In addition, eugenol stimulated the nuclear translocation of Nrf2 as well as the subsequent induction of ARE-mediated GST and QR activities. Therefore, eugenol has a potent protective effect against DMBA-induced genotoxicity, presumably by induction of DMBA detoxification. These results suggest that eugenol has potential as a chemopreventive.

609 CHEMOPREVENTION IN MICE BY 2-ARYL/ALKYL SELENAZOLIDINE-4(R)-CARBOXYLIC ACIDS.

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In epidemiology and human supplementation studies, as well as many animal models, selenium has shown chemopreventive activity. The efficacy of a series of 2-aryl/alkyl selenazolidine-4(R)-carboxylic acids (SCAs), compounds designed as selenocysteine pro-drugs, in reducing NNK [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone] -induced lung adenomas in mice, a model for tobacco-related lung tumorigenesis, has been investigated. Prior studies have shown 2-oxoSCA but not 2-methylSCA or SCA itself to be chemopreventive. In the present study, mice received AIN-76A diet supplemented with the selenazolidines at 15 ppm Se for one month prior to carcinogen administration and during the subsequent four months of tumor development. 2-ButylSCA, 2-cyclohexylSCA and 2-phenylSCA reduced mean lung tumor numbers from the 10.9 ± 0.9 seen in unsupplemented controls to 4.7 ± 0.9 , 5.3 ± 1.0 and 2.8 ± 0.5 respectively. 2-(2-Hydroxyphenyl)SCA was not active in reducing tumor numbers. Repeating the study but with selenazolidine supplementation beginning 3 days after carcinogen administration, only the 2-butylSCA and 2-cyclohexylSCA were chemopreventive. In a third study in which selenazolidine supplementation was only for the period one month prior until 3 days after carcinogen administration, only 2-butylSCA and 2-phenylSCA were chemopreventive. Both retained chemopreventive activity when the supplementation period was shortened further to 9 days prior until 2 days prior to carcinogen administration. The reductions in tumor numbers were 40% for 2-phenylSCA and 44% for 2-butylSCA. With a 2 days prior until 3 days after supplementation regimen, the reductions in tumor numbers, 26% for 2-phenylSCA and 17% for 2-butylSCA, were not statistically significant. Thus dietary supplementation with selenazolidines shows promise as a chemoprevention strategy. For at least two 2-substituted SCAs, short-term rather than continuous supplementation is sufficient to demonstrate chemopreventive activity.

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610 A NOVEL NUTRIENT MIXTURE INHIBITS CELL PROLIFERATION, MMP SECRETION AND INVASION IN HEP G2 HEPATOMA CELL LINE.

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Introduction:

A novel nutrient mixture (NM) containing lysine, proline, ascorbic acid and green tea extract has exhibited anti-tumor activity *in vivo* and *in vitro*. In this study we investigated the effect of NM on Hep G2, human hepatoma cell line.

Objective:

NM has been shown to possess a wide range of pharmacological properties, and is one of the most promising chemo preventive agents for cancer. To provide a better understanding of the preventive effect of NM on liver cancer, we examined NM for its effect on proliferation, MMP secretion, invasion and morphology.

Methods:

Hep G2 cells (ATCC) were cultured in MEM medium supplemented with 10% FBS and antibiotics. The cells were then challenged with NM at 0, 10, 50, 100, 500 and 1000 μ g/ml concentration in triplicate at each concentration. Cell proliferation was assessed by MTT assay, MMP activity by gelatinase zymography, invasion through Matrigel and morphology by H&E staining. Cells were also treated with PMA for MMP-9 induction.

Results:

NM was slightly toxic at 100- μ g/ml concentration and significant at 500 and 1000 μ g/ml concentration. NM showed a dose dependent inhibition of MMP-9 in PMA-induced cells, with virtual total inhibition at 500- μ g/ml concentration. Invasion through Matrigel was inhibited by 30%, 80% and 100% at 50, 100 and 500 μ g/ml. H&E staining showed no morphological changes below 500 μ g/ml. Conclusions:

These results suggest that NM has therapeutic potential as a new anti-tumor agent.

611 CALCIUM-INDEPENDENT PHOSPHOLIPASE A₂ AS A NOVEL TARGET FOR THE INHIBITION OF CANCER CELL GROWTH.

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We recently demonstrated that inhibition of Ca²⁺-independent phospholipase A₂ (iPLA₂) decreased cellular proliferation in correlation with decreased expression of phosphatidylcholines (PtdCho). However, the mechanisms involved in these processes, and the effect of iPLA₂ inhibition on chemotherapeutic-induced alterations of cell growth were not studied. Thus, this study determined the ability of iPLA₂ inhibitors to alter the effect of chemotherapeutics on cell growth and death in multiple cancer cell lines. Inhibition of total iPLA₂ activity using bromoenol lactone (BEL, 0-5 μ M) decreased cell growth by 20%, decreased 14:0-16:0 and 16:0-16:0 PtdCho, and increased chemotherapeutic-induced cell death in lung, neural, and prostate cancer cells exposed to cisplatin and vincristine (1-50 μ M). Interestingly, the effect of BEL was only evident in cells undergoing log phase growth, and was not evident when confluent cultures were treated. Transfection of cells with siRNA against iPLA₂ β or γ demonstrated that inhibition of iPLA₂ β , but not iPLA₂ γ , decreased cell growth 30-40%. These data were verified using the pharmacological inhibitors R-BEL (microsomal iPLA₂ γ inhibitor) and S-BEL (cytosolic iPLA₂ β inhibitor). To investigate the mechanisms involved in these processes the effect of BEL (5.0 μ M) on the progression of prostate cells through the cell cycle was studied. Exposure of cells to BEL increased the amount of cells in G1 while decreasing the amount of cells in S-Phase. Incubation with cisplatin or vincristine induced a G2-M arrest. However, pretreatment with BEL inhibited this arrest and increased the amount of cells in G1 and sub-G1. These data suggest that iPLA₂ inhibition decreases cell growth by decreasing the progression of cells from the G1 to the S-phase of the cell cycle, that the effect of iPLA₂ inhibitors and chemotherapeutics on cancer cell growth are additive, and that iPLA₂ may be a novel target for the inhibition of cancer cell growth.

612 GENE EXPRESSION PROFILING IN CHEMOPROTECTING ACTION OF CAFFEIC ACID PHENETHYL ESTER (CAPE) ON THE INITIATION IN A RAT HEPATOCARCINOGENESIS MODEL.

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CAPE is a natural honeybee hive product that has shown anti-carcinogenic properties in the modified resistant hepatocyte model when is administered on initiation stage. The information about its action mechanism is limited. The aim was to characterize the effect of CAPE on gene expression profile on initiation stage of hepatocarcinogenesis by microarrays analysis. Three groups of male adult Fisher-344 rats were administered with one ip dose of CAPE (20 mg/kg), diethylnitrosamine (DEN) (200 mg/kg) and DEN plus CAPE 12 h before DEN administration. Animals were sacrificed 12 h after CAPE dosing, 24 h after DEN and 12 and 24 h after DEN+CAPE. Liver was removed and processed to obtain microsomes and for microarrays analysis using a cDNA slide with 28800 genes (Transcriptome-Biochips Platform of Genopole Toulouse Midi-Pyrenees). CAPE alone did not produce any alteration in gene expression. At 24 h DEN treatment altered 160 genes, 91 over-expressed and 69 down-expressed including the diminution of several cytochrome-P450 (CYP) isoforms such as CYP2C11 and 3A1. DEN+CAPE treatment at 24 h altered 352 genes, 100 over-expressed and 252 down-expressed including the diminution of CYPs involved in DEN metabolism such as CYP1A1, 2B1 and 2E1. These isoforms were validated by enzymatic activities. In CAPE group CYP1A1, 1A2 and 2B enzymatic activities were reduced. In DEN+CAPE group CYP1A1 and 1A2 were decreased at 12 h and at 24 hr CYP1A1 diminished and CYP1A2 was incremented. These results suggest that CAPE produces a general down-expression in the gene expression profile including CYP isoforms involved in DEN bioactivation. In addition, support the proposal that chemoprotective effect of CAPE may be by hepatic CYP regulation in chemical hepatocarcinogenesis.

613 MRP-MEDIATED GLUTATHIONE DEPLETION AS A POTENTIAL NEW STRATEGY OF CANCER CELL SENSITIZATION.

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New approaches in cancer therapy focus on better tumor cell targeting strategies. Compounds such as L-S,R-buthionine sulfoximine (BSO) have been developed to deplete cancer cells of their antioxidant stores thereby sensitizing them to pro-oxidant agents. The major draw-back to this type of therapy is an inherent lack of specificity. Since multi-drug resistance proteins (MRPs) are known to be over expressed in tumor cells and can co-transport glutathione (GSH), we have chosen to target this pathway as a selectivity solution. We previously reported that two prototypic flavonoids, i.e. 2',5'-DHC and chrysin, are efficient inducers of GSH depletion in A549 cells. Herein, we report that, in these cells, cisplatin induces a differential expression of mitochondrial respiratory chain proteins and that the potentiation effect of cisplatin's toxicity induced by these flavonoids is associated with intracellular GSH depletion and mitochondrial dysfunction. Compared to BSO, the effects of the two flavonoids were greater. Our data suggests that the efficacy of the cisplatin/flavonoid combination results, in part, from a synergy of pro-oxidant effects, the first by disrupting the formation of the mitochondrial respiratory chain and the second by decreasing the antioxidant defenses of the cell. MRP-mediated GSH depletion may reveal an adjunctive and specific approach to sensitize cancer cells to pro-oxidant treatment. Whereas chrysin induced GSH depletion is known to be mediated by MRP1, MRPs triggered by 2',5'-DHC remain to be identified.

614 SINGLE AND COMBINED EFFECTS OF BENZENE AND DIABETES ON TOXIC AND CARCINOGENIC ACTIVITY OF NDMA IN RABBIT LIVER- PROTECTIVE EFFECT OF DIALLYL SULFIDE.

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Benzene is an occupational and environmental toxicant. Benzene causes acute myelogenous leukemia. Metabolism of benzene by CYP2E1 is required for its hemopoietic toxicity. Nitrosodimethylamine (NDMA) is a procarcinogen. NDMA is converted to its carcinogenic form after it is activated by CYP2E1 dependent NDMA N-demethylase. Induction of CYP2E1 by diabetes was identified in rabbit liver (Arinc, E., Arslan, S., Adali, O., 2005). The goal of this study was to assess interactive effect of benzene and diabetes status on CYP2E1 expression and its associated NDMA N-demethylase activity. We also investigated whether diallyl sulfide (DAS), protected bioactivation thus, carcinogenicity of NDMA by decreasing CYP2E1 protein amount and its associated activity. NDMA activity was found to be increased significantly (p<0.001) in diabetic or benzene-treated rabbits. The combined effects of benzene and diabetes were found to be additive. Similar trend was elicited when CYP2E1 expression was quantitated. CYP2E1 protein immunostaining intensity was increased upto 7.5-fold in the combined case. Results of regression analysis indicated that there was a significant correlation (r = 0.98, p<0.001) between NDMA N-demethylation and relative CYP2E1 staining intensity in all four cases. DAS, a constituent of garlic has been shown to be anticarcinogenic. However, no data is available concerning the influence of DAS on rabbit cytochrome P450 dependent enzymes. A single dose of DAS in corn oil (250 mg/kg body weight) was given intragastrically to rabbits. When DAS was given after benzene treatment, NDMA N-demethylase activity was decreased to its basal level. Similarly, CYP2E1 contents of the microsomes obtained from DAS treated rabbits were also decreased. Thus, DAS reversed hazardous action of benzene, possible carcinogenicity of NDMA by decreasing CYP2E1 protein amount and its associated enzyme activity.

615 A FAR ENHANCER IN THE CYTOCHROME P450 3A4 GENE FUNCTIONS AS A HIGH DENSITY-REGULATORY MODULE IN RESPONSE TO THE PREGNANE X RECEPTOR AND FUNCTIONALLY RELATED PROTEINS.

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Cytochrome P-450 3A4 (CYP3A4) is the most abundant P450 enzyme and metabolizes more than half of drugs and other xenobiotics. The expression of CYP3A4 is markedly induced by a variety of structurally dissimilar chemicals. The pregnane X receptor (PXR) is recognized as a major regulator for the induction. It has been established that PXR transactivates the CYP3A4 gene by physically binding two elements (DR3 and ER6) in the distal and proximal promoter regions, respectively. Very recently, a farther upstream region (FUR) was found to support constitutive

expression of CYP3A4. Motif analysis revealed that this region contains multiple elements recognized by PXR or functionally related proteins such as hepatocyte nuclear receptor-4 (HNF-4). It was, therefore, hypothesized that FUR represents a high density-regulatory module that supports transactivation activity of multiple nuclear receptors. In order to test this hypothesis, reporter and electrophoretic mobility shift assays were conducted. Consistent with the hypothesis, fusion of FUR to the CYP3A4 proximal promoter markedly increased the reporter activity by PXR activator rifampicin. The increase, however, was significantly abolished when the putative PXR element (F-ER6) was selectively disrupted. Electrophoretic mobility shift assay detected a shifted band toward biotinylated F-ER6 probe, and the shifted band was super-shifted by an antibody against PXR. In addition, selective disruption of individual HNF-1 and -4 elements in FUR resulted in decreased reporter activity, although the magnitude of the decrease was less than that with the F-ER6 disrupted reporter. These findings functionally define FUR as a regulatory module that supports coordinate activity of multiple transcription factors.

616 FUNCTIONAL ANALYSIS OF THE CYP1A1 PROMOTER/ENHANCER REGION FROM DANIO RERIO (ZEBRAFISH).

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The induction of CYP1A1 by the Ah receptor (AHR) is used as an indicator of environmental exposure to halogenated aromatic hydrocarbons, typified by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The upstream flanking region of a CYP1A1 gene from zebrafish contains 8 putative core xenobiotic response elements (XREs) organized in two distinct clusters. The region between -580 to -187 contains XRE 1-3 while the region between -2608 to -2100 contains XRE 4-8. Only XRE 1, 3, 4, 7, and 8 exhibit TCDD-dependant association of AHR-ARNT complexes when evaluated by gel shift assays. To evaluate this finding in more detail, luciferase constructs were generated that contained both regions (-2608/-2100Ur construct). Mutagenesis of individual XREs confirmed that TCDD-mediated induction could occur when a single XRE was mutated, but maximal induction required XRE 4, 7, and 8 although each did not function in an additive manner. To determine whether non-functional XREs could be made functional, in vitro mutagenesis was used to change nucleotides within the core XRE or in the flanking regions to mirror those of 4, 7 and 8. Interestingly, there were no changes in the level TCDD-induced luciferase activity with any of the changes made. To investigate the role of other transcription factors in CYP1A1 induction, in silico analysis was used to identify Sp1, HNF-3, and CREB binding sites and in vitro mutagenesis was utilized to knock them out within the -2608/-2100Ur construct. The results show that the absence of the Sp1 and HNF-3 binding sites cause a significant reduction of both basal and induced levels of CYP1A1 while the mutation of the putative CREB binding site had no effect. Collectively, these results show that the inability of certain XREs to confer TCDD-mediated gene induction is likely not sequence specific but may involve the inaccessibility of the region containing the binding site. Furthermore, maximal induction of CYP1A1 may require factors other than AHR/ARNT binding at the enhancer region. Supported by ES10991 (RSP).

617 COMPREHENSIVE GENE EXPRESSION ANALYSIS IN MALE NRF2KO MICE TREATED WITH 2, 3, 7, 8-TETRACHLORO DIBENZO-P-DIOXIN (TCDD).

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Nrf2 (nuclear factor erythroid 2-related factor 2) is an essential transcription factor for inducible and/or constitutive expression of a group of Phase II detoxification and antioxidant enzymes. On the other hand, TCDD has been known as an environmental contaminant and a potent agonist of AhR (aryl hydrocarbon receptor) that regulates Phase I enzymes such as CYP1A1 and CYP1A2. Here, we analyze comprehensive gene expression induced by TCDD in livers of male Nrf2 knockout mice to clarify Nrf2-dependent transcriptional regulation. Forty micrograms of TCDD per kg body weight were administered orally to five Nrf2(-/-) mice and five Nrf2(+/+) mice, which were produced by intercrossing Nrf2(+/-) mice. Total RNA was extracted from the livers 24 hrs after the administration. DNA microarray analysis was performed using the CodeLink mouse 20K array. Fold change values with the p-value of less than 0.01 were adopted as statistically significant data in each group of the Nrf2(-/-) and Nrf2(+/+) mice for network analysis using KeyMolnet afterwards. CYP1A1 and CYP1B1 were significantly up-regulated over 2-fold in both Nrf2(-/-) and Nrf2(+/+) mice treated with TCDD, while Nrf2, NAD(P)H quinone dehydrogenase (NQO1), glutathione S-transferases (GSTs) were up-regulated by TCDD in the Nrf2(+/+) mice, but not in the Nrf2(-/-) mice. Keap1, the negative regulator of Nrf2, was significantly down-regulated by TCDD

only in the Nrf2(-/-) mice. Furthermore, the network analysis implies that transcriptional regulation of E2F(Transcription factor E2F) and TCF(T cell specific transcription factor) is involved in the TCDD-treated Nrf2(-/-) mice. Taken together, our observations suggest that the up-regulation of gene expression of NQO1 and GSTs is induced by TCDD depending on Nrf2 regulation and that E2F and TCF transcription factors are activated by TCDD in the absence of Nrf2.

618 REGULATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR1 (VEGFR1) EXPRESSION BY SPECIFICITY PROTEINS 1, 3 AND 4 IN PANCREATIC CANCER CELLS.

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Vascular endothelial growth factor receptor 1 (VEGFR1) is expressed in cancer cell lines and tumors and, in pancreatic and colon cancer cells activation of VEGFR1 is linked to increased tumor migration and invasiveness. Tolfenamic acid, a non-steroidal anti-inflammatory drug, decreases Sp protein expression on Panc-1 and L3.6pl pancreatic cancer cells, and this was accompanied by decreased VEGFR1 protein and mRNA and decreased luciferase activity on cells transfected with constructs (pVEGFR1) containing VEGFR1 promoter inserts. Comparable results were obtained in pancreatic cancer cells transfected with small inhibitory RNAs for Sp1, Sp3 and Sp4, and all three proteins bound to GC-rich elements in the VEGFR1 promoter. These results demonstrate that VEGFR1 is regulated by Sp proteins and that treatment with tolfenamic acid decreases expression of this critical angiogenic factor. Moreover, in vitro studies in Panc-1 cells demonstrate that activation of VEGFR1 by VEGF-B to increase mitogen-activated protein kinase 1/2 phosphorylation and cell migration on collagen-coated plates is also inhibited by tolfenamic acid. Thus, targeted degradation of Sp proteins is a highly effective for inhibiting VEGFR1 and associated angiogenic responses in pancreatic cancer.

619 TGF-B1 SUPPRESSES IL-2 PRODUCTION DURING PRIMING OF NAIVE, BUT NOT DURING RE-CHALLENGE OF PREVIOUSLY ACTIVATED CD4⁺ T CELLS: A ROLE FOR CHROMATIN STRUCTURE.

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When naive CD4⁺ T cells are activated they secrete interleukin (IL)-2 and proliferate. During a secondary immune challenge, IL-2 production is faster and greater in magnitude. This is a consequence of an increased frequency of responding cells, a lower threshold for their activation, less dependence on accessory co-stimulation, and an increased ability to respond to a wider range of antigen presenting cells. We now demonstrate stable chromatin remodeling of the IL-2 locus during priming and resistance to suppression by TGF-b1 during re-challenge. We also demonstrate that TGF-b1 targets the -300 bp minimal essential regulatory region of the IL-2 promoter and suppresses IL-2 transcription in naive CD4⁺ T cells in a Smad3-dependent manner. This effect is abrogated in effector and memory CD4⁺ T cells. We correlate non-responsiveness to inhibition by TGF-b1 with decreased Smad3 binding to the proximal IL-2 locus, despite the presence of functional Smad3 signaling in previously activated CD4⁺ T cells. The differential response to suppression by TGF-b1 reported here provides a mechanism by which TGF-b1 may function in a T cell intrinsic manner to facilitate anergy induction in self-reactive T cells while concomitantly sustaining a source of IL-2 for active suppression of immune responses by T regulatory cells to control inflammation and maintain peripheral self-tolerance.

620 THE NON-PSYCHOACTIVE CANNABINOID, CANNABIDIOL, SUPPRESSES T CELL FUNCTION THROUGH INHIBITION OF NUCLEAR FACTOR OF ACTIVATED T CELLS (NFAT).

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Marijuana is derived from the Cannabis Sativa plant. Although there are over 60 cannabinoid compounds in the plant, Δ⁹-tetrahydrocannabinol (THC) is the primary psychoactive congener of marijuana and binds to the two known cannabinoid receptors, CB1 and CB2. THC has exhibited immunosuppressive actions both in vivo and in vitro. Interestingly, another predominant cannabinoid from the plant,

cannabidiol (CBD), possesses low affinity for both cannabinoid receptors, yet also exhibits immunosuppressive actions. A major target of immune suppression by CBD is interleukin-2 (IL-2) as demonstrated in PMA/Io-stimulated mouse splenocytes. The focus of these studies was to determine the mechanism by which CBD suppressed IL-2. Using purified T cells from mouse splenocytes, CBD suppressed PMA/Io-stimulated IL-2 production, similar to the transplant drug cyclosporin A. Consistent with the observation that CBD does not possess high affinity for CB1 or CB2, CBD also suppressed PMA/Io-stimulated IL-2 from purified T cells derived from CB1/CB2 null mice. IL-2 transcription is regulated by several transcription factors, including NFAT, activator protein-1 (AP-1), and nuclear factor κ B (NF- κ B). Using the human Jurkat T cell line, in which CBD suppressed PMA/Io-stimulated IL-2 production, NFAT- and AP-1-luciferase reporter constructs were transiently transfected and induced with PMA/Io in order to examine the effect of CBD on transcriptional activation. CBD suppressed both PMA/Io-induced NFAT- and AP-1-luciferase in a concentration-dependent manner. These results are consistent with the mechanism by which other plant-derived, synthetic and endogenous cannabinoids suppress IL-2 and other functional immune endpoints. (Supported in part by NIH DA007908).

621 INVOLVEMENT OF MITOCHONDRIAL AND DEATH RECEPTOR PATHWAYS IN CHOLESTEROL SECOALDEHYDE-INDUCED APOPTOSIS IN MURINE H9C2 CARDIOMYOCYTES.

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Cholesterol secoaldehyde (3 β -hydroxy-5-oxo-5,6-secocholestan-6-al or ChSeco) is known to occur *in vivo* in humans and is associated with atherosclerosis, Alzheimer's disease and Lewy body dementia. Previous studies from our laboratory have shown that ChSeco induces apoptosis in H9c2 cardiomyocytes. In this study, we investigated the downstream signaling mechanisms that culminate in apoptosis. Quantitative RT-PCR analysis revealed that ChSeco activates mitochondrial (intrinsic) and death receptor (extrinsic) pathways. The mitochondrial pathway was shown to be of rapid onset and it involved disruption of transmembrane potential ($\Delta\psi_m$), generation of reactive oxygen species (ROS), increased Bax/Bcl-2 ratio, and decreased oxygen consumption. The loss of $\Delta\psi_m$ was followed by formation of permeability transition pore (release of cytochrome *c*), activation of caspase 9, and up-regulation of Apaf1. The extrinsic pathway involved up-regulation of Fas and FasL. The significance of this pathway was also evident based on activation of procaspase 8. Thus, it appears that a combined activation of intrinsic and extrinsic pathways resulted in activation of effector caspases 3 and 7 and cleavage of poly(ADP-ribose)polymerase. Inhibitors of caspases 8 and 9 decreased the activity of caspase 3, and the cell death was partially prevented by a pancaspase inhibitor, Z-Val-Ala-Asp-fluoromethyl ketone. Antioxidants such as N-acetyl-L-cysteine and Trolox significantly inhibited the ChSeco-induced cell death. In summary, we conclude that ChSeco induces apoptosis by pathways involving both mitochondria and death receptor. The enhanced ROS production and a 'cross-talk' between the two pathways are thought to play a role in ChSeco-induced apoptosis in H9c2 cardiomyocytes. [Funding support from NIEHS ES 10018 and NIH BRIN program of NCRR (grant number P20 RR6456) to RU is acknowledged. Corresponding author: rao_uppu@subr.edu]

622 DEVELOPMENT OF A LIMITED TRANSCRIPT PANEL FOR THE DETECTION OF STRIATED MUSCLE TOXICITY.

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Striated muscle (skeletal and cardiac) toxicity has been an issue for a number of marketed drugs such as the statins and fibrates, and thus represents a target organ toxicity for which new predictive screening tools would be desirable. Traditional clinical chemistry markers for muscle injury, including creatine kinase (CK) and AST, although useful, have limitations. For example, they can be relatively insensitive owing to limited half life in the circulation, they enter the circulation only after tissue injury and are thus not premonitory, and are nonspecific in terms of their ability to distinguish the muscle fiber type affected. Herein, we describe the identification and development of a limited panel of gene transcripts that correlates well, on a sample by sample basis, with skeletal muscle injury detected using histopathology. Importantly, the selected transcripts can identify muscle injury in muscles of differing fiber types that are mediated by compounds directed against differing biological targets. The transcript panel, comprised of transcripts representing several muscle injury mechanistic domains, increases the likelihood of correctly identifying muscle injury induced by compounds with differing mechanisms of action. We also demonstrate: 1) the predictive potential of the transcript panel by showing significant transcriptional modulation of panel members at muscle toxicant doses where no associated pathology was described and, 2) the potential utility of the transcript

panel in an early *in vitro* compound screening paradigm, using a myotube cell line model. Collectively, the data demonstrate that transcript profiling can be used to study myotoxicity in a rapid, facile manner and with improved biological insight, as compared to current methods.

623 CROSS-SPECIES ASSOCIATION MAPPING IDENTIFIES GENETIC RISK FACTORS FOR LIVER TOXICITY.

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A major challenge to defining human sensitivity to pharmaceutical agents is a lack of validated rodent models that reflect the genetic diversity of a population. We hypothesized that a panel of inbred mouse strains can be successfully used to define genetic factors that contribute to differential sensitivity to toxicity. In this work, a bedside-to-bench-to-bedside paradigm was explored using acetaminophen (APAP)-induced liver injury as a model exposure. Recent human studies show that the maximum recommended daily dose of APAP may cause liver injury (Watkins et al, JAMA, 2006). This phenomenon was confirmed in an independent cohort of 60 healthy volunteers who received placebo (n=13) or APAP (1,000 mg every 6 hrs for 7 days; n=47) in a randomized clinical trial. Marked differences in individual sensitivity to liver injury due to APAP, as evidenced by elevations in serum ALT greater than 3-fold from the baseline, were detected in 17% of subjects. To identify genetic susceptibility factors, we utilized a panel of 36 inbred mouse strains. Following an acute dose of APAP (300 mg/kg, i.g., n=4/strain), we observed a large degree of strain-specific sensitivity to liver damage. Whole genome association analysis of sensitivity to APAP-induced liver injury identified significant phenotypic correlation within six genomic intervals. Targeted sequencing of candidate regions revealed that polymorphisms in *Ly86*, *CD44*, *CD59a*, and *Capn8* genes correlate strongly with the degree of liver injury in the mouse. The potential importance of these genes in controlling the severity of APAP-induced liver injury was validated in the human cohort. We demonstrate that by using a genetically diverse panel of inbred mice and genetic association analysis, disease-relevant markers of toxicity can be identified and used to predict risk or identify novel therapies in people. Supported by: U19-ES11391, STAR-RD832720, T32-ES07126, N01-ES-35513, N01-ES-25497, N01-ES-65406.

624 CO-EXPOSURE OF MICE TO TROVAFLOXACIN AND LIPOPOLYSACCHARIDE LEADS TO AN ALTERED INFLAMMATORY RESPONSE IN A MOUSE MODEL OF IDIOSYNCRATIC LIVER INJURY.

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Concurrent inflammation caused by lipopolysaccharide (LPS) renders rats susceptible to hepatotoxicity from several drugs known to cause idiosyncratic adverse drug reactions (IADRs) in humans. Among these is trovafloxacin (TVX), a fluoroquinolone antibiotic. We explored whether this hepatotoxic TVX/LPS interaction occurs in mice and characterized the interaction by measuring inflammatory cytokines in plasma. Treatment with TVX alone up to 200 mg/kg did not increase plasma alanine aminotransferase (ALT) activity, a biomarker of liver injury. When mice were exposed to a nontoxic dose of LPS (2×10^6 EU/kg) 3 hours after TVX, a dose-dependent increase in ALT activity was observed with significant liver injury occurring at 150 mg/kg TVX. Examination of histology corroborated hepatocellular necrosis in the TVX/LPS-treated mice. Levofloxacin, a fluoroquinolone antibiotic that does not cause human IADRs, did not interact with LPS to cause liver injury. A detailed examination of the time-course of injury revealed significant increases in ALT activity by 4.5 and peak activity by 15 hours after LPS treatment in TVX/LPS-treated mice. TVX/LPS-treated mice had an accentuated plasma TNF α peak compared to LPS-treated mice. Increases in IL-10, interferon γ , monocyte chemoattractant protein 1 (MCP-1), and IL-6 were also greater in TVX/LPS-treated mice compared to LPS-treated mice. In summary, TVX/LPS cotreatment caused pronounced hepatotoxicity in mice, and the cytokine profiles in LPS-treated mice were significantly enhanced by TVX cotreatment at times near the onset of liver injury. (Supported by NIH grant DK061315.)

625 ANALYSIS OF HEPATIC AND RENAL MULTIDRUG-RESISTANCE ASSOCIATED PROTEIN EXPRESSION IN TYPE 1 DIABETIC MICE.

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Insulin-deficient type 1 diabetic (DB) mice are resistant to hepatic and renal toxicity by acetaminophen and S-1,2-dichlorovinyl-L-cysteine, respectively. This resistance is associated with increased hepatic and renal cellular proliferation. Recent

studies suggest that altered expression of hepatobiliary transporters influences susceptibility of liver to chemical injury. Transporters such as the multidrug resistance-associated proteins (Mrp) are critical for the hepatic and renal elimination of chemicals and metabolites. In this study, we examined the hepatic and renal expression of Mrp1-6 in DB mice and investigated whether treatment with the antimetabolic drug, colchicine (CLC), alters Mrp expression patterns in DB mice. Type 1 diabetes was induced in male Swiss-Webster mice with a single dose of streptozotocin (STZ, 200 mg/kg ip). Of these, one group of mice received 2 mg/kg CLC on day 8 and 1.5 mg/kg on day 10 after STZ or vehicle treatment. Livers and kidneys were collected on day 11 for western blot analysis. Hepatic expression of Mrp1 (2-fold), Mrp3 (3-fold), Mrp4 (3.6-fold) and Mrp5 (2-fold) proteins was higher in DB mice compared to controls. Similarly, DB mice showed increased renal Mrp1 (2.5-fold), Mrp4 (1.8-fold) and Mrp5 (5.5-fold) proteins. No change in Mrp2 or Mrp6 expression was observed in either tissue, while Mrp3 was undetectable in kidneys of any treatment group. Expression of Mrp1, Mrp3 (liver), Mrp4 and Mrp5 in livers and kidneys from DB mice receiving CLC was similar to control non-diabetic mice. Blockade of hepatic and renal cell division prevented over-expression of Mrp proteins in DB mice suggesting an association between cell proliferation and transporter expression. Up-regulation of Mrps along with enhanced cell proliferation in DB livers and kidneys may influence pharmacokinetics of drugs and endogenous compounds as well as susceptibility to chemical toxicity.

626 INDUCTION OF HEPATIC MRP3 AND MRP4 TRANSPORTERS BY ACETAMINOPHEN TREATMENT: ROLE OF THE TRANSCRIPTION FACTOR NRF2.

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Transcription factor nuclear factor E2-related factor 2 (Nrf2) regulates detoxification and antioxidant genes following electrophile exposure and oxidative stress. Mice deficient in Nrf2 exhibit lower expression of detoxification and stress genes along with higher susceptibility to acetaminophen (APAP) hepatotoxicity. Toxic doses of APAP significantly induce the basolateral efflux transporter multidrug resistance-associated proteins 3 and 4 (Mrp3 and 4) in mouse liver. The purpose of this study was to determine if Nrf2 participates in Mrp3 and Mrp4 induction by APAP. APAP (200-400mg/kg i.p.) was administered to wild-type (WT) and Nrf2-null (Nrf2^{-/-}) mice. Hepatic gene and protein expression of Mrp3 and Mrp4, and toxicity (serum ALT activity and histopathology) were assessed 24 and 48 hrs later. As expected, hepatotoxicity was greater in Nrf2^{-/-} compared to WT mice. APAP treatment increased hepatic Mrp3 mRNA (5-fold) and protein (1.3-fold) in WT mice. Constitutive Mrp3 protein expression was reduced in Nrf2^{-/-} mice to 20% of WT controls and was not induced with APAP. Similarly, APAP treatment induced Mrp4 mRNA (3.6-fold) and protein (4.5-fold) in WT mice. Mrp4 induction was significantly less in APAP-treated Nrf2^{-/-} mice. Mrp1 mRNA and protein were increased 2-fold in both genotypes suggesting that transcription and translation are functional in Nrf2^{-/-} livers despite greater toxicity. These results show that Nrf2 activation by APAP regulates Mrp3 and Mrp4 expression. Coordinated expression of detoxification and transporter genes by Nrf2 during APAP hepatotoxicity is a mechanism by which hepatocytes limit intracellular accumulation of potentially toxic chemicals.

627 THE ROLE OF DIFFERENCES IN EXPRESSION OF PPAR α BETWEEN MOUSE STRAINS IN PLEOTROPIC RESPONSE TO PEROXISOME PROLIFERATORS.

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Peroxisome proliferators are non-genotoxic rodent liver carcinogens. Studies in peroxisome proliferator activated receptor (PPAR) α -null mice show that this receptor is required for PP-induced liver tumor formation. Inter-species (rodent to human) variability in liver carcinogenesis response to PPs is thought to be, at least in part, a result of differential expression of PPAR α in liver. Thus, we hypothesized that the magnitude of PP-induced response should correlate with expression of PPAR α in liver. A panel of inbred mouse strains that have widely varying levels of basal PPAR α expression in liver was selected for this study. Mice were fed either control or WY-14,643 containing (0.1% w/w) diets for up to 5 wks. Changes in liver and

body weight, cell proliferation, clinical chemistry and a number of molecular endpoints were assessed. No significant inter-strain differences in WY-induced cell proliferation, or liver-to-body weight ratio were observed. However, we observed marked differences in PP-induced alterations in serum triglycerides, glucose, and creatinine, and time-dependent weight loss which suggests that a wide variation in stimulation of fatty acid and energy metabolism in response to PPs exists between strains. We conclude that despite varying receptor expression and inter-strain differences in responses to PPs, the uniform increase in liver cell proliferation in all strains of mice suggests that differences in PPAR α expression alone are not indicative of the potential long-term effects of these compounds (Supported by ES11391, ES11660).

628 HEPATIC MRP4 INDUCTION FOLLOWING ACETAMINOPHEN EXPOSURE IS DEPENDENT ON KUPFFER CELL FUNCTION.

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Increased expression of efflux transporters has been documented with hepatic damage of varying etiologies. The multidrug resistance-associated protein 4 (Mrp4) is the most significantly up-regulated transporter in mouse liver after toxic administration of acetaminophen (APAP). Mrp4 levels are also increased in human liver following APAP overdose. While expression profiles of transporters following APAP hepatotoxicity are well-characterized, the signaling pathways contributing to these changes remain unknown. We hypothesize that Kupffer cell (KC)-derived mediators participate in the regulation of hepatic transporters. To investigate this, C57BL/6J mice were pretreated with liposomal clodronate (0.1 ml i.v.) to deplete KCs and then challenged with APAP (500mg/kg i.p.). Hepatic Mrp4 protein expression was determined by western blot and immunohistochemistry. At 72 hrs after APAP dosing Mrp4 levels were increased by 33-fold in mice receiving empty liposomes. Immunohistochemistry showed that Mrp4 staining was confined to centrilobular hepatocytes. Depletion of KCs by liposomal clodronate increased susceptibility to APAP hepatotoxicity. Remarkably, KC depletion completely prevented Mrp4 induction by APAP. These findings show that KCs protect the liver from APAP toxicity and that KC mediators released in response to APAP are responsible for Mrp4 induction. Enhanced Mrp4 expression may represent a compensatory response to enhance the elimination of mediators of tissue injury and bile constituents from an injured liver. Alternatively, Mrp4 induction might augment the secretion of signaling molecules for tissue repair from injured hepatocytes.

629 LIPOPOLYSACCHARIDE INDUCED CYTOKINE, CHEMOKINE AND ACUTE-PHASE PROTEIN RESPONSE IN KUPFFER CELL DEPLETED RAT LIVER SLICES.

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Kupffer cells play an important role in the hepatotoxicity induction of a number of xenobiotics and are the main cellular mediators of the effects of lipopolysaccharide (LPS) in the liver. To elucidate the role of Kupffer cells in the pathogenesis of liver injury, specific Kupffer cell inactivating or depleting agents such as gadolinium chloride (GdCl₃) or liposome-encapsulated dichloromethylene diphosphonate (Cl₂MDP) have been used. To identify the appropriate treatment and dose that will deplete the Kupffer cells from rat liver, rats were intravenously injected with 10mg/Kg of GdCl₃, or 0.2 mL or 1 mL of Cl₂MDP liposomes. Histological examination of the liver after iv administration of India ink (2mL/Kg) showed that Cl₂MDP liposomes (1 mL) caused the maximum decrease in the number and function of Kupffer cells in liver tissue. To investigate the role of Kupffer cells in LPS-induced cytokine, chemokine and acute-phase protein production in the liver slice model, Sprague-Dawley rats were intravenously treated with either 3mL/Kg phosphate buffered saline (PBS), PBS liposomes or Cl₂MDP liposomes. Precision-cut liver slices were prepared and cultured for 4h or 24h in medium containing 0, 1, 10, and 100 μ g/mL LPS. LDH, NO, various cytokines and chemokines and the acute-phase proteins AGP, A2M, ceruloplasmin, haptoglobin, hemopexin and thio-statin were analyzed in the medium. In control liver slices, LPS treatment caused a dose dependent increase in NO, IL-1 α , IL-1 β , IL-6, IL-10, IFN- γ , TNF- α , CINC-1 and MIP-2 at both 4 and 24 h and caused a dose dependent increase in GM-CSF only at 24 h. The acute phase proteins increased with time in the culture medium and no further increase was observed with the LPS treatment. LPS treatment of Kupffer cell depleted liver slices showed a significantly reduced induction of chemokines and cytokines compared to control liver slices. Thus, depletion of Kupffer cells prior to liver slice preparation may provide a tool to assess the role of inflammatory mediators on xenobiotic toxicity in vitro.

630 TROGLITAZONE-INDUCED HEPATIC NECROSIS IN AN ANIMAL MODEL OF SILENT GENETIC MITOCHONDRIAL ABNORMALITIES.

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Troglitazone, a first-generation thiazolidinedione antidiabetic drug, was withdrawn from the market due to an unacceptable risk of idiosyncratic hepatotoxicity. The underlying mechanisms are unclear; normal healthy rodents tolerate high doses of troglitazone without developing liver injury, but in vitro models suggest a mitochondrial hazard at high concentrations. Therefore, we explored whether genetic mitochondrial abnormalities might sensitize mice to the hepatic adverse effects of troglitazone. We used heterozygous superoxide dismutase 2 (*Sod2*^{+/−}) knockout mice as a model, as these mice develop clinically silent mitochondrial stress. Troglitazone was administered daily for 4 weeks at doses comparable to human exposure (0, 10, or 30 mg/kg/d, ip). We found that troglitazone caused overt liver injury in the high-dose group, manifested by increased serum ALT activity and mid-zonal foci of hepatic necrosis which were congruent with TUNEL-positive cells, in *Sod2*^{+/−}, but not wild-type, mice. Hepatic mitochondria isolated from troglitazone-treated mice exhibited decreased activities of aconitase (by 45%) and complex I (by 46%) and increased (by 58%) protein carbonyls, indicative of enhanced mitochondrial oxidant stress. Furthermore, mitochondrial glutathione levels were increased by troglitazone as a compensatory response. Finally, in cultured hepatocytes isolated from untreated *Sod2*^{+/−} (but not wild-type) mice, troglitazone caused a concentration-dependent increase in superoxide anion production as demonstrated with the mitochondria-targeting fluorescent probe MitoSOX Red. In conclusion, prolonged administration of therapeutic doses of troglitazone can superimpose oxidant stress, potentiate mitochondrial damage, and induce hepatic necrosis in mice with genetically compromised mitochondrial function. These data are consistent with our hypothesis that inherited or acquired mitochondrial abnormalities may be one of the contributing determinants of susceptibility to troglitazone-induced idiosyncratic liver injury.

631 ARSENIC-INDUCED TRANSFORMATION OF HUMAN UROGENITAL PROGENITOR CELLS.

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Inorganic arsenic is a known human carcinogen and is associated with various cancers of the urogenital system (UGS) including the prostate, urinary bladder, and kidney. Our recent data indicate that transplacental arsenic exposure induces tumors and proliferative lesions throughout the UGS of the progeny mice. This suggests that during fetal exposure arsenic may target an undifferentiated, stem/progenitor cell population to induce malignant transformation. Here, we examined the effects of long-term arsenic exposure on the human progenitor cell line, WPE-stem. The WPE-stem cells were isolated from the immortalized, non-tumorigenic, normal human prostate-derived heterogeneous epithelial cell line, RWPE-1. Both cell lines were continuously exposed to a non-toxic concentration (5 μ M) of arsenic for 18 or more weeks. The WPE-stem cells became transformed after only 18 weeks of exposure, as determined by a 470% increase in MMP-9 activity. In comparison, transformation of the RWPE-1 cells took almost twice as long (30 weeks), and they showed only a 220% increase in MMP-9 activity. Additionally, compared to untreated controls, the transformed WPE-stem cells exhibited significant increases in invasion and colony forming efficiency, both markers of malignant transformation. Transformed RWPE-1 cells were not invasive and did not form colonies. An examination of differentiation markers suggests that arsenic may cause aberrant differentiation as a prelude to malignant transformation. These data suggest that: 1) arsenic may indeed target a stem/progenitor population during transplacental carcinogenesis; 2) arsenic-induced cell transformation appears to occur by altering various differentiation pathways of normal UGS cells; and 3) arsenic transformation of progenitor cells occurs much faster and results in a more aggressive malignant phenotype compared to the transformed "mature" cells. Experiments to determine in vivo tumorigenicity of arsenic-transformed progenitor cells are in progress.

632 THE ROLE OF MEMBRANE TRANSPORTERS IN THE CELLULAR METABOLISM OF ARSENIC.

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Arsenic (+3 oxidation state) methyltransferase (AS3MT) catalyzes methylation of inorganic arsenic (iAs) in humans. In this pathway iAs is converted to mono-(MAs) and dimethylated (DMAs) metabolites containing either As(III) or As(V).

Because toxicities and metabolic fates of these metabolites differ, the overall toxicity associated with exposure to iAs depends on the pattern and extent of its conversion to metabolites. In a clonal human urothelial cell line expressing rat AS3MT (UROtsa/F35) iAs(III) is more cytotoxic than in parental UROtsa cells that neither express AS3MT nor methylate iAs. Increased cytotoxicity of iAs(III) in UROtsa/F35 cells has been associated with production and retention of MAs. Here, we examined the roles of membrane transporters, multidrug resistance associated proteins (MRPs), and P-glycoprotein (P-gp), in production and retention of iAs metabolites in UROtsa and UROtsa/F35 cells. Retention and methylation of arsenicals were examined in cells in which MRPs or P-gp were inhibited or in untreated cells after exposure to 1 μ M iAs(III), MAs(III), or DMAs(III) for 2 or 24 h. After 2-h exposure, inhibition of MRP or P-gp significantly increased MAs(III) accumulation in either cell line and slightly increased DMAs production in MAs(III)-treated UROtsa/F35 cells. MRP and P-gp inhibition also increased MAs(III) accumulation in UROtsa cells after 24-h exposure. Neither cellular retention nor methylation rate was affected in UROtsa/F35 cells which converted MAs(III) to DMAs. Retention and methylation rates were unaffected in UROtsa or UROtsa/F35 cells exposed to iAs(III) or DMAs(III) for 2 or 24 h. Hence, MRPs or P-gp may play a key role in efflux of MAs(III), a toxic intermediate in iAs metabolism, in cells with limited capacity to convert MAs(III) to DMAs. (This abstract does not reflect EPA policy.)

633 THE INHIBITORY EFFECTS OF LEAD ON BONE FORMATION ARE MEDIATED BY AN INDUCTION OF SCLEROSTIN IN OSTEOBLASTS.

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Sclerostin is a newly identified regulatory factor that controls bone formation. It has been shown to be a potent repressor of osteoblast activity and may be responsible for mediating the mechano-transduction signals of osteocytes. Sclerostin is known to be produced by both osteocytes and osteoblasts. A number of laboratories have demonstrated that lead exposure in bone leads to a decrease in osteoblast function and a depression in bone formation. Our recent results indicate that the mechanism for this effect is due to a rapid and large upregulation of sclerostin in osteoblasts. Immunohistochemical evaluation of sclerostin expression was performed in mouse long bones and in fracture callus using a commercially available antibody with good specificity. Gene expression of sclerostin was measured in osteoblasts with RT-PCR. Osteoblasts were treated with lead at concentrations ranging from 16 nM to 20 μ M for periods of 24 hours.

We were able to show sclerostin expression in both osteocytes and osteoblasts in normal long bones as well as fracture callus. As would be predicted where bone formation was required to heal the fracture, sclerostin levels were low. They returned to normal after 15-20 days of healing. Osteoblasts that were exposed to lead dramatically upregulated sclerostin expression (by as much as 8 fold) with statistically significant effects occurring at concentrations of 2-4 micromolar and above. The effects were most pronounced in progenitor osteoblasts. Osteoblasts that were allowed to differentiate in culture or were treated with differentiating were not as sensitive to the effects of lead.

Lead exposure has been proposed as a risk factor for osteoporosis and as an inhibitor in fracture healing. The data in this report show that these effects may be mediated by upregulation of the bone-formation-repressor, sclerostin. Moreover, the effect of lead appears to be more pronounced in the less differentiated osteoblasts, implying that committed bone stem cells may be a key target for this heavy metal toxin.

634 LEAD INDUCES OXIDATIVE STRESS IN ARTICULAR CHONDROCYTES: A MOLECULAR MECHANISM FOR THE GENESIS OF OSTEOARTHRITIS.

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Osteoarthritis (OA) is the single most widely distributed musculoskeletal affliction in humans. As a number of reports in the literature have documented the effect of lead exposure on cartilage degeneration in animals and joint pain in humans, we believe lead may be key risk factor for OA. From the data in this study we hypothesize that the effect of this toxin may be mediated by altering the oxidative stress to articular chondrocytes.

Articular chondrocytes were propagated in culture and exposed to lead (80 nM to 2.0 μ M). Generation of oxidizing free radicals was quantified with the dye, DCFDA. Phenotype changes in the chondrocytes were monitored by measuring the expression of type X collagen, caspase 3 and 8. Free radical production, glutathione peroxidase (GPX), superoxide dismutase (SOD-1) and catalase activity were used to assess the balance of oxidative stress on the cells.

Chondrocytes show a statistically significant increase in the production of reactive oxygen species after exposure to lead. Singlet oxygen and superoxide radicals as well as hydrogen peroxide levels were all increased in a time and dose dependent fashion. This was accompanied by an increase in DCFDA conversion. These effects happened within minutes of lead addition. GPX and catalase were inhibited by lead at low concentrations (0.4 micromolar). The resultant increase in oxidative stress induces a phenotypic change in the articular chondrocytes characterized by large increases in type X collagen and the caspases. Interestingly, prolonged exposure of the chondrocytes to lead (24 hours) induced a six fold increase in SOD-1 expression. We interpret this observation as an attempt by the cells to resist the change in the oxidative milieu.

Articular chondrocytes that are exposed to lead alter their redox environment, tipping the balance toward oxidative stress. This induces specific phenotypic changes characteristic of cartilage breakdown that will progress to OA.

635 PARTICULATE CR(VI)-INDUCED DNA DOUBLE STRAND BREAK MISREPAIR LEADS TO NEOPLASTIC TRANSFORMATION.

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DNA double strand breaks (DSBs) are one of the most dangerous types of DNA damage. Defects in DSB repair can cause the loss or amplification of chromosomal material and tumorigenesis. Hexavalent chromium (Cr(VI)) is a potent respiratory toxicant and carcinogen with widespread human exposure. The most toxic and carcinogenic form of Cr(VI) is the particulate such as lead chromate (LC), which deposits and persists in the respiratory tract after inhalation. LC particles induce DSBs in human lung cells. The hMRE11-RAD50-NBS1 protein complex has emerged as a central player in the human cellular DNA-damage response, and recent observations suggest that these proteins are at least partially responsible for linking DSB damage detection to DSB-repair and cell cycle-checkpoint functions. Using the comet assay, we found that LC induced concentration-dependent increases in DSBs in cultured human lung cells with 0.1, 0.5, 1, and 5 µg/cm² LC inducing 1.5, 2, 4.9 and 10.2 percent increase in tail DNA relative to control, respectively. The repair of LC-induced DSBs occurred within 24 h. This repair required MRE11 as reducing Mre11 expression by siRNA inhibited repair. We confirmed these observations by treating MRE11 deficient skin cells with LC and found that the repair of these lesions was greatly reduced in these cells as well. To link the DSB to carcinogenesis, we developed a model of neoplastic transformation using MRE11 deficient cells. A 5 day exposure to LC induced loss of cell contact inhibition in a concentration-dependent manner with 0, 0.1, 0.5 and 1 µg/cm² lead chromate inducing 1, 78, 44, 103 growth foci in 20 dishes, respectively. The transformed cells developed through a series of sequential steps, including altered cell morphology and anchorage-independent growth. These data indicate that MRE11 protects cells from LC-induced DNA DSBs and neoplastic transformation. This work was supported by NIEHS grant ES10838 (J.P.W.)

636 MANGANESE INDUCES RAPID AND SELECTIVE LYSOSOME TRAFFICKING AND DEGRADATION OF THE GOLGI MEMBRANE PROTEIN GPP130.

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We used rat neuronally derived GABAergic (AF5) cell and HeLa cell models to investigate the effect of Mn exposure on intracellular trafficking. Cross-reactivity in an immunoblot analysis of AF5 cells revealed that Mn exposure (100 – 150µM) caused the rapid (within 4 hr) depletion of an initially unknown protein with an apparent MW of ~95kDa. Mass spectroscopy identified this protein as Golgi Phosphoprotein 4 (GPP130). GPP130 is a Golgi-localized integral membrane protein that cycles to endosomes where it may mediate endosome-to-Golgi retrieval of other cycling Golgi proteins (Natarajan and Linstedt, 2004). Mn-induced GPP130 degradation was neither recapitulated by the protein synthesis inhibitor cyclohexamide nor blocked by the proteasome inhibitor PSI suggesting a post-translational and proteasome-independent mechanism. After exposure to Mn, GPP130 underwent a dramatic redistribution from its normal Golgi localization to endosomal and lysosomal structures and then, by 2-4 h, was significantly depleted from all cellular structures. In support of the model that Mn had induced trafficking of GPP130 to lysosomes where GPP130 was then degraded the degradation was blocked by the lysosome hydrolase inhibitor chloroquine. Importantly, the normal localization and abundance of endosome and Golgi markers, including the GPP130-related protein GP73 whose Golgi localization depends on endosome-to-Golgi retrieval, indicated that Mn specifically affected GPP130 and did not generally disrupt membrane trafficking. Thus, cellular toxicity resulting from Mn exposure involves, in part, induced trafficking and degradation of the Golgi protein GPP130.

637 CADMIUM INDUCES STAT3 ACTIVATION BY A MECHANISM DEPENDENT ON NADPH OXIDASE ACTIVITY.

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Oxidative stress elicited by Cd is a likely contributor to hepatotoxicity. Cd can activate some oxidative stress-sensitive transcription factors that are responsible for regulating gene expression such as AP-1 or Nrf2, however STAT3 is not fully characterized. The aims of this study were to determine the activation of STAT3 pathway induced by Cd-oxidative stress and to address the participation of STAT3 in Hsp70 production in HepG2 cells. Cells were treated with 5 µM CdCl₂ for 0.25, 0.5, 1, 2, 3, 4 or 5 h. Cadmium induced 4-fold Stat3 activation after 1 h treatment, determined by nuclear protein Western blot. To explore the ROS source for Cd-induced STAT3 activation, inhibitors for different ROS generating systems were used. Only DPI, an inhibitor of NADPH oxidase, decreased STAT3 activation. NADPH oxidase activity, was then determined. Cd induced 3-fold the activity of this enzyme. To confirm if NADPH oxidase-derived ROS were involved in STAT3 activation, SOD-peg and Catalase-peg were added as antioxidants. Treatment with Catalase-peg reduced significantly STAT3 activation. MAPK pathway is activated by oxidative stress. In order to explore if MAPK pathway is involved in STAT3 activation, we pretreated HepG2 cells with 20µM of PD98059 (Erk inhibitor), SB203580 (p38 inhibitor) or SP600125 (JNK inhibitor). Only Erk and JNK inhibitors abrogated Cd-induced STAT3 activation. Finally, in order to figure out some physiologic relevance in Cd-induced STAT3 activation, we explored Hsp70 expression, a Cd-induced protein. Pretreatment with a specific STAT3 peptide inhibitor, abrogated Cd-induced Hsp70 expression. Our data suggest that Cd activates NADPH oxidase activity which induces ROS production leading Erk and JNK activation, these MAPK promote STAT3 phosphorylation that can induce a protective mechanism against Cd toxicity.

CONACYT P 39618-M.

638 GENE EXPRESSION PROFILE IN RESPONSE TO POTASSIUM DICHROMATE-INDUCED TOXICITY IN HUMAN DERMAL FIBROBLASTS.

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Several adverse health effects including irritant and allergic contact dermatitis have been reported among workers following occupational exposure to chromium containing compounds. To understand the molecular mechanisms responsible for the dermal toxicity of chromium, a differential gene expression profile of human skin fibroblasts exposed to a cytotoxic concentration of hexavalent potassium dichromate [Cr(VI)] was studied. Skin fibroblasts were exposed to 5 micromolar Cr(VI) for time intervals up to 24-hours and a differential gene expression profile was studied using the human Toxicology and Drug Resistance Microarray (Super Array Inc.). Of the 280 genes represented on the array, 32 were found differentially expressed in the fibroblasts exposed to Cr(VI). In general, genes involved in stress response, cell cycle control, drug metabolism, apoptosis and growth were found differentially expressed in the Cr(VI) exposed fibroblasts. The molecular mechanisms responsible for the Cr(VI)-induced differential gene expression were investigated using heme oxygenase 1 (HO-1) as the model gene. HO-1 gene expression was significantly higher in the Cr(VI) exposed cells compared with the control cells. Pre-exposure of dermal fibroblasts to actinomycin D (inhibitor of transcription) and N-acetyl cysteine (scavenger of reactive oxygen species) blocked the Cr(VI)-induced overexpression of the HO-1 gene. Similarly, modulating the intracellular glutathione level by pre-treating cells with either BSO or glutathione significantly influenced the potential of Cr(VI) to induce the expression of the HO-1 gene. Similar results were obtained when experiments were conducted using a luciferase reporter gene expression system containing the HO-1 gene promoter. In conclusion, our results demonstrate that the cellular glutathione level may be an important determining factor in the dermal toxicity induced by hexavalent chromium.

639 DEVELOPMENT OF A BASE SET OF TOXICITY TESTS AS A COMPONENT OF A NANOPARTICLE RISK MANAGEMENT FRAMEWORK.

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The development of a risk management framework for nano or ultrafine particle-types requires a base set of hazard data. Previously, we have suggested "parallel tracks" as a strategy for conducting nanoparticle research. Mechanistic studies on "representative" nanoparticles could be supported by governmental agencies. Alternatively, for commercial nanoparticles, the EHS framework would include a minimum base set of toxicity studies, supported by the companies that are develop-

ing that nano-based product. The minimum base set could include: substantial particle characterization, pulmonary toxicity studies, acute dermal toxicity and sensitization studies, acute oral and ocular toxicity studies, along with screening type genotoxicity, and aquatic studies.

We report here the toxicity results of a base set of tests on a newly developed, well-characterized, ultrafine rutile TiO₂ (uf-TiO₂) particle-type. In vivo pulmonary toxicity studies demonstrated low inflammatory potential and lung tissue toxicity. Acute dermal irritation studies in rabbits and local lymph node assay results in mice indicated that uf-TiO₂ was not a skin irritant or dermal sensitizer. Acute oral toxicity studies demonstrated very low toxicity and uf-TiO₂ produced short-term ocular conjunctival redness in 1 of 3 rabbits. Genotoxicity tests demonstrated that uf-TiO₂ was negative in both the Bacterial Reverse Mutation Test and In Vitro Mammalian Chromosome Aberration Test in Chinese Hamster Ovary Cells. The results of aquatic toxicity screening studies demonstrated that uf-TiO₂ exhibited low concern for aquatic hazard in un aerated, 48-hour, static acute tests using the water flea, *Daphnia magna*; exhibited low concern for aquatic hazard in un aerated, 96-hour, static acute tests using the rainbow trout, *Oncorhynchus mykiss*; and exhibited medium concern in a 72-hour, acute test using the green algae *Pseudokirchneriella subcapitata*.

In the aggregate, most of the tests demonstrated low hazard potential in mammals or aquatic species following acute exposures to ultrafine rutile TiO₂ particles.

640 TOXIC EFFECTS OF NANOCOPPER ON ZEBRAFISH, DANIO RERIO.

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With increasing utilization of metallic nanomaterials there is the potential for environmental release of these materials. Despite this, little is known about potential adverse effects of these nanomaterials on ecologically important species. Because many of the metals being used as particles are toxic in soluble forms, it is important to determine if nanomaterials elicit responses that differ from soluble metals. To investigate this question, adult female zebrafish were exposed to 50nm copper particles at concentrations up to 1.5 mg/L for 48 hours. When added to water, copper particles aggregated and mean particle volume was 20 microns, although large numbers of smaller particles (less than 1 micron) remained in the water column. Copper nanoparticles were less acutely toxic to adult female zebrafish than copper sulfate (the 48-hr LC50 for nanocopper = 1.5 mg/L, vs. 0.25 mg/L for copper sulfate). The observed lethal effects of particle exposure appear to be primarily mediated by the particles, not dissolution. In tanks treated with 1.5 mg/L copper particles, only 0.1 mg/L of dissolved copper was present at 48 hours, equivalent to a concentration of copper sulfate producing 15% mortality. This conclusion was supported by differences in biochemical and molecular changes following exposure to the two forms of copper. While BUN/ALT levels, gene expression patterns in liver, and liver histopathology showed similar responses to both forms of copper, the observed gene expression responses were markedly different in gill samples, indicating that the particles induced a different transcriptome level response than did copper sulfate. Specifically, HIF-1 and HSP 70 were upregulated 10-fold by nanocopper, but not soluble copper. We therefore conclude that copper nanoparticles exert a toxic effect on zebrafish gill that is not solely due to dissolution of the particles. This work is supported by NSF grant BES-0540920.

641 BI-VALVE MOLLUSKS AS VALUABLE MODELS FOR CHARACTERIZING NANOPARTICLE TOXICITY.

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There are numerous potential environmental risks of engineered nanoparticles that are not yet well-characterized or understood. Nanoparticles may be introduced into aquatic environments during production processes and also as a result of release following their use in electronic and biological applications. Filter-feeding bivalve mollusks spend their lives removing particles so they are potentially very valuable as model species for characterizing nanoparticle bioavailability and interactions with basic cellular processes. The purpose of these studies was to characterize the toxicity of various nanoparticle preparations (e.g. quantum dots and fullerenes) using various exposure regimes with whole animal exposure, isolated hepatopancreas cells, and embryos of oysters, *Crassostrea virginica*. Nonfunctionalized QDs composed of a Cd/Se core surrounded by layers of Zn (Evident Technologies) were used to evaluate potential hepatotoxicity using a lysosomal destabilization following whole animal and isolated cell exposures. Significant increases in toxicity were observed, similar to levels observed with cadmium chloride concentrations ranging from 10 to 1000 ug/l. Similar toxicity studies were also conducted using fullerene preparations, solubilized using either toluene or THF. Significant toxicity was observed with both preparations, and the toluene extracts were more toxic than the THF extracts with both hepatopancreas assays and embryo development assays.

Fluorescent confocal microscopy and electron microscopy were used to verify the accumulation of the nanoparticles inside hepatopancreas cells and embryos. These kinds of basic studies are essential for addressing the potential impacts of nanoengineered particles on fundamental cellular processes as well as aquatic organisms.

642 IN VIVO TOXICITY EVALUATIONS OF GOLD NANOPARTICLES: PROACTIVELY DESIGNING SAFER NANOMATERIALS.

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Gold nanoparticles varying in composition, core size and surface functionalization have been proposed for many applications in nanoscience. It has largely been assumed that gold nanoparticles would be biologically inactive given that both the molecular and bulk forms of gold are benign; however, this assumption has not been formally tested. Embryonic zebrafish were chosen as a dynamic whole animal assay to reveal whether gold nanoparticles perturb developing systems. To investigate the importance of size and shape on the toxic potential of nanomaterials, we chose two core sizes (0.8 and 1.5nm) with one of three surface groups (neutral, positive and negative). Embryonic zebrafish were exposed for five days and were evaluated for mortality, morphological malformations and developmental progression. Core size and surface functionalization both influenced toxicity. Gold nanoparticles functionalized with TMAT caused a significant increase in mortality at 10 parts per million (ppm) for 1.5 nm particles and 400 parts per billion (ppb) for 0.8 nm particles. Embryos exposed to 1.5 nm TMAT functionalized nanoparticles also displayed increased incidences of malformations at 50ppm. Exposure to MES functionalized particles did not result in increased mortality at concentrations up to 250 ppm; however, concentrations of 10 and 50 ppm did result in increased incidences of malformation for 1.5 and 0.8nm particles, respectively. MEE surface functionalization was benign for both size particles. Results obtained from these studies demonstrate that integrating the biological assessment of nanomaterials with their development can be used to direct the rational development of safer, less hazardous nanomaterials.

643 IN VITRO STUDIES OF SINGLE-WALLED CARBON NANOTUBES: A TOXICOGENOMICS APPROACH.

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Engineered nanomaterials are characterized as having at least one dimension of 1 – 100 nm. Early reports show contradicting results as to the toxicity of unmodified single-walled carbon nanotubes (SWNT). Here, an *in vitro* toxicogenomics approach is used to assess the toxicity of these nanomaterials. Human epidermal keratinocytes (HEK) and normal human bronchial epithelial (NHBE) cells were cultured for 24 hours with cytotoxic doses of SWNT, silica (SiO₂) and carbonyl iron (CI). Cells from individual treatments were harvested at each timepoint and snap frozen to -80 °C. Total RNA was isolated from these cell pellets and cRNA probes were synthesized and hybridized against gene expression microarrays. All microarray experiments were done in triplicate. To compare expression profiles from the two cell systems, only the 3,464 common unique genes were considered in this work. Gene expression profiles of significantly expressed genes (2-fold change or greater) from HEK cells were nearly 1 – 2 orders of magnitude greater than that of the NHBE cells. Using hierarchical clustering and principal components analysis, the largest variation in the gene expression values were between the skin and lung cells, regardless of nanomaterial treatment. However, at cytotoxic doses, treatment with CI gave the highest gene activity in the HEK cells while treatment with SiO₂ gave the highest activity in the NHBE cells. HEK cells treated with SWNT showed a 4-fold increase in expression for the genes that encode for human IL-8 and cytokine subfamily B (Cys-X-Cys) agreeing with previous citations of particulate exposure. Potential biomarkers of other annotated and unannotated genes were also found.

644 SIZE AND SURFACE PROPERTIES DETERMINE THE INTERNALIZATION PATHWAY OF NANOPARTICLES BY TYPE II ALVEOLAR EPITHELIAL CELLS.

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Accumulating observations suggest that inhaled nanoparticles (NPs) exert harmful effects on human health to a greater extent than larger particles. The effects have been attributed, in part, to NPs large surface-area-to-mass ratio, leading to increased reactivity and oxidative stress. However, it is not yet clear what role specific

physical and chemical surface properties have in modulating the interaction of NPs with the living cell. Although large agglomerates of NPs are found within cells, it is thought that they are formed as NPs accumulate within specific cellular compartments, or as the result of experimental conditions. The initial interaction of NPs with the cell in vivo must occur at the level of individual or small NP aggregates, if the increase in surface-area plays a role in NP reactivity and toxicity. Little is known, however, about the mechanisms that underlie the internalization process of individual NPs. Here we applied time-lapse single-molecule fluorescence imaging techniques to investigate the properties that govern the initial interactions of individual NPs with cultured type II alveolar epithelial cells (C10). Using amorphous silica particles that were tagged with a small number of fluorescent molecules, we compared 500 nm particles with 100 nm or smaller particles, and naked particles with surface-modified particles. We found that 500 nm and 100 nm particles could enter the cells through completely different pathways, and that their route was dependent on specific surface modifications. Our findings support the idea that the size and surface properties of particles determine their initial interactions with alveolar epithelial cells, and suggest that the differential effects of particles with different sizes might originate from their different internalization pathways. Supported by the U.S. DOE under Contract DE-AC05-76RL01830, and conducted in EMSL, DOE User's Facility.

645 TARGETING NANOMATERIALS TO CELL SURFACE RECEPTORS: IMPLICATIONS FOR DEFINING RELEVANT MODES OF ACTION AND CHRONIC TOXICITY.

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Nanomaterials delivered via intravenous administration are cleared by phagocytes located in the liver and spleen with evidence for persistent retention in hepatic tissues. Here we investigated the uptake of SWCNTs by scavenger receptors in RAW 264.7 macrophage cells and associated effects on the inflammatory response to endotoxin (LPS). LPS (1 ng/ml, 24 hr) increased cyclooxygenase-2 (Cox-2) expression, a key mediator of inflammatory responses, in RAW 264.7 cells. Cotreatment of RAW cells with LPS and SWCNTs solubilized by sonication in normal growth medium resulted in the inhibition of LPS-mediated Cox-2 expression, while sonication in an emulsifying agent (Pluronic F-127) was without effect. Fucoidan blocked the ability of SWCNTs to inhibit LPS-mediated Cox-2 expression, implicating scavenger receptors in this process. Expression of class A and B scavenger receptors in RAW cells was confirmed by Western Blot. Alexa 647-labeled SWCNTs were internalized by RAW cells and observed to colocalize with lysosomes, as determined using a lysotracker green dye. Lysosomal localization of Alexa 647-labeled SWCNTs was dramatically reduced by fucoidan and polyinosinic acid, consistent with a role for scavenger receptors in this process. Albumin was identified as the major serum protein binding to SWCNTs. These observations indicate that SWCNTs bind to scavenger receptors in a manner that is dependent upon interaction with serum components indicating that the method of nanomaterial resuspension can dramatically effect their molecular targeting. We hypothesize that nanomaterials that reach systemic circulation have the potential to form nanomaterial-albumin complexes that will be recognized as damaged albumin by scavenger receptors. This hypothesis has plausible implications for the persistent retention of nanomaterials in scavenger receptor rich tissues and chronic toxicity. This research was supported by U.S. DOE funding (Contract DE-AC05-76RL01830).

646 NANOPARTICLES STIMULATE INTRACELLULAR AND EXTRACELLULAR RADICAL SPECIES.

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Nanomaterials are being identified for a number of applications ranging from cosmetics to circuitry. With the increase in production and use comes an increase for potential human and environmental exposures which when combined with nanoparticle hazard presents a potential increase in risk. However, the mechanism of action is not clearly understood. There has been some research suggesting that nanoparticles are capable of eliciting their damage via oxidative damage. This research addresses the questions concerning how much of the oxidative damage is due to the intrinsic activity of the particle, how much is due to stimulation of in vitro models, and how these effects might be additive or synergistic. Rat derived pulmonary or neuronal cell lines were cultured and exposed to copper, titanium dioxide, and aluminum nanoparticles in the presence of fluorescent dyes to detect reactive oxygen species (ROS). Additionally, cell endocytosis was blocked to determine the importance of uptake in ROS generation. Results from the studies reveal that

nanoparticles are capable of producing both intracellular and extracellular ROS; soluble ions did not elicit the same response as the particle. These particle effects are sustained and can be measured even after free particles were removed. Active uptake of particles produces a greater intracellular response compared to particles that are only able to act at the cell surface. We can conclude that nanoparticles do possess intrinsic radical producing activity; which appears to be supplemented by cellular ROS eventually triggering an antioxidant response. These effects varied with both particle and cell type with neuronal cells exhibiting the greatest response to the most benign particles. Those particles that possess the greatest ROS activity were generally the most cytotoxic to the cells; providing evidence that ROS production may be a mechanism of action by which nanoparticles exert toxicity.



647 APPROACHES TO MANAGE ETHICAL CONFLICTS IN RESEARCH.

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Researchers have identified funding and non-financial factors as potential sources of conflicts for research conducted in or funded by academia, industry, governmental and non-governmental organizations. For example, different organizations may introduce conflicts by retaining control over publication rights for research findings, or by increasing the probability of renewing grants for those researchers who obtain "positive" results. Disclosure and transparency are important tools for the management of conflicts associated with sources of research funding. Current guidelines on conflict of interest from the National Institutes of Health and others most commonly focus on disclosure requirements for financial conflicts of interest and less frequently cover other tools for management of conflicts, such as control of publication rights or comprehensive documentation of research protocols and results. If adopted, these tools may serve to increase the reliability and credibility of research results. Four speakers with research experience in a broad range of setting will address ethical conflicts and potential management tools. University researchers will discuss management of financial and non-financial conflicts and important considerations for conducting privately-funded research in a university setting. A private sector researcher will discuss potential conflict of interest at contract research laboratories and a government regulator will discuss agency procedures to verify research integrity. The presentations will be followed by an open discussion of methods to manage ethical conflicts in research.



648 BEYOND THE SINGLE POINT: THE USE OF DOSE AND TIME IN GENOMIC DATA FOR MECHANISTIC-BASED RISK ASSESSMENT.

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As gene expression profiling becomes more common, a growing number of studies have examined gene expression responses over a wide dose range and/or an extended time course. Such data beg two questions. Can gene expression give insight into the adverse events that occur as a result of chronic and/or lower exposures? Can this data identify environmentally relevant effects and distinguish those not associated with toxicity (e.g., adaptive responses) vs those that are associated with toxicity (i.e., mal-adaptive effects)? The discussants will first describe several exemplar studies where multiple dose and/or time points were collected for genomic analysis, covering agents of environmental concern (furans, estrogen and fungicide conazoles) as well as of pharmaceutical interest (acetaminophen). The roundtable will then address questions posed, in the context of gene expression responses that are both continuous and discontinuous over time and dose, and mechanisms that come into play at different stages of the exposure continuum.



649 DOSE-RELATED CHANGES IN THE TRANSCRIPTOMIC RESPONSE OF CELLS AND TISSUES TO GENOTOXIC STRESS.

B. Gollapudi. *Dow Chemical Company, Midland, MI.*

Exposure of cells and tissues is expected to set into motion a series of events, including gene expression changes, to cope with the stress. A number of variables, such as the dose, sampling time, and cell and tissue source have been shown to influence the transcriptomic response in experimental models. This presentation will discuss data from in vitro and in vivo studies that examines the utility (or lack thereof) of transcriptomic responses as indicators of exposure to genotoxic/carcinogenic materials at environmentally relevant dose levels.

650 GENOMIC ASSESSMENT OF DOSE RESPONSES TO CONAZOLE FUNGICIDES.

S. Hester. *NHEERL, U.S.-EPA, Research Triangle Park, NC.* Sponsor: C. Corton.

Acute responses to chemical exposure that are below a toxic threshold can provide data on genes and pathways involved in the action of a chemical or drug. We will present dose response analysis of an Affymetrix dataset with low, non-tumorigenic and tumorigenic doses for a class of triazole-containing fungicides containing 3 conazoles with varied toxicities. Distinct dose-dependent transcriptional profiles were associated with each chemical and these dose profiles could be correlated with other toxicologic endpoints. Tumorigenic doses were associated with defined transcriptional profiles which could differentiate these conazoles. The analyses will highlight an approach to examine the effects of dose and how to interpret these findings in a biological context.

651 MICROARRAY ANALYSIS OF THERAPEUTIC AND TOXIC DOSES OF ACETAMINOPHEN IN RAT LIVERS.

M. Orr. *CDER, FDA, Silver Spring, MD.*

A global snapshot of the molecular changes induced by either therapeutic or toxic doses of acetaminophen allows one to determine if the gene expression responses are unique between an efficacious or a toxic dose of acetaminophen (APAP). This study provides molecular evidence that the low (0.1g/kg) dose of APAP is not inducing toxicity in rats. In addition, this study provides evidence that more cell cycle regulatory genes were dysregulated at earlier time points (3 and 6 hr) while the majority of the phase II genes were dysregulated at 24 hrs following exposure to the high dose of APAP (2g/kg). Based upon microarray generated gene expression information: Can therapeutic index be determined using toxicogenomic data? Preliminary evidence indicates it is possible at the mechanistic level since gene expression profiles are distinct between non-toxic and toxic doses of APAP. Clearly, we would need to collect more toxicogenomic data from dose-ranging studies for multiple compounds to shed further light on this topic.

652 DOSE AND TIME RESPONSE OF ESTROGEN-INDUCED GENE EXPRESSION CHANGES IN THE DEVELOPING REPRODUCTIVE SYSTEM.

G. Daston. *Procter & Gamble Company, Cincinnati, OH.*

Early development is likely to be the life stage that is most susceptible to endocrine-mediated toxicity, but the functional and structural effects of early exposures tend to be latent. We have used dose-response data to confirm that the transcript profile is robust across dose levels, and to shed light on the controversy about the existence of non-monotonic low-dose effects of estrogens on male reproductive development. Our results, using three model estrogens and a 4-6 orders of magnitude spread of dosages, indicates a monotonic dose-response curve for estrogens, with a clear non-observable transcriptional effect level.

653 GRANTSMANSHIP FORUM: FUNDING OPPORTUNITIES AT NIH.

V. S. Vaidya¹ and E. M. Faustman². ¹*Medicine-Renal, Harvard Medical School, Boston, MA* and ²*Env & Occup Hlth Sciences, Institute for Risk Analysis & Risk Communication, Seattle, WA.*

Extramural funding has always been a backbone of academic research and NIH is the leading federal institute to fund extramural research grants with a first year (FY) 2006 budget of \$ 28,740 million. Although traditionally toxicology researchers have received funding from a wide spectrum of the 20 NIH institutes, the NIEHS and NIGMS have been of particular interest in terms of funding chemical toxicology and environmental health related research projects. Similarly, a number of toxicology researchers are also actively pursuing research related to use of genomics and bioinformatics for mechanistic identification of genes, proteins and molecular pathways to identify mechanisms of toxicity. This makes National Human Genome Research Institute (NHGRI) very attractive for potential grant opportunities.

The objective of this forum is to present funding opportunities to toxicology researchers specifically focused on NIH grant opportunities by NIEHS, NIGMS, and NHGRI and will indicate recent announcements for request of applications, novel research directions, and new funding mechanisms for e.g.: NIH Pathway to Independence (PI) Award (K99/R00), the NIEHS Outstanding New Environmental Scientist (ONES) Award, etc.

This session will be chaired by Dr. Elaine Faustman and co-chaired by Dr. Vishal Vaidya. The session will highlight grant opportunities at NIEHS, NIGMS and NHGRI. Dr. Anne Sassaman is the Director of extramural research and training at NIEHS and will discuss the new funding initiatives by the NIEHS

including a discussion of the recent ONES awards. Dr. Richard Okita is the Program Director at the NIGMS and will highlight the novel funding opportunities from the NIGMS for young and established investigators. Dr. Mark Guyer is the Director of the division of extramural research at NHGRI and is responsible for overseeing the NHGRI extramural program. Dr. Guyer will share the new funding opportunities by NHGRI with an emphasis on scientific activities to advance genomics research in environmental health and medicine.

654 UNDERGRADUATE RESEARCH AS A SPRINGBOARD INTO RESEARCH CAREERS.

J. B. Tarloff. *Pharmaceutical Sciences, University of the Sciences in Philadelphia, Philadelphia, PA.*

In recent years, SOT has begun to develop and sponsor educational programs targeted at recruiting undergraduate students who may be interested in pursuing toxicology as a career. We believe that one of the best ways to recruit undergraduate students into graduate education is to expose them to well-designed and productive research programs. Undergraduate research benefits the students involved by fostering the development of problem solving and critical thinking skills. Also, when undergraduate students successfully participate in laboratory discussions or scientific presentations, they develop confidence and raise their self-esteem levels. Undergraduate research programs benefit the laboratories involved, providing a pipeline for capable undergraduate research assistants, mentoring opportunities for graduate students, desirable learning outcomes for accreditation agencies, and ultimately a pool of graduates who are highly qualified for graduate study or employment in research laboratories. Programs and initiatives for undergraduate research in toxicology are scattered throughout the country and information about these opportunities is not readily available. Through this workshop, we will highlight several different programs using undergraduate students in research settings, discussing the key components for successful undergraduate research experiences: (1) a well-designed project that yields unambiguous data, (2) time and patience of the principal investigator, and (3) financial remuneration, particularly for summer experiences. For anyone with access to undergraduate students, whether or not they are currently involved in these types of educational research experiences, this workshop will provide the basics for development of a program that will serve as first steps for qualified undergraduate students interested in research careers.

655 UNDERGRADUATE RESEARCH: OPENING THE DOOR TO A CAREER.

K. E. Stine. *Department of Biology/Toxicology, Ashland University, Ashland, OH.*

The value of integrating research into undergraduate science education has been recognized, and has long been promoted by groups such as the Council on Undergraduate Research, Sigma Xi, and Project Kaleidoscope. Over the past fifteen years, the Department of Biology/Toxicology at Ashland University has undergone a significant transition, moving from considering undergraduate research as a peripheral component of undergraduate education to considering it as a focal point of the department. The results of this change have been widely perceived as beneficial, both for the students and for the department. Over the past five years, more than 60 students within the department have participated in research, most of whom had no prior experience in the laboratory, and little or no idea that a research career was desirable or even possible for them. However, given the opportunity to participate in a research project (designing and carrying out experiments, analyzing data and even presenting findings at national meetings or in publications), many of these students became highly motivated to seek out careers in research. Furthermore, even students not directly involved in a laboratory or field project at Ashland have been influenced by the experience of their peers into also pursuing research careers. This presentation will describe the undergraduate research experience at Ashland University, and how this experience is integrated into the university's undergraduate toxicology, biology, and environmental science programs. The focus will be on the impact of research opportunities on undergraduate students in toxicology, including influence on career choice, graduate school acceptance, and job searches. The benefits to faculty of involving undergraduates in their toxicology research will also be discussed, as well as the practical considerations that are necessary in setting up an appropriate niche for undergraduates in a research program. Finally, options for funding undergraduate research in toxicology will be addressed.

656 TURNING STUDENTS INTO SCIENTISTS: SUMMER RESEARCH EXPERIENCES AT THE UNIVERSITY OF THE SCIENCES IN PHILADELPHIA.

J. B. Tarloff. *Pharmaceutical Sciences, University of the Sciences in Philadelphia, Philadelphia, PA.*

The Academic Research Enhancement Award (AREA grant, R15) is designed to stimulate investigator-initiated research at colleges and universities that lack significant NIH funding. In addition, the awards are intended to stimulate participation

by both undergraduate and graduate students in research projects. We have supported undergraduate students in research programs funded through the AREA mechanism for the past three summers. The experiences have been invaluable for both students and mentors. Previously, student participated in research during the academic year. Time constraints for students and faculty members significantly impacted the quantity and quality of the research. The AREA program allows concentrated effort for students at a time when faculty members are unencumbered by teaching and service requirements. As part of their curriculum, undergraduate students at USP do simple laboratory experiments and learn basic calculations. Summer research helps bring these experiences in to perspective and reinforces students' skills. Participation of undergraduate students in research projects also gives graduate students opportunities to serve as mentors and advisors. We have found several key points that are critical in designing a meaningful summer research experience for undergraduate students. Intensive participation in the training process by the faculty member is important, as is helping the students understand the hypothesis underlying the specific research project on which the student is working. Laboratory meetings help all the participants see how the separate pieces fit into a whole picture. As undergraduate students gain confidence in their research and problem-solving skills, they develop positive attitudes toward their entire educational experience. Students who complete summer research experiences are extremely successful in securing graduate school admission or full-time research employment following graduation.

i 657 UNDERGRADUATE RESEARCH PROGRAMS AT THE CIIT CENTERS FOR HEALTH RESEARCH (CIIT).

D. C. Dorman. *CIIT Centers for Health Research, Research Triangle Park, NC.*

In 1989, CIIT developed a Summer Intern Program to complement its already broad-based post-doctoral and pre-doctoral training missions. CIIT's cooperative education program was subsequently developed to give undergraduate students the opportunity to integrate their academic studies with related job experience at CIIT. These programs are designed to acquaint promising undergraduates with biomedical research in one of CIIT's research focus areas (respiratory biology, inhalation toxicology, endocrine biology, reproductive toxicology, molecular biology, computational biology, and risk assessment). Students are exposed to many aspects of modern research including literature review, hypothesis formulation, conduct of laboratory experiments, data analysis and interpretation. Professional students (e.g., students enrolled in veterinary medicine) have also participated in the training program. Program participants are supervised by CIIT staff scientist, postdoctoral fellow, or senior staff member. All students also receive orientation in the use of animals in biomedical research as well as health and safety and quality assurance and control training. At the conclusion of the summer, Interns generally present their findings at a seminar attended by CIIT scientists and staff. Research conducted by CIIT undergraduate researchers is critical to the overall research mission of the Institute and often forms the basis of many presentations at national scientific meetings and ultimately publications. Participating students often go on to complete advanced training upon completion of their undergraduate degree. A locally competitive hourly salary is provided to all program participants. Funding for this program is primarily provided through the prospective mentors research grants or contracts. Advertisement of these positions occurs through the institute's website (www.ciit.org) and several internship guides (e.g., Peterson's Guide to Internships). The application deadline is March 1st of the year prior to the summer program and on-line application through CIIT's website is strongly encouraged.

i 658 STUDENT INTERNSHIPS: AN IMPORTANT ELEMENT IN SCIENTIFIC DEVELOPMENT.

M. D. Aleo. *Drug Safety R&D, Pfizer Global R&D, Groton, CT.*

Undergraduate internships in academia and industry can be difficult to obtain, but are extremely beneficial to students, faculty sponsors, and academic/industrial sponsors. Laboratory-based experiences in the undergraduate environment are typically confined to cookbook experiments using basic laboratory procedures, instruments and concepts that are designed to produce a successful outcome within a short period of time. Internships, like independent research experiences, challenge students to learn and be successful, even through failure, in the real world of scientific research. Students are exposed to scientific projects, concepts, techniques, and equipment that expands their experience base. This presentation will focus on the following core elements of helping students obtain internships in an academic research or corporate toxicology setting: understanding the three major types of internship experiences, finding internship opportunities, the application process (timing, application and interview), along with general information regarding eligibility requirements for employment, stipends, housing and travel expenses. The

benefits of internship opportunities will be discussed from the perspective of the student, sponsoring faculty member and academic/industrial sponsor. Information regarding internship opportunities directly targeting minority applicants will also be presented.

659 NOVEL COMPUTATIONAL STRATEGIES FOR REDUCING CHEMICAL RESIDUES IN FOOD OF ANIMAL ORIGIN.

R. E. Baynes¹, J. E. Riviere¹ and A. L. Craigmill². ¹PHP, North Carolina State University, Raleigh, NC and ²Environmental Toxicology, University of California, Davis, CA.

Animal-derived foods can be contaminated with drugs or toxicants at some stage prior to slaughter. One strategy to prevent the entry of contaminated food products from entering the food chain is to allow the clearance of these harmful chemicals from the animal's body to safe levels prior to slaughter. These safe levels refer to concentrations of chemical in the food item that will not cause adverse effects in humans.

Regulatory methods require utilization of classical pharmacokinetic and statistical analyses of drug residue data from target tissues. When the drug concentration has reached the safe level, this is deemed the withdrawal time. If the withdrawal time is adhered to, residue levels in the food item should be safe for human consumption. These computational methods are however limited to the use of approved drugs in approved livestock species at the approved dose, and approved route of administration. Departure from any of these approved conditions will very likely result in violative drug residues. The farmer or veterinarian may be forced to use the drug in an unapproved manner to adequately treat the animal. Animals are also accidentally exposed to environmental contaminants as evidenced by the accidental PBB contamination of livestock feed in Michigan in 1974. This is believed to have resulted in adverse health effects in humans who had consumed dairy or meat products from similarly exposed livestock animals.

The purpose of this workshop is to not only review these cases and the regulatory framework, but to share with participants past, current, and future computational strategies that will significantly limit the presence of harmful chemical residues in meat and dairy products. This workshop will focus computational methods that are based on sound pharmacokinetic and human health risk assessment principles. These approaches also closely examine pharmacokinetics of the drugs/toxicant in livestock within a deterministic and probabilistic context, while limiting exposure to the most sensitive human population.

660 FLUORIDATION: SCIENCE MEETS POLICY: AN HISTORICAL PERSPECTIVE.

S. G. Gilbert¹, D. R. Taves² and J. Doull³. ¹INND, Seattle, WA, ²Self, Redmond, WA and ³Department of Pharmacology Toxicology & Therapeutic, University of Kansas Medical Center, Kansas City, KS.

Fluoridation of drinking water is a controversial topic that highlights the intersection of science and policy in a struggle to balance the benefits and hazards of fluoride exposure. Historically, water fluoridation demonstrates that these controversies can be so intense that it is difficult to get impartial scientific debate on the issues involved. In 2002 the National Academy of Science (NAS) was asked to assess the adequacy of current EPA maximum contaminant level goals (MCLG) of 4 ppm to protect children and adults from adverse effects of fluoride exposure. The March 2006 Report of NAS Committee about fluoride is historic for two reasons. It included scientists who are opposed to fluoridation of the water supplies and it was unanimous in concluding that the EPA maximum contaminant level goal (MCLG) of 4-ppm fluoride is too high. This re-evaluation did not address the issue of benefit or risk of fluoridation.

The history of fluoride is rich and fascinating. It started with dental fluorosis studies that led to the idea of adding fluoride to the drinking water to decrease caries just prior to WWII. Fluoride, toxic and highly reactive, was critical in manufacturing uranium and plutonium for the first atomic weapons during WWII. Early research on the hazards and possible benefits of fluoride exposure was started at the University of Rochester in the 1930s by Harold Hodge, a founder and first president of the Society of Toxicology. It was during this period that the Public Health Service (and later the CDC) switched from a role of requiring a demonstration of safety to one of promoting fluoridation of drinking water. The story of fluoridation demonstrates the complexity of the interaction of science and government policy. This symposium will place the current controversy surrounding fluoride in an historical context and examine critical issues in science and policy making in order to develop a better scientific basis for re-assessing the risks and benefits of fluoridation.

i 661 NEW FRONTIERS IN ENVIRONMENTAL SCIENCES AND HUMAN HEALTH: IMPLEMENTATION UPDATE ON THE NIEHS STRATEGIC PLAN.

D. A. Schwartz, S. A. Newton, W. J. Martin, J. R. Bucher and A. Sassaman. *NIEHS/NIH, Research Triangle Park, NC.*

The NIEHS has begun work to implement its new Strategic Plan, "New Frontiers in Environmental Sciences and Human Health." With the Plan, the NIEHS is changing the way it approaches research, increasing the integration of clinical sciences into environmental health and the translation of basic and clinical research in environmental health into disease prevention strategies, therapeutics, and public health practice. NIEHS is emphasizing a dual approach balancing the work of interdisciplinary teams of scientists along with the very best individual investigator-initiated research. The vision of the NIEHS, supported by the Plan's goals, is to prevent disease and improve human health by focusing our research efforts on using environmental sciences to understand human biology and human disease. The Plan identifies seven major goals for the NIEHS: promoting clinical research in environmental health; using environmental sciences to understand human biology; fostering integrative approaches; promoting community-linked research, both domestically and globally; developing new approaches for studying the biology and measurement of exposure; creating new models for training the next generation of environmental health scientists; and fostering more widespread and effective partnerships to improve both the quality of the Institute's research and its ultimate public health impact. In this informational session, NIEHS and NTP leadership will discuss the Plan, describe recent accomplishments, present new program initiatives to advance the Plan's goals, and invite discussion from participants.

i 662 AIR POLLUTION AND ATHEROSCLEROSIS: IMPACT ON VASCULAR OXIDATIVE STRESS, DYSLIPIDEMIA, AND REMODELING.

M. J. Campen¹ and L. Chen². ¹Toxicology, Lovelace Respiratory Research Institute, Albuquerque, NM and ²Environmental Medicine, New York University, Tuxedo Park, NY.

Cardiovascular disease remains a major health concern in the Western World, representing greater than 25% of all-cause mortality. Atherosclerosis, an inflammatory disease of the vasculature that causes or complicates many forms of cardiovascular disease, is greatly influenced by genetics and lifestyle, but environmental factors such as air pollution may cause or promote ongoing disease. Recent animal studies have begun to shed light on epidemiological findings of air pollution-related morbidity and mortality due to ischemic heart disease. Atherosclerosis is a complex disease, characterized by dyslipidemia, oxidative stress, inflammation, and vascular remodeling. Dysregulation of vascular cells can lead to remodeling and the development of vascular lesions or plaques that can obstruct flow in vital organs and erode or rupture to reveal procoagulant factors and induce intravascular clotting. This symposium will explore mechanisms by which air pollutants, mainly particulate matter, can contribute to the vascular lesion progression via each of these various facets of atherogenesis. Studies presented will explore both biological pathways as well as components of air pollution that drive the vascular pathology. A more thorough understanding of the interactions between inhaled toxins and systemic vascular dysfunction may help identify susceptible individuals, highlight potential therapeutic targets, and determine contributing components of the complex pollutant mixture.

i 663 COMPARATIVE PROGRESSION OF ATHEROSCLEROSIS IN APOE^{-/-} MICE EXPOSED TO ETS AND CAPS.

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There are many similarities in the nature and extent of the risks that have been associated with the inhalation of environmental tobacco smoke (ETS) and ambient air fine particulate matter (PM_{2.5}). Population-based risk estimates for chronic exposures are significantly elevated for both of these complex mixtures for lung growth in children, and for cardiovascular disease and lung cancer in adults. For both complex mixtures, the effects seen in population studies have been questioned on the basis of the paucity of toxicological evidence establishing biological plausibility at the relatively low levels of ETS and PM at which humans have been exposed. In this study, we exposed ApoE knockout mice susceptible to atherosclerosis development to ETS at a nominal concentration of 500 ug/m³ for 6 hr/day, 5 day/wk, for 6 months. Atheroma progression and plaque characteristics (lesion cellularity, lipid core formation, and extent of macrophage and T-cell infiltration) were

evaluated using non-invasive ultrasound biomicroscopy (UBM) and immunohistopathology and morphometric analysis. The results were compared to our previous subchronic CAPs inhalation study to evaluate whether ETS and CAPs caused comparable levels of alterations in atherogenesis.

i 664 CARBON NANOTUBE RESPIRATORY EXPOSURE AND RISK FROM SYSTEMIC EFFECTS.

P. P. Simeonova, Z. Li and A. Erdely. *TMBB, HELD, NIOSH, CDC, Morgantown, WV.*

The most attractive features of nanomaterials including their small size, large surface area, and reactivity might also be the main factors for their toxicity. In this regard, nanoparticles may induce not only higher damage at the penetration site but also can lead to unexpected distant responses as a result of their translocation and reactivity through the body. Our research efforts are currently directed to evaluate the cardiovascular effects, including vascular inflammation, blood cell coagulation status, atherosclerosis, as well as the related molecular mechanisms associated with respiratory exposure to different forms of carbon nanotubes (CNT) using animal models. We demonstrated that pulmonary exposure to multiple doses of single wall-CNT induces severe lung toxicity and accelerates the progression of atherosclerosis in ApoE^{-/-} mice. This response is accompanied by oxidative modification in the vascular wall and induction of markers which facilitate blood coagulation. The atherogenic effects might be a result of a systemic effects related to the lung toxicity and/or translocation of nanotubes into the systemic circulation. The accumulation of toxicological data on engineered nanomaterials will allow for development of adequate risk assessment and regulations.

i 665 IN VIVO ENDOTHELIAL RESPONSE OF TIE2-GFP/APOE DEFICIENT MICE TO WHOLE DIESEL EXHAUST.

J. Maresh¹, M. J. Campen², M. D. Reed² and R. V. Shohet¹. ¹John A. Burns School of Medicine, University of Hawaii, Honolulu, HI and ²Toxicology, Lovelace Respiratory Research Institute, Albuquerque, NM.

Air pollution accelerates atherogenesis and cardiovascular disease. We examined the effect of diesel exhaust on endothelial gene expression in vivo, utilizing hypercholesteremic transgenic mice. Tie2-GFP mice expressing green fluorescent protein regulated by the endothelial-specific promoter Tie2 were crossed with apolipoprotein E knockout mice to generate Tie2-GFP/ApoE null mice. Tie2-GFP and Tie2-GFP/ApoE mice were exposed to diesel exhaust for 6 hours/day for 3 or 30 days at measured levels of diesel engine particulates from a Yanmar engine (0.3 and 1.0 mg/m³). Within 24 hours of exposure, aortae (pooled from 4 animals) were dissected from the valve to the iliac bifurcation and rapidly processed by proteolytic dissociation followed by fluorescence activated cell sorting to yield > 50,000 cells of > 95% purity. Changes in abundance of endothelial transcripts were then examined comprehensively by microarray techniques. RNA representing > 10,000 cells was subjected to amplification by the Ovation system (Nugen, CA), prior to labeling and hybridization to arrays created from the Operon V3 long oligo set. Following each exposure level, several genes detected by microarray and confirmed by real-time PCR were consistently dysregulated by > 2-fold. After 3 days of exposure at 1.0 mg/m³, increased expression of aryl sulfotransferase 1a and epoxide hydrolase 1 may reflect in vivo response to conditions of oxidative stress, and increased abundance of metal response element transcription factor-2 likely reflects the delivery of particulate-derived metals into the circulation. After 30 days of exposure at either 0.3 or 1.0 mg/m³, dysregulation of gene expression of type 2 angiotensin II receptor, sulfotransferase-related protein 3, and beta-carotene-9',10'-dioxygenase was potentiated in the ApoE deficient mice. The gene regulation identified in these experiments implicates pathways important in enhancement of atherosclerosis by diesel exhaust.

i 666 ENVIRONMENTAL OXIDANT EXPOSURE CAUSES MITOCHONDRIAL DAMAGE ASSOCIATED WITH INCREASED ATHEROGENESIS.

S. W. Ballinger. *Pathology, University Alabama at Birmingham, Birmingham, AL.* Sponsor: M. Campen.

The etiology of atherosclerosis is understood as an inflammatory process of the artery wall involving oxidative stress exacerbated by metabolic defects in cholesterol or glucose metabolism. Stress factors such as diet, diabetes and smoking are well recognized as environmental or metabolic factors that play roles in atherogenesis. More recently, it has become clear that environmental oxidant exposure increases the risk for cardiovascular disease development. One potential mechanism by which environmental oxidant exposure acts to increase cardiovascular disease risk is

by increasing damage to cellular organelles. Mitochondria are important cellular organelles with critical functions related to energy production, programmed cell death, growth, and redox signaling. In this regard, our laboratory has studied the impact of two forms of environmental oxidant exposure (cigarette smoke and ozone) on atherogenesis, mitochondrial damage and function. These studies reveal that neonatal and childhood exposure to cigarette smoke significantly increases the level of adult mitochondrial damage and furthermore, decreases vascular endothelial function in exposed animals (mice and primates). Additionally, our studies have shown that decreased mitochondrial antioxidant capacity increases the impact of environmental oxidant exposure (cigarette smoke) on atherogenesis. Finally, studies investigating the impact of ozone exposure on the cardiovascular have shown that both acute and chronic ozone exposure induces significant mitochondrial DNA damage, and significantly increases atherogenesis. Consequently, these studies support the hypothesis that environmental oxidants increase cardiovascular disease risk by causing mitochondrial damage that manifests in "mitochondrial dysregulation". Mitochondrial dysregulation will ultimately impair vascular cell function via energetic and cell signaling mechanisms. Current and future studies investigating the impact of environmental oxidant exposure on "mitochondrial dysregulation" are underway.



667 EXPOSURE TO WHOLE GASOLINE ENGINE EMISSIONS RESULTS IN ALTERATIONS OF MOLECULAR PATHWAYS INVOLVED IN PROGRESSION OF ATHEROSCLEROSIS.

A. K. Lund, T. Knuckles, J. Seagrave, C. Obot Akata, J. McDonald and M. J. Campen. *Department of Toxicology, Lovelace Respiratory Research Institute, Albuquerque, NM.*

Cardiovascular disease is currently the leading cause of death in the US, with atherosclerosis being responsible for approximately half of all CVD-related deaths. Epidemiological studies have reported an association between environmental air pollution and increased rates of cardiovascular morbidity and mortality. Remodeling of arterial extracellular matrix is a crucial step in the progression of atherosclerosis; therefore we investigated markers of vascular remodeling and inflammation in a model of atherosclerosis-prone ApoE^{-/-} mice, exposed to gasoline exhaust emissions. The aortic biomarkers examined included members of the matrix metalloproteinase (MMP) family and endogenous inhibitors of MMPs, TIMPs. Additionally, reactive oxygen species (ROS) and vascular peptides, endothelin-1 (ET-1) and heme-oxygenase (HO-1), levels were assessed. 10-wk old male ApoE^{-/-} mice (n=8 per conc.), on a high fat diet, were exposed via inhalation for 6 h/d, 7 d/wk, for a period of 1, 3, 7 days (acute: only control and 50 µg PM/m³ exposures) or 7 wks (chronic), to either filtered air, 8, 40, or 60 µg PM/m³, or a particle-filtered exposure (matched by NO_x and CO levels of 60 µg PM/m³). Exposure to gasoline emissions resulted in an increase in aorta MMP-2 and -9 mRNA by d7 in acute studies; chronic studies showed an exposure-related increase in aortic expression of MMP-3, -7, and -9. Aortic TIMP2 mRNA expression was significantly elevated by d1 of exposure, with dose-related increases in chronic exposures. Aorta ET-1 mRNA was significantly elevated by exposure d3, with further increases observed in chronic exposures; whereas HO-1 mRNA was found elevated in all chronic exposure levels. TBARs and nitrotyrosine histochemistry of exposed aortas were indicative of a chronic oxidative stress. Such findings suggest that exposure to gasoline emissions can alter molecular pathways involved in vascular remodeling, inflammation, and the progression of atherosclerosis.



668 EFFECTS OF ACROLEIN EXPOSURE ON ATHEROSCLEROSIS IN MICE: ROLE OF DYSLIPIDEMIA.

D. J. Conklin, S. Srivastava and A. Bhatnagar. *Institute of Molecular Cardiology, University of Louisville, Louisville, KY.*

Environmental pollutant exposure represents an important but understudied cardiovascular disease risk factor. Acrolein is a highly reactive and ubiquitous aldehyde pollutant present in industrial and automobile exhaust, environmental tobacco smoke, drinking water, and several foods. Thus, we asked if acrolein exposure modifies or contributes to classical cardiovascular disease risk factors. Using C57Bl/6 and apoE^{-/-} male mice (8-14 weeks old) as models of normal and at-risk human populations, we determined whether acrolein exposure affects plasma lipids and lipoproteins. Acrolein exposure within the range of expected human exposure (0.1-5 mg/kg; oral dose) in C57Bl/6 mice induced significant dose- and time-dependent changes in plasma lipids (cholesterol: mg/dl, control = 64.4±2.1; treated = 115.3±9.0, n=10,9, respectively; phospholipids: mg/dl, control = 132.5±5.9; treated = 230.7±30.4, n=10,7, respectively; and triglycerides: mg/dl, control = 67.5±6.3; treated = 382.1±114.3, n=10,9, respectively). These changes were positively correlated with the degree of alteration in lipoprotein electrophoretic mobil-

ity on Agarose gels. Additionally, chronic exposure of apoE^{-/-} mice fed high-fat diet to acrolein (0.1-2 mg/kg; 7-8 weeks) also elevated plasma cholesterol and enhanced atherosclerosis lesion area in thoracic aorta. These results suggest that acute and chronic acrolein exposures promote dyslipidemia and exacerbate atherogenesis, possibly due to direct lipoprotein modification. Acrolein exposures are likely to contribute to the increased cardiovascular disease risk associated with exposure to environmental pollutants.



669 INTRODUCTION.

N. I. Kerkvliet² and S. B. Pruett¹. ¹*Cellular Biology & Anatomy, LSU Health Sciences Center, Shreveport, LA and* ²*Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.*

As immunology advances, immunotoxicologists often find that new concepts or methodologies are invaluable in identification and characterization of mechanisms of immunotoxicity. Results presented here provide examples of recent investigations that illustrate this principle. The identification of T regulatory cells and the identification of toll-like receptors and their signaling pathways are new findings in basic immunology that have been exploited to better understand mechanisms of action of immunotoxicants that have been the subject of investigation for many years. The role of T regulatory cells is further considered (along with the role of co-receptors on antigen presenting cells) in xenobiotic-mediated modification of the types of immune response (Type 1 vs. Type 2) induced by a particular antigen. Finally, a completely new concept involving the role of physical interactions between intracellular kinases and ribosomes will be discussed as a mechanism of action for immunotoxic biological toxins on macrophages. These presentations all involve the use of new immunological concepts in understanding the mechanisms of action of immunotoxicants. The results also serve to illustrate that immunotoxicology studies complement basic immunology studies by determining the practical relevance of new basic mechanisms of immunology or new methodologies.



670 TOLL-LIKE RECEPTOR SIGNALING AS A TARGET OF DISPARATE IMMUNOTOXICANTS.

S. B. Pruett. *Cellular Biology & Anatomy, LSU Health Sciences Center, Shreveport, LA.*

Toll-like receptors (TLR) have been characterized within the last 8 years as major receptors for recognition of "danger" signals in the form of pathogen associated molecular patterns (PAMPs). They play critical roles in innate immunity, in the guidance of acquired immunity toward Th1 or Th2 (by dendritic cells), in hematopoietic responses associated with infection, and possibly in some harmful manifestations of inflammation such as cardiovascular disease. However, they have only recently begun to be evaluated as potential targets for immunotoxicants. Two compounds with disparate structural and functional characteristics produce similar alterations in cytokine production mediated through TLR3 and TLR4. The effects of ethanol and sodium methylthiocarbamate on cellular signaling events leading to cytokine production and on downstream events in innate immunity illustrate that differing alterations in signaling can yield similar outcomes. These results suggest a need for a comprehensive, systems biology approach to fully explain and predict the effects of altered signaling on downstream outcomes.



671 INDUCTION OF T REGULATORY CELLS AS A MECHANISM OF AHR-MEDIATED IMMUNOSUPPRESSION.

N. I. Kerkvliet, C. J. Funatake and N. B. Marshall. *Env. Molec. Toxicology, Oregon State University, Corvallis, OR.*

TCDD, acting through the Ah receptor, is one of the most potent immunosuppressive chemicals known. However, despite extensive research, the immunological mechanisms underlying the immunosuppression have remained elusive. Recent studies have shown that AhR activation in T lymphocytes induces their differentiation to a regulatory (Treg) cell phenotype, providing a new perspective on the mechanism of AhR-mediated immune suppression. Current data suggest that AhR activation in T cells enhances some known pathways for Treg generation/function (TGFB, IL-10 and IL-2) as well as unique changes not previously described in Treg populations. Since Treg are involved in the control of autoimmune diseases, transplantation tolerance, and anti-tumor immunity, and also inhibit both Th1 and Th2 dependent immune responses, elucidation of the signaling pathways that are activated by the AhR in Treg holds the promise of pharmacologic exploitation of this novel immunosuppressive pathway for clinical use.

**672 MODIFIED IMMUNE RESPONSES THROUGH EFFECTS ON CO-STIMULATION.**

R. Pieters, S. Nierkens, F. van Wijk and C. de Haar. *Institute for Risk Assessment Sciences, Immunotoxicology, and Immunology Division, Utrecht University, Utrecht, Netherlands.*

Cell-bound costimulatory molecules are crucial in naive T cell activation, in particular CD40, CD80 and CD86 on the antigen-presenting cell (APC) side, and CD40L, CD28 and CTLA-4 on the T cell side of the immunological synapse. Importantly, CD28 is a stimulatory molecule, whereas CTLA-4 is regulatory and also constitutively expressed on regulatory T cells. Recently, the role of these costimulatory molecules became the focus of immunotoxicological research, particularly because these molecules may not only have a role in stimulation of immune responses to xenobiotics but also in immune regulation. Food allergy responses, airway responses to particulate matter, as well as local lymph node responses (LLNA and PLNA) all appear to be controlled by costimulatory molecules. Simultaneous blockade (using monoclonal antibodies) of CD80 and CD86 or CD40-CD40L interaction was most effective in inhibition of these responses, whereas CD80 by itself and CTLA-4 (with high binding affinity to CD80) had clear regulatory effects. In particular, blockade of CTLA-4 led to enhanced IgE response, suggesting a potential role of regulatory T cells in dampening immune responses induced by xenobiotics. Expression of costimulation may eventually provide early parameters for hazard assessment. However, knowledge of the role of costimulation and the cells involved (such as regulatory T cells) may also be included in methods to prevent adverse immune reactions.

**673 RIBOSOME-MEDIATED STRESS RESPONSE TO BIOLOGICAL TOXINS IN THE MACROPHAGE.**

J. J. Pestka, H. B. Bae and M. Li. *Microbiology and Molecular Genetics, Food Science and Human Nutrition, Center for Integrative Toxicology, Michigan State University, East Lansing, MI.*

Major advances in understanding how the immune system is regulated have been derived from increased understanding of intracellular signaling pathways within distinct leukocyte populations. These signaling cascades are directed by protein kinases and impact proteome expression by modulating transcription, mRNA stability and transcript selection for translation. Macrophages are known to play a critical role in innate immune function and regulation of the acquired immune response. Aberrant activation or apoptosis of macrophages can contribute to chronic inflammatory disease and immune suppression, respectively. Biological toxins which interfere with protein synthesis including the trichothecene mycotoxins (Fusarium, Stachybotrys), Shiga toxins (E. coli, Shigella) and ricin (castor bean) have been demonstrated to induce proinflammatory cytokine and chemokine expression as well as apoptosis in cultured macrophages and rodent models. Using molecular and bioinformatic strategies developed for studying intracellular signaling cascades, it has been learned that, in the macrophage, the ribosome functions as a toxin sensor as well as a scaffolding site for phosphorylation and activation of several protein kinases upstream of the mitogen-activated protein kinases (MAPKs). These events drive several intracellular signaling cascades that selectively mediate transcription, mRNA stability and translation of genes associated with innate immune function and apoptosis. Research on this ribosome-mediated stress response indicates the immune system is a primary target of ribotoxic chemicals that might be encountered via natural food or airborne contamination or as a result of chemical terrorism. Such studies inform risk assessment/management efforts and will yield strategies for preventing and/or treating toxic effects in persons exposed to these chemical agents.

**674 MECHANISTIC BIOMARKERS AND INNOVATIVE METHODOLOGIES TO DETECT ACUTE KIDNEY INJURY.**

V. S. Vaidya¹ and D. P. Jones². ¹*Medicine-Renal, Harvard Medical School, Boston, MA* and ²*Medicine-Pulmonary Division, Emory University, Atlanta, GA.*

Acute kidney injury is a common disease and is associated with a high mortality rate. Although there has been a significant progress in understanding the mechanisms of ischemic or toxic forms of acute kidney injury (AKI) in animal models, translation of these findings to therapeutics useful in clinical practice remains challenging. One of the predominant reasons for the slow translation of the results from bench to bedside, especially in the treatment of existing AKI, is the absence of reliable and sensitive biomarkers of injury in animal and human studies. The FDA's critical path initiative has reinforced the need for additional biomarkers to predict drug toxicity in preclinical studies which can act as surrogate endpoints, and/or aid in making efficacious and cost-saving decisions or terminating drug development more quickly.

The objective of this symposium is 3-fold: First to give a mechanistic insight of metabolomic and genomic approaches adopted to discover new biomarkers and discuss the validation of both technologies with respect to various animal models of renal injury. Second aim is to describe the use of novel high throughput technologies such as a multiplexing tool kit based on microbead technology, which is capable of quantitating multiple biomarkers in the same biological sample at one time. Finally, this symposium will also discuss the use of zebrafish as a highly novel tool for mechanistic toxicology and the evaluation of kidney injury in predictive toxicology that may improve our ability to identify effective therapeutic agents for kidney injury and eliminate nephrotoxic compounds early in the drug development process.

This session will be Chaired and Co-Chaired by Drs. Jones and Vaidya respectively. Dr. Vishal Vaidya will give a brief introduction of the session and the first presenter will be Dr. Dean Jones followed by Dr. Donna Mendrick. The third presenter will be Dr. Vaidya followed by Dr. Joseph Bonventre. All the speakers are confirmed.

**675 METABOLOMIC APPROACHES TO DISCOVER PREDICTIVE AND MECHANISTIC BIOMARKERS OF TOXICITY.**

D. P. Jones. *Medicine-Pulmonary Division, Emory University, Atlanta, GA.*

During drug development, unprecedented and novel toxicities arise, and even after introduction of effective drugs into use, other toxicities can become apparent due to drug-genome, drug-environment and drug-drug interactions. Such toxicities can be difficult to detect and monitor, and the mechanisms of toxicity can be obscure, further complicating biomarker discovery and development. Metabolomics is a discipline/method that is well suited to understand environmental, especially toxicologic, influences on living systems. This presentation will focus on human metabolomics research with chemically defined diets to simplify discovery of sensitive metabolic pathways and metabolic response patterns. Two strategies will be discussed, use of 1H-NMR spectroscopy, which provides means to rapidly screen for changes affecting macronutrient metabolism as well as renal function, and liquid chromatography-Fourier-transform mass spectrometry (LC-FT/MS) which provides a similarly rapid and more sensitive detection, but has not yet been standardized for routine use. Both approaches can be applied to a range of biofluids and tissue extracts. Data from different biostatistical and bioinformatics approaches will be used to illustrate extraction of useful toxicologic information from these information-rich technologies.

**676 PHARMACOGENOMIC TECHNOLOGY: A HIGH THROUGHPUT APPROACH FOR PRE-CLINICAL AND CLINICAL RENAL BIOMARKER DISCOVERY.**

D. L. Mendrick. *Gene Logic Inc., Gaithersburg, MD.*

New biomarkers are needed to provide insight into a drug's potential to induce toxicity prior to irreversible damage (the so-called leading or predictive biomarkers). Clearly, better biomarkers of organ toxicity are needed in both preclinical and clinical arenas to protect human safety. Pharmacogenomics opens the door to new discoveries because one can measure the response of thousands of genes at once. However, the measurement of thousands of events at one time adds noise to any system, making false discovery a real problem. This presentation will focus on in silico approaches that can be used to discover and qualify potential biomarkers that bridge preclinical and clinical testing to provide better tools to identify safety risks to patients. Various issues with respect to data-mining, cross platform comparison and validation of the genomic results will also be discussed.

**677 NOVEL METHODS TO QUANTITATE URINARY BIOMARKERS FOR EARLY DETECTION OF NEPHROTOXICITY.**

V. S. Vaidya. *Medicine-Renal, Harvard Medical School, Boston, MA.*

As much as sensitive and specific biomarkers are needed to detect kidney injury, highly sensitive and high throughput methodologies are also needed to quantitate these biomarkers. We have cloned a potential biomarker for AKI named Kidney Injury Molecule-1 (Kim-1) which is upregulated more than any other known protein after renal injury. Kim-1 is a type 1 transmembrane protein that is not detectable in normal kidney tissue but is expressed and shed in the urine at very high levels in dedifferentiated proximal tubule epithelial cells in human and rodent kidneys after ischemic or toxic injury. The challenge now is to design innovative methods to quantitate Kim-1 in the urine which can be used as a sensitive, specific, high

throughput and noninvasive technique in preclinical and clinical detection of kidney injury. This presentation will focus on the development, evaluation and validation of ELISA's and microbead based assay to quantitate urinary Kim-1 in various preclinical and clinical models of acute kidney injury.



678 ZEBRAFISH AS A NOVEL TOOL FOR MECHANISTIC TOXICOLOGY AND HIGH THROUGHPUT SCREENING OF KIDNEY INJURY.

J. V. Bonventre. *Medicine-Renal, Harvard Medical School, Boston, MA.* Sponsor: V. Vaidya.

Zebrafish is a well established model in the study of developmental biology. More recently, zebrafish have been used in models of disease, such as myocardial injury and gastrointestinal lipid metabolism. Taking advantage of the ease of husbandry and wealth of established molecular biology methodology adds versatility to the model. Based on the translucency of zebrafish larvae we developed a fluorescent compound based method to determine changes in larval zebrafish glomerular filtration. We validated this method in a model of nephrotoxicity in larval zebrafish using gentamicin or cisplatin. The small size of larval zebrafish in combination with fluorescent markers make 96-well based high-throughput assays possible. This presentation will be about use of zebrafish as a novel tool for early detection of acute kidney injury and mechanistic toxicology.



679 PIGS AND THE PUBLIC'S HEALTH: AN ASSESSMENT OF HOG FARMING IN NORTH CAROLINA.

D. J. Crawford-Brown¹, C. Williams² and A. M. Jarabek³. ¹*Department of Environmental Sciences and Engineering, University of North Carolina at Chapel Hill, Chapel Hill, NC.* ²*Department of Animal Science, North Carolina State University, Charlotte, NC* and ³*National Health and Environmental Effects Research Laboratory, Research Triangle Park, NC.*

Hog production in North Carolina has recently transformed from a system dominated by small independent farmers to a large-scale agribusiness, and is one example of an expanding class of confined animal feeding operations (CAFOs) which produce high concentrations of chemical and biological waste. Exposure scenarios include two categories: routine releases and releases during extreme climatic events. The hog CAFOs in NC are concentrated in the coastal plain region that is sensitive due to low-lying flood plains and high water tables. Officials in the state are attempting to understand the benefits that such operations might bring in the face of a declining tobacco and manufacturing base relative to the costs from ecosystem impacts and the reduction of human health and quality of life for those working or living nearby hog CAFOs. Ecological epidemiological and occupational studies suggest a variety of respiratory, gastrointestinal, and neurological health effects due to exposures from CAFOs. These results have spurred an effort to locate more environmentally-sustainable technologies that will reduce these risks. As part of that effort, NC and other states have attempted to apply risk assessment approaches to develop health-based quantitative limits that the technologies must reach with respect to the various contaminants. This symposium will describe the public health problems posed by CAFOs and then consider how toxicological sciences can contribute to the risk assessment effort by translating mechanistic data from in vitro and animal test systems to inform inferences made from observations at the population level in humans. Both chemical and microbial contaminants will be considered by a panel of experts from relevant toxicological disciplines.



680 AN OVERVIEW OF COMMUNITY HEALTH CONCERNS RELATIVE TO CONCENTRATED ANIMAL FEEDING OPERATIONS.

K. J. Donham. *Occupational and Environmental Health, University Of Iowa College Of Public Health, Iowa City, IA.* Sponsor: A. Jarabek.

The proliferation of animal feeding operations (AFO's) has been a phenomenon influenced by economic policy forces associated with our global economy. The observed increased intensity, concentration, specialization and consolidation in livestock and poultry production has been occurring over the past three decades. The US EPA has a specific definition of AFO's that includes the confinement of animals for at least 45 days in a 12 months period, and in a structure or lot where vegetation or crops will not grow. The term: Confined Animal Feeding Operation (CAFO) is used to describe AFO's over a specified size that are a risk as a point source for water pollution, and therefore require regulation (U.S. EPA 2002). In regard to water, air, and solid waste pollution, CAFO's have many of the same concerns as described for other agricultural operations. However, there are special concerns in regards to CAFO's because of the sheer size, the concentration of animals, feed, manure (usu-

ally handled in liquid form), dead animals, flies, and associated gases, particulates, odors and odorants, and infectious diseases all concentrated on a small land area. There are concerns that the manure cannot be recycled without pollution on such a small area, and that local and regional air and water quality suffers. Public concerns relative to adverse consequences of livestock production have been increasingly voiced since the late 1960s (Thu 1998). Numerous regional, national and international conferences have been held on the subject since 1994 (Merchant 2002; Thu 1995). An in-depth review of the literature has been recently published (Donham 2000). One reason large scale livestock production has raised concern is that it has separated from family farming and has developed like other industries in management, structure, and concentration. The magnitude of the problem is highlighted by the following facts relative to the U.S.



681 PUBLIC HEALTH IMPACTS OF INDUSTRIAL SWINE PRODUCTION.

S. Wing. *Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC.* Sponsor: A. Jarabek.

Industrialization of animal agriculture and development of confined animal feeding operations (CAFOs) have resulted in complex ecological impacts at local, regional and global scales. Swine CAFO confinement buildings, waste lagoons, and spray fields contribute to global greenhouse gases and affect local levels of respirable particulate matter, ammonia, hydrogen sulfide, and volatile organic compounds. Chemical and biological contamination of ground and surface waters also occur. Swine CAFOs may threaten public health conditions of host communities through occupational exposures, local air and water pollution, malodor, and threats to healthy community development. In North Carolina swine CAFOs have been located disproportionately in poor communities of color where they may contribute to existing health disparities. Although adverse impacts of confinement building exposures on respiratory health of CAFO workers have been well documented, fewer studies have evaluated impacts on neighbors, including susceptible populations such as children and the elderly. Several epidemiological studies link swine CAFOs to respiratory and gastrointestinal symptoms, immune function, asthma, and impaired quality of life in neighboring communities. Future studies could provide better exposure and outcome assessment using longitudinal study designs.



682 POTENTIAL HEALTH EFFECTS OF ODOROUS AERIAL EMISSIONS FROM SWINE PRODUCTION WASTE: ROLE OF VOCs AND PARTICULATES.

S. S. Schiffman. *Department of Psychiatry, Duke University Medical Center, Durham, NC.* Sponsor: A. Jarabek.

Complaints of health symptoms from ambient odors have become more frequent in communities with confined swine facilities. Health complaints include eye, nose, and throat irritation, headache, nausea, diarrhea, hoarseness, sore throat, cough, chest tightness, nasal congestion, palpitations, shortness of breath, stress, drowsiness, and alterations in mood. While these symptoms usually occur at the time of exposure and typically remit after a short period of time, health symptoms in sensitive individuals such as asthmatic patients may persist for longer periods. This presentation will cover two projects dealing with potential health effects of odorous emissions from swine operations. First, the conclusions of a workshop to assess the current state of knowledge regarding the health effects of ambient odors will be presented; this workshop was cosponsored by the Environmental Protection Agency (EPA) and National Institute on Deafness and Other Communication Disorders (NIDCD). Second, the results of an exposure study will be described in which 48 healthy adult volunteers were exposed for one hour to diluted swine air at a level that could occur at varying distances downwind from a confined swine operation both within and beyond the property line. This study was performed in an exposure chamber located at North Carolina State University's Field Laboratory. Objective measures of blood pressure, temperature, heart rate, respiratory rate, lung function, nasal inflammation, secretory immunity, mood, attention, and memory were correlated with objective measures of air quality. Ratings of perceived (self-reported) health symptoms were also obtained.



683 HOGS AS SOURCES OF ENTERIC AND OCCUPATIONAL ILLNESSES IN HUMANS.

R. Holley. *Food Science, University of Manitoba, Winnipeg, MB, Canada.* Sponsor: A. Jarabek.

In contrast with the US, there is a relationship between pork consumption and pork-associated foodborne illnesses in several European countries including Holland and Denmark where hog densities are high (1500 & 500 animals/km², re-

spectively). In the latter two countries pork is the meat species consumed in the greatest amount and it is a major source of human enteric illness. All human *Yersinia* and most *Salmonella* Typhimurium infections in these two countries are attributed to pork consumption. In Belgium and Norway raw or undercooked pork are the main sources of yersiniosis in man. The two organisms are highly prevalent in Dutch and Danish swine, but more recently improved slaughterhouse hygiene in these countries and in Norway has resulted in declines of pathogen occurrence in hog carcasses. In Holland and Norway decline in the frequency of *Yersinia* contamination has been coincident with the reductions in human clinical cases of yersiniosis. In the US and Canada high pig density and pork production have not increased known numbers of cases of pork-associated illnesses. In both countries pork consumption ranks third after poultry and beef. Cases of human illness attributed to pork fluctuate yearly at about 15% of the total in the US despite relatively high frequencies of pathogens in pigs and retail pork (7.4 to 31.5% and 1.3 to 15.6% respectively). While hog density in North Carolina at 465 animals/km² is close to that of Denmark, the difference in pathogen effects are probably explained by the consumption of well-cooked pork in North America. Improper manure handling can lead to direct contamination of produce, water supplies, animals or even humans. Outbreaks of illness associated with fresh produce and water supplies have risen in recent decades and evidence suggests there may be a connection with improper manure handling in some instances. Increased produce consumption and better reporting of foodborne illness cases have also contributed to this change. Occupational hazards (aerosols, antibiotic resistant bacteria) faced by agricultural laborers and those employed in waste treatment operations are largely uncharacterized.

 684 ECOLOGICAL IMPACTS AND RAMIFICATIONS OF ATMOSPHERIC NITROGEN DEPOSITION TO ESTUARINE AND COASTAL WATERS: THE CONCERN ABOUT AGRICULTURAL AMMONIA EMISSIONS.

H. W. Paerl¹, D. R. Whitall², B. L. Peierls¹ and R. L. Dennis³. ¹University of North Carolina Chapel Hill, Morehead City, NC, ²NOAA, Silver Spring, MD and ³U.S. EPA, Research Triangle Park, NC. Sponsor: A. Jarabek.

Atmospheric deposition of nitrogen (AD-N) is a major source of externally-supplied or new nitrogen (N) to N-sensitive estuarine and coastal waters. In European and North American coastal waters downwind of anthropogenic emission sources, AD-N contributes from 10 to over 40 percent of new N loading, and can be the single largest source of new N impacting these waters. In developing regions of the world, AD-N is one of the most rapidly expanding sources of new N. In North Carolina, AD-N deposition has at least doubled since the 1970's, initially as a result of urbanization (chiefly NO_x), but more recently due agricultural growth (NH_x and organic N). Recent growth and intensification of animal operations in coastal regions (e.g., Mid-Atlantic coastal plain) have been linked to increasing amounts of NH_x deposition, according to a 3 decadal analysis of the National Atmospheric Deposition Program (NADP) wet deposition network. Ammonia emitted from these operations accounts for approximately half the AD-N in nearby Atlantic estuarine and coastal waters. The ecological ramifications of this growing N source in these N-sensitive waters include eutrophication, water quality, habitat and food web alterations. The role of AD-N in eutrophication dynamics depends on sources, chemical forms and amounts of N and interactions with other atmospheric nutrient sources (Fe). Ecological effects of AD-N are dependent on physical conditions, including stratification, residence time, and optical properties of receiving waters. AD-N and other atmospheric nutrient sources may promote major biotic changes now apparent in these waters, including the proliferation of harmful algal blooms, declines in water quality (hypoxia) and loss of fisheries habitat.

 685 ADVANCES IN THE EFFICACY OF CHELATION TREATMENT TO ALLEVIATE NEUROCOGNITIVE EFFECTS OF LEAD AND AUTISTIC SPECTRUM DISORDERS.

D. Smith. *Environmental Toxicology, University of California, Santa Cruz, CA.*

Clinical and animal model studies showing adverse neurological effects of low level exposures to metals such as lead and mercury underscore the need to devise efficacious intervention strategies to augment primary prevention efforts. Chelation therapy is one such intervention strategy that has been used in the treatment of metal poisoning in children, and it is now being advocated by some for the treatment of neurological disorders, most notably autism spectrum disorders (ASD). However, few clinical or animal model studies have thoroughly investigated the efficacy of chelation to alleviate the adverse neurological effects of lower level metal exposure. Further, the practice of using chelation therapy to treat ASD is becoming more common, though the overall benefits and risks of this therapy are not well known. This workshop will highlight recent clinical and animal model studies to

address the relative benefits and risks associated with chelation treatment for metal poisoning, and the efficacy of chelation in general and succimer treatment in particular in the treatment of ASD.

 686 INTRODUCTION; THE EFFICACY OF SUCCIMER CHELATION FOR REDUCING TARGET TISSUE METAL LEVELS.

D. Smith. *Environmental Toxicology, University of California, Santa Cruz, CA.*

Chelation therapy has been used to treat metal poisoning in children for over 50 years. Chelation treatment is also now being advocated by some for the treatment of neurological disorders, most notably autism spectrum disorders (ASD). This presentation will provide an introduction of the workshop speakers, and a presentation of data on the pharmacokinetics of succimer, and its efficacy to reduce target tissue metal levels. Particular emphasis will be placed on the relationship between treatment regimen duration, blood and brain lead reductions, and the extent to which decreases in blood lead levels predicts reductions in brain lead in rodent and non-human primates. A number of studies in rodents and non-human primates have indicated that succimer-mediated reductions in blood lead levels greatly overestimate reductions in brain lead levels, suggesting that treatment regimens designed to bring about an acceptable reduction in blood lead may not achieve sufficient reductions in brain lead levels to achieve the desired efficacy.

 687 CHELATION THERAPY, CEREBRAL DEVELOPMENT AND NEUROPSYCHOLOGICAL FUNCTIONS IN LEAD POISONED CHILDREN.

K. Dietrich, PhD. *Department of Environmental Health, University of Cincinnati College of Medicine, Cincinnati, OH.* Sponsor: D. Smith.

It is unclear whether chelation therapy can prevent or reverse the neurodevelopmental sequelae of lead toxicity. Our objective is to determine whether chelation therapy with succimer in children with referral blood lead levels between 20 and 44 ug/dL at 12 to 33 months of age confers neurodevelopmental benefits. The Treatment of Lead Poisoned Children (TLC) study is a randomized, double-blind, placebo-controlled trial that was conducted between September 1994 and June 2003 in Philadelphia, PA; Newark, NJ; Cincinnati, OH; and Baltimore, MD. 780 children between the ages of 12 to 33 months were randomized to the active drug and placebo groups stratified by clinical center, body surface area, blood lead level, and language spoken at home. At 7 years of age, 647 subjects remained in the study. Up to 3 26-day courses of succimer or placebo therapy were administered depending on response to treatment in those who were given active drug. Scores on standardized neuropsychological measures that tap cognition, behavior, learning and memory, attention, and neuromotor skills were measured. Chelation with succimer lowered average blood lead levels for approximately six to 12 months but resulted in no benefits with respect to cognitive, behavioral or neuromotor endpoints. These follow-up data confirm our previous finding that the TLC regimen of chelation therapy is not associated with neurodevelopmental benefits in children with blood lead levels between 20 and 44 ug/dL (0.96-2.17 umol/L). In conjunction with new data from the Cincinnati Lead Study cohort that demonstrate cortical gray matter loss and dysfunctional cerebral organization in response to blood lead levels in this range, these TLC results emphasize the importance of taking primary environmental measures to prevent exposure to lead.

 688 SUCCIMER CHELATION IMPROVES LEARNING, ATTENTION AND AROUSAL REGULATION IN LEAD-EXPOSED RATS BUT PRODUCES LASTING COGNITIVE IMPAIRMENT IN THE ABSENCE OF LEAD EXPOSURE.

B. Strupp, PhD. *Nutritional Sciences, Cornell University, Ithaca, NY.*

There is growing pressure for clinicians to prescribe chelation therapy at only slightly elevated blood lead levels. However, very few studies have evaluated whether chelation improves cognitive outcomes in lead-exposed children, or whether these agents have adverse effects that may affect brain development in the absence of lead exposure. The present study was designed to answer these questions, using a rodent model of early childhood lead exposure and treatment with succimer. Results indicate that lead exposure produced lasting impairments in learning, attention, inhibitory control, and arousal regulation, paralleling the areas of dysfunction seen in lead-exposed children. Succimer treatment of the lead-exposed rats significantly ameliorated learning, attention, and arousal regulation, although the efficacy of the treatment varied as a function of the lead exposure level and the specific functional deficit. In contrast, succimer treatment of rats not previously exposed to lead produced lasting and pervasive cognitive and affective dysfunction

comparable in magnitude to that produced by the higher lead exposure regimen. These findings suggest that it may be possible to identify a succimer treatment protocol that improves cognitive outcomes in lead-exposed children. However, they also suggest that succimer treatment should be strongly discouraged for children who do not have elevated tissue lead levels.



689 THE CLINICAL USE OF DETOXIFICATION AND CHELATION THERAPY IN CHILDREN WITH AUTISM SPECTRUM DISORDERS.

K. Bock. *Rhinebeck Health Center, Rhinebeck, NY*. Sponsor: D. Smith.

We are in the midst of an epidemic of autism spectrum disorders, with an incidence that has increased from 1 in 2,500 to 1 in 166. Numerous theories exist as to why this has occurred, ranging from better diagnosis to thimerosal induced neurotoxicity. I believe we are looking at an underlying genetic predisposition/vulnerability, in concert with environmental insults, including multiple toxicants. These toxicants include, but are not limited to, mercury, lead, and arsenic; as well as multiple chemicals, including persistent organic pollutants. Recent research has pointed to the synergistic toxicity of combinations of chemicals, and this is known to be true for heavy metals as well. Adverse effects of these toxicants in children with autism spectrum disorders include neurotoxicity, immunotoxicity, and metabolic imbalances. These metabolic imbalances include abnormalities in the methylation and transsulfuration pathways, most likely exacerbated by genetic polymorphisms coding for various enzymes in these pathways, including MTHFR, and glutathione-S-transferase. Clinical detoxification strategies, including methylcobalamin, n-acetyl-cysteine, reduced glutathione; and chelating agents such as DMSA and calcium EDTA, will be discussed in terms of their roles in a comprehensive biomedical treatment program which addresses these toxicities and imbalances, and coupled with intensive therapeutic interventions, may help these children on their road to recovery.



690 USE OF THIOL CHELATORS IN CHILDREN WITH AUTISM SPECTRUM DISORDERS: CLINICAL AND THEORETICAL IMPLICATIONS.

J. Bradstreet. *International Child Development Resource Center, Melbourne, FL*. Sponsor: D. Smith.

In the late 90's, the FDA asked all agencies to report the potential sources of mercury present in various commercially available medical and cosmetic products. This resulted in the observation that the Thimerosal present in many vaccines regularly exceeded the ATSDR and EPA guidelines. Methylmercury is a well described neurodevelopmental intoxicant, however less was known concerning the toxicology of ethylmercury. Further, emerging data indicated fetal exposure patterns were placing one in six children at risk of mercury toxicity at the time of birth. Thus, as this information became disseminated to physicians, some clinicians attempted to quantify relative body burdens of mercury in children with autism spectrum (ASD) using oral succimer (DMSA or Chemet®). One published study demonstrated significantly more mercury excretion during a provocation challenge of autistic children than age matched neurotypical children. Subsequently, Thimerosal has been reduced or eliminated in many vaccines – effectively changing the toxic exposure pattern. However, mercury likely represents a useful and effective model of studying environmental factors interacting with genomic vulnerability. This presentation will review the common practice of using thiol chelators in ASD, and discuss the ongoing controlled studies as well the results of provocation urine studies. This will be integrated into a discussion of observed and published abnormalities of the methylation and transsulfuration pathways in the ASD population.



691 ENVIRONMENTAL INFLUENCES ON THE OVARY AND HYPOTHALAMIC PITUITARY GONADAL AXIS.

W. N. Jefferson¹ and J. Flaws². ¹Laboratory of Molecular Toxicology, NIEHS, Research Triangle Park, NC and ²Department of Veterinary Biosciences, University of Illinois, Urbana, IL.

A functional ovary is critical to successful conception, regular menses, regulation of circulating steroid hormones and can potentially help regulate risk of serious female reproductive diseases. A variety of environmental components have been implicated in alteration of ovarian function and such events as early menopause, polycystic ovarian syndrome, and infertility/subfertility. In addition, transgenic mouse models lacking estrogen receptor α or β illustrate the ovary as both an endocrine organ and endocrine target for estrogens. Consequently, ovarian function is especially vulnerable to insult by endocrine disrupting chemicals. Developmental exposure to estrogenic chemicals such as genistein causes altered ovarian differentiation and function

contributing to reduced fertility later in life. Exposure to environmental aryl hydrocarbon receptor (AhR) ligands such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) can accelerate reproductive senescence in female primates involving changes in neuroendocrine and ovarian function. The pesticide methoxychlor (MXC) causes antral follicle-specific toxicity, characterized by a decreased number of healthy antral follicles, and an increased percentage of antral follicles undergoing atresia. Exposure to the occupational chemical 4-vinylcyclohexene diepoxide (VCD) is used as a chemical-induced mouse model for menopause characterized by ovarian failure. This symposium will highlight the roles that environmental effectors can have on the ovary and the impact that these effects can have on human health and reproduction.



692 CHRONIC ACTIVATION OF THE ARYL HYDROCARBON RECEPTOR PATHWAY AND REPRODUCTIVE SENESCENCE.

B. Petroff. *Center for Reproductive Sciences, University of Kansas Medical Center, Kansas City, KS*.

The aryl hydrocarbon receptor (AhR) pathway is an evolutionarily conserved, widely expressed orphan receptor pathway that is activated by many environmental and experimental toxicants and carcinogens. The transition to reproductive senescence (i.e. the menopause in primates) in females involves changes in neuroendocrine and ovarian function and can be accelerated by exposure to environmental AhR ligands such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). We will discuss studies addressing the possible mechanisms behind the impact of acute vs. chronic exposure to AhR ligands on the loss of reproductive function with age. Through endocrinological and molecular study, we have attempted to determine when developmentally and where anatomically females are particularly susceptible to endocrine disruption by TCDD during chronic exposures in rats. This work may help characterize and eventually prevent premature loss of reproductive function in smokers and residents of polluted regions. (Supported by Kansas City Area Life Sciences Institute and NIH RO1 ES12916)



693 DEVELOPMENTAL EXPOSURE TO ESTROGENS AND EFFECTS ON OVARIAN MATURATION.

W. Jefferson. *Laboratory of Molecular Toxicology, NIEHS, Research Triangle Park, NC*.

It has been previously shown that developmental exposure to estrogenic chemicals during critical stages of reproductive tract differentiation causes adverse consequences on female reproductive system. The naturally occurring phytoestrogen genistein found in soy-derived products is an example of an environmental estrogen that has been shown to cause altered murine ovarian differentiation and function as well as subfertility/infertility. Mice treated neonatally on days 1-5 with genistein (0.5 – 50 mg/kg/day) by subcutaneous injection exhibit altered ovarian differentiation characterized by inhibition of oocyte nest breakdown leading to the formation of multi-oocyte follicles (MOFs). This malformation is mediated by estrogen receptor β (ER β) since mice lacking ER β do not develop MOFs following neonatal genistein treatment. We have also observed limited ovulatory function in adult mice treated with the highest dose of genistein as evidenced by reduced numbers of corpora lutea. This effect is rescued with exogenous gonadotropin treatment suggesting altered hypothalamic/pituitary/gonadal signaling in genistein treated mice. Reproductive function is also altered with early reproductive senescence in the lower genistein groups and infertility in the highest group. To further characterize female infertility following neonatal genistein, we examined the effects on oocyte quality in culture. Female mice were treated with exogenous gonadotropins (PMS & HCG) and bred to proven control males. Oocytes were collected, counted, and placed in culture. Fertilized oocytes were determined by the appearance of the second polar body and followed to determine progression to 2-cell, 4-cell and blastocyst stages of development. Oocytes from genistein treated mice exhibited lower fertilization rates and reduced ability to develop to the blastocyst stage suggesting poor oocyte quality. These data suggest that exposure to estrogenic chemicals like genistein during critical stages of differentiation can cause long term consequences on ovary differentiation and subsequent reproduction.



694 ENDOCRINE, PARACRINE & AUTOCRINE ACTIONS OF ESTRADIOL IN OVARIAN FUNCTION.

J. F. Couse. *Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC*. Sponsor: W. Jefferson.

The ovaries produce the bulk of circulating estradiol in females of reproductive age. The endocrine actions of estradiol in the hypothalamic-pituitary axis are well known to be necessary for determining the appropriate pattern of gonadotropin secretion, and are therefore obligatory to ovarian function. In contrast, putative paracrine and autocrine actions of estrogen signaling in the ovary are less understood, largely because in vivo studies of intra-ovarian estrogen action are impeded

by the inability of pharmacological reagents to overcome the immense level of endogenous estrogens within growing follicles. Hence, the advent of gene-targeted mice that lack estradiol synthesis (CYP19-null) or the nuclear estrogen receptors (ER α or ER β) has introduced renewed opportunities in this area of study. The divergence of ovarian phenotypes in each model has expanded our appreciation of the multiple sites at which estrogen signaling impinges upon ovarian function. For example, ER α -null females are anovulatory due to their inability to produce a gonadotropin surge, reflecting the loss of endocrine estradiol actions in the hypothalamic-pituitary axis; but also exhibit constitutively elevated ovarian steroidogenesis due to the loss of ER α -mediated paracrine actions in thecal cells. In contrast, ER β -null females are oligovulatory due to the lack of ER β -mediated autocrine functions that enhance the granulosa cell response to follicle stimulating hormone. CYP19-null ovaries more closely mimic those of ER α -null females but also exhibit features seen only in mice lacking both ER forms. These models nicely illustrate the ovary as both an endocrine organ and endocrine target for estradiol. Consequently, ovarian function must be considered especially vulnerable to insult by endocrine disrupting chemicals. A better understanding of the endocrine, paracrine and autocrine roles of estradiol in ovarian function will improve our abilities to predict the mechanisms by which the ovary is most susceptible to toxic insult and the manifestations that may result.

695 TOXICANT INDUCED FOLLICULAR ATRESIA.

J. A. Flaws. *Department of Veterinary Biosciences, University of Illinois, Urbana, IL.*

Females are born with a finite number of primordial follicles. Although some primordial follicles mature to the antral stage and release eggs for fertilization, greater than 95% undergo atresia. Atresia is an apoptotic process that is regulated by conserved factors, including members of the Bcl-2 family of proteins and oxidative stress pathway. Over time, the ovary becomes devoid of follicles because they are depleted through the processes of atresia or ovulation. Once the ovary is devoid of follicles, the female becomes infertile and is said to have entered reproductive senescence. Any chemical that increases atresia of follicles could increase the rate at which follicles are depleted from the ovary and result in premature ovarian failure. This talk will review chemicals that cause atresia of ovarian follicles. Particular emphasis will be placed on the pesticide methoxychlor (MXC) because it causes antral follicle-specific toxicity, characterized by an increased percentage of antral follicles undergoing atresia versus controls. While previous work indicates that MXC increases atresia of antral follicles, it is important to know whether the increased atresia is due to a direct effect of MXC on antral follicles, or an indirect effect caused by toxicity to other components of the ovary or other tissues that contribute to ovarian function (i.e., the hypothalamus or pituitary). Further, it is important to know the mechanisms by which MXC induces atresia. This talk will present information on the direct effects of MXC on the ovary as well as information on the role of the Bcl-2 family and oxidative stress pathway in MXC-induced atresia. Such information may improve our understanding of toxicant-induced atresia and help in the development of treatments for toxicant-induced atresia. Supported by NIH R21 ES1306, RO1 ES 012893, and Colgate Palmolive.

696 OOCYTE DEPLETION AND EARLY MENOPAUSE.

P. B. Hoyer. *Department of Physiology, University of Arizona, Tucson, AZ.*

A variety of types of health risks are associated with menopause in women. Therefore, it is important to identify environmental influences that damage ovarian follicles and might be capable of causing early menopause. This talk will review classes of environmental chemicals that have been shown to damage ovarian follicles in laboratory animals. It is also important to develop animal-based studies aimed at better understanding menopause-associated disorders. Therefore, this talk will review available animal models for menopause, focusing on ovariectomized and genetically altered animals. Particular attention will be paid to a chemical-induced mouse model for menopause. In this model, ovarian failure induced by the occupational chemical 4-vinylcyclohexene diepoxide (VCD) is gradual, and the animal retains residual ovarian tissue. Thus, the VCD-treated animal model is highly relevant to women undergoing a natural progression through the peri-menopausal transition and into post-menopause. Finally, physiological end points seen in this model that mimic menopause-associated disorders will be presented. (Supported by AG021948)

697 HOW CAN BIOLOGICALLY-BASED MODELING OF ARSENIC KINETICS AND DYNAMICS INFORM THE RISK ASSESSMENT PROCESS?

E. M. Kenyon and H. El Mastri. *ORD/NHEERL/ETD/PKB, U.S. EPA, Research Triangle Park, NC.*

Quantitative biologically-based models describing key events in the continuum from arsenic exposure to the development of adverse health effects provide a framework to integrate information obtained across diverse research areas. For example,

genetic polymorphisms in arsenic metabolizing enzymes can lead to differences in target tissue dosimetry for key metabolites causative in toxic and carcinogenic response. This type of variation can be quantitatively incorporated into pharmacokinetic (PK) models and used together with population based modeling approaches to evaluate the impact of genetic variation in methylation capacity on dose of key metabolites to target tissue. The PK model is an essential bridge to the pharmacodynamic (PD) models. A particular benefit of PD modeling for arsenic is that alternative models can be constructed for multiple proposed modes of action for arsenicals. Genomics data will prove useful for identifying the key pathways involved in particular responses and aid in determining other types of data needed for quantitative modeling. These models, when linked with PK models, can be used to better understand and explain dose- and time-response behaviors. This in turn assists in prioritizing modes of action with respect to their risk assessment relevance and future research. This type of integrated modeling approach can form the basis for a highly informative mode-of-action directed risk assessment for inorganic arsenic. This workshop will address both practical and theoretical aspects of integrating PK and PD data in a modeling framework, including practical barriers to its application.

698 HUMAN GENETIC POLYMORPHISMS: BIOMARKERS OF VARIABILITY IN ARSENIC METABOLISM.

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Variability in the metabolism of xenobiotics between humans is well established as a determinant of variability in toxicity resulting from the xenobiotic exposure. The presence of variant DNA sequences in genes involved in the relevant biochemical pathways is capable of contributing to this inter-individual variability in metabolism by potentially altering the intrinsic enzyme activity, the quantity, the localization, or the timing of the appearance/disappearance of the gene's protein product. By reducing unexplained variation in the data, the use of DNA polymorphisms as biomarkers can inform both the underlying biological model, as well as pharmacokinetic and pharmacodynamic models. We recently performed a comprehensive screen for DNA biomarkers of arsenic metabolism in 135 human subjects from Sonora, Mexico, exposed to drinking water arsenic concentrations ranging from 5.5 ppb – 43.3 ppb. The screening study focused on three genes with literature-validated functionality in arsenic metabolism, glutathione-S-transferase omega-1, purine nucleoside phosphorylase and arsenic (III) methyltransferase (AS3MT). Within the entire human population a cluster of tightly linked polymorphic sites in AS3MT was strongly associated with urinary dimethylarsenic : monomethylarsenic (D/M) ratio. Closer inspection of the data revealed that this association was only present in the children (7-11 years), and not in the adults (> 18 years). Interpretation of the genetic data required consideration of the number and location of all polymorphisms within AS3MT, the number and location of co-occurring polymorphisms in the cluster with statistical association to urinary D/M ratios, the ethnic ancestry of the subjects, and the relevant covariates within the subjects. This study provides an illustrative example of the complexities surrounding the successful application of DNA biomarkers to inform quantitative biologically-based models.

699 DEVELOPMENT OF A HUMAN PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODEL FOR INORGANIC ARSENIC AND ITS MONO- AND DIMETHYLATED METABOLITES.

H. A. El-Mastri¹ and E. Kenyon². ¹NHEERL, U.S. EPA, Research Triangle Park, NC and ²NHEERL, U.S. EPA, Research Triangle Park, NC.

A physiologically-based pharmacokinetic (PBPK) model was developed to estimate levels of arsenic and its metabolites in human tissues and urine after oral exposure to either arsenate (AsV) or arsenite (AsIII). The model consists of interconnected individual PBPK models for AsV, AsIII, monomethylarsenic acid (MMAV), and dimethylarsenic acid (DMAV). Reductions of MMAV and DMAV to their respective trivalent forms are also described in the overall model. Each PBPK model was constructed using flow limited compartments describing the mass balance of the chemicals in GI tract (lumen and tissue), lung, liver, kidney, muscle, skin, heart, and brain. The choice of tissues was based on physiochemical properties of the chemicals (solubility), exposure routes, and target and metabolic sites of inorganic arsenic and its metabolites. Metabolism of inorganic arsenic in liver was described as a series of reduction and oxidative methylation steps, in addition to the inhibitory influence of the metabolites on methylation. The inhibitory effects of AsIII on the methylation of MMAIII to DMA, and MMAIII on the methylation of AsIII to MMA were assumed to follow a noncompetitive mechanism. Most of the parameters used in the model were estimated from literature. The remaining parameters

were calibrated and evaluated using cumulative levels of inorganic arsenic and its metabolites in urine of exposed people. The calibrated and evaluated human PBPK model can be used, when combined with Monte-Carlo simulations, in identifying population-based distributions for some sensitive parameters (such as metabolic rate parameters differing due to genetic polymorphisms) and address the impact of these distributions on the human risks to arsenic exposure. This abstract does not necessarily



700 COMPUTATIONAL MODELING TO EVALUATE CANDIDATE MODES OF ACTION FOR THE CARCINOGENICITY OF ARSENIC.

R. Conolly, M. Breen and M. Zhao. *U.S. EPA, Research Triangle Park, NC.*

Several modes of action, including cytotoxicity, direct genotoxicity, and oxidative stress, have been proposed to explain carcinogenic effects of arsenic. The goals of our work are to (1) develop biologically-based computational models of candidate modes of action to better understand dose- and time-response behaviors and (2) prioritize the modes for relevance to risk assessment. The value of computational modeling lies in its ability to provide non-intuitive insights into dynamic behaviors. This value can only be realized if the model accurately reflects knowledge about the modes of action. Close consultation between computational modelers and arsenic experimentalists is required to ensure that the model incorporates this knowledge. Identification of the appropriate level of biological detail is also important. There is little point in incorporating large amounts of molecular-level information if corresponding data for parameterization and comparison with model predictions are lacking. Thus, to a large extent, the availability of data determines the level of biological detail. These considerations guide development of a qualitative map of key events for each mode. The quantitative version of the model is then developed by specifying the appropriate functional relationships between the components of the qualitative model and the associated parameter values. Ideally, parameters are set to measured values, though some values may be inferred by optimization. Lack of sufficient, relevant data, however, hinders successful translation of the qualitative model into its quantitative counterpart. Once the quantitative models for candidate modes are available, they will be ranked according to their relative abilities to reproduce time-course and dose-response data for endpoints of regulatory interest such as bladder cancer. Use of the preferred model for risk assessment will first require analysis of uncertainty, as the uncertainty of the biologically-motivated model should be less than that of its default-based counterpart. This presentation may not reflect the official policies of the U.S. EPA.



701 INTEGRATION OF GENOMIC DOSE-RESPONSE INFORMATION IN A MODE OF ACTION DIRECTED RISK ASSESSMENT FOR INORGANIC ARSENIC.

H. Clewell. *CIIT Centers for Health Research, Research Triangle Park, NC.*

It has been suggested that the dose-response for the carcinogenicity of inorganic arsenic (AsI) is highly nonlinear, with an effective threshold, due to an apparent mode of action involving inhibition of DNA repair. In the absence of a fully developed biologically based dose-response model for AsI carcinogenicity, it would be of value to be able to use a margin-of-exposure approach as an alternative to the default linear option. This presentation will describe a proposed approach for conducting a margin of exposure risk assessment for AsI using *in vitro* dose-response information on the expression of genes associated with the obligatory precursor events for tumorigenesis. The cellular effects of AsI can be understood as the superposition of highly-specific direct interactions of AsI with critical proteins containing vicinal dithiols, such as those involved in DNA repair, overlaid against a background of chemical stress, including proteotoxicity and depletion of non-protein sulphydryls, that induces an array of cellular responses common to many types of chemical and non-chemical stressors. The enhanced impact of DNA repair inhibition under conditions of oxidative stress, inflammation, and proliferative pressure leads to a situation in which the cell is no longer able to maintain the integrity of its DNA prior to division. Genomic dose-response data on AsI is consistent with data on other chemical stressors, in that there appears to be a transition from an adaptive state at low concentrations to an impaired state at higher concentrations. In the proposed risk assessment approach, the concentration at which this transition occurs in the human target cells (e.g., bladder epithelium) would be used as the point of departure for the margin-of-exposure determination. The margin of exposure would be selected to consider both variability in the human population (e.g., polymorphic variation) and uncertainty in the mode of action. A key aspect of this approach is the use of *in vivo* biomarker data from populations with known exposure to AsI to anchor the *in vitro* dose-response.



702 USING MODELING TO INFORM THE RISK ASSESSMENT PROCESS FOR ARSENIC.

B. D. Beck and A. S. Lewis. *Gradient Corporation, Cambridge, MA.*

This presentation will discuss how pharmacokinetic (PK) and pharmacodynamic (PD) models for inorganic arsenic (As_i) and organoarsenicals can inform the risk assessment process from an applied perspective. Present risk assessment methods for As_i rely primarily on data from highly exposed non-US populations, with limited consideration given to population variability and potential non-linearities in dose-response. Use of PBPK models to estimate dose to target should reduce uncertainty in extrapolating across populations, particularly with respect to addressing the role of nutritional deficiencies. However, uncertainties in identifying relevant metabolite(s) as a function of tumor type and in interpreting whether impairments in the first methylation step are causal or a result of disease are some of the obstacles to the application of PBPK models. Ongoing PD models for As_i, such as the genomic approach being developed between CIIT, EPRI, and EPA, may provide an elegant way to incorporate biological understanding into the dose-response process and should enhance the epidemiologically-based models. Challenges include the lack of a well-validated animal model for As_i, particularly for bladder and lung cancer, adding uncertainty to the interpretation of genomic changes with respect to tumor development (e.g., what changes are on the causal pathway, how much change is required for tumor development?). In contrast to As_i, dimethylarsinic acid (DMA), an organic arsenic compound, is a recognized animal carcinogen, causing bladder tumors in rats with a relatively well-understood mode of action (cytotoxicity, likely due to DMAV metabolites including DMAIII, followed by regenerative hyperplasia). PBPK and PD modeling for DMA carcinogenesis may have more near-term applicability. In fact, benchmark dose (BMD) modeling has been applied to the critical step in DMA carcinogenesis (increased DNA synthesis) to develop BMD parameters for use in risk assessment. PBPK modeling, particularly to the extent that it incorporates hemoglobin binding of DMA in the rat and its impact on dose, should add precision to cross-species extrapolations for this compound.

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COMPARISON OF CORROSITEX AND EPIDERM™ IN VITRO SKIN CORROSION SCREENING ASSAYS TO IN VIVO CORROSIVITY RESULTS.

D. M. Wilson, T. T. McMullin, H. L. Kan, R. M. Golden and W. T. Stott. *Dow Chemical, Midland, MI.*

A battery of chemicals was objectively compared in the EpiDerm™ (MatTek Corp., Ashland, OH) and Corrositex (InVitro International, Irvine, CA) *in vitro* corrosivity screens to existing *in vivo* data. EpiDerm™ consists of cultured, normal human epidermal keratinocytes in a differentiated epidermal model with organized basal, spinous and granular layers, and a stratum corneum containing intercellular lamellar lipid layers analogous to those found *in vivo*. Corrosivity is assessed as relative cytotoxicity at 3-min and 1-hr as the mitochondrial reduction of a tetrazolium salt. Corrositex uses a bio-barrier membrane comprised of a hydrated collagen matrix and supporting filter membrane on top of a vial filled with a chemical indicator dye, with corrosivity assessed according to breakthrough time. A panel of 12 reference chemicals recommended in OECD Guideline 431 for laboratory validation procedures was used [six corrosive: 1,2-diaminopropane; acrylic acid; 2-tert-butylphenol; potassium hydroxide; sulfuric acid; octanoic acid; and six non-corrosive: 4-amino-1,2,4-triazole; eugenol; phenethyl bromide; tetrachloroethylene; isostearic acid; and 4-methylthio-benzaldehyde], as well as 15 proprietary chemicals [nine corrosives and six non-corrosives *in vivo*, comprised of one alcohol, three organic acids, seven amines, two acrylic acid derivatives, and two halogenated herbicides]. EpiDerm™ exhibited 96% *in vivo* concordance (26 of 27), with 1 of 27 giving a borderline false negative for an organic acid. Corrositex exhibited 70% (19 of 27) *in vivo* concordance; with 3 of 27 false negatives (two organic acids and one halogenated herbicide), 2 of 27 false positives (both were amines), and 3 of 27 that didn't qualify due to lack of reactivity with the indicator dye. Erroneous results weren't restricted to false negatives, positives or by chemical class. One organic acid was a false negative for both *in vitro* assays, with the borderline EpiDerm™ result suggesting potential corrosivity *in vivo*. In conclusion, EpiDerm™ gave near-perfect concordance with *in vivo* corrosivity data.

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IN VITRO PREDICTION OF OXAZOLIDINONE-MEDIATED BONE MARROW TOXICITY.

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Oxazolidinones are antibiotics that inhibit bacterial protein synthesis. Daily dosing in rats is associated with selective depletion of immature erythroids and reticulocytes by day 5, followed by a 60-80% rebound on day 14. Binding of erythropoi-

etin (Epo) to its receptor EpoR plays a role in erythropoiesis, a multi-stage process requiring proliferation and differentiation of erythroblasts into reticulocytes. The purpose of this study was: (1) to develop an *in vitro* model that predicts *in vivo* reticulocyte reduction and subsequent tolerance by oxazolidinones and (2) to investigate the mechanism of toxicity. Treatment with the Epo-responsive erythroid cell line HCD57 with Epo resulted in a dose-dependent increase in proliferation (EC50=22pM). Addition of oxazolidinones did not affect Epo potency, but reduced proliferative efficacy from 100% to 40% in the absence of cytotoxicity. Oxazolidinones mediated a dose-dependent decrease in proliferation (IC50s: 200nM to 10µM) and were 100-fold less potent in the myeloid cell line FDCP-1 compared to HCD57 cells thus confirming *in vivo* erythroid-specific toxicity. Comparison of *in vitro* IC50s and *in vivo* reduction of reticulocytes (20-90%) in mice for 17 compounds established a linear correlation ($r^2=0.83$), indicating that the *in vitro* assay is predictive of *in vivo* findings. Treatment of cells for 21 days resulted in a dose-dependent suppression of growth for 7 days, followed by a rebound that mimicked *in vivo* effects. Mechanistic studies targeting EpoR find that the compounds have no effect on EpoR protein as determined by western blot analysis nor on EpoR synthesis measured by *in vitro* transcription and translation of the receptor. Binding of the ^{125} I-Epo to its receptor in transfected Cos cells or HCD57 cells was also not modulated by the compounds. In conclusion, HCD57 cells can be used to screen oxazolidinone compounds for bone marrow toxicity, but the mechanism by which the compounds mediate their effects is yet to be determined.

705 POST-IMPLANTATION RAT WHOLE-EMBRYO CULTURE ASSAY: *IN VITRO* – *IN VIVO* EXTRAPOLATION.

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The postimplantation rat Whole-Embryo Culture (WEC) assay is intended to assess embryotoxicity and was agreed by the ECVAM (European Centre for the Validation of Alternative Methods) Scientific Advisory Committee (ESAC) as a scientifically validated test. The protocol uses parameters such as IC50 values for malformations in purely statistically derived prediction models for classification as *non*, *weak*, or *strong* embryotoxic. However, its biological relevance and validity is under discussion. Further, *in vitro* – *in vivo* extrapolation remains a critical issue in quantitative risk assessment.

Therefore, in the present study, the feasibility of extrapolation of *in vitro* to *in vivo* dose-response relationships was explored. For each of 20 compounds the available *in vivo* studies were reviewed using the Benchmark Dose (BMD) approach by estimating the dose at which 10% foetal reduction and/or 10% prenatal deaths were observed (defined as BMD10). Similarly, for the *in vitro* studies the Benchmark Concentration was assessed for a 5% effect (BMC05). The results indicate that predicting the *in vivo* BMD from the *in vitro* BMC was not possible within one to two orders of magnitude. The large uncertainty in the *in vivo* BMD values is due to large variations in study design (prenatal exposure window, route of exposure, developmental effects at all dose-levels) and limits the possibility to assess extrapolation of the *in vitro* BMC values. Estimated (in *in vivo*) AUC and Cmax values were nearly linearly related to the external dose, indicating that in these cases including toxicokinetics would not improve the correlation. Conclusively, *in vitro* – *in vivo* extrapolations should be further evaluated for application in testing strategies by gathering developmental toxicity studies based on identical protocols.

Keywords: Whole-Embryo Culture, Quantitative *in vitro* – *in vivo* extrapolation

706 *IN VITRO* TOXICITY ASSESSMENT OF PHOTOPOLYMERIZABLE EMBOLIC RESINS.

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The present research is part of a multidisciplinary study to achieve a more controlled embolization of vascular defects utilizing photopolymerized acrylates that might be both safer and easier to deploy than current fluids. A mixture of photopolymerized Bisphenol-A-Glycidyl dimethacrylate (BisGMA) and CD582, an alkoxyated cyclohexane dimethanol diacrylate monomer, were assessed for cytotoxicity *in vitro* utilizing trypan blue cell viability assay. Compounds alone, together, and with the photoinitiators were tested to compare toxicity of the different combinations. Rat brain endothelial cells (RBE4) passages 10 to 15 were utilized in the study. Concentrations of approximately 1.6 million cells per well, plated in six-well plates were used. Configuration of design included four different concentrations of the mixtures, and two controls. Assessments were performed for three and six hour

periods. Repetitions of the process were performed to attain statistical relevance. At its highest concentration on the three hours period, CD582 alone exhibited a 73% viability compared to 93% for the controls. However, at six hours cell death with CD582 increased dramatically (to 100%). The presence of the photopolymerizing agent decreased cell death caused by CD582 at both three and six hours for the highest concentration (to 75% & 62% viability, respectively), but had no statistical effect on cell viability or cell toxicity for the BisGMA+photopolymerizing agent mixture, nor for the BisGMA+CD582+ photopolymerizing agent mixture. Overall, toxicity of the mixtures was less than the toxicity of the compounds alone. Different dilutions followed a pattern of decreased toxicity, presumably due to dilution factors of the mixtures, but the decreases were not significant over the concentration ranges tested.

707 TRANSFER OF LEAD ACETATE THROUGH AN *IN VITRO* BLOOD-BRAIN BARRIER SYSTEM.

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Lead-induced neurotoxicity remains a problem for humans and animals. Effects of this heavy metal are dependent on its ability to reach the brain. Transport of lead acetate was examined in an *in vitro* blood-brain barrier (BBB) system consisting of rat endothelial (RBE4) cells and astrocytes. These cells were cultured in Minimal Essential Medium (MEM) with 10% fetal bovine serum and in MEM with 10% heat-inactivated horse serum and placed on the upper and lower sides, respectively, of 0.45 micron inserts coated with collagen type I and placed in 6-well plates. Lead acetate in concentrations of 0.1, 1, 10 and 100 micromolar were placed inside the insert, on the endothelial cells. After 14 hours of exposure, electrical resistance of the BBB and lead concentration in cell culture medium under the barrier was determined. A concentration-related decrease in resistance from the control value of 210-390 ohm.cm² was seen, with 118-130 ohm.cm² measured at the highest lead concentration. Lead (215 ppb) was detected in the medium under the insert of cells exposed to 100 micromolar lead. Separate experiments indicated that RBE4 cells exposed to concentrations as high as 100 micromolar lead still had greater than 90% viability, as indicated by lack of uptake of trypan blue dye. Microscopic visualization of the co-cultures suggested that endothelial cells directly exposed to the lead sustained more damage (indicated by morphological change) as the concentration of lead increased. These experiments indicate that this *in vitro* system has potential value in studies examining lead transport into the nervous system.

708 DETERMINATION OF LC50 AND VISUAL ASSESSMENT OF COMPOUND INDUCED DEVELOPMENTAL TOXICITY ON ZEBRAFISH BODY, LIVER, INTESTINE AND HEART.

W. L. Seng¹, C. Parng¹, K. A. Augustine² and P. McGrath¹. ¹Phylonix Pharmaceuticals, Inc., Cambridge, MA and ²Reproductive Toxicology, Bristol-Myers Squibb Pharmaceutical Research Institute, Hopewell, NJ. Sponsor: C. Zhang.

Twelve blinded compounds provided by BMS were evaluated for developmental toxicity in zebrafish embryos by a LC50/Visual Assessment protocol designed by Phylonix. The goals of this study were to use zebrafish embryos as a model to assess median lethal dose concentration (LC50) and to assess body morphology and liver, intestine, and heart abnormalities after compound treatment.

Embryos were exposed continuously to compounds for 4 days from 24 hours post-fertilization (hpf) to 120 hpf. Total mortality for each compound was assessed every 24 hours. To determine the range for the effective concentration, embryos were initially treated with 5 concentrations one log apart: 0.01, 0.1, 1.0, 10 and 100 µM. If the LC50 was not within the initial test concentration range, additional concentrations were tested. Morphology of the body, heart, liver and intestine were assessed by microscopic examination.

The data from the assay was evaluated for strength in predictivity and accuracy based on their respective *in vivo* teratogenicity properties. Results from the evaluated test set suggested that the assay was good (>70% <80%) for specificity and excellent (>80%) for sensitivity. In this regard, there was a 75% success rate in positively identifying *in vivo* non teratogenic compounds and a 100% success rate in positively identifying *in vivo* teratogens, respectively. The accuracy for predicting relative teratogenic potency of the compounds was excellent with a 92% success rate. This study demonstrates an approach for simple and direct primary assessment of developmental toxicity in zebrafish. In summary, the results of the LC50/Visual Assessment Pilot study demonstrate that the zebrafish embryo assay format (ZE-TAXTM) is a valuable model for conducting predictive teratogenicity studies, presenting excellent concordance with a 92% success rate in positively identifying teratogenic and non teratogenic compounds from the evaluated test set.

709 ZEBRAFISH AS A MODEL VERTEBRATE FOR CARDIOTOXICITY.

A. J. Hill, S. Albert, B. Richards and J. Tinsley. *VASTox Plc, Abingdon, Oxfordshire, United Kingdom.*

Drug discovery is an increasingly expensive and risky process with high rates of safety related attrition often at late stages of R&D. Withdrawals of drugs from the market, especially those affecting the cardiovascular system, demonstrate the difficulties of predicting safety pharmacology at pre-clinical stages.

The zebrafish is a valuable in vivo model for expediting prioritisation of candidate drugs for development and flagging toxicity very early in the R&D process. Strong conservation of genetic pathways, increasing availability of disease models, its small size and ease of administration of compounds (micrograms) to the developing embryo, make zebrafish a very attractive, cost-saving vertebrate model for toxicity testing.

VASTox plc has undertaken validation of a cardiotoxicity assay using a set of thirty compounds targeting mechanisms known to affect the human heart (e.g. various ion channel blockers; HERG blockers; adreno- and muscarinic receptor agonists and antagonists). Larval zebrafish were exposed at a range of doses via the water in 24-well plates for 0-3 h and assessed using a CCD digital camera. Rates of atrial (A) and ventricular (V) contraction were determined; thereby revealing any tachycardia, bradycardia and changes in the A:V ratio.

The effects on zebrafish of known cardioactive compounds showed complete concordance to other higher order vertebrates. This included a specific phenotype indicative for the detection of HERG blockers. These data suggest the zebrafish is a suitable model for assessing drugs for toxicity at early stages of development.

710 DETERMINATION OF XENOBIOTIC INTRINSIC CLEARANCE IN FRESHLY ISOLATED HEPATOCYTES FROM RAINBOW TROUT (ONCORHYNCHUS MYKISS) AND RAT AND ITS APPLICATION IN BIO-ACCUMULATION ASSESSMENT.

X. Han, D. L. Nabb, R. T. Mingoia and C. Yang. *DuPont Haskell Laboratory, Newark, PA.*

Bioaccumulation in fish is a dynamic equilibrium of various processes that involve fish uptake, storage, and elimination of xenobiotics. Elimination via fish biotransformation of xenobiotics is one of the key processes that could be evaluated in an in vitro system to improve the performance of the prediction of xenobiotic bioaccumulation potentials. In this study, values of intrinsic clearance (CL_{int}) of seven reference compounds [atrazine, molinate, 4,4-bis(dimethylamino)benzophenone, 4-nonylphenol, 2,4-di-tert-butylphenol, trifluralin, benzo(a)pyrene] in hepatocytes freshly isolated from rainbow trout and rat were determined using a substrate depletion approach. Atrazine was metabolized in rat hepatocytes with an CL_{int} value of 3.81 (+/-) 1.96 mL/h/106 cells, whereas in trout hepatocytes, no apparent metabolism was observed within the incubation period (4 h). Intrinsic clearance values for all other compounds were 5.5- to 78.5-fold lower in trout hepatocytes than those in rat hepatocytes. Trout hepatic clearance (CL_H) values were extrapolated from the CL_{int} values using scaling factors and a well-stirred liver model. Biotransformation rate constants (k_M) of the compounds in trout were subsequently estimated and used as inputs to a kinetic model for the prediction of bioconcentration factors (BCF) in fish. Compared to the predicted BCF values that were obtained without consideration of fish biotransformation, the inclusion of estimated k_M values significantly improved fish BCF prediction for the reference compounds. Our work provided a framework for future bioaccumulation assessment of xenobiotics using combined information of the physical-chemical properties of the compounds and the biotransformation potentials of the compounds in fish.

711 PBDE AND HEXABROMODODECANE LEVELS IN THE U.S. POPULATION AND THE ENVIRONMENT AND PRELIMINARY PBDE ESTIMATES OF INTAKE FROM FOOD, DUST AND AIR.

A. J. Schecter¹, O. Paepke², R. Harris¹, A. Musumba¹, V. Ramakrishnan¹ and N. Shah¹. ¹*Environmental Sciences, University of Texas School of Public Health, Dallas, TX* and ²*Eurofins -ERGO, Hamburg, Germany.*

PBDE brominated flame retardants have been rapidly increasing the past few decades in US human milk and blood at the same time dioxin and PCB levels have been declining. Current US blood and milk levels vary from 4-400 ppb, lipid adjusted, in our studies. In household dust we find levels from 705 to 69,283 ppb with a median of 2,507. These are the highest levels reported worldwide. Food levels are highest in fish, median 616, range 11-3726 pg/g ww/day, then meat, median 190, range 39-1378 pg/g ww, and dairy, median 32, range 8-683 pg/g ww/day. However, dietary intake in the USA is highest from meat because of its predominance in the US diet. US adult dietary intake is approximately 1.0 ng/kg/day. Indoor air levels

are higher than outdoor levels. In our study, the indoor air level mean is 590, outdoors 125 and car 283 pg/cubic meters. We calculate 307 ng/kg/day dietary intake for nursing infant and 1.0 for adults. Air PBDE intake is about 0.109 ng/kg/day for adults. Median dust PBDE intake for adults is 7 ng/day with a range from 0.265 to 346. Under different intakes estimates within the range of levels measured, either diet or dust could contribute more towards PBDE body burden. This is unlike dioxins where food of animal origin is the route of almost all exposure. Hexabromocyclododecane was found at much lower levels in over 20 individually analyzed human milk samples than PBDEs and the alpha congener only was detected, unlike the commercial mixture where the gamma congener predominates.

712 LEAD EXPOSURE IN CHILDREN LIVING IN A SMELTER COMMUNITY FROM NORTHERN MEXICO: A FIVE YEARS FOLLOW-UP AFTER ENVIRONMENTAL INTERVENTION.

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In 1997 we described the largest environmental problem due to lead exposure in children in Latino America associated with a smelter complex in Torreon City, northern Mexico. In 2001, our studies and other performed by the CDC estimated that more than 32,000 children were at risk to have ≥ 10.0 µg/dL of Pb in Blood (PbB). As a consequence the Smelter Company began an environmental intervention program which consisted in abatement of lead emissions and cleaning up the neighbourhoods around the smelter from contaminated dusts. In 2001 we began a follow up study in 569 children and measuring PbB concentrations at 0, 6, 18 and 60 months. Median (range) for PbB concentrations in each sampling time were: 0 months: 9.40 (2.3 - 43.82) µg/dL; 6 months: 8.65 (2.90 - 41.45) µg/dL; 18 months: 8.37 (2.4 - 35.8) µg/dL; 60 months: 4.51 (1.43 - 30.27) µg/dL. The decrease of PbB with respect to time fitted a linear regression model with a slope of 1.116 (µg/dL)/year ($p < 0.00001$). Equation: $y = -1.116(x) + 10.73$. Also, the significance of the relationship between lead and Zinc-protoporphyrin was gradually reduced, suggesting a relieve in this biomarker of biochemical effect. Although the tendencies of PbB values are encouraging, still there are neighbourhoods with prevalences of lead poisoning in children (PbB > 10.0 µg/dL) higher than 25 %, which means that more aggressive environmental interventions are needed to protect children from the well known adverse effects of lead.

713 POULTRY PRODUCTION AND ARSENIC IN DRINKING WATER FROM GROUNDWATER IN THE EASTERN AND SOUTHERN SHORE COUNTIES OF MARYLAND.

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Arsenicals are utilized in poultry feeds for both prophylactic and growth promoting purposes. As a consequence, arsenic is excreted in waste and arsenic-containing broiler waste is applied to cropland in broiler producing areas as manure. This practice results in the environmental release of inorganic arsenic species into soils, and possibly groundwater. No previous studies have examined the association between land-applied arsenic-containing broiler waste and arsenic in drinking water from groundwater. This study utilized geographic analysis and multivariate modeling to examine this issue. Total arsenic levels were measured in tap water samples from private wells in the Eastern Shore of Maryland, a site of intensive poultry production and waste disposal, and the Southern Shore of Maryland, an area devoid of commercial broiler farms. Concentrations of arsenic in drinking water samples (Range: below DL-42 ppb) were evaluated in terms of two land use variables - percent cropland and the density of broiler operations. These variables were summarized within 1 Km radius of each drinking water sample. Results show that log arsenic concentration is significantly associated with percent cropland, after adjusting for other predictors ($p < 0.005$). No association was observed with broiler operation density. As further evidence for the role of broiler waste disposal, this association was observed in the region of the Eastern shore characterized by extensive crop production rather than broiler farms. These results are not unexpected, since poultry houses do not accumulate as much waste as land where wastes are frequently disposed. These data suggest that the use of arsenicals in broiler feed and the land application of arsenic-containing broiler waste may introduce arsenic into groundwater/drinking water in poultry producing areas. Research supported by a grant from the Cigarette Restitution Fund, Maryland.

714 DIETARY INTAKE OF PBDES BASED ON CONSUMPTION OF CATFISH IN SOUTHERN MISSISSIPPI.

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Polybrominated diphenyl ethers (PBDEs) are used as flame retardants in a wide variety of consumer products. Concerns surrounding these compounds are primarily due to their ubiquitous presence in the environment as well as human tissues, coupled with evidence indicating the potential for adverse effects. Despite these concerns, relatively few studies are available in the literature which characterize and quantify intake by humans. In this study, PBDE concentrations in Southern Mississippi catfish were measured in an effort to determine background levels and daily intake of these compounds from a primary food source for residents in this region of the United States. Sixty-one wild catfish samples were collected from local fisherman in March 2006 from five locations in Southern Mississippi and 28 farm-raised catfish samples were purchased from seven grocery markets. All samples were analyzed by Alta Analytical for 43 PBDEs (mono- through deca- congeners) using high resolution gas chromatography-mass spectrometry. PBDE concentrations and congener profiles varied by fish type and location. The average ΣPBDE concentration in all samples was 3.3 ng/g (wet weight); wild-caught catfish fillets had an average concentration of 3.85 ng/g whereas farm-raised catfish fillets had an average PBDE concentration of 0.45 ng/g. Using a range of consumption rates for catfish only, average PBDE concentrations, and an average body weight of 70 kg, intake values ranged from approximately 14-66 pg/kg bw for all fish types measured, 16-76 pg/kg bw for wild catfish fillets and 2-9 pg/kg bw for farm fillets. These results provide a reasonable data set describing the current background levels and daily intake of PBDEs from consumption of catfish representative of the Southeastern region of the United States. This abstract does not represent EPA policy.

715 OCCUPATIONAL EXPOSURES ASSOCIATED WITH PETROLEUM-DERIVED SOLVENTS CONTAINING TRACE LEVELS OF BENZENE.

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Benzene may be present as a trace impurity or residual component of mixed petroleum solvents due to refining processes. In this presentation, we review the physical and chemical properties of various petroleum solvents and characterize the typical airborne concentrations of benzene associated with the handling or use of low-level benzene-containing products in the United States. Our assessment was based on (1) a review of the benzene content and flammability hazard of various petroleum solvents, (2) a review and synthesis of the publicly available literature on measured airborne concentrations of benzene associated with products that have typically contained <0.1% benzene since the late 1970s or early 1980s, and (3) indoor exposure modeling to predict breathing zone 8-hr TWA airborne concentrations of benzene under several hypothetical scenarios involving the use of benzene-containing mineral spirits. Our analysis showed that petroleum-derived products that may have historically contained trace levels of benzene, such as paints and paint solvents, printing solvents and inks, cutting and honing oils, adhesives, mineral spirits and degreasers, and jet fuel could have produced average TWA airborne concentrations of benzene that ranged from 0.002 to 0.2 ppm under standard use scenarios. Higher air concentrations (up to 1 or 2 ppm benzene) have occasionally been reported for specific products, particularly if the bulk content of the product was unknown (or suspected of being greater than 1%) or the sampling was performed under "worst case" solvent use patterns or workplace conditions. The overall weight-of-evidence indicates that only certain low volatility solvents (such as mineral spirits) can be safely used in spray applications or unventilated workplace conditions, and that petroleum-based products containing <0.1% benzene are not expected to produce 8-hr TWA airborne concentrations that exceed current occupational exposure limits under nearly all anticipated product use scenarios.

716 AIRBORNE DUST EXPOSURE ASSESSMENT FOR AUTOMOTIVE BODY SHOP WORK INVOLVING RESIN FILLERS AND ABRASIVES.

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Airborne dust exposures were assessed for two body shop workers who performed routine body repair work using two different resin filler products and similar abrasive products (sandpapers and powered grinders). This pilot study compared the

composition of total and respirable atmospheric dusts associated with use of the resins and abrasive products with the composition of the original products. Observers documented the timing and nature of tasks performed, and general air flow characteristics. Personal and area samples were collected for total dust, respirable dust, total aluminum oxide and titanium dioxide, and both total and respirable talc. Scanning electron microscopy (SEM) was used to identify the physical characteristics of atmospheric dusts. A continuous dust monitor measured peak exposures from grinding, sanding, or compressed air blowing. Personal total dust concentrations ranged from 0.44 to 18 mg/m³ (background 0.1), while respirable dust ranged from 0.04 to 0.53 mg/m³. Airborne talc levels averaged 0.4% of total dust and < 1.6% of respirable dust, but comprised 10-45% by weight of the resin fillers. Titanium dioxide averaged < 0.9% of atmospheric total dust, but comprised 1-10% of the resin fillers. Airborne aluminum oxide (from abrasives) was not detected. SEM samples indicated most of the atmospheric total dust was comprised of irregular particles > 5 microns in diameter. Despite large differences in talc content between the two resin products, there was no significant difference in airborne respirable dust or talc levels during use. These findings suggest that airborne dusts generated during auto body work are largely comprised of larger particles > 5 microns, and that composition of these airborne dusts differs from the composition of the resin mixtures before curing.

717 AIRBORNE ASBESTOS MEASUREMENTS AND RISK ASSESSMENT FOR AGRICULTURAL ACTIVITIES ON AN EASEMENT WITH INSULATION DEBRIS FROM A PETROLEUM PIPELINE.

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The Arapahoe Pipeline Corridor is a 150 mile easement that contained a former petroleum pipeline spanning through agricultural and ranch lands in northeast Colorado and southwest Nebraska. The pipeline was removed for recycling but debris from the pipeline insulation comprised of chrysotile coated with tar was left in place. After the larger surface debris was removed, a study was undertaken to determine whether upper bound exposures during relevant agricultural land uses on the easement would pose appreciable cancer risk to local farmers or ranchers. A public record survey and numerous personal interviews were conducted to identify land use characteristics and activity patterns relevant to the easement. An airborne asbestos measurement study was carried out to assess potential upper bound exposures from activities that disturbed easement soils: tractor tilling of soils; harvesting with a combine; tractor mowing of grass; and driving a pick-up truck on the easement. NIOSH Method 7400 and the TEM asbestos structure counting rules of AHERA were applied for 49 air samples including personal samples of vehicle drivers and high volume area samples for each soil disturbance activity. The TEM results showed only 1 of 43 valid samples with detectable asbestos (1 chrysotile fiber, 1.4 x 0.11 microns, 0.0017 TEM f/ml). The data were validated and statistically analyzed assuming asbestos to be present at one half the detection limit for non-detects. Three upper bound agricultural exposure scenarios were formulated to simulate local farmer impacts from the easement, and the USEPA cancer potency factor of 0.23 per PCM f/ml was applied. Each 40 year exposure scenario showed upper bound cancer risks below 1 per 10 million from easement-related asbestos exposures. These findings suggest that small amounts of asbestos pipe wrap debris are unlikely to pose an appreciable inhalation cancer risk for common agricultural activities that disturb the impacted soils.

718 AIRBORNE ASBESTOS MEASUREMENTS AND RISK ASSESSMENT FOR AN ATHLETIC FIELD WITH TRANSITE BOARD DEBRIS FROM BARRICKS DEMOLITION.

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Barricks with chrysotile-containing transite board siding at a U.S. Coast Guard facility in Michigan were demolished and most of the debris removed several years before the land was donated to a city government for use as an athletic field. After the larger surface debris (> 2 sq. in.) was removed, a study was undertaken to determine whether upper bound exposures during relevant athletic field uses of this property would pose appreciable cancer risk to the athletic field users and maintenance personnel. An airborne asbestos measurement study was carried out to assess potential upper bound exposures from activities that disturbed the impacted soils: annual soil aeration, grass mowing, and use as a soccer field. NIOSH Method 7400 and the TEM asbestos structure counting rules of AHERA were applied for 56 air samples including personal samples of equipment operators/players and high volume area samples for each soil disturbance activity. The TEM results showed that

none of the 53 valid samples had detectable asbestos (detection limits 0.0017-0.043 TEM f/ml). The data were validated and statistically analyzed assuming asbestos to be present at one half the detection limit for non-detects. Three upper bound exposure scenarios were formulated to simulate field use and maintenance activities that disturb the impacted soils, and the USEPA cancer potency factor of 0.23 per PCM f/ml was applied. Upper bound cancer risk estimates were well below 1 per million for asbestos exposures related to field user or maintenance worker scenarios. These findings suggest that small amounts of transit board debris are unlikely to pose an appreciable inhalation cancer risk for common activities that disturb impacted soils at an athletic field.

719 RIBEPEIX: A COMPUTER PROGRAM TO OPTIMIZE SOME HEALTH RISKS AND BENEFITS RELATED TO FISH CONSUMPTION.

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In recent years, and based on the importance of fish as a part of a healthy diet, there has been a notable promotion of fish and seafood consumption. For cardiovascular risk reduction in the general population, prestigious institutions such as the American Heart Association has suggested (among other dietary recommendations) to consume fish, especially oily fish, at least twice a week. Oily fish are rich in the omega-3 polyunsaturated acids EPA (eicosapentaenoic) and DHA (docosahexaenoic), which have shown to have protective effects in preventing coronary heart disease among other important health benefits. However, a number of recent studies performed in different regions and countries have shown that fish consumption may be also a potential source of exposure to chemical pollutants, some of them with well known adverse effects on human health. Recently, we determined in 14 edible marine species (the most consumed in Catalonia, Spain) the concentrations of EPA and DHA, as well as those of a number of chemical contaminants: Cd, Hg, Pb, polychlorinated dibenzo-p-dioxins and furans, PCBs, hexachlorobenzene, polycyclic aromatic hydrocarbons, polychlorinated naphthalenes, polybrominated diphenylethers and polychlorinated diphenylethers. To quantitatively establish the intake of these pollutants (risks) versus that of EPA + DHA (benefits), we designed a simple computer program, RIBEPEIX. The concentrations of EPA, DHA, and the chemical pollutants have been used as database of the program. The goal of this presentation is to show to the audience how RIBEPEIX may be used as an easy tool to optimize fish consumption: most suitable species, frequency of consumption, and size of meals. RIBEPEIX can be useful not only for professionals (cardiologists, general physicians, nutritionists, etc.), but also for the general population, as it will be available (free) in Internet.

720 DOCOSAHEXAENOIC ACID CONSUMPTION SUPPRESSES MULTIPLE CREB PHOSPHORYLATION PATHWAYS IN MURINE PERITONEAL MACROPHAGES.

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Fish oil ingestion has been reported to have value in suppressing several chronic inflammatory diseases. Consumption of docosahexaenoic acid (DHA), a major component of fish oil, inhibits induction of interleukin-6 (IL-6) expression by the trichothecene deoxynivalenol (DON) in peritoneal macrophages by decreasing binding activities of transcription factors, most notably cAMP regulated element binding protein (CREB). The purpose of this study was to test the hypothesis that the activities of CREB kinases are impaired in macrophages from DHA-fed mice. Peritoneal macrophages were collected from B6C3F1 mice that were fed for 4 to 6 wk with AIN-93G diet supplemented with 6% high oleic acid safflower oil (control diet) or 6% fish oil (DHA diet). Isolated peritoneal macrophages were treated with DON (250 ng/ml), forskolin (50 μ M) or ionomycin (5 μ M) to activate CREB through protein kinase B (PKB or Akt), mitogen and stress activated protein kinase (MSK) and ribosomal S6 kinase (RSK) (by DON), protein kinase A (PKA) (by forskolin) and calcium/calmodulin-activated protein kinase (CaMK) (by ionomycin) pathways respectively. Proteins were extracted at different time points (0, 15 and 30 min after treatment) and analyzed by Western analysis. Induction of CREB phosphorylation by DON, forskolin or ionomycin at 15 and 30 min was suppressed in macrophages from DHA-fed mice. DON-induced phosphorylation of Akt, MSK and RSK as well as Akt kinase activity were also inhibited in macrophages from mice consuming DHA. Taken together, the data suggest that DHA consumption alters macrophage programming rendering them less capable of CREB activation through multiple pathways induced by chemical stressors. DHA suppression of CREB-activated IL-6 expression might be a critical mechanism for the prevention of inflammatory and autoimmune diseases (Supported by Public Health Service Grants DK58833).

721 CARDIOMYOPATHIC CHANGES INDUCED BY 2-DEOXYGLUCOSE, A POTENTIAL MIMETIC OF CALORIE RESTRICTION.

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This study was designed to test whether the glucose analogue, 2-deoxy-D-glucose (2-DG), which may mimic some of the effects of caloric restriction is safe and effective with chronic use. Caloric restriction (CR) has been proven to extend life span, delay the incidence of several age-related diseases, thereby leading to healthy aging. To effectively obtain the desired beneficial effects of CR, 30% to 40% reduction in food intake is necessary. If a compound such as 2-DG can mimic this effect, then the unlikely paradigm of consuming 30% to 40% less (30-40% CR) can be negated. We used three different concentrations of 2-DG (0.04%, 0.2% and 0.6% w/w in the chow) from two different sources (Sigma and Regent). Two different rat strains (Fisher-344 and Brown Norway) were administered 2-DG in their ad libitum diets for six weeks at the concentrations mentioned. A control group for each strain was included as well. Cardiac muscle was assessed for several parameters by histopathology on hematoxylin and eosin (H&E) and scored on a scale of 0 to 4. A score of 0, which is typical of normal cardiac muscle and a score of 4 which is typical of cardiomyopathic changes was attributed to the sections. The cardiomyopathic changes in the 4+ category included observations such as very thin fibers, loss of fibers, distinct vacuolations dispersed in several fibers, distinct loss of striations and general architecture, expanded interstitial space, or infiltration of inflammatory cells and fibroblasts. Substantial cardiomyopathic changes were seen in both strains at 0.6% 2-DG. However, the changes were more pronounced in Fisher-344 rats. Both 0.2% and 0.04% groups in both strains showed cardiomyopathic changes with score ranges from 0 to 1.33. The control groups showed no observable cardiomyopathic changes on histopathology. We conclude that 2-DG is toxic at the 0.6% and 0.2% doses and we need to see longer exposure with more animals to determine if the low dose would be toxic as well.

722 DERIVATION OF AN ACCEPTABLE DAILY INTAKE (ADI) USING A BENCHMARK DOSE (BMD) APPROACH FOR A VETERINARY PHARMACEUTICAL RESIDUE IN FOOD-PRODUCING ANIMALS.

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The Committee for Veterinary Medicinal Products (CVMP) in the European Agency for the Evaluation of Medicinal Products (EMA) has traditionally relied on NO(A)ELs to establish ADIs for residues of veterinary pharmaceuticals found in the edible tissues of food-producing animals. Recently, an ADI was proposed based on the BMD approach for firocoxib use in horses. Horses are considered a food-producing animal in the EU. Firocoxib is an orally administered non-steroidal COX-2 anti-inflammatory drug used for the control of pain, inflammation, and lameness. Based on the comprehensive set of laboratory animal safety studies conducted in support of EU registration, developmental toxicity in rabbits and reproductive toxicity in rats were considered the most appropriate for establishing a point-of-departure for the ADI. Given dose-responses for the most sensitive endpoints and effects at or near the lowest dose levels tested, the BMD approach was considered to be an appropriate and precise method for assessing potential human health risk. Several measures of fetal viability (rabbit developmental toxicity study) and pup mortality (one-generation rat reproductive toxicity study) were modeled to calculate BMDs and benchmark dose limits (BMDLs). The NLogistic Model provided the best goodness-of-fit for both data sets. The most conservative BMDL was 0.34 mg/kg/day from the rabbit developmental toxicity study based on a 1% Extra Risk Level. Use of a 200-fold uncertainty factor (UF) was recommended, resulting in a proposed ADI of 1.7 μ g/kg/day. An independent assessment of the reproductive and developmental data was conducted in the EU and an alternative BMD proposed. An unconstrained Weibull model resulted in a more conservative BMDL of 0.043 mg/kg/day based on the rat reproductive toxicity data. The CVMP applied a UF of 200 to this BMDL and proposed an ADI of 0.215 μ g/kg/day, which is pending final European Commission approval.

723 PHARMACOKINETIC AND DEVELOPMENTAL SAFETY EVALUATION STUDIES OF METHYLSULFONYLMETHANE IN RATS.

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Methylsulfonylmethane (MSM) is sold as a dietary supplement to promote joint health. Studies were undertaken to determine the pharmacokinetics of acute MSM in male rats, and its effect in rats when administered orally to pregnant rats during the period of major organogenesis (days 6 through 20).

Male Sprague-Dawley rats were administered a single oral dose of [35S]-MSM (500 mg/kg) and blood levels of radioactivity were determined at different time points for up to 48 hours, and tissue, urine, and fecal radioactivity levels were measured at different time points for up to 120 hours. Oral [35S]-MSM was rapidly and efficiently absorbed with a mean T_{max} of 2.1 hours, and a $t_{1/2}$ of 12.2 hours. Approximately 86% of the dose was recovered in the urine after 120 hours. No quantifiable levels of radioactivity were found in any tissues after 120 hours, indicating complete elimination of [35S]-MSM. Radioactivity concentrations seen in the soft tissues were similar to that seen in the central blood compartment, with a mean MSM blood concentration of 63.7 $\mu\text{g eq/ml}$. In the subsequent developmental study, four groups of 24-25 timed-bred primiparous female rats were administered 0, 50, 500, or 1000 mg MSM/kg/day via gavage on gestation days 6 through 20. Maternal feed consumption, body weight, body weight gain, uterus weight and corrected body weight/body weight gain were unaffected by treatment. No evidence of maternal toxicity or litter differences was detected. No evidence of fetal mortality, toxicity, or growth and structural alterations was observed at any dose level. The results of this study suggest that [35S]-MSM is rapidly absorbed, well distributed, and completely excreted from the body, and has a no-observed-adverse-effect level (NOAEL) for maternal and developmental toxicity of at least 1000 mg/kg/day.

724 ASSESSING TCDD WASTING SYNDROME IN AN *IN VIVO* OBESITY MODEL.

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TCDD is a by-product of incineration commonly found as a microcontaminant in the food supply. The TCDD wasting syndrome, characterized by prolonged weight loss, has been examined for decades. Much of this work has focused on high dose *in vivo* and *in vitro* studies. This body of work suggests that TCDD inhibits adipogenesis and that these effects are not rescued by high fat diet. We hypothesized that a low dose of TCDD during a developmentally sensitive window would induce the TCDD wasting syndrome in mice on high and low fat diets. FVB/NJ females were dosed with 1 $\mu\text{g/kg}$ TCDD or vehicle on gestational day 12.5. At birth, cages were placed on standardized low or high fat diets. Body weights were measured weekly through weaning and monthly thereafter. Percent body fat was measured at 5 weeks, 3 and 6 months of age. Fasting blood glucose was measured at 5 weeks, 4 and 6 months of age. Body weights were higher in mice on high fat diet compared to low fat diet throughout the lifespan evaluated. However, TCDD exposure did not alter their body weights. High fat diet increased percent body fat at 3 months and blood glucose at 4 months. Again TCDD had no effect on these measures. In summary developmental low dose TCDD has no overall impact on classic wasting syndrome phenotypes, both during the time period in which TCDD is expected to be in the tissue and long after it is expected to be cleared. This longitudinal study of the affect of low dose TCDD on the wasting syndrome suggests that the wasting syndrome is a high dose phenomenon. (THIS ABSTRACT DOES NOT REFLECT AGENCY POLICY.)

725 CHEMICAL AND TOXICOLOGICAL EVALUATION OF FUMONISIN B₁ IN EXTRUDED CORN GRITS.

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Extrusion cooking reduces fumonisin concentrations in corn but its effect on fumonisin toxicity is unknown. Batches of corn grits were contaminated by spiking with fumonisin B₁ (FB₁) (SG, 43 ppm) or by fermentation (FG1, 46 ppm FB₁; FG2 48 ppm FB₁) and then extruded with and without the addition of glucose (10% w/w). FB₁ concentrations of extruded SG (E-SG), extruded FG1 (E-FG1) and extruded FG2 (E-FG2) were 34 (31% reduction), 29 (37%), and 51 ppm (no reduction). Glucose increased reductions: FB₁ concentrations in E-SG plus glucose (E-SG+), E-FG1 plus glucose (E-FG1+) and E-FG2 plus glucose (E-FG2+) products were 9.8 (77% reduction), 5.9 (87%), and 11.7 ppm (76%), respectively. Significant amounts (20-38 ppm) of the FB₁-glucose reaction product, *N*-(deoxy-D-fructosyl-1-yl) FB₁ were found only in the glucose-supplemented products. SG, FG1 or FG2 (50% w/w in rodent chow) and equivalent weights of each extruded material were fed to male Sprague-Dawley rats for 4 weeks to assess toxicity. Control groups were fed (50% w/w) uncontaminated grits or extruded uncontaminated grits. Relative kidney weights of rats fed E-FG2+ (0.83% BWt) and control diets (0.79-0.82% BWt) were similar and greater than those of the other groups (0.72-0.76% BWt). Microscopic kidney lesions were typical of those caused by

FB₁. Those found in rats fed E-FG1+ were scored as less severe (mean score = 1.2) than those from groups fed FG1 or E-FG1 (3.0). Comparison of kidney lesions from the SG, E-SG, and E-SG+ or the FG2, E-FG2 and E-FG2+ groups revealed no differences; their histopathology scores averaged 2.6-3.0. In summary, extrusion with glucose significantly reduced FB₁ in the grits (>75%) but had an inconsistent effect on *in vivo* toxicity. Extrusion must be further evaluated to determine if it is a suitable method for reducing fumonisins in foods.

726 TOXICOLOGICAL EVALUATION OF *FUSARIUM VERTICILLIOIDES* CULTURE MATERIAL NIXTAMALIZED IN THE PRESENCE AND ABSENCE OF CORN MATRIX.

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Cooking-steeping corn in alkaline water (nixtamalization) reduces the amount of detectable fumonisin B₁ (FB₁) in masa and tortilla products. However, reductions might be overestimated if FB₁ reacts with the corn matrix to form undetectable reaction products. The purpose of this study was to investigate if corn matrix facilitates the reduction of measurable FB₁ during nixtamalization and reduces toxicity of the cooked material. *Fusarium verticillioides* culture material (CM) was nixtamalized in the absence (NCM) and presence (NCMC) of ground uncontaminated corn. Sham nixtamalization (cooking-steeping in water) of the CM without (SCM) and with corn (SCMC) was also done. Equivalent weights of each material were added to rodent chow and fed to male SD rats for 1 (n=3/group) or 3 weeks (n=5/group) to evaluate toxicity. Control groups were fed chow plus uncontaminated corn (UC), chow plus nixtamalized uncontaminated corn (NUC), or uncooked CM. FB₁ concentrations of the diets were: UC = 0.15, NUC = 0.16, CM = 9.08, NCM = 2.08, NCMC = 0.49, SCM = 1.19 and SCMC = 0.95 ppm. Hydrolyzed FB₁ concentrations were: UC = 0.07, NUC = 0.09, CM = 0.25, NCM = 1.27, NCMC = 1.60, SCM = 0.55 and SCMC = 0.37 ppm. No differences in body weights, food consumption or relative kidney weights were found during the feeding study. Apoptotic kidney lesions typical of fumonisins were found in all groups except UC and NUC. Compared to the CM group (mean apoptotic cell counts at week 3 = 207 $\pm$ 105 (SD)), fewer apoptotic renal epithelial cells were found in rats from all other groups. Furthermore, apoptosis counts in the NCMC group (22 $\pm$ 8) were less than those of the NCM group (44 $\pm$ 12). Apoptosis counts of the SCM and SCMC groups were similar, averaging 17 $\pm$ 5 and 19 $\pm$ 6. These findings suggest that the corn matrix facilitated reduction of measurable FB₁, possibly through matrix binding, and further reduced toxicity of nixtamalized CM.

727 GENE EXPRESSION PROFILING AND DIFFERENTIATION ASSESSMENT IN PRIMARY HEPATOCYTE CULTURES, ESTABLISHED HEPATOMA CELL LINES, AND LIVER TISSUES.

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Primary hepatocytes are frequently used as a viable *in vitro* model for the liver *in vivo*. However, the culture conditions reported vary considerably, with associated variability in performance. In this study, we used microarray gene expression profiling to evaluate the differentiation character of a highly defined serum-free two-dimensional sandwich culture model for primary human hepatocytes, one that configures hepatocytes with collagen I as the substratum together with a dilute extracellular matrix (Matrigel) overlay and nanomolar levels of dexamethasone. Expression profiles were compared to those exhibited by human liver tissues and by cultures of the hepatoma-derived cell lines HepG2 and Huh7. Analyses using the PANTHER database (ABI) identified biological processes significantly overrepresented among probe sets differentially expressed in the *in vitro* systems vs. those in human liver. Categories relevant to liver function, such as 'detoxification' and 'steroid metabolism,' exhibited altered function in the *in vitro* models. Within each category, hepatocytes maintained expression unchanged from the liver for more than 77% of the probe sets, representing such genes as *CYP2B6*, *CYP3A4*, *ABCB11*, and *SLC22A1*. In contrast, HepG2 and Huh7 cells were unchanged from the liver tissues for fewer than 48% and 55% of these probe sets, respectively. Further, hierarchical clustering of the hepatocytes, but not the cell line samples, changed from donor-specific to treatment-specific when the probe sets were filtered to focus on the phenobarbital (PB)-inducible genes, indicating that only the hepatocytes exhibited responsiveness to PB. These data support the use of a highly defined 2-D primary hepatocyte culture system that establishes gene expression endpoints closely resembling that of liver, including maintenance of responses to inducer challenge. Supported by NIEHS ES11387 and NIGMS GM66411.

728 PREDICTING IN VIVO OUTCOMES FROM IN VITRO mRNA PROFILING.

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In vivo rodent studies are used during the lead optimization phase of drug development to evaluate the toxicity of new candidate drugs. These studies require a significant amount of test article and live phase resources, making them impractical for screening large numbers of compounds earlier in development. Alternatively, *in vitro* assays employing rat primary hepatocytes have been evaluated as surrogates for short term *in vivo* toxicity studies using biochemical, morphological, and genomic endpoints. Although mechanistic data obtained *in vitro* has proven useful in resolving issues with individual molecules, the application of genomics to the rat primary hepatocytes model in compound screening has been more elusive. Here, we demonstrate that although mRNA profiles from *in vitro* studies did not correlate well with *in vivo* profiles, similar signaling and response pathways were modulated based on functional grouping of altered transcripts. For example, treatment with a PPAR coagonist, resulted in 1027 transcriptional changes *in vivo* and 813 changes *in vitro*, with only 33 transcripts in common. When evaluated from a mechanistic view, we observed a significantly higher concordance in GO Biological Process terms, which is one method for functional grouping of array data. These changes indicate that both *in vivo* and *in vitro*, hepatocytes undergo similar physiological responses to xenobiotics despite minimal overlap in the individual genes modulated by compound treatment. From these studies, we have demonstrated that gene expression in primary rat hepatocytes can be used to identify compound associated hepatic liabilities, thus enabling prioritization of molecules to bring forward for *in vivo* evaluation in drug development.

729 GENE PROFILING IN WILD TYPE AND PPAR α NULL MICE EXPOSED TO PFOA.

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Perfluorooctanoic acid (PFOA) is a perfluoroalkyl acid used in a variety of commercial applications. Concerns have been raised because PFOA is ubiquitous in the environment and can be detected in human tissues. PFOA is a rodent carcinogen and a developmental toxicant in mice. While the mode of action (MOA) of PFOA toxicity is not clear, this compound is a PPAR α agonist and a peroxisome proliferator. For this reason, liver tumors in rodents are thought to be related to PPAR α signaling. However, PFOA also increases liver weight in the PPAR α null mouse. To further study the MOA of PFOA, wild type and PPAR α null mice were orally dosed with either PFOA (1 or 3 mg/kg), Wy14,643 (50 mg/kg), or vehicle for 7 days. Wy14,643 was included as a known PPAR α agonist. Animals were euthanized, livers weighed, and liver samples collected for preparation of total RNA. Gene profiling analysis was conducted using Applied Biosystems microarrays on 4 animals per group. In wild type mice, Wy14,643 induced many of the predicted PPAR α related changes in genes associated with lipid metabolism, sterol biosynthesis, bile acid biosynthesis, retinol metabolism, and inflammation. Wy14,643 had little effect on the PPAR α null mouse. On the other hand, while it was clear from the response of the wild type mice that the majority of PFOA related changes were mediated through PPAR α , changes in gene expression were nonetheless observed in the knockout animal. These included 177 genes that were similarly altered in both wild type and knockout mice. Among the up-regulated genes were several Cyp2 and Cyp3 genes, Acyl CoA dehydrogenase (long chain), Cyclin D1, Aox1, Dbi, Aldh1a7, and Cyp4a10. Included in the group of down-regulated genes were Complement components 4A and B, Complement factors B and H, Cathepsin C, and Baat. These results indicate that while PFOA influences certain biological processes such as lipid homeostasis, inflammation, and cell cycle progression via a PPAR α mediated MOA, there also appears to be a non-PPAR α related component to this response. This abstract does not necessarily reflect EPA policy.

730 PREDICTIVE PROFILING OF HEPATOTOXICITY USING GENOMIC CLUSTER AND NETWORK ANALYSIS.

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In the present study, in order to estimate the usability of genomic approaches to predict hepatotoxicity with short-term treatment, we treated male rats with acetaminophen (APAP), carbon tetrachloride (CL), amiodarone (AD) or tetracycline at toxic doses. At 6 or 24 hours post dosing, their livers were removed and used for pathological examination and genomic analysis. Histopathologically, no noteworthy changes were observed at 6 hr, but some were detected at 24 hr except in the AD group. Regarding the genomic analysis, to confirm the reliability of the

GeneChip data, we performed hierarchical cluster analysis by using S-plus. The individual microarray data were classified clearly into 5 treatment-related clusters at 24 hr as well as at 6 hr when no remarkable morphological changes were detectable. In the gene expression analysis using GeneSpring, transcription factor genes, as well as oxidative stress- and lipid metabolism-related genes, were markedly affected in all treatment groups at both time points when compared with their expression in the control group. Finally, we investigated gene networks involving the above affected genes by using Ingenuity Pathway Analysis. Down-regulation of lipid metabolism-related genes regulated by SREBP1 was observed in all groups at both time points, whereas up-regulation of oxidative stress-related genes regulated by Nrf2 was found in the APAP and CL treatment groups. From the above findings, for the application of genomic approaches to predict hepatotoxicity, we considered that cluster analysis for classification and early prediction of hepatotoxicity and network analysis for investigation of toxicological biomarkers would be useful. Currently, we investigate the protein expression of above two transcription factors in order to support our consideration.

731 THE SEARCH FOR TOXICOGENOMICS BIOMARKERS BRIDGING BETWEEN *IN VIVO* AND *IN VITRO*: ANALYSIS OF THE HEPATOTOXICITY OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS.

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Gene expression analysis opens new perspectives in toxicology to identify mechanisms of toxicity and biological response and to improve toxicity assessment. Toxicogenomics project (TGP), a 5-year project started in 2002, is a collaborative project by NIBIO, NIHS and 15 pharmaceutical companies in Japan. The goal of our project is to construct a large-scale database of over 150 drugs exposed to rats (*in vivo*, *in vitro*) and to human (*in vitro*), to develop a system that will forecast the toxicity of new chemical in the early stage of drug development, hopefully by *in vitro* experiments. TGP database integrates gene expression data analyzed by Affymetrix GeneChip with traditional toxicological endpoints including blood biochemical, hematological, and pathological data.

In the present study, to search for genes, which consistently changed *in vivo* and *in vitro*, gene expression data of rat livers and hepatocytes treated with non-steroidal anti-inflammatory drugs (NSAIDs; aspirin, diclofenac, ibuprofen, indomethacin, naproxen, and sulindac) were selected. By multivariable analysis of these expression data, 45 probe sets that showed dose-dependent expression changes by the exposure to NSAIDs were extracted. These 45 probe sets contained endoplasmic reticulum (ER) stress markers (erp70, grp94, etc.). Our results are in accordance with previous reports, that long-term treatment of NSAIDs causes apoptosis through ER stress in various types of cells, and this is independent of their cyclooxygenase activity. We suggest that the *in vitro* expression profile of these 45 probe sets could be used to predict *in vivo* phenotypes caused by long-term treatment of NSAIDs, and that they would be practical biomarkers in drug research and development. At present, pathological examination by electron microscopy is in progress confirming the occurrence of ER stress in rat livers treated with single administration of NSAIDs.

732 HEPATOCELLULAR INJURY AND GENE EXPRESSION ANALYSIS IN LIVERS OF RATS CO-TREATED WITH LIPOPOLYSACCHARIDE AND CHLORPROMAZINE.

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Idiosyncratic drug reactions are adverse responses that typically occur in a small fraction of the population taking therapeutic drugs. Chlorpromazine (CPZ) is a phenothiazine drug that was associated with idiosyncratic hepatotoxicity during its use as an antipsychotic drug. Previously, we demonstrated that cotreatment of rats with small doses of lipopolysaccharide (LPS) and CPZ produces liver injury that mimics human idiosyncratic responses to this drug. In this study we tested the hypothesis that unique gene expression changes occur in livers near the time of onset of injury in rats treated with LPS plus CPZ. Rats were treated with a nonhepatotoxic dose of LPS (1.1X 10⁶ EU/kg, iv) or its saline vehicle. Two hours thereafter they were given either CPZ (70mg/kg, ip) or its saline vehicle. They were killed 2 or 6 h after drug treatment, and serum and liver samples were collected. Alanine aminotransferase (ALT) activity, an indicator of hepatic parenchymal cell injury, increased significantly in sera of LPS/CPZ-cotreated rats compared to rats receiving

LPS or CPZ alone. Microarray analysis was performed on RNA isolated from livers. Principal component analysis and hierarchical clustering of gene expression distinguished rats treated with LPS/CPZ or CPZ from those treated with LPS alone. Expression of a large number (>7,000) of genes was changed by CPZ, irrespective of LPS cotreatment. Expression of many inflammatory genes, including NF-kappa B, several chemokines, Kruppel-like factor 4 and MMP9, were changed by LPS/CPZ treatment. These results suggest that gene expression changes after cotreatment with LPS and CPZ are driven largely by CPZ, but LPS cotreatment affects expression of several genes potentially related to the hepatotoxic response. (Supported in part by GM075865.)

733 GENE EXPRESSION ANALYSIS OF LIVERS FROM RATS CO-TREATED WITH LIPOPOLYSACCHARIDE AND AFLATOXIN B1.

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Aflatoxin B1 (AFB1), causes both acute hepatotoxicity and hepatic neoplasia in exposed humans and rats. Previous studies in rats demonstrated that a small, noninjurious dose of lipopolysaccharide (LPS) augments the hepatotoxicity of AFB1 through activation of inflammatory cells and production of soluble inflammatory mediators. We tested the hypothesis that hepatic gene expression changes could be distinguished among vehicle-, LPS-, AFB1- and AFB1/LPS-treated rats before the onset of liver injury in the AFB1/LPS-treated rats. Male, Sprague-Dawley rats (250-350 g) were treated with 1 mg AFB1/kg, ip or its vehicle (0.5% DMSO/saline) and 4 h later with either E. coli LPS (7.4 x 10⁶ EU/kg, iv) or its saline vehicle. Animals were sacrificed at 6 h and 12 h after AFB1 treatment, and liver samples were collected for evaluation of liver injury and isolation of RNA. Hepatic parenchymal cell injury, as estimated from increases in serum alanine aminotransferase (ALT) activity, was observed only in rats cotreated with AFB1/LPS and only at 12 h. Hierarchical clustering of gene expression data from the 6-h (i.e. before the elevation of ALT) grouped animals distinctly according to treatment. Less distinct clustering was observed at the 12-h. Relative to treatment with AFB1 or LPS alone, AFB1/LPS cotreatment altered hepatic expression of numerous genes, including those encoding products involved in inflammation and cell death, such as early growth response gene-1, monocyte chemoattractant protein-1, Cxcl2, and interleukin-6. These data suggest that enhanced expression of inflammatory genes by cotreatment with AFB1 and LPS contributes to liver injury (Supported in part by GM075865).

734 TOXICOGENOMICS AND PROTEOMICS ANALYSES FOR HEPATOCARCINOGENICITY OF THE STRUCTURAL ISOMERS, 2, 4- AND 2, 6-DIAMINOTOLUENE IN RATS.

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The aromatic amines, 2,4-diaminotoluene (2,4-DAT) and 2,6-diaminotoluene (2,6-DAT) are structural isomers that are mutagenic in Ames test (+S9) but have different results in rat carcinogenicity bioassays. In the rat liver UDS and RDS tests, only 2,4-DAT was reported to be positive. To elucidate the difference, rat hepatocarcinogenicity using both DATs, toxicogenomics with GeneChip, and proteomics with 2D-DIGE analyses were performed. Male Sprague-Dawley rats (5 wk) were administered by oral gavage with 2,4-DAT (2, 10, 50 and 250 mg/kg) or 2,6-DAT (1.6, 8, 40 and 200 mg/kg) and sacrificed 3, 6, 9, 24 and 48 hrs after the dose to obtain the liver and serum samples. According to serological and liver histological examinations, no toxicity was observed except for 2,4-DAT-administered group at the maximum dosage (250 mg/kg). In gene and protein expression profiling, both DATs affected aromatic compound-inducible P450 (CYP1A1), steroid metabolism, and cytosolic fatty acid beta-oxidation. However, ATM-p53 (DNA damage/repair), Keap1/Nrf2 (oxidative stress response) signaling pathways, and liver regeneration (cell proliferation, cytoskeleton, ribosome biogenesis, protein folding etc.) were activated at 2,4-DAT in the 50 and 250 mg/kg groups as compared to the 2,6-DAT groups. Activation of these pathways may be reflected in the results of the rat liver UDS and RDS by 2,4-DAT. In addition, 2,4-DAT caused a decrease of Sult1c1/2, which is known to be involved in carcinogenesis of the aryl amine compound, 2-AAF. We hypothesized that Sult1c1/2 may play an important role for DAT-induced rats hepatocarcinogenicity, that is, 2,4-DAT was activated to an electrophilic metabolite that react with DNA and/or protein by sulfate conjugation, whereas 2,6-DAT lacks metabolic activation.

735 DETECTION OF POSSIBLE TOXIC RESPONSE AT DOSE LEVELS BELOW NOAEL FROM DOSE-DEPENDENT CHANGES IN GENE EXPRESSION PROFILE.

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The use of toxicogenomic approaches in the evaluation of candidates for the drug discovery decision-making process and in the prediction of toxicity at an early stage of drug development is widely accepted. However, there are few reports that describe any relationship between dose-dependent gene expression and toxicological findings, including histopathological changes. In the present study, we examined dose-dependent effects on gene expression of various hepatotoxic drugs including fibrates (clofibrate and gemfibrozil). The gene expression profile was then compared with changes in the conventional toxicological parameters. Each drug was administered in three oral doses (low, middle and high) by single dosing or by repeated dosing for 4 days to male SD rats, aged 7 weeks. At necropsy 6 hours after the final administration, blood samples were drawn for hematology and biochemistry examinations, and the livers were collected for gene expression analysis and histopathology. DNA microarray analysis revealed that genes related to beta-oxidation of fatty acid metabolism were up-regulated dose dependently from the low dose to high dose level in the single and repeated dose studies of fibrates and benzobromarone, which is reported to have adverse effects similar to those of fibrates. These genes are considered to be important for peroxisome proliferation through PPARalpha-mediated signaling. After clofibrate and benzobromarone treatment, changes in the gene expression profile were associated with histopathological findings in the liver (eosinophilic and other proliferative changes) at the high dose level in the repeated dose study, but not at the low dose level. It was concluded from these results that dose-dependent changes in gene expression profile could allow detection of possible toxic response even in the absence of conventional toxicological findings such as histopathological changes.

736 MULTIFACTOR CLUSTER ANALYSIS OF GENDER, CHEMICAL, AND DOSE EFFECTS UPON GENE EXPRESSION IN TCDD AND AROCLOR 1254 EXPOSED RATS.

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The toxic equivalency quotient (TEQ) methodology is used to estimate human health risks from exposure to dioxins and PCBs. Since mixtures comprise varying quantities of full agonists, partial agonists, and antagonists of the aryl hydrocarbon receptor (AHR), the integrated biological activity of such mixtures may show dependencies on more complex factors than just the presence of the AHR. Thus, the TEQ approach likely provides an over simplistic and imprecise estimation of the true potency of TEQ mixtures that would be better determined by testing the actual mixture. The current study evaluates the relative importance of chemical, dose, and gender on gene expression response to Aroclor 1254 and TCDD. Rats (n=4) were administered corn oil or 0.3 or 3.0 ug/kg TCDD or 0.6, 6.0, or 60 mg/kg Aroclor 1254 mg/kg po for 3 consecutive days. Day 4 hepatic RNA was screened using Affymetrix RG U34 arrays. An in-house (GE) linear model was used to generate a discovery gene set fulfilling both a 2-fold change and 0.005 q-value cutoff requirements in at least 2 exposure groups. The discovery dataset, representing a total of 107 probe sets or ~73 rat genes, was dominated by enzymes involved in xenobiotic/steroid/lipid metabolism. Distinct clusters displayed significant gender (i.e., Cyp3a13), chemical (i.e., Comt, Cyp2b), dose effects (i.e., Nqo1, Cyp1b1), and various interactions among these 3 variables (i.e., Igfbp1, Hgf). This approach may have utility for characterizing sets of responsive genes and in identifying which of multiple experimental factors are most important in determining the profile of both AHR-dependent and AHR-independent responses.

737 ASSESSING AHR-MEDIATED GENE EXPRESSION EFFECTS IN A NOVEL HUMAN LIVER ADULT STEM CELL LINE.

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Primary and continuous cell lines are valuable in vitro models that have been widely used to provide insights into mechanisms of toxicity. The recent availability of human hepatic adult stem cells provides an innovative in vitro model that may

more accurately predict *in vivo* toxicity. The human liver adult stem cell line, HL1-1, was used to assess gene expression effects elicited by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Expression of the aryl hydrocarbon receptor (AhR) in HL1-1 cells was verified by quantitative real-time PCR (QRT-PCR) and Western blot analysis. For temporal studies, cells were treated with 10 nM TCDD or vehicle (DMSO) for 1, 2, 4, 8, 12, 24 or 48 hrs. Cyp1a1 was induced >100-fold between 2 to 48 hrs, with an EC50 value of 8.30 nM, in dose response studies using QRT-PCR. Global gene expression responses of cells treated with TCDD in the absence and presence of cycloheximide (CHX) were conducted using custom human cDNA microarrays representing 7,844 unique genes to identify primary responses. Empirical Bayes analysis identified 78 and 200 active genes that were differentially regulated by TCDD at 4 and 12 hr, respectively, with 77 potential primary based on CHX co-treatment studies. Comparison to CHX studies in murine Hepa1c1c7 cells identified 4 differentially regulated orthologous genes that are putative conserved primary responses. Species-dependent and cell line differences likely contribute to the low overlap, but also provides further evidence of species-specific AhR regulons. Supported by NIGMS grant R21 GM075838.

738 METABOLOMIC ANALYSES OF LIPID AND AQUEOUS LIVER EXTRACTS IN TCDD-TREATED MICE.

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The hepatic metabolomic response of immature ovariectomized C57BL/6 mice to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was examined using thin layer chromatography (TLC), 13C, 31P, and 1H NMR (14.1 T), and high-pressure liquid chromatography (HPLC). Mice received either sesame oil or 30 ug/kg TCDD by gavage and were sacrificed at 168 hr post dose. TCDD induced a significant increase in liver weight with marked cytoplasmic vacuolization accompanied by individual cell apoptosis and foci of mixed populations of inflammatory cells consisting mainly of mononuclear cells and some neutrophils. Oil Red O staining indicated vacuolization was due to lipid accumulation and TLC of lipid extracts revealed a 2.5-fold increase in triacylglycerides (TAG). The 13C lipid spectra were subdivided into 1024 integral regions (bins) for Principal Component Analysis (PCA), which yielded two distinct data clusters (treated and control) in the PCA scores plot (PC1 vs. PC2). Spectral examination showed: (1) cholesterol levels were unaffected, (2) TAG were increased 3.5-fold (20.8 ± 1.9 vs. 6.0 ± 0.3 umol/g liver), and (3) omega-3 fatty acids were decreased by 22% (treated vs. controls, p<0.005). 31P NMR phospholipid analyses showed that TCDD increased phosphatidylcholine 24%, while phosphatidylethanolamine and cardiolipin each decreased 16% (p<0.05). HPLC of lipid extracts identified >3-fold increases in 16:0, 18:1n9, 18:2n6, 18:3n3, 20:2n, 20:3n6, 20:3n9 and 22:5n3 fatty acids. 1H and 31P NMR of aqueous extracts also displayed a clear separation in the PCA scores plots. Thus in addition to eliciting changes in transcription (Boverhof, et al. Toxicol Sci 85, 1048 (2005)), TCDD also induces alterations in hepatic aqueous molecules and lipids. Relationships among changes in genes transcripts and metabolites are being sought. Funded by RO1 ES013927.

739 COMPARATIVE TOXICOGENOMICS OF AH RECEPTOR MEDIATED HEPATOTOXICITY IN C57BL/6 MICE.

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Temporal microarray analyses were performed on hepatic tissue from orally gavaged immature female C57BL/6 mice in order to evaluate similarities and difference in gene expression profiles between 2,3,7,8-TCDD (TCDD) and 2,3,7,8-TCDF (TCDF). Time course studies out to 168 h were conducted with 300 µg/kg TCDF, 30 µg/kg TCDD or sesame oil. Gene expression was monitored using cDNA microarrays containing 13,362 features, representing 8,516 genes. Empirical Bayes analysis identified 342 differentially expressed genes following treatment with TCDF. Comparison of TCDF and TCDD differential gene expression identified 192 genes which exhibited common responses; 140 common responses exhibited comparable profiles (correlation coefficients > 0.3). In general, TCDF was less potent when compared to TCDD at later time points, with maximal efficacy occurring primarily at 12 h. QRT-PCR verification of Cyp1a1 and Ngq1 induction were consistent with differences in efficacy observed in microarray results. TCDF elicited hepatocytes cytoplasmic vacuolization consistent with lipid accumulation at 24 h, was more severe at 72 h, but significantly subsided by 120 and 168 h compared to

TCDD. Hepatic TCDF levels were significantly lower than TCDD after 24 h, with maximal levels attained at 12 h. This was followed by a gradual decrease between 18 and 168 h, suggesting pharmacokinetic differences may account for the lower accumulation of hepatic lipids and gene expression activity elicited by TCDF at later time points. The declining TCDF responses observed after 24 h relative to TCDD, due to metabolism and/or excretion, suggests that TCDF's TEF of 0.1 may overestimate its hepatic toxicity. Funds provided by The DOW Chemical Company.

740 COMPARATIVE TOXICOGENOMIC ANALYSIS OF THE HEPATOTOXIC EFFECTS OF TCDD IN SPRAGUE DAWLEY RATS AND C57BL/6 MICE.

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Comparative temporal and dose-response microarray analyses were performed on hepatic tissue from immature, ovariectomized Sprague Dawley rats and C57BL/6 mice to further characterize conserved and species-specific mechanisms of TCDD-mediated toxicity. For temporal studies, rats and mice were gavaged with 10 or 30 µg/kg of TCDD, respectively, and sacrificed after 2, 4, 8, 12, 18, 24, 72 or 168 hrs while dose-response studies were performed at 24 hrs. Hepatic gene expression was monitored using custom cDNA microarrays containing 8,567 (rat) or 13,361 (mouse) cDNA clones. Affymetrix data from male rats treated with 40 µg/kg TCDD were also included to expand the species comparison. In total, 3,087 orthologous genes were represented in the cross-species comparison. Comparative analysis identified 33 orthologous genes that were commonly regulated by TCDD as well as 185 rat-specific and 225 mouse-specific responses. Functional annotation identified conserved gene responses associated with xenobiotic/chemical stress and amino acid and lipid metabolism. Rat-specific gene expression responses were associated with cellular growth and lipid metabolism while mouse-specific responses were associated with lipid uptake/metabolism and immune responses. The common and species-specific gene expression responses were also consistent with complementary histopathology, clinical chemistry, hepatic lipid analyses and reports in the literature. These data expand our understanding of TCDD-mediated gene expression responses and indicate that species-specific toxicity may be mediated by differences in gene expression. This may explain the wide range of species sensitivities and will have important implications in risk assessment. Funded by NIH R21 GM75838.

741 COMPARATIVE TOXICOGENOMIC EXAMINATION OF THE HEPATOTOXIC EFFECTS OF 3, 3', 4, 4', 5-PENTACHLOROBIPHENYL (PCB126) AND 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) IN C57BL/6 MICE.

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Polychlorinated biphenyls (PCBs) are persistent environmental pollutants with potent dioxin-like properties that elicit a wide range of effects in humans and wildlife. The most toxic congener, 3,3',4,4',5-pentachlorobiphenyl (PCB126), was chosen to characterize hepatic temporal gene expression responses in immature ovariectomized C57BL/6 mice to further elucidate its mechanisms of toxicity. Mice were gavaged with 300 µg/kg PCB126, or vehicle (sesame oil) and sacrificed after 2, 4, 8, 12, 18, 24, 72 or 168 hrs. Comparisons were also made to mice treated with 30 µg/kg 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to identify conserved responses and assess the appropriateness of the PCB126 Toxic Equivalence Factor (TEF) of 0.1. Hepatic gene expression was monitored using a 13361 feature cDNA microarray representing 8516 unique genes. PCB126 induced the differential expression of 285 features (243 unique genes) at one or more time points, as determined by an empirical Bayes approach. Functional gene annotation from public databases with complementary histopathology, clinical chemistry and hepatic triglyceride analysis identified gene expression responses consistent with the observed physiological effects including responses involving oxidative stress, differentiation, gluconeogenesis and fatty acid uptake and metabolism. Hepatic PCB126 levels were also determined in order to associate differential gene expression to tissue levels. Despite comparable hepatic TEQ levels and early gene expression responses, collectively the data indicate significant differences in efficacy between PCB126 and TCDD suggesting that the PCB126 TEF of 0.1 is an over estimate of its potency. This work was funded by Superfund P42ES04911.

742 COMPARATIVE ONTOLOGICAL ANALYSIS OF HEPATIC GENE EXPRESSION PROFILES FOLLOWING 52 WEEKS OF CHRONIC EXPOSURE TO PCB126, PCB153 AND A BINARY MIXTURE OF PCB126 AND PCB153.

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Our laboratory conducted a series of gene array experiments to evaluate toxicogenic effects in the liver of female Sprague Dawley rats exposed for 52 weeks to PCBs in recently completed NTP studies (2004, 2006). Our studies were conducted in an effort to understand the relationship between the toxicogenic responses of the liver to PCBs and the pathological endpoints seen with chronic exposure to these toxins; which include non-neoplastic lesions, hepatocellular adenoma and cholangiocarcinoma in rats exposed to the AHR ligand PCB126, but not to the non-AHR ligand PCB153. Interestingly, co-administration of PCB126 and PCB153 potentiated the effects of PCB126. This is not explained by the pharmacokinetics of PCB126, which exhibits decreased hepatic retention when co-administered with PCB153 (NTP, 2004). In an effort to identify the genes which may be responsible for the potentiation of hepatotoxicity seen with co-exposure to PCB126 and PCB153, we have conducted ontological analysis on genes differentially expressed following exposure to PCB126 and PCB153, alone and in combination. Utilizing Ingenuity Pathway Analysis software we identified gene clusters that may be contributing to the lesions seen in the liver. The following list of biological functions significantly associated with differentially expressed genes will read as follows; biological function followed in parenthesis by the number of genes associated with said function differentially expressed by PCB126, the binary mixture and PCB153, respectively. A few of the biological functions that are relevant to genes differentially expressed by these compounds include cancer (108, 136, 18), cell death (100, 127, 15), inflammatory disease (27, 46, 4), endocrine system disorder (4, 25, 7), cell cycle (7, 24, 8) and lipid metabolism (42, 56, 29). These analyses will help in efforts to evaluate the mechanisms and risks associated with real world exposure to mixtures of PCBs.

743 PHENOTYPIC TRANS-DIFFERENTIATION OF HEPATOCYTES TO BILIARY CELLS AFTER BILE DUCT INJURY INDUCED BY METHYLENE DIANILINE.

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Cholestatic liver diseases (CLD) are life threatening liver complications with suppression of biliary epithelium (BE). Recent studies from our laboratory demonstrate that hepatocytes transdifferentiate to BE cells in case of impaired biliary regeneration following bile duct ligation and subsequent administration of biliary toxicant methylene dianiline (DAPM). Present study is aimed at determining if hepatocytes transdifferentiate into BE after structural destruction of bile ducts induced by DAPM alone. Immunohistochemistry of biliary specific cytokeratins such as AE1/AE3 and CK19 conducted on liver sections of male F344 rats treated with DAPM (50 mg/kg) revealed that periportal hepatocytes acquire biliary markers during bile duct repair indicating hepatocyte transdifferentiation. When Chimeric rats carrying dipeptidyl peptidase IV (DPP-IV)-positive hepatocytes (DPP-IV) were treated with DAPM (50 mg/kg, every 2 days, 3 times), bile ductules bearing the donor hepatocyte marker DPP-IV were evident within 30 days after the DAPM treatment indicating hepatocyte transdifferentiation. To investigate the mechanisms of transdifferentiation, an in vitro organoid culture of rat hepatocytes was utilized where hepatocytes to BE transdifferentiation has been previously demonstrated. Growth factors known to be involved in differentiation including HGF, EGF, VEGF, PDGF, SCF, MSP, FGF-a, FGF-b, and FGF-8b were tested for their ability to promote hepatocyte transdifferentiation. None of the growth factors except HGF and EGF induced formation of BE despite the presence of cognate receptors. Microarray analysis of the organoid cultures indicated upregulation of various HGF and EGF targets such as osteopontin, CXCR chemokine LIX, CXCR receptor LCR1, chemokine orphan receptor 1, cyclin D1, and CD44. These data suggest that HGF and EGF signaling is responsible for transdifferentiation of hepatocytes to biliary cells. Moreover, this study has revealed hepatocyte transdifferentiation as a novel therapy against CLD.

744 HEPATIC GENE EXPRESSION PROFILE AND LIPID HOMEOSTASIS IN MICE EXPOSED TO ETHANOL.

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Ethanol is a well-known hepatotoxicant that induces cumulative liver damage, which includes steatosis, steatohepatitis, and cirrhosis. In the present study, we investigated the effects of acute and chronic ethanol exposure on gene expression in

the mouse liver using the Applied Biosystems Mouse Genome Survey Microarray. For acute exposure, A single oral dose of 0.5 or 5 g/kg ethanol was administered to male ICR mice, and liver samples were obtained after 6, 24, and 72 h. For chronic exposure, mice were fed regular Lieber-Decarli ethanol diet ad libitum and liver samples were obtained after 1, 2 and 4 weeks. Histopathological evaluation showed typical fatty livers in the high-dose group at 24 h (acute) as well as in 2 and 4 weeks (chronic). In these groups serum AST, ALT and triglyceride levels were slightly elevated. Microarray analysis identified 28 genes as being ethanol-responsive, in that their expression levels differed significantly from the controls (two-way ANOVA; $p < 0.05$), after adjustment by the Benjamini-Hochberg multiple testing correction; these genes displayed more than 2-fold induction or repression. The expression of genes that are known to be involved in fatty acid synthesis was examined. The transcript for lipogenic transcription factor, sterol regulatory element (SRE)-binding factor 1 (SREBF1), was upregulated by ethanol exposure. Of the genes known to contain SRE or SRE-like sequences and to be regulated by SRE-binding protein 1, those encoding malic enzyme (ME1), ATP-citrate lyase (ACLY), and fatty acid synthase (FASN) in the cytosol, and stearoyl-CoA desaturase (SCD1) in the endoplasmic reticulum, were induced by ethanol. Quantitative real-time PCR confirmed the changes in the expression levels of the selected genes. Overall, our results suggest that the global changes in hepatic gene expressions that follow acute and chronic ethanol exposure can be used as biomarkers for ethanol induced hepatotoxicity.

745 TOXICOGENOMICS STUDY OF FLUTAMIDE USING CHIMERIC MICE WITH HUMANIZED LIVER.

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Flutamide, a non-steroidal antiandrogen, is widely used in the treatment of prostate cancer. However, flutamide occasionally causes severe liver dysfunction resulting in hepatitis, liver necrosis or cholestasis. Although rats treated with flutamide show liver enlargement, no serious liver injury has been reported in rats in pre-clinical studies. To investigate the cause of human-specific adverse effects, we studied the effect of flutamide on chimeric mice with near-completely humanized liver. Chimeric mice were generated by transplanting human hepatocytes into urokinase-type plasminogen activator-transgenic SCID mice (uPA/SCID mice). Both rat and chimeric mice were dosed with flutamide (30 & 300 mg/kg daily) and their liver gene expression profiles compared after 6h, 24h and 8 days. Rats treated with flutamide for 8 days showed an 80% increase in liver mass. However, no other significant changes in histopathology were observed in either species at earlier time points. After 24 h, flutamide strongly induced Cyp1a1 in rat liver and by 8 days induced other aryl hydrocarbon receptor (AhR) regulated genes including Cyp1a2 and NADPH quinone oxidoreductase (NQO1). AhR regulated genes remained largely unchanged after flutamide treatment in chimeric mice liver. Reports that reduced expression levels of AhR regulated genes are involved in the onset of flutamide-induced human specific hepatic injury and our microarray data suggests that induction of these genes protects rats from flutamide-induced hepatotoxicity. In addition, we found several differences in the expression levels of ADME related genes which may further explain the human-specific hepatic injury induced by flutamide. This study demonstrates that chimeric mice with humanized liver are useful models for the investigation of mechanisms involved in human drug-induced hepatotoxicity.

746 INVESTIGATION OF HEPATOCELLULAR CARCINOMA IN MALE RATS USING EARLY GENE EXPRESSION PROFILING.

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The carcinogenic potential of RO-XXX was investigated in vivo using a 2-year oral gavage carcinogenicity study in Sprague-Dawley rats. RO-XXX treatment induced hepatocellular adenomas in males and females ($p \leq 0.001$) and hepatocellular carcinoma in male rats ($p \leq 0.008$); a no-effect level for hepatocellular neoplastic changes was not achieved in male rats. Despite these findings, no previous in vivo studies in rats raised hepatocarcinogenic concerns. In addition, all genotoxicity testing results for RO-XXX were negative, including: Ames assay, in vitro mouse micronucleus test, human lymphocyte chromosome aberration test, in vivo mouse and rat bone marrow micronucleus test, in vivo kidney and liver rat comet assays, and SHE cell assay. Further development of RO-XXX was halted and subsequent work has focused on identifying the molecular mechanism of hepatocellular neoplastic transformation. In an attempt to gain further insight into the mechanism of carcinogenesis for RO-XXX, we examined gene expression in rat liver samples from animals treated with RO-XXX in a 4-day repeat dose study. Samples were profiled

using Affymetrix RG230v2 microarrays and the Iconix ToxFx data analysis service. The significantly perturbed genes from RO-XXX treated rats were enriched for genes associated with lipid and fatty acid metabolism, and electron transport. The aryl hydrocarbon receptor and RXR signaling pathways were also significantly affected. An apparent absence of DNA damage and a lack of changes in DNA repair genes suggest an epigenetic mechanism of carcinogenicity.

747 GENDER DIFFERENCES IN THE CANINE LIVER IN RESPONSE TO PHENOBARBITAL.

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The antiepileptic drug phenobarbital (PB) has a long history of use in preclinical investigations as a prototypical inducer of drug metabolizing enzymes (DMEs) and is known to increase total serum cholesterol levels in rodents, canines and humans. The present investigation evaluated the effect of gender on liver gene expression in beagle dogs using Affymetrix GeneChip® Canine Genome 2.0 Arrays following treatment with PB. Animals in the PB-treated group (n=3 per gender) received via oral gavage (p.o.) 10mg/kg/day on treatment Days 1 and 2 and 30 mg/kg/day on treatment Days 3-7. Vehicle control animals (n=3 per gender) received deionized water via oral gavage (p.o.). Animals were sacrificed, and livers were removed for histopathological examination and gene expression analysis at 7 days post-study initiation. Histopathological examination showed mild centrilobular hypertrophy in the PB-treated animals from both genders. Likewise, up-regulation in mRNA levels of various DMEs was observed in both genders following treatment. However, there were significant differences in expression perturbations between genders in the effect of PB exposure on cholesterol biosynthesis pathway constituents. Specifically, the expressions of HMG-CoA reductase and squalene monooxygenase, the rate-limiting enzyme genes in cholesterol biosynthesis, were up-regulated in male dogs but not in females. These findings suggest that while both genders respond to PB treatment with increased mRNA levels of various DMEs, gender differences arise at the gene expression level in the effect of PB treatment on cholesterol biosynthesis pathways, implying that male dogs have a greater potential for cholesterol accumulation than female dogs. While it is unknown as to whether these differences between genders in the canine response to PB treatment translates to humans, it does serve to illustrate the importance of addressing potential gender differences in preclinical animal models and to prioritize understanding how such information may be impactful in the clinical setting.

748 IDENTIFICATION OF LIVER GENE EXPRESSION CHANGES ASSOCIATED WITH HIV PROTEASE INHIBITOR-INDUCED HYPERLIPIDEMIA IN SENSITIVE AND RESISTANT STRAINS OF RATS.

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A number of HIV protease inhibitors (PI) have been associated with elevations in serum triglycerides and cholesterol in patients. The standard preclinical animal species, such as Sprague-Dawley (SD) rats, are not sensitive to HIV PI-induced hyperlipidemia, making mechanistic studies and the identification of new PIs not associated with dyslipidemia a challenge. The purpose of this study was to employ a panel of rat strains with controlled genetic diversities in order to identify rat strains that reflect the hyperlipidemia associated with PIs in humans. Ritonavir was administered to Fischer 344 (F344), SD, and 12 PharmGenixTM panel (PG1-PG12) rats for 5 days. Following drug treatment, blood samples were evaluated for changes in serum lipids. Increases in triglyceride and cholesterol levels were observed in the PG2 strain with ritonavir treatment, while the lipid levels in the other strains remained near baseline. Livers from the sensitive rats and the Sprague-Dawley rats were then evaluated for differences in gene expression profiles. CYP7A1, a key enzyme for cholesterol metabolism, was down regulated in the sensitive strain and up regulated in the resistant strain. A number of genes involved in the lipid synthesis and metabolism were also regulated differently between the two rat strains. Collectively, these results suggest novel potential mechanisms for this clinically relevant HIV PI-induced hyperlipidemia.

749 EFFECTS OF AFLATOXIN ON HEPATIC GENE EXPRESSION IN A POULTRY MODEL.

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Aflatoxin is a toxic secondary metabolite produced by the fungi *Aspergillus flavus* and *A. parasiticus*. Ingredients of livestock and poultry feed are often contaminated with aflatoxin. Aflatoxin affects many species including humans, dogs, cats, pigs,

cattle, and poultry, with liver being the major organ affected. A chicken model was used to evaluate the effect of aflatoxin on hepatic gene expression. Seventy five day-old male broiler chicks were assigned to three dietary treatments (5 replicates of 5 chicks each) from hatch to day 21. The diets contained 0, 1 and 2 mg/kg aflatoxin/kg of feed. Feed intake, body weight gain, liver weights and serum chemistry were evaluated at the end of the study and liver samples were collected in RNase free tubes and stored at -80°C. Aflatoxin reduced ($P \leq 0.05$) feed intake, body weight, serum total proteins, serum calcium and phosphorus but increased ($P < 0.01$) liver weights in a dose dependent manner. Microarray experiments were conducted using chicken long oligo arrays, to identify the changes in hepatic gene expression in chicks fed 0 (control) and 2mg/kg aflatoxin/kg of feed. A loop design was followed for microarray experiments with four technical and four biological replicates per treatment group. RNA was extracted from liver tissue and its quality was determined using gel electrophoresis. High quality RNA was purified from DNA contamination, reverse transcribed to cDNA and was used for microarray hybridizations. Microarray data will be analyzed using a 2-step ANOVA model using GenePix and MAANOVA softwares, and the differentially expressed genes will be identified using SAM, TIGR, and Cluster softwares. The microarray data will be validated using real time PCR. It is hypothesized that genes associated with antioxidant, detoxification and immune systems will be affected along with many unidentified pathways in birds fed aflatoxin compared to controls.

750 TRANSCRIPTOME CHANGES IN HEPG2 CELLS EXPOSED TO COPPER: GO ANALYSIS AND HIERARCHICAL CLUSTERING.

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Copper is an essential trace element that serves as an important catalytic cofactor for cuproenzymes. It has been shown that copper has the potential to generate free radicals and oxidize cellular components through its redox activity. We have previously shown that copper activates transcription through both metal- and oxidative stress-responsive signal transduction pathways. Our hypothesis is that copper modulates the activity of multiple intracellular signal transduction pathways to affect transcription, which ultimately disrupt normal development. HepG2 cell transcriptomes were analyzed using Agilent human microarrays, and differentially expressed genes identified for cells exposed to copper (100, 200, 400 and 600 μ M) for 4, 8, 12 and 24-h. We mapped differentially up-regulated genes onto Gene Ontology categories using GenMAPP, and then performed hierarchical clustering (average linkage) with Cluster. Hierarchical clustering shows that as toxicity increases, the number of significantly enriched ($p \leq 0.01$) GO categories increase and that there is a change in the types of categories across the 16 conditions. Metal ion binding and homeostasis, TGF- β receptor signaling, anti-apoptosis, IL 1 receptor binding and glutathione synthesis were significantly enriched all across 16 conditions with higher z values at lower concentrations (100 and 200 μ M). Regulation of transcription, insulin-like growth factor binding, LDL receptor binding and immune cell activation were enriched only at higher concentrations (400 and 600 μ M) for all exposure time points. Morphogenesis and immune response are GO categories enriched at most conditions except at lower concentrations after 8 h exposure. Significantly enriched GO categories for down-regulated genes were cellular lipid metabolism, mRNA processing, regulation of DNA replication, phosphoinositide-mediated signaling, oligosaccharide metabolism and DNA packaging. We will perform investigation of potential signal transduction pathways based on mapped biological and molecular pathways.

751 LABORATORY DIET ALTERS GENE EXPRESSION IN MICE.

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Nutritional studies in laboratory animals have long shown that various dietary components can contribute to health consequences, but the effects of diet alone have not been carefully considered in toxicogenomic whole animal studies. In this study, C57BL/6J mice (n=5 per group) were raised for five weeks on either a standard lab chow diet LRD-5001 or a purified, low-arsenic AIN-76A diet as part of a larger study examining the effects of arsenic (As) in diet or drinking water on gene expression. Affymetrix Mouse Whole Genome 430 2.0 microarrays were used to assess gene expression changes in the liver. Using a robust analysis and a two-fold change cutoff, 360 genes were found to be differentially expressed. Many of these genes are involved in Phase I and Phase II metabolism, cholesterol metabolism, immune response, electron transport, ubiquitination, nitric oxide biosynthesis, glutathione metabolism, estrogen signaling, G-protein coupled receptor signaling, and transcriptional regulation. We found, using ICP-MS, that the AIN-76A diet con-

tained 5 ppb total As, while the LRD-5001 diet contained a mixture of inorganic and organic As at a total concentration of 290 ppb. Chronic human exposure to As has been known to cause a variety of health problems, and many of the genes found to be differentially expressed in this study are known to have roles in As metabolism and toxicity. Thus, the substantial gene expression differences between these diets may confound or contribute to investigations of effects of As or other toxicants. Previous published studies on As toxicogenomics in mice have used standard lab chows, containing fish meal (a probable source of As) as the primary protein source and have not considered the amount of As naturally found in the chow. These results indicate that diet must be carefully controlled in whole animal toxicogenomic studies. (NIH-NIEHS P42 ES07373)

752 GENE EXPRESSION CHANGES IN RAT PANCREATIC ACINAR CELLS ISOLATED FROM RATS ADMINISTERED WYETH 14, 643 OR AMMONIUM PERFLUOROOCTANOATE.

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The nongenotoxin ammonium perfluorooctanoate (APFO, 300ppm in the diet) has been shown previously to induce pancreatic acinar cell tumours in rats. The aim of this study was to assess whether procarcinogenic gene expression changes that were identified previously in whole pancreas tissue in response to APFO and Wyeth 14,643 (Wy) treatments were taking place in acinar 'target' cells. Male Sprague Dawley rats (6-7 weeks old) were fed either the potent pancreatic carcinogen Wy in the diet at 50ppm (a carcinogenic dose) or APFO at 300 ppm for up to 4 weeks. Pancreatic acinar cells were isolated and purified using collagenase digestion and centrifugation. Transcription profiling analysis was performed on acinar cells after 1, 7 and 28 days of dosing using a rat whole genome 60mer oligonucleotide array containing about 44,000 genes. 'Signature' gene lists of significantly ($p < 0.01$) regulated genes for each treatment were compared using LuminatorR software. Time course analysis of the previously identified carcinogenesis-associated genes Early Growth Response 1 (EGR1), Growth Arrest and DNA Damage 45 alpha (GADD45alpha), Thymine Glycosylase and TPA-associated Sequence II (TISII) indicated that the gene expression changes observed in whole pancreas did not occur in purified acinar cells. By contrast, the metabolic genes Phosphoenolpyruvate Carboxykinase (PEPCK) and Dual Specificity Phosphatase 6 (DUSP6), both markedly regulated by Wy and APFO in whole pancreas, were changed also in the acinar cells by both treatments in a similar fashion across time. These results suggest that either procarcinogenic gene expression changes that were observed with these treatments in whole pancreas were selectively negated by the work-up procedure for purification of the acinar cells, or that the initial procarcinogenic changes are taking place in a different (non-acinar) cell population. This work was funded by Plastics Europe

753 COMPARISON OF ENDOTHELIAL CELL PROLIFERATION IN LIVER AND ADIPOSE TISSUE IN B6C3F1 MICE, F344 RATS, AND HUMANS.

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A new class of drugs called peroxisome proliferator-activated receptor gamma (PPAR- γ) agonists has been developed for use in the treatment of diabetes. Vascular tumors were increased in animals treated with some of these drugs. In a 2-year bioassay, troglitazone, a member of this class of drugs, caused an increased incidence of hemangiosarcomas in male and female mice but not in rats. Rodents treated with these drugs also had increased interscapular brown fat with an increased rate of endothelial cell proliferation. Hemangiosarcomas, arising from the endothelial cells are rare in rats and humans with a generally poor prognosis. However, the B6C3F1 mouse has a high rate of spontaneous hemangiosarcoma formation in all tissues. The mechanism of hemangiosarcoma induction is not well understood. The objective of this study was to determine the endothelial cell proliferation rate in normal liver and adipose tissue of B6C3F1 mice, F344 rats, and humans to aid in the investigation of the genesis and development of hemangiosarcomas, and to help determine the relevance of the increased vascular proliferation rate of endothelial cells in drug-treated rodents in assessing the risk of these drugs in humans. We determined the proliferative index (PI) in untreated mice, rats, and humans, of endothelial cells in normal liver, brown fat (rats and mice only) and white fat by dual immunohistochemical staining for CD-31 (endothelial cell marker) and Ki-67 (proliferation marker). The PI was highest in mice and lowest in humans. The difference in the PI was statistically significant when the mouse was compared to the human and rat (except for female rat white fat) and when the rat was compared to the human. The male PI was significantly increased when compared to the female PI in the liver tissue in mice and rats and in the white fat tissue in mice. The

increased rate of spontaneous or PPAR- γ agonist-induced hemangiosarcoma formation may be related to the increased normal proliferation rate of endothelial cells in this strain of mouse compared to rats and humans.

754 MODULATION OF AHR-DEPENDENT SIGNALING BY PPARB/D.

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Peroxisome proliferator-activated receptors (PPARs) are ligand activated transcription factors, and each PPAR isoform (α , β (also referred to as δ), and γ) regulates specific target genes involved in a multitude of biological processes. Previous evidence has shown PPAR β can attenuate skin tumorigenesis, and this could be due to differences in the metabolism of the carcinogen. While treating primary keratinocytes with 7,12-dimethylbenz[a]anthracene (DMBA) induces expression of cytochrome P450 1A1 (CYP1A1) or CYP1B1 mRNA or protein in wild-type cells; surprisingly, this effect is not found in similarly treated PPAR β -null cells. This expression profile was confirmed upon treatment with other polycyclic aromatic hydrocarbons (PAHs), and this effect does not appear to be due to differences in the relative level of the AhR, which is known to mediate changes in CYP expression. Interestingly, PAHs also cause an increase in the expression of mRNA encoding heme oxygenase I in wild-type keratinocytes, and this effect does not occur in similarly treated PPAR β -null keratinocytes. This demonstrates that the relative ability of PAH to induce CYPs in the presence (wild-type) or absence (null) of PPAR β also correlates well with the induction of heme oxygenase; a phase II enzyme that is not known to be regulated by the aryl hydrocarbon receptor (AhR). The accumulation or ineffective clearance of chemical carcinogens can also lead to altered formation of protein or DNA adducts. Consistent with this idea, preliminary data suggest that DNA adduct formation is significantly lower in PPAR β -null primary keratinocytes treated with benzo[a]pyrene (B[a]P). These findings suggest a novel mechanism where PPAR β modulates AhR-dependent signaling and alters the carcinogenic effect induced by PAH, likely through modifying the balance between bioactivation and clearance of PAH. (Supported by CA89607, CA97999, CA124533 [J.M.P], ES011834 [G.H.P], and the NSF GRFP)

755 EPIGENETIC EFFECTS OF THE PEROXISOME PROLIFERATOR WY-14, 643 IN MOUSE LIVER.

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Peroxisome proliferators are potent rodent liver non-genotoxic carcinogens. The mode of action of these agents in rodents includes increased cell proliferation, decreased apoptosis, secondary oxidative stress and other events; however, it is not well understood how peroxisome proliferators trigger the plethora of the molecular signals leading to cancer. Epigenetic changes have been implicated in the mechanism of liver carcinogenesis by a number of environmental agents. Short-term treatment with peroxisome proliferators induced hypomethylation of DNA and proto-oncogenes in mouse liver, events that were suggested to correlate with a burst of cell proliferation. In the current study, we investigated whether or not long-term exposure to peroxisome proliferators is accompanied by a sustained epigenetic change. Male SV129 mice were fed a control, or WY-14,643-containing (1000 ppm) diet for 1 wk, 5 wks or 5 mo. Treatment with WY-14,643 lead to progressive global hypomethylation of liver DNA as determined by an HpaII-based cytosine extension assay with the maximum effect reaching ~200% at 5 mo. Likewise, trimethylation of histone H4 lysine 20 and H3 lysine 9 was significantly decreased at all time points. Since the majority of cytosine methylation in mammals resides in repetitive DNA sequences, we measured the effect of WY-14,643 on the methylation status of major and minor satellites, as well as in IAP, LINE L1 and LINE L2 elements. Exposure to WY-14,643 resulted in gradual loss of cytosine methylation in major and minor satellites, IAP, LINE L1 and LINE L2 elements. Interestingly, the majority of the epigenetic changes were not correlated with the temporal effects of WY-14,643 on cell proliferation, especially at the 5 mo time point, and no effect on c-myc promoter methylation was observed. These data indicate the importance of epigenetic alterations in the mechanism of action of peroxisome proliferators.

756 PPARALPHA-DEPENDENT GENE EXPRESSION CHANGES IN THE MOUSE LIVER AFTER EXPOSURE TO PEROXISOME PROLIFERATORS.

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Peroxisome proliferators (PP) are a large class of structurally diverse chemicals that mediate their effects in the liver mainly through the PP-activated receptor α (PPAR α). Development of PP induced hepatocarcinogenesis in mouse liver is

known to be dependent on PPAR α but downstream events related to the alteration in cell proliferation and apoptosis are not well defined and are needed for mode of action analysis. Using BrdU labeling of replicating hepatocytes, we first characterized cell proliferation effects in wild-type and PPAR α -null mice at 12, 24, 48, and 96 hrs after initial exposure. Cell proliferation peaked at 48 hrs in wild-type mice. PPAR α -null mice did not exhibit changes at any of the time points measured. Gene expression changes after the 12 hr treatment were assessed using Affymetrix U74Av2 gene chips. Significance Analysis of Microarray data showed that ~500 genes were significantly different between the treated and untreated groups at a 0.05 False Discovery Rate and almost all of the genes were dependent on PPAR α for regulation. To further understand the biological significance of these alterations, we compared the gene expression profiles to those from historical experiments assembled in a PP Compendium containing Affymetrix gene chip data from rats and mice exposed to different PP. Sets of genes commonly regulated by PP were identified at different time points and after exposure to different PP. These genes fell into a number of categories known to be regulated by PPAR α strengthening the argument that many PP cause effects in the liver through PPAR α . This abstract does not necessarily reflect EPA policy.

757 IDENTIFICATION OF COMMON GENES AND PATHWAYS REGULATED BY PPARALPHA ACTIVATORS.

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Exposure to peroxisome proliferators (PPC) leads to alterations in hepatocyte growth and apoptosis, increases in liver to body weights (LW/BW) and liver tumors. There is strong evidence that PPC cause many of these effects through the nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR α). The molecular events that occur after PPAR α activation and alteration in liver growth are not well characterized. To help identify common genes and pathways that may be mechanistically linked to PPC-induced carcinogenesis, we have compared the transcript profiles of the livers of wild-type or PPAR α -null mice exposed to a number of PPC using Affymetrix chip data. A number of PPC which all induce liver cancer were compared in this study including WY-14,643 (WY) and di(2-ethylhexyl) phthalate (DEHP) which require PPAR α for increases in LW/BW and perfluorooctanoic acid (PFOA) which causes increases in LW/BW in both wild-type and PPAR α -null mice. Transcript profiling was performed using either mouse U74Av2 or 430_2 Affymetrix chips. Significantly altered genes were identified using Rosetta Resolver. Under similar exposure conditions (7 consecutive daily gavages) a number of common functional categories of genes were identified including those involved in fatty acid oxidation and transport, peroxisome biogenesis, proteome maintenance, coagulation, complement cascade and oxidative stress as well as those linked to cell growth control. Almost all of these common genes were dependent on PPAR α for changes in transcript levels as the changes were not observed in PPAR α -null mice. Each chemical also altered a unique set of genes. For WY all of these genes were dependent on PPAR α . These results indicate that the model PPC WY, does not alter gene expression in the mouse liver independently of PPAR α , and that all PPC examined alter a smaller unique set of genes. Future work will be directed toward characterizing the functional significance of the changes in the cell growth genes commonly regulated by many PPC. This abstract does not necessarily reflect EPA policy.

758 THE ROLE OF KUPFFER CELLS IN HEPATOCARCINOGENESIS.

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The induction of chemically induced hepatic neoplasia is a multi-step process involving DNA damage and cell proliferation. Recent research has suggested a role for nonparenchymal cells, specifically Kupffer cells, as mediators of cell proliferation by tumor promoters. Kupffer cells, the resident macrophage of the liver, upon activation, secrete bioactive products including cytokines and reactive oxygen species that may participate in hepatocarcinogenesis through inducing oxidative stress and damage, and altering signaling cascades; events that may ultimately lead to hepatocellular proliferation. Our group has examined the role of the Kupffer cell on hepatocellular growth using both in vitro and in vivo models. In vitro, basal levels of DNA synthesis in purified hepatocyte cultures (lacking Kupffer cells) were reduced compared to standard (non-purified) hepatocyte cultures in both the mouse and rat. In addition, the hepatic tumor promoter Wyeth 14,643 failed to induce DNA synthesis in pure hepatocytes. Together, these results suggest that Kupffer cells act as mediators of DNA synthesis in hepatocytes. Activation of p38 MAP kinase has been shown to participate in cellular responses to external stress signals, and invokes signaling cascades that lead to altered cell growth. Wyeth 14,643 induced phosphorylation of p38 in standard hepatocyte cultures, but not in pure he-

patocyte cultures. In vivo, male B6C3F1 mice treated with LPS (0.25mg/kg; 1 or 2x/week) increased the number of Kupffer cells (~150 – 170%) and increased DNA synthesis in hepatocytes (~147%) over control levels. Conversely, Kupffer cell depletion via liposomal delivery of clodronate reduced the number of Kupffer cells (~80%) and both basal and LPS-induced DNA synthesis in the liver (57% and ~67% decreases, respectively). These data demonstrate that Kupffer cell activation stimulates hepatocellular growth while depletion of Kupffer cells inhibits hepatocellular growth, results that provide further support for the involvement of Kupffer cell in hepatocarcinogenesis.

759 DIET ACIDIFICATION HAS NO EFFECT ON PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR (PPAR) OR EPIDERMAL GROWTH FACTOR (EGF) SIGNALING IN RAT URINARY BLADDER UROTHELIUM.

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We previously reported prevention of urolithiasis and associated rat urinary bladder tumors by urine acidification (via diet acidification) in male rats treated with the dual PPAR α/γ agonist muraglitazar. Because the potential for urine acidification to alter urothelial PPAR or EGF signaling was not examined, we evaluated urothelial PPAR expression and signaling, cell proliferation, and/or epidermal growth factor receptor (EGFR) expression and signaling in rats fed either a normal or an acidified diet for 5, 18, or 33 days. A subset of rats in the 18-day study also received 63 mg/kg of the PPAR γ agonist pioglitazone daily for the final 3 days to directly assess the effects of diet acidification on responsiveness to PPAR γ agonism. Urothelial PPAR expression and signaling were evaluated in the 18- and 33-day studies by immunohistochemical assessment of PPAR protein levels (33-day study only) and quantitative real-time polymerase chain reaction (qRT-PCR) measurement of PPAR-regulated gene expression. In the 5 day study, EGFR expression and signaling were evaluated by immunohistochemical staining of total and phosphorylated EGFR protein and qRT-PCR measurement of egfr and akt2 mRNA levels. Diet acidification did not alter urothelial PPAR α or γ receptor levels, expression of PPAR α - or γ -regulated genes, EGFR protein levels or phosphorylation, egfr or akt2 expression, or urothelial cell proliferation. Moreover, diet acidification had no effect on pioglitazone-induced changes in urothelial PPAR γ -regulated gene expression. From this work, we conclude that urine acidification does not prevent PPAR γ -induced bladder tumors by altering PPAR or EGFR expression or signaling in rat bladder urothelium.

760 UROTHELIAL CARCINOGENESIS IN THE URINARY BLADDER OF MALE RATS TREATED WITH MURAGLITAZAR, A PPAR α/γ AGONIST: EVIDENCE FOR UROLITHIASIS AS THE INCITING EVENT IN THE MODE OF ACTION.

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Muraglitazar, a PPAR α/γ agonist, dose dependently increased urinary bladder tumors in male HSD rats administered 5, 30, or 50 mg/kg/day for up to 2 years. To determine the mode of tumor development, rats were treated daily for up to 21 months at doses of 0, 1, or 50 mg/kg while being fed either a normal or 1% NH₄Cl-acidified diet. Muraglitazar-associated, time-dependent changes in urine composition, urothelial mitogenesis and apoptosis, and urothelial morphology were assessed. In control and treated rats fed a normal diet, urine pH was generally ≥ 6.5 , which facilitates formation of calcium- and magnesium-containing solids, particularly in the presence of other prolithogenic changes in rat urine. Urinary citrate, an inhibitor of lithogenesis, and soluble calcium concentrations were dose dependently decreased in association with increased calcium phosphate precipitate, crystals and/or microcalculi; magnesium ammonium phosphate crystals and aggregates; and calcium oxalate-containing thin, rod-like crystals. Morphologically, sustained urothelial cytotoxicity and proliferation with a ventral bladder predilection were noted in treated rats by month 1 and urinary carcinomas with a similar distri-

bution occurred by month 9. Urothelial apoptotic rates were unaffected by muraglitazar treatment or diet. In muraglitazar-treated rats fed an acidified diet, urine pH was invariably <6.5, which inhibited formation of calcium- and magnesium-containing solids. Moreover, dietary acidification prevented the urothelial cytotoxic, proliferative, and tumorigenic responses. Collectively, these data support an indirect pharmacologic mode of urinary bladder tumor development involving alterations in urine composition that predispose to urolithiasis and associated decreases in urine soluble calcium concentrations.

761 THE EFFECTS OF CIGARETTE SMOKE INHALATION ON THE BLADDER UROTHELIUM OF FEMALE C57BL/6 MICE.

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Cigarette smoking is the major environmental risk factor for bladder cancer in humans. The exact mechanism is unknown but the potent bladder carcinogens, aromatic amines, are present in cigarette smoke and are believed to be involved. Increased cell proliferation, caused by a direct mitogenic effect or in response to cytotoxicity, is a well-accepted mode of action for tumor formation, and may play a role in cigarette smoke-induced bladder cancer. Hyperplasia of the bladder epithelium has been found in human cigarette smokers. We examined the effects of inhalation exposure to smoke in a whole body exposure (Teague) system, 2 hours/day, 5 days/week, on the urothelium of female C57BL/6 mice. In study 1, the effects of smoking were determined at 1 week and 1 and 3 months by light microscopy and by Ki-67 labeling index (LI). Reversibility was examined by treating for 1 week or 1 month followed by 3 weeks or 2 months, respectively, of no treatment. In study 2, the effects of treatment were determined at 1 and 5 days by light microscopy, and bromodeoxyuridine (BrdU) LI. Reversibility was examined by treating for 5 days followed by no treatment for 2 days. In both studies, there was no increase in urothelial hyperplasia by light microscopy. However, in study 1, the Ki-67 LI of the urothelium was significantly increased in the smoking group compared to the control group at all time points. The Ki-67 LI in the reversibility group was similar to control at 1 month and significantly lower than the control at 3 months. In study 2, the BrdU LI in the smoking group was similar to control on day 1 but was significantly increased compared to the control at 5 days. The LI in the reversibility group was increased compared to control but the increase was not statistically significant. Exposure of female C57BL/6 mice to cigarette smoke by inhalation induced an increase in urothelial cell proliferation which was reversible upon cessation of exposure.

762 EFFECT OF TREATMENT WITH SODIUM ARSENATE (ASV) OR SODIUM ARSENITE (ASIII) IN THE DRINKING WATER ON THE BLADDER UROTHELIUM OF MALE AND FEMALE F344 RATS AND C57BL/6 MICE.

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Based on epidemiological data, inorganic arsenic (arsenate and arsenite) in the drinking water is carcinogenic to the urinary bladder in humans. The lack of a good animal model has made studies of the mechanism of inorganic arsenic carcinogenesis in humans difficult. Previous long-term studies in rats and mice with inorganic arsenic were negative although these studies were done prior to the development of today's standards for 2-year bioassays. We evaluated the effect of treatment with 100 ug/g AsV or AsIII in the drinking water for 2 weeks on the urothelium of male and female rats and mice using light microscopy. There was a significant decrease in body weight gain in AsV-treated rats and the female mice and in AsIII-treated rats and mice. This may indicate that the arsenic doses used were toxic, or the decreased body weight gain may be related to an aversion to drinking the arsenic-containing water. By light microscopy, there was a significant increase in the incidence of hyperplasia in the urothelium of AsV-treated rats, with the female more sensitive than the male. There were no changes in the urothelium of AsV-treated mice. In the AsIII-treated animals, there was a significant increase in urothelial hyperplasia in the treated rat and mouse groups, with the females more sensitive than the males of each species. The urothelial changes in the AsV-treated rats confirm a previous report from our laboratory of increased urothelial hyperplasia in AsV-treated female rats and indicate that the rat may be useful in studies evaluating the mode of action of arsenic-induced urinary bladder cancer in humans. However, significant differences in the metabolism of arsenic in the rat compared to humans should be taken into account. Based on the changes in the bladder urothelium of AsIII-treated mice

and the similarity in arsenic metabolism in mice compared to humans, the mouse may provide a more appropriate animal model for mechanistic studies of AsIII-induced urinary bladder cancer.

763 INDUCTION OF URINARY BLADDER PATHOLOGY IN MALE AND FEMALE C3H MICE EXPOSED TO SODIUM ARSENITE FROM GESTATION THROUGH YOUNG ADULTHOOD.

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Epidemiology studies suggest that chronic exposure to inorganic arsenic is associated with cancer of the skin, urinary bladder and lung as well as the kidney and liver. Recently, an in utero animal model was developed to characterize the carcinogenic properties of inorganic arsenic (Waalkes et al., 2003). A brief exposure to sodium arsenite in utero (gestation day 8 to 18, GD8-18) increased liver, lung, adrenal, ovarian and uterine tumors in C3H and/or CD-1 mice. In the study described here, C3H mice were exposed to 85 ppm inorganic arsenic in their drinking water in utero (GD8-18) or a similar brief exposure post-pubescent (postnatal day 57 to 67, PD57-67) or a prolonged continuous exposure through both stages of development (GD8-PD67). At PD67, groups (n=5) of male and female mice from control and all treatment groups were examined pathologically. A treatment-related increase in eosinophilic granules of the urinary bladder transitional epithelium was observed in all male and female mice of the continuous (GD8-PD67) and post-pubescent groups (PD57-67). These eosinophilic granules are representative of degradative products in response to injury. The severity of bladder lesions was greatest in the GD8-PD67 group followed by the PD57-67 group. One female mouse in the in utero only exposure group also demonstrated minimal eosinophilic granule accumulation in the bladder epithelium. Uterine hyperplasia was observed in two female mice in the GD8-PD67 group and one female mouse in the PD57-67 treatment group. These results demonstrate that a brief exposure, in utero or post-pubescent, to sodium arsenite is sufficient in inducing aberrant changes in urinary bladder and/or uterine pathology in mice and that prolonged exposure from gestation through young adulthood increases the severity/incidence of these lesions. This abstract does not necessarily reflect EPA policy.

764 ARL HYDROCARBON RECEPTOR (AHR): DUAL FUNCTIONS, ONE FOR TUMOR PROMOTION THROUGH DRUG METABOLISM AND THE OTHER FOR TUMOR SUPPRESSION.

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Ahr plays a role of tumor promoting function via xenobiotic activation of cytochrome p4501A1. However, accumulated current data contrarily suggest its tumor suppressive function, such as an activation of p27, association with Rb gene for cell-cycle arrest, etc., although direct evidence has never yet been reported by lifetime observation studying the tumorigenicity. Aim of the present study is to establish a CYP1A1-independent, Ahr-mediated tumor suppressive function using Ahr knockout (KO) mice. **Materials and methods:** The generation of homozygous Ahr KO (Ahr^{-/-}) mice, 129/SvJ, is described elsewhere (Mimura, 1997). Twenty-four mice of Ahr^{+/+}, 23 of Ahr^{+/-}, and 18 of Ahr^{-/-} male mice used in this study were monitored their spontaneous tumor development throughout the lifetime. Survival curves were applied to Gompertzian law of mortality to examine life spans and mortality rates. Cell kinetic study of hemopoietic stem-cell compartment was also conducted. **Results and Discussions:** The mean life span of the Ahr^{-/-} group was 756 days, which was significantly shorter than those of Ahr^{+/+} (855 days) and wild type mice (890 days) (Kaplan-Meier/logrank test: P<0.002 and p<0.0001, respectively). The shortened life span in Ahr^{-/-} mice was based on an early onset of hemopoietic neoplasms, and an early and high incidence of hepatoma development in the Ahr^{-/-} group. The histological spectrum of hepatomas observed among the groups was essentially identical. Because of the early onsets of lymphomas and hepatomas observed in Ahr^{-/-} mice, and the faster cell cycle in the hemopoietic progenitor cells, the biological function of Ahr is implied to be a tumor suppression, and the function of aryl hydrocarbon receptor in the hemopoietic progenitor cell is to keep the stem cell compartment be quiescent and stabilized from risky stem-cell cycling and perturbation.

765 FORMATION OF REACTIVE OXYGEN SPECIES (ROS) IN TCDD- AND ESTRADIOL-TREATED LIVER CELLS.

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was classified as a human carcinogen. TCDD acts as a liver carcinogen in female but not in male Sprague-Dawley rats. Its effects are mediated by the aryl hydrocarbon receptor (AhR). The activated AhR enhances the transcription of a wide spectrum of genes including cytochromes P450 (CYP) 1A1, 1A2, and 1B1. There is good evidence for a role of estrogens in the mechanism of TCDD-induced hepatocarcinogenesis in the rat showing estrogen-dependent formation of 8-oxo-2'-deoxyguanosine (8-oxo-dG) in female rat liver DNA. It was postulated that estrogens can act as genotoxic procarcinogens. In particular, the catechol estradiol metabolite 4-hydroxyestradiol, formed by CYPs, can generate DNA damaging ROS via redox cycling and the corresponding quinone may cause DNA damage.

We examined the formation of ROS using the 2',7'-dihydrodichlorodifluorescein diacetate (H2DCFDA) fluorescence assay after incubation of hepatoma cells or rat primary hepatocytes with TCDD and/or estradiol (E2). The amount of 8-oxo-dG in DNA of hepatoma cells was measured by HPLC-MS/MS. In addition, we analysed the amount of 8-oxo-dG in hepatic DNA from mice deficient in 7,8-dihydro-8-oxoguanine-DNA glycosylase (Ogg1 protein) and Cockayne syn-drome B (Csb) using a modified alkaline elution technique.

Induction of CYPs by TCDD was able to increase ROS formation in rodent but not in human HepG2 hepatoma cells. Co-incubation of TCDD with 100 nM E2 did not markedly enhance TCDD-induced ROS formation. 8-oxo-dG levels in the DNA of H4IIE cells, HepG2 cells and rat hepatocytes after TCDD treatment were in good agreement with ROS formation and induction of CYP1A activity. However, in vivo treatment with TCDD (96 h) had no effect on steady-state levels of fpg-sensitive base modifications neither in wild-type nor in mice deficient in the repair of 8-oxo-dG.

766 ARYL HYDROCARBON RECEPTOR (AHR) MODULATES VANADATE INDUCTION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN CULTURED TRAMP MOUSE PROSTATE.

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AhR, hypoxia inducible factor-1 α (HIF-1 α) and aryl hydrocarbon receptor nuclear translocator (ARNT also named HIF-1 β) are PAS family transcription factors. AhR and HIF-1 α dimerize with ARNT to alter gene expression. The impetus for the present study was the finding that AhR null (*Ahr*^{-/-}) transgenic adenocarcinoma of the mouse prostate (TRAMP) mice develop prostate tumors with greater frequency than AhR wild-type (*Ahr*^{+/+}) TRAMP mice. This suggests that *Ahr* inhibits prostate carcinogenesis. Induction of prostate epithelial hyperplasia is not altered by *Ahr* genotype, but macroscopic tumor development is inhibited in AhR wild-type (*Ahr*^{+/+}) TRAMP mice. In TRAMP mice, prostatic hypoxia stabilizes HIF-1 α which induces expression of VEGF facilitating blood vessel migration into prostatic ducts, a prerequisite for tumor growth. Since ARNT is a common dimerization partner of AhR and HIF-1 α , we tested the hypothesis that AhR in wild-type (*Ahr*^{+/+}) TRAMP mice inhibits prostate tumor formation by competing with HIF-1 α for ARNT, thereby inhibiting VEGF production. At 140 days of age, prostates from *Ahr*^{+/+}, *Ahr*^{-/-} and *Ahr*^{-/-} C57BL/6J TRAMP mice were minced and incubated in culture media containing graded concentrations of vanadate, an inducer of phosphatidylinositol 3-kinase and the HIF-1 α /ARNT pathway and subsequent VEGF expression. Vanadate induced VEGF protein in a dose-dependent fashion in *Ahr*^{+/+} and *Ahr*^{-/-} TRAMP prostate cultures, but not in *Ahr*^{+/+} cultures. Western blot analysis demonstrated that vanadate induced upstream proteins in the signaling cascade to a similar extent in TRAMP prostates of each *Ahr* genotype, evidenced by Akt phosphorylation. These findings suggest that inhibition of prostate carcinogenesis in *Ahr*^{+/+} TRAMP mice may result from increased AhR-ARNT dimerization reducing the amount of ARNT available to interact with HIF-1 α , thereby reducing VEGF production and tumor growth. (Supported by NIH grants CA095751, ES01332 and ES12352).

767 ASSESSMENT OF 4-CHLOROBIPHENYL *IN VIVO* USING THE BIG BLUE RAT MODEL: MUTAGENICITY, GENE EXPRESSION, AND HISTOLOGY.

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Toxicological studies of polychlorinated biphenyls (PCB) have traditionally focused on receptor-mediated effects. However, the toxicity of lower-chlorinated PCB congeners via metabolic pathways should not be ignored. PCB3 (4-chlorobiphenyl) is

detected in urban air when careful analysis is undertaken to measure this semi-volatile congener. We hypothesize that PCB3 causes DNA mutations directly via metabolic transformation to an electrophilic species, or indirectly by increasing cellular oxidative stress. To investigate the *in vivo* mutagenicity of PCB3, male and female Fischer 344 BigBlue® rats, transgenic for the lacI gene, were injected intraperitoneally with PCB3 (600 μ mol/kg), 4-hydroxy-PCB3 (4-HO-PCB3, 400 μ mol/kg), 3-methylcholanthrene (3-MC, 300 μ mol/kg, positive control), or corn oil (negative control) once per week, for 4 weeks. Animals were killed 17 days after the last injection and the mutant frequency (MF) of the liver lacI gene was determined using E. coli plated in agar containing X-Gal. In male rats 3-MC induced a 4-fold increase of the MF of the lacI gene in the liver. The MF in male PCB3-treated animals was also significantly elevated. In contrast, 4-HO-PCB3 induced a non-significant doubling of the MF. Examination of the livers showed no morphological or histological toxicity in PCB-treated animals, excluding general toxicity as cause for the increased MF with PCB3. Microarray analysis of gene expression in the livers revealed changes in 3-MC, but not in PCB-treated animals. LacI mutations were sequenced and examined to determine the type of mutational event. Transversions and frameshift mutations dominated in 3-MC and PCB-mutants compared to controls. In female rats 3-MC induced just over a 2-fold increase in MF and PCB3-treatment resulted in only a slight increase in MF. This study demonstrates for the first time the mutagenicity of a PCB *in vivo*. (Supported by ES013661, DAMD17-02-1-0241, R-82902102-0 and CHEEC)

768 ALTERATION OF GROWTH REGULATION DURING THE PROMOTION OF HEPATOCARCINOGENESIS BY PCBs IN MICE LACKING THE P50 SUBUNIT OF NF- κ B.

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Polychlorinated biphenyls (PCBs) are persistent ubiquitous chemical compounds which bioaccumulate and act as liver tumor promoters in rodents. Previous studies have shown that the promotion of hepatocarcinogenesis by PCB-153 was diminished when NF- κ B activation is impaired by the absence of p50. The present study examined the effect on growth regulation in mice that lack the NF- κ B p50 subunit. Wild type and p50^{-/-} male mice were injected with DEN (90 mg/kg) and then subsequently injected biweekly with 27 injections with corn oil or 300 μ mol/kg of 3,3',4,4'-tetrachlorobiphenyl (PCB-77) or 20 biweekly injections of 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153). Mice were administered bromodeoxyuridine in the drinking water for 6 days before euthanasia for the measurement of cell proliferation. Controls not receiving DEN were also studied. In DEN-treated mice, cell proliferation in normal hepatocytes was significantly increased in mice receiving PCB-153; this increase was inhibited in p50^{-/-} mice. PCB-77 did not affect cell proliferation in normal hepatocytes. In hepatic tumors, the rate of cell proliferation was much higher than in normal hepatocytes, but was not affected by PCB treatment or p50 deletion. The rate of apoptosis, as measured by the TUNEL assay, was significantly higher in normal hepatocytes from p50^{-/-} mice receiving PCB-77 compared to wild-type mice receiving PCB-77. The apoptotic index was slightly lower in wild-type mice receiving PCB-153 than in control wild-type mice or p50^{-/-} mice administered PCB-153. In hepatic tumors, the rate of apoptosis was lower than in normal hepatocytes, but was not affected by PCB treatment or p50 deletion. Taken together, these data indicate that the absence of the NF- κ B p50 subunit alters the proliferative and apoptotic changes in mouse liver in the response to PCBs. (Supported by ES013661, ES07380, and the Kentucky Agricultural Experiment Station.)

769 MODULATION OF EXPRESSION OF MDM2 AND MATRIX METALLO PROTEINASES (MMPs -9, -10, -12) DURING DIMETHYLNITROSAMINE (DMN)-INDUCED PROGRAMMED AND UNPROGRAMMED LIVER CELL DEATH.

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The rapid multiplication of cells in the early phase of a tumor usually does not cause serious disease as long as the tissue growth remains confined to its original loci. In contrast, malignant cells migrate from their original location, invade surrounding normal tissue areas, and ultimately disseminate throughout the body. Remodeling of the surrounding extracellular matrix (ECM) proteins facilitates this process. It appears that proteolytic enzymes, such as Plasmin and MMPs play an instrumental role in degrading the surrounding ECM, thereby "clearing a path" for tumor cell migration. Although the precise role of high-level expression of distinct MMPs during malignant tumor invasion appears to be more or less settled, *in vivo* modulation of MMPs during hepatocarcinogen-induced programmed and unpro-

grammed cell death remains unknown to date. Therefore, in order to investigate the role of MDM2 and MMPs in the liver, toxic doses of DMN (0, 25, 50 and 100 mg/kg, ip) were administered to male C3H mice for 24 hours. Liver samples were subjected to biochemical analysis. Besides classic cell death parameters (determination of serum ALT activity, oxidative stress, genomic fragmentation, expression of bcl-XL, p53), expression of MDM2, MMPs-9, -10 and -12 (Gelatinase-B, Stromelysin-2, Metalloelastase) were also analyzed by western blot in the liver homogenates. Results show that DMN induced dose-dependent increases in the expression of MDM2 and MMPs, and expression of these genes strongly correlated with the elevated levels of the toxic markers (ALT in U/L: Con-75 and 100 mg DMN-3830; malondialdehyde nmol/g.liv: Con-22 and 100 mg/Kg DMN-102; DNA fragmentation: Con-100% and 100 mg/Kg DMN-375%). In conclusion, this study for the first time, has shown involvement of MDM2 and MMPs during DMN-induced acute liver injury suggesting a possible contributory role for these proteins in the propagation of drug and chemical-induced organ toxicities.

770 GLOBAL GENE-EXPRESSION PROFILING OF TAMOXIFEN-INDUCED RAT HEPATOCARCINOGENESIS.

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Tamoxifen is a non-steroidal anti-estrogen used for the treatment of breast cancer and, more recently, as a chemopreventive agent in healthy women at high risk of developing breast cancer. On the other hand, tamoxifen is a potent hepatocarcinogen in rats, with both tumor initiating and promoting properties. Recently it has been demonstrated that, in addition to the genotoxic components, tamoxifen-induced hepatocarcinogenesis in rat is characterized by substantial epigenetic changes. Considering the crucial role of epigenetics in regulation of gene expression, the present study was undertaken to investigate whether or not epigenetic changes induced by long-term exposure of rats to genotoxic hepatocarcinogen tamoxifen are accompanied by a sustained alteration in gene expression profile. Female F344 rats were fed a control or tamoxifen-containing diet (420 ppm) for 24 weeks. The global gene expression profile was determined in liver tissues after 12 and 24 weeks of exposure to tamoxifen. Differentially expressed genes involved distinct cellular pathways for lipid biosynthesis and metabolism, hormone metabolism, cell signaling, oxidative-stress response genes, cell proliferation and apoptosis. In addition, we identified three different patterns of gene expression associated with temporal changes in progression of the carcinogenic process: 1 - differentially expressed genes specific to 12 week time-point only; 2 - differentially expressed genes specific to 24 week time-point only; 3 - common genes differentially expressed after 12 and 24 weeks of exposure. Importantly, differentially expressed genes include those playing an important role in cancer development, such as *ABCC3*, *BRCA2*, *PPAR α* , *RBP*, *ETS-1*, *PDGFC*, *GDF15*, *pFKB1*, *GAS6*, and *LIFR*. These data indicate the importance of global gene expression profiling for elucidating the molecular mechanisms of tamoxifen-induced changes related to carcinogenesis with the potential to be used as early biomarkers for carcinogenicity.

771 LASOFOXIFENE AND OTHER SERMS INDUCE GENE TRANSCRIPTION PROFILES CONSISTENT WITH ESTROGEN RECEPTOR AGONISM IN THE MALE RAT KIDNEY.

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Lasofloxifene is a selective estrogen receptor modulator (SERM), having receptor affinity comparable to that of the endogenous hormone 17 β -estradiol (E2). In a two-year carcinogenicity study, an increased incidence of renal tumors was observed in lasofloxifene-treated male rats, but not in female rats or mice of either sex. Examination of the metabolism and genotoxicity of lasofloxifene suggests that tumor formation does not result from either direct DNA damage or a sex or species selective difference in exposure. Small numbers of renal tumors have also been detected in male rats following long-term administration of other estrogenic compounds (raloxifene, droloxifene, tamoxifen and chlorotrianisene). In order to test the hypothesis that a hormone-mediated mechanism leads to renal tumors, SERMs were evaluated for estrogen receptor (ER) agonist activity in the male rat kidney using transcriptomics. Renal expression profiles were generated utilizing Affymetrix rat genomic arrays and tissues from male rats dosed daily for 15 days with E2, or E2 plus ICI-182,780 (ICI), an ER antagonist. From this experiment, 190 Estrogen Responsive Fingerprint (ERF) genes were identified and incorporated into a custom Affymetrix FlexArray that also included 1,420 control genes for normalization. A study was undertaken that included E2 and 17 α -ethinylestradiol (EE) as positive controls, haloperidol as a negative control, and the SERMs lasofloxifene, ta-

moxifen and raloxifene. Detailed statistical analysis of the data from the ERF array revealed that lasofloxifene did indeed regulate ERF gene expression, and that the lasofloxifene profile was most similar to that of raloxifene, closely followed by tamoxifen. The results from this experiment demonstrate that SERMs have ER agonism activity in the male rat kidney and therefore that a hormone-mediated mechanism could explain the renal tumors observed in the two-year carcinogenicity study with lasofloxifene.

772 ESTROGENIC STATUS MODULATES DMBA-MEDIATED HEPATIC GENE EXPRESSION AND CARCINOGENICITY: MICROARRAY-BASED ANALYSIS.

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Estrogenic status in women influences the metabolism and toxicity of polycyclic aromatic hydrocarbons (PAH). The objective of this study was to examine the influence of estradiol (E2) on 7,12 dimethylbenz(a)anthracene (DMBA), a PAH and ligand for aryl hydrocarbon receptor, mediated changes on hepatic gene expression. Sprague-Dawley rats were ovariectomized at post natal day 50 and infused with E2 using osmotic pumps for 14d at 5 μ g/kg/day. Changes in hepatic gene expression were examined 24h following gavage with 50mg/kg DMBA. To understand the mechanism of DMBA-mediated hepato-carcinogenicity in the presence of E2, microarray analysis (Affymetrix Rat 230 2.0 GeneChip) was performed. 225 genes were down-regulated while 54 genes were up-regulated significantly (± 2 fold, $P < 0.05$) by DMBA treatment. Expression of 520 genes was altered by the combination of E2 and DMBA. Hierarchical clustering revealed that expression of 24 genes (including *egf*, *gh receptor*, *leptin receptor*, *igfbp1*) was reversed by the co-treatment of E2 and DMBA compared to DMBA alone. Gene ontology associated regulation of 303 genes involved, for example, in transcriptional regulation (including *ahr*, *hepatic nuclear factor4*, *sp1*, *igfbp3*), protein modification, xenobiotic and stress response, uniquely with the interaction of E2 and DMBA, but not by the either treatment alone. Results from *real-time* RT-PCR confirmed a significantly ($P < 0.05$) higher hepatic *cyp1a1* and *cyp1b1*, pro-carcinogen metabolizing enzymes, mRNA expression in the groups co-treated with E2 and DMBA as compared to DMBA alone, suggesting increased DMBA-mediated mutagen activation, and hence carcinogenesis, in the presence of E2. These data expand our understanding of the synergistic, additive and interactive effects of E2 on PAHs-mediated carcinogenicity. Supported by USDA #6251-51000-005-02S.

773 IDENTIFICATION OF GENES THAT MAY PLAY A KEY ROLE IN PHENOBARBITAL (PB)-INDUCED MOUSE LIVER TUMORIGENESIS DUE TO ALTERED DNA METHYLATION.

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The epigenetic DNA modification 5-methylcytosine regulates tissue-specific gene expression. Abnormal patterns of DNA methylation facilitate altered gene expression underlying tumorigenesis. PB, a nongenotoxic rodent hepatocarcinogen, alters DNA methylation during tumor promotion. Our laboratory devised a method, involving methylation-sensitive restriction digestion, arbitrarily-primed PCR, and capillary electrophoretic separation of the PCR products, that detects treatment-related regions of altered DNA methylation (RAMs), i.e., hyper-, hypo-, and "new" methylations in multiple portions of the genome, simultaneously. Male B6C3F1 mice, sensitive to PB-induced liver tumorigenesis, and the relatively resistant C57BL/6 strain were treated with PB, 0.05% (w/w) in drinking water for 2 or 4 wk prior to analysis of methylation status. RAMs that were unique to the B6C3F1, especially those that carried forward from 2 to 4 wk, are deemed possible key RAMs involved in tumorigenesis (Bachman et al., 2006). The goal of the current study is to annotate the key RAMs. PCR products representing key RAMs were cloned, sequenced, and annotated via the UCSC BLAT sequence alignment tool. A third of the 138 unique PB-induced RAMs have been annotated. Genes that may play key roles during mouse liver tumorigenesis include: Ephrin-B2 (EfnB2) encodes a ligand that activates a signaling pathway which plays an essential role in angiogenesis and vessel maturation and is hypomethylated at 2 wk; tyrosine kinase non-receptor 2 (Tnk2) whose overexpression in human cancer cells causes an increase in cell motility, invasiveness, and metastasis, is hypomethylated at 4 wk; and protein tyrosine phosphatase, receptor type O (Ptpro), a potential tumor suppressor gene whose overexpression delayed re-entry of human cells into the cell cycle and increased susceptibility of the cells to apoptosis, is hypermethylated at 4 wk. In this fashion we are striving to discern novel genes that may play a role in tumorigenesis due to altered methylation.

774 EFFECT OF DIET AND *HELICOBACTER HEPATICUS* INFECTION ON THE DETECTION OF LIVER CARCINOGENICITY IN B6C3F₁ MICE FOLLOWING TWO-YEAR DERMAL EXPOSURE TO TRIETHANOLAMINE.

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Nomination for NTP characterization of the carcinogenicity of triethanolamine (TEA) was based on the likelihood of human exposure and the potential for metabolism to the carcinogen, *N*-nitrosodiethanolamine. An initial two-year study in mice was repeated due to the detection of *Helicobacter hepaticus* (*H. hepaticus*), which was associated with hepatitis in males. Male (0, 200, 630, or 2000 mg/kg) and female (0, 100, 300, or 1000 mg/kg) B6C3F₁ mice were topically administered TEA in both studies, but the diets differed between the first (NIH-07 diet) and second (NTP-2000 diet) studies. A PCR-based method was used to evaluate tissues for the presence of *H. hepaticus*. In the initial study, significant increases in hepatocellular adenomas (HAD) and multiple hepatocellular adenomas (MHAD) were found at the high dose in both sexes, and in hepatocellular carcinomas at 300 mg/kg in females. In the second study, a significant increase in hepatic hemangiosarcomas was found in males exposed to 630 mg/kg. The incidence of HAD, although lower in controls when compared to the first study, was not affected by TEA exposure. Significant increases in HAD at all dose levels and in MHAD at 300 and 1,000 mg/kg were found in females. The incidence of liver tumors in controls was decreased two-fold when compared to the initial study. Observed control liver tumor incidences in females were similar to those predicted by a statistical model for each diet. The first study was considered *inadequate to evaluate carcinogenicity* due to uncertainty over the role of *H. hepaticus* infection in the observed liver tumor incidences. In the second study, liver tumor data provided *equivocal* (males) and *some* (females) *evidence of carcinogenic activity*. While the presence of *H. hepaticus* did not markedly affect the overall conclusions of the study, the lack of a HAD response in males was likely due to the absence of *H. hepaticus* infection and hepatitis. The decrease in control liver tumor formation, which was attributed to improved diet, enhanced the sensitivity of the assay in females.

775 CHILD-SPECIFIC REFERENCE VALUES FOR SCHOOL SITE RISK ASSESSMENT ARE BASED ON PHARMACODYNAMIC AND PHARMACOKINETIC DIFFERENCES BETWEEN CHILDREN AND ADULTS.

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California Health and Safety Code 901(g) requires the Office of Environmental Health Hazard Assessment (OEHHA) in the California Environmental Protection Agency (Cal/EPA) to identify chemical contaminants at school sites that may be of concern because of child-specific exposure and child-specific physiological sensitivities. These chemicals are found in soil and indoor dust of new schools constructed in urban or agricultural settings. The lack of guideline toxicity studies in juvenile animals has required a choice between trying to identify and apply a child-specific safety factor appropriate for all chemicals to an existing guideline study or to search for peer-reviewed experimental studies in immature animals to develop child-specific reference doses (chRDs). The Integrated Risk Assessment Branch (IRAB) found that data are more available on pharmacokinetic (PK) age-related comparisons than for pharmacodynamic (PD) comparisons. Mechanism of action research usually provides the only PD data for chemicals that alter maturation in developing organs compared to causing overt toxicity to a mature organ. IRAB will present three neurotoxins - chlorpyrifos, dieldrin and toluene - to exemplify different effects on developing brain compared to mature brain. They also illustrate the attributes of toxicity in juvenile animals: 1) toxicity is chemical specific and the mechanism of toxicity in young animals may differ from that in adults, 2) adverse effects during development alter mature function but produce no visible pathology, and 3) effects on developing animals are usually irreversible, whereas the same exposures to adult animals cause transient effects. IRAB will also show the PD or PK aspect of each finalized chRD (cadmium, chlordane, heptachlor, heptachlor epoxide, manganese, and nickel) and "public review" chRDs (atrazine, chlorpyrifos, DDT, deltamethrin, dieldrin, endosulfan, lead, methoxychlor, and toluene) and compare juvenile and mature organ endpoints.

776 MICROARRAY ANALYSIS OF OFLOXACIN-INDUCED CHONDROTOXICITY IN MALE JUVENILE RATS.

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To investigate the molecular details of chondrotoxicity due to the quinolone antibacterial agent ofloxacin (OFLX), this agent was orally administered once at 900 mg/kg to male juvenile (3 weeks old) Sprague-Dawley rats, and then gene expres-

sion profiles in the articular cartilage of the distal femur were measured at 2, 4, 8 and 24 h post-dose with Affymetrix GeneChip Rat Genome 230 2.0 Array. Some of genes were confirmed by a quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) analysis. As a result, 134 probes showed a statistical difference in expression and an up- or down-regulation at least 1.5-fold in the OFLX-treated group as compared with the vehicle group. Based on hierarchical clustering, these probes were classified into the following 6 groups: group 1, inflammatory and cell death-related genes (22 probes, up-regulated at 4 and 8 h post-dose); group 2, stress response genes (22 probes, up-regulated at 8 and 24 h post-dose); group 3, matrix-degrading protease and protease inhibitor genes (46 probes, up-regulated at 24 h post-dose); group 4, endoplasmic reticulum-related genes (4 probes, down-regulated at 4 and 8 h post-dose); group 5, interleukin-related genes (15 probes, up-regulated at 2 h post-dose); group 6, matrix synthesis and protection-related genes (25 probes, down-regulated throughout the study period). Among these genes, significant increases in tumor necrosis factor receptor superfamily, member 12a (Tnfrsf12a, group 1), prostaglandin-endoperoxide synthase 2 (Ptgs2, group 1), matrix metalloproteinase 3 (MMP3, group 3) and interleukin 6 receptor (Il6r, group 5), and decreases in gremlin 1 homolog, cysteine knot superfamily (Grem1, group 6) and hyaluronan synthase 2 (Has2, group 6) were observed in the qRT-PCR analysis. In conclusion, the initial event of OFLX-induced chondrotoxicity was considered to be increases in cytokines and prostaglandins in the cartilage, followed by the activation of cartilage disruption and disturbance of cartilage protection.

777 GENDER BASED PRELIMINARY PEDIATRIC STUDIES FOR SERUM AMYLOID A (SAA) AND C-REACTIVE PROTEIN (CRP).

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Proinflammatory response is a key to many toxicological exposures. Knowing the acute phase reactants baseline levels are critical to defining the initial response state in risk groups, such as children. Serum Amyloid A (SAA) and C-Reactive protein (CRP) increase in inflammatory, infectious and neoplastic disorders. Pediatric SAA data are limited. Our study objectives were to measure the SAA and CRP levels in non-infected male and female children and to compare them to children with acute viral or bacterial infections. We found significant gender differences in SAA values within the non-infected group (males median = 4.81 µg/mL, females median = 9.77 µg/mL; p<0.05). Despite the finding, these differences are not considered clinically relevant. We examined SAA values separately in males and females. For males, SAA values were significantly elevated in the bacterial infection group (151 µg/mL; p<0.0001) compared to non-infected controls. Significantly elevated SAA levels were also found in males with viral infection (27.6 µg/mL; p = 0.005). In males, SAA levels, therefore, appear to be of value in the diagnosis of infection, and may also be of value in differentiating between viral and bacterial infections. In contrast, females had no differences in SAA levels between the three groups examined. By comparison, there were no gender differences in the CRP non-infected group concentrations. CRP levels were found significantly higher in the bacterial group (0.42 mg/dl) as compared to the non-infected group (0.09 mg/dl, p<0.001), as well as in the viral infection group compared to the non-infected group (0.34 mg/dl, p=0.004). In summary, for males, SAA and CRP levels can be indicative of generalized infection, and comparison between the two may be useful in the early differentiation between viral and bacterial infections. Further studies are needed to verify these preliminary findings. In females, pre- and post-menstrual studies are required to further evaluate our current data.

778 ARSENIC AND DIELDRIN MIXTURES CAUSE A DELAY OF MATURATIONAL INDICES IN NEONATAL AND JUVENILE RATS.

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The objective of this study is to identify potential toxic effects of sodium arsenite (As) and dieldrin (Ded) mixtures in neonatal and juvenile rats using in-life data and hormone levels as end points. After acclimation, plug positive rats were randomly assigned to 1 of 6 Groups (Gp) (16/Gp) and orally gavaged from Gestation day (GD)7 through lactation with As-Ded-mixtures as follows: Gp 1 [0-0], Gp 2 [0.5-0.34], Gp 3 [1.0-0.69], Gp 4 [2.0-1.38], Gp 5 [4.0-2.75] mg/kg/day. After weaning, neonates were gavaged from postnatal day (PND) 22 through PND80 with the same dose that their respective dams received. Litter weights were determined at PND 0, 7, 14, and 21. Biweekly body weights, weekly feed consumption, daily clinical observations, hormones, and maturational indices were documented in the F1 male and female offspring.

Neonatal mortalities were greatest in the higher doses (Gp 4 & 5). On PND0 there was a trend toward shortening of the anogenital distance (AGD) in all male pups exposed to the mixtures; the difference was significant in Gps 3, 4, and 5. Female pups showed a similar effect in Gps 3 & 5 (PND 0 & 4). There was a delay in preputial separation in males and vaginal opening in females. Incisor eruption and eye opening were delayed in both males (Gps 3 & 5) and females (Gps 4 & 5). These data suggest a general delay in maturation of both sexes but a specific increased tendency toward femaleness following exposure to As-Ded mixtures. (Supported by MHPF/ATSDR Cooperative Agreement # U50/ATU473408-01).

779 ENHANCED IMMUNOSUPPRESSION OF THE PLAQUE FORMING CELL (PFC) ASSAY IN JUVENILE B6C3F1 MICE EXPOSED TO 1, 2,5, 6 DIBENZANTHRACENE (DBA) AS COMPARED TO ADULT MICE.

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Numerous findings have suggested that the increased prevalence of childhood illnesses such as cancer, infection, and allergy may be due to environmental exposures. One such cause may be maternal smoking or passive smoke exposure. Known immunotoxicants in cigarette smoke and environmental pollution include polycyclic aromatic hydrocarbons such as DBA. The objective of these studies was to evaluate the immunosuppressive effects of DBA on various stages of immune system development. Adult mice were administered DBA in corn oil at dose levels of 158, 500, 1580, and 5000 µg/kg sc for 28 days. Immunosuppression was not observed at levels less than 5000 µg/kg in the following immune parameters: NK cell activity, anti-CD3 antibody-mediated proliferation and mixed-leukocyte response. In contrast, holistic assays such as the PFC assay and the delayed type hypersensitivity response were significantly suppressed at dose levels of 500 µg/kg and greater. Mice exposed to DBA in utero and through lactation showed neither immunosuppressive nor sex differences among the immune parameters tested when evaluated at weaning, postnatal day (PND) 21. Transference of DBA metabolites from mother to pup is suggested by HPLC analysis of milk extracted from PND 8 pups. In contrast, juvenile mice administered DBA beginning on PND 21 at dose levels from 0.25 to 2500 µg/kg for 28 days demonstrated a dose-dependent suppression (43-79%) of the PFC assay, statistically significant at or above the 2.5 µg/kg dose level. Neither immunosuppressive nor sex differences were observed among the various other immune parameters evaluated. Collectively, these studies indicate that the juvenile life stage in B6C3F1 mice is the most vulnerable to DBA-induced immunotoxicity with a 200-fold enhancement in immunosuppression of the PFC response as compared to adult mice. These studies provide insight into how environmental contaminants, such as DBA, may impact children's health. Sponsored by NIEHS F31 ES-014111 and in part by NIEHS N01 ES-05454.

780 TOXICITY STUDY OF ULTRA-VIOLET LIGHT ABSORBER 2-(3', 5'-DI-TERT-BUTYL-2'-HYDROXYPHENYL)-5-CHLOROBENZOTRIAZOLE (DBHCB) IN RATS DURING THE PRE-WEANING PERIOD.

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DBHCB is an ultraviolet light absorber widely used in polyolefines, polyvinyl chloride, polycarbonates, polyurethane, plexiglass and many kinds of paint. We also showed that toxicological changes in the liver, including increase in weight, were noted in male rats, but not in female rats, given DBHCB at 250 mg/kg and higher for 14 days by gavage, from the age of six weeks (SOT, 2007). In the present study, the toxicity of DBHCB was investigated in pre-weaning rats. Crj:CD(SD) rats were given DBHCB by gavage at a daily dose of 0, 250 or 500 mg/kg during the pre-weaning period, postnatal days (PNDs) 4-21. Four litters per group were used in this study. All littermates (4 males and 4 females) were assigned to the same dosage group. On PND 22, rats were necropsied. In dams, no effects of DBHCB were observed on general condition, body weight, food consumption, or in necropsy findings. In pre-weaning rats, decrease in body weight was observed in both sexes of the DBHCB-treated groups. In pre-weaning rats of both sexes in the DBHCB-treated groups, increases in absolute and relative weights of the liver, increases in the levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and total bilirubin in plasma, and histopathological changes in the liver, were noted. These data indicate that there were no sex differences in liver toxicity between males and females when DBHCB was given to rats during the pre-weaning period. Supported by the grant from the Japanese Ministry of Health, Labour and Welfare.

781 INTERPRETATION OF BIOMONITORING DATA FOR TOLUENE: USE OF PBPK MODELING.

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Previously published rat and human physiologically-based pharmacokinetic (PBPK) models for toluene inhalation from the literature were modified to incorporate an oral route of exposure. The models were used to simulate blood level profiles in rats and adult humans for a) exposure regimens that serve as the basis for the derivation of the current US EPA IRIS RfD and RfC values (0.08 mg/kg-d and 5 mg/m³, respectively), and b) chronic exposures at the RfD and RfC. The impact of differing levels of uncertainty factors used in the derivation of the IRIS benchmarks was assessed. The models were also used to evaluate maximal metabolic rate achieved under various exposure scenarios as surrogates for maximal metabolite concentrations, which may be relevant to kidney toxicity observed in rodent bioassays. Simulated human exposures at the RfC and the RfD resulted in blood level profiles that differed substantially, both in toluene blood levels achieved and in temporal profiles. The influence of differences in children's metabolic capability compared to adults was also evaluated based on the published literature. Infants and young children develop CYP2E1 capability gradually, resulting in higher predicted blood toluene levels in infants and young children than adults for the same external exposures. Based on the modeling and evaluation of underlying data, the blood level associated with chronic exposure at the RfC in a typical adult (approximately 17.5 µg/L) appears to provide a reliable benchmark as a safe blood level of toluene. This benchmark can be used to assess measured levels of toluene in blood collected during biomonitoring studies in children and adults by calculating a hazard index as a ratio of measured blood levels to the RfC-associated benchmark blood level. Blood levels of toluene measured in a previous study of school-aged children in an urban community corresponded to hazard indices of less than 0.02 using this approach, suggesting that ambient exposures to toluene are well below levels of health concern.

782 COMBINED REPEATED DOSE TOXICITY STUDY WITH THE REPRODUCTIVE/DEVELOPMENTAL TOXICITY SCREENING TEST OF ULTRAVIOLET ABSORBER 2-(3', 5'-DI-TERT-BUTYL-2'-HYDROXYPHENYL)-5-CHLOROBENZOTRIAZOLE (DBHCB) IN RATS.

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DBHCB is widely used as a UV absorber within many kinds of polymer products. The possibility of these chemicals entering the biological system has aroused great concern about their toxic potential. However, very little information is available for the toxicity. It is important to gain the toxicological information for investigating the possible influences on human health. In this study, the repeated dose toxicity and reproductive toxicity of DBHCB was evaluated in rats. Crj:CD(SD)IGS rats were given DBHCB by gavage at dose of 0, 2.5, 25 or 250 mg/kg/day. Male and female rats were dosed beginning 28 days before mating and each female rat was mated with a male rat of the same dosage group. Males were dosed for total of 56-57 days, and females were dosed to day 3 of lactation throughout the mating and pregnancy period. The remaining control and high dose groups of rats were kept without treatment for 14 days. No effects on general condition, body weight, food consumption or reproductive/developmental parameters were observed. Significant decrease in the number of red blood cells and increase in the number of neutrophilic leukocytes at 250 mg/kg/day, and increases in the albumin levels, albumin/globulin ratio and alkaline aminotransferase levels at 25 mg/kg/day and higher were noted in males. Significantly increased levels of albumin levels were also found in males at 250 mg/kg/day after recovery period. The absolute and relative weight of the liver was noted in males at 25 mg/kg/day and higher. No change in the parameters of hematology and blood chemistry and liver weight was observed in females of any group. No significant changes in histopathological examination of the organs, including the liver, were found. This study was supported by the grant from the Japanese Ministry of Health, Labour and Welfare.

783 AN INTRA-NASAL IRRITATION ASSESSMENT OF ANTI-BACTERIAL OINTMENT ALONE OR IN COMBINATION WITH MUPIROICIN VERSUS BACTROBAN NASAL IN RABBITS.

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The purpose of this study was to evaluate the potential irritating effects of an anti-bacterial (REP8839) ointment alone or in combination with Mupirocin versus Bactroban Nasal in rabbits and to assess reversibility of any changes following a 14-day recovery period. Five treatment groups of six/sex received dose levels of 1, 2, or

4% REP8839, 2% Bactroban Nasal, and 2% REP8839/2% Mupirocin combination. One additional group served as the vehicle control. The test articles or vehicle was administered to the external nares, twice a day at approx. 8 hr intervals between the doses for 21 consecutive days, at a dose volume of 100 µL per naris/dose. The external nares were reflected back and the mucosal lining was evaluated and scored for erythema and edema within 30 to 60 minutes following the first dose each day. Blood samples were collected from all animals at designated time points on Day 21 of the study to assess exposure levels. Cross sections of the nasal tract were performed in all the groups for microscopic evaluation; these were labeled as nose level A-D, which consisted of cross sections trimmed from just posterior to the upper incisors, anterior to the incisive papilla up to the first upper molar to the second upper molar.

Mucosal scoring of the narice did not reveal any edema or erythema in any of the dose groups of REP8839 alone or the combination product and as well as with Bactroban Nasal. Minimal plasma exposure was observed in the rabbits (<5 ng/mL). The REP8839 groups did appear to have dose-responsive exposure (from below the limit of quantitation to 5 ng/mL with 1%, 2%, and 4% REP8839, respectively). Microscopic changes on the nasal sectioning noted in these animals were infrequent and considered incidental findings unrelated to administration of the test articles. Doses of up to 4% of REP8839 or 2% of the combination product as well as 2% Bactroban Nasal were not found to induce mucosal irritation. Additionally, no delayed effects were observed in the recovery animals.

784 DEVELOPMENT OF A MURINE PNEUMONIA MODEL FOR VACCINE TESTING.

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Streptococcus pneumoniae is a common respiratory pathogen and a major cause of morbidity and mortality in humans, especially infants under 2 and people over 60 years of age. Current conjugate vaccines (eg. PREVNAR, Wyeth) have limited serotype coverage (4, 6B, 9V, 14, 18C, 19F and 23F). The purpose of this study was to develop a mouse pneumonia model suitable for testing novel vaccine test articles. Two pneumococcal strains, TIGR4 (serotype 4) and D39 (serotype 2) were passaged intraperitoneally through mice and stored at -80°C. Bacteria for infection were thawed rapidly, cultured in Todd Hewitt Broth to an O.D. of 0.6, harvested by centrifugation and resuspended in PBS, pH 7.4. The in vivo portion of the study was performed in an AAALAC accredited barrier facility applying Laboratory Biosafety Level 2 (BSL 2) precautions in compliance with applicable laws and regulations under IACUC oversight. One hundred µL of log phase bacteria was administered intranasally to isofluorane anesthetized mice. In Phase A, mice (n=5/group) were administered saline, TIGR4 or D39 strains at 7X10E7, 3.5X10E8, 7X10E8 CFU/mL on day 1. In Phases B and C, mice (n=10/group) were dosed subcutaneously with saline or PREVNAR (3.2 µg/mouse) on days 1 and 15 and then infected with D39 or TIGR4 intranasally on day 22. Body weight and time to death in hours (including moribund sacrificed animals) were monitored. All groups showed a continued increase in body weight during the study. In Phase A, mean time to mortality averaged 60 hr (TIGR4) and 46 hr (D39) and was similar at all three inoculum levels. In Phases B and C, PREVNAR was protective against TIGR4 (time to death 382 hr compared to saline control 79 hr) but only partially against D39 (mean time to death 144 hr compared to saline control 106 hr) as expected. The difference in time to death among saline controls between Phase A and Phase B and C was attributed to mice being 3 weeks older and heavier body weight. In conclusion, a viable murine *S. pneumoniae* model suitable for novel vaccine screening studies was developed.

785 COMPARISON OF THE IN-VIVO PRIMARY DERMAL IRRITATION STUDY IN RABBITS WITH THE IN-VITRO TECHNIQUE OF THE CORROSITEX SYSTEM: CORROSIVE NATURE OF FOURTEEN TEST ARTICLES.

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Assessment of the irritation and corrosive nature of test materials have been historically required by regulatory agencies. Rabbit Primary Dermal Irritation assay is performed routinely for this purpose. Recently progress in in-vitro testing methods and the wide commercial availability of certain in vitro models have enabled refinement, reduction and replacement of animal models. The purpose of this study was to determine the potential irritant and/or corrosive effects of several test articles on the skin of rabbits and correlate these data with the in-vitro assay (Corrositex System) which is presently accepted by regulatory agencies like Department of Transportation (DOT).

Fourteen test articles, (classified as Packing Group I, II or III) were subjected to the in vitro technique of the Corrositex System and were subsequently tested for dermal irritation in New Zealand White rabbits (applied to three sites on the clipped dorsal trunk for a period of 3 and 60 minutes and 4 hours).

The results were as follows;

- 1) Both assays predicted two test materials to be non-corrosive.
 - 2) The Corrositex System could not be utilized in 2 of 14 instances since the test articles did not qualify for the assay. The in-vivo assay indicated 1 as a severe irritant and 1 as a corrosive.
 - 3) The in-vitro results indicated 10 test articles to be non-corrosive. The in-vivo assays indicated those 10 test articles to be corrosive.
- Based on the results of this comparison, the correlation of the in-vitro to the in-vivo was very low (2/14). Since in 10 of 14 instances, false negative responses were obtained in the in vitro system, further evaluation of the system is necessary before the in-vivo assay can be ruled out as the benchmark standard for actual dermal exposure.

786 ELUCIDATING SIGNALING NETWORKS IN THE PERI-PUBERTAL TESTIS FOLLOWING SERTOLI CELL TOXICANT-INDUCED INJURY.

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Children born small for gestational age (SGA) have a significantly elevated risk of cardiovascular and metabolic diseases in adulthood; however, data is limited on how SGA may impact reproductive development. Studies indicate that altered intrauterine growth increases the risk of congenital hypospadias, cryptorchidism, and testicular cancer approximately 2- to 3- fold. Evidence for these outcomes points towards alterations in the normal function of both the Sertoli and Leydig cells. Animal and human studies suggest that impaired peri-pubertal growth can affect testis size and function into adulthood. Therefore, elucidating signaling networks which modulate testis growth and identifying environmental toxicants which impinge upon these pathways will significantly impact environmental health. The Akt gene family effects testis growth. Akt1-deficient mice are born small for gestational age and have significantly smaller testes throughout their lifetime, and the germ cells of these mice are more sensitive to ionizing radiation. Our preliminary data indicate that the Akt1 signaling pathway plays a significant role in maintaining peri-pubertal testicular homeostasis. We found a striking sensitivity to germ cell apoptosis in vivo in the testis of Akt1-deficient mice exposed to Mono-2-ethylhexyl-phthalate, MEHP, a peri-pubertal Sertoli cell toxicant; and a reduction in testis size and reproductive potential in mice exposed to 6-N-propylthiouracil (PTU), a thyroid toxicant which targets Sertoli cell proliferation and differentiation. Based on these findings, we hypothesize that the Akt gene family plays a critical role in peri-pubertal testis homeostasis and that toxicants which target the Sertoli cell at this developmental window provide a crucial link between the environment and the etiology of male reproductive disease.

787 104-WEEK SAFETY EVALUATION ON IC TAG (MICROCHIP) IMPLANTED SUBCUTANEOUSLY IN RAS H2 MICE.

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We have reported that IC Tags, an animal identification tool, had no effect on a 26-week carcinogenicity test of rasH2 mice (Urano et al. in press). In this study, we investigated the chronic effect on IC Tags on growth, survival rate, and pathological findings in rasH2 mice with IC Tags implanted subcutaneously for a longer period. A 104-week observation was performed on a total of 600 mice including 150 male and female rasH2 mice (Tg mice) each, and 150 male and female non-Tg mice from the same litter as the rasH2 mice divided into a IC Tag implanted group (implanted group) and non-IC tag implanted group (non-implanted group). In the implanted group, all mice were implanted with IC Tags with an exterior finish of polypropylene subcutaneously at 7 weeks of age with a special needle, and no implantation was performed in animals in the non-implanted group. All mice were housed in plastic cages at up to five animals per cage until 104 weeks of age. Clinical observation and body weight measurement were performed every day and once every other week, respectively. A pathological examination was performed on all mice. Body weight in the implanted group was significantly higher than that in the non-implanted group in females of both Tg and non-Tg mice. The survival rate showed no difference between the implanted group and non-implanted group in Tg and non-Tg mice at 52 weeks of age. However, a slight increase in the survival rate in males in the implanted group was shown compared with the non-implanted group at 104 weeks of age. In the pathological examination, there were no specific changes induced by the IC Tag except for a subcutaneous mass observed in a female in the implanted group of Tg mice. From these data, we conclude that strong effects were not shown in rasH2 mice with IC Tags implanted for long periods.

788 INDUCED ATRIAL FIBRILLATION IN THE ISOLATED RABBIT HEART.

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Atrial fibrillation (AF) is common cardiac arrhythmia that can precipitate potentially lethal events such as ventricular arrhythmias and stroke. Currently, most AF models involve whole animals. We have developed a method to induce sustained AF ex vivo. Isolated hearts from New Zealand White rabbits of either sex were perfused with low potassium Krebs-Henseleit buffer (2.5 mM K⁺; normally 5.9 mM K⁺). After a period of equilibration, 300 μ M acetylcholine chloride (ACh), followed by 50 μ M epinephrine, was introduced into the perfusate solution. Following approximately 5 minutes of ACh/epinephrine exposure, AF was triggered with bursts of pacing consisting of 6000 beats per minute, through the right atrium, lasting approximately 15 seconds. These bursts of pacing were repeated until AF did not spontaneously revert. In 8 hearts, 7 developed sustained AF. The average number of episodes of burst pacing needed to generate persistent AF was 2.9. One heart did not develop AF after 20 attempts and was deemed not inducible. Left ventricular function appeared independent of atrial function in this model. A subset of hearts (n=3) were exposed to 10 μ M flecainide (a known treatment for AF) after AF was established. Within 6 minutes after exposure to flecainide, AF subsided in these hearts indicating that this model is responsive to pharmacologic intervention. Our model provides a promising method that may be used to determine the efficacy of therapies directed against AF.

789 SUPPRESSION OF HEPATIC FIBROSIS BY THE RADIX OF PLATYCODON GRANDIFLORUM.

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Herbal medicines are increasingly being utilized to treat a wide variety of hepatic disease processes. We previously reported that aqueous extract from the roots of *Platycodon grandiflorum* A. DC (Campanulaceae), Changkil (CK), had hepatoprotective effects against acetaminophen induced liver injury. In the present study, we assayed the preventive and therapeutic effects of CK on experimental hepatic fibrosis induced by dimethylnitrosamine or carbon tetrachloride in rats. Rats were given a single intraperitoneal injection of 20 mg/kg dimethylnitrosamine or 0.5 ml/kg carbon tetrachloride twice weekly for 4 weeks. In each model, CK was given orally at 10-200 mg/kg daily for 4 weeks. CK reduced the hepatic levels of malondialdehyde, a production of lipid peroxidation and partially prevented the marked decrease in body weight and reduced the mortality rate. The degree of fibrosis was evaluated by image analysis and also by measurements of collagen and hydroxyproline content in the liver. CK treatment significantly decreased the dimethylnitrosamine- or carbon tetrachloride-induced collagen and hydroxyproline contents. Immunohistochemical examination showed that CK reduced the deposition of type I and III collagen and the expression of α -smooth muscle actin in the liver in a dose-dependent manner. These findings indicate that CK suppress the induction of hepatic fibrosis and suggest that CK might be useful therapeutically in hepatic fibrosis/cirrhosis.

790 FEASIBILITY OF MULTI-FOCAL ELECTRO-RETINOGRAPHY IN THE CYNOMOLGUS MONKEY.

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Introduction: Humans and primates are unique in sharing a central point of best visual acuity that is a result of their macula lutea. The macula is an area of the retina that is specifically susceptible to degenerations, toxicities and photo-toxicities that can cause legal blindness. The multifocal ERG (mERG) is an important new development that enables objective topographic evaluation of the visual function of the outer layer of the posterior retina, thus highlighting the vision of the macula. The mERG is non-invasive and can be performed on repeated occasions within the in-life phase of any toxicology study. Method: Cynomolgus monkeys were tested using a combined mERG-SLO system (Roland Retiscan version 3.1; Rodenstock SLO). They were anesthetized using ketamine and xylazine injection and were placed in the prone position within a stereotaxic device (Crist Instruments, Hagerstown, MD, USA). Pupils were dilated using 1% tropicamide and 1 % atropine topical drops. The cornea of the eye being tested was anesthetized with 0.5% proparacaine drops. A wire speculum to maintain the ocular aperture and then the specially designed wide aperture monopolar platinum mERG electrode with a scleral flange was inserted. Macula position was maintained by direct control of eye position in relation to the mERG stimulus field as viewed on the SLO video screen. Results: First order P1 amplitude and latency data were recorded. The primary outcome measures of this study were taken from the mERG "ring" printout of 19 hexagonal field positions arrayed with a central peak and surrounded by two concentric rings of successively lower amplitude. Conclusion: The data demonstrate good centration of the macula and proof of concept for the mERG as a first order toxicology testing procedure in the cynomolgus monkey.

791 ASSAY DEVELOPMENT FOR INSULIN AND GROWTH HORMONE ASSESSMENT IN CYNOMOLGUS MONKEYS.

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The purpose of this study was to establish analytical methods using an enzyme-linked immunoassay (ELISA) for serum insulin and growth hormone (GH) in non-human primates commonly used in toxicity testing. To determine the induction of these markers, including glucose, cynomolgus monkeys were overfed on the first day (20 biscuits at 09:00, and 20 biscuits at 16:00). On the second day, animals were fed a normal meal of 20 biscuits/day (15 biscuits at 07:00, and 5 biscuits at 16:00). On the third day, no food was provided (fasting condition) until completion of the oral glucose tolerance test (OGT). Blood samples were processed and the serum analyzed for insulin, growth hormone and glucose on Days 2 and 3 at defined intervals for a total of 7 time-points on each day. After the 7th blood draw on the third day, glucose was administered to all animals by oral gavage (at 2g/kg body weight as 34% aqueous solution). Blood samples for serum were taken at approximately 30 minute intervals for another 3 hours (for a total of 6 time-points). Constant serum glucose levels were observed on Day 2 and Day 3 before the OGT. Following administration of the glucose, there was a sharp transient serum glucose increase which lasted for approximately 1 hr. Serum insulin levels on day 3 were constant before the OGT test, and increased thereafter, correlating with glucose level increases within the same transitory period. Growth hormone increased after OGT with minimum time lag to glucose/insulin increase. The increase in growth hormone was unexpected and was attributed to handling stress. These data suggest that the analytical methods following the OGT are efficient tools to assess insulin and glucose level changes in cynomolgus monkeys.

792 APPLICATION OF OBJECT BASED IMAGE ANALYSIS AND VIRTUAL MICROSCOPY TO QUANTIFY PANCREATIC ISLET MASS IN A MOUSE GENETIC MODEL OF DIABETES.

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Evaluation of drug effects on pancreatic islet mass can be measured biochemically by measurement of insulin content, or morphometrically using unbiased stereology or semiquantitative image analysis of immunohistochemically-stained tissue sections. However, these methods are often very labor intensive and demonstrate high inter-animal variability, thus limiting their practical use in drug discovery. Two recently developed imaging technologies, virtual microscopy and automated image analysis, offer the potential of overcoming these problems. Combined, they allow for capturing of an area of interest at one magnification and its analysis in greater detail at a higher magnification. These two technologies were used to quantify insulin expression in the Wolframin mouse model which has been shown to have progressive islet degeneration and loss of insulin staining (Ishihara et al). Insulin area and insulin staining intensity were determined in wild type and Wolframin Knock-out mice. As previously reported for this model, islet area and insulin staining were decreased in KO mice (0.75X wild type values). In addition, this object-based image analysis approach allows easy partitioning and segmentation within the islet based upon intensity of cell staining (not easily done with standard pixel-based image analysis systems), leading to additional information to assess preclinical efficacy with islet sparing therapies. Furthermore, the effects of potential therapies on islet integrity may be an important toxicologic endpoint for assessing pancreatic toxicity in preclinical safety studies. Finally, these methods have broad applicability to other tissue-based image analysis questions.

Ishihara et al, Human Molecular Genetics, 2004, Vol. 13, No. 11

793 THE RELATIONSHIP BETWEEN BLOOD GLUCOSE LEVELS AND AMPHETAMINE-INDUCED DOPAMINE DEPLETION.

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The mechanism through which amphetamine causes a long-lasting dopamine depletion remains unknown. In rodents, amphetamine has also been shown to cause an immediate increase in blood glucose levels followed by a marked period of hypoglycemia. As glucose is the main energy source for the brain, the hypoglycemia

caused by amphetamine may play an important role in amphetamine's dopaminergic toxicity. In a preliminary study, we found that pretreatment with dextrose counteracted the marked period of amphetamine-induced hypoglycemia and afforded some protection against dopamine depletion. In the present study, we demonstrated a significant decrease in blood glucose levels in mice that then shows a slow recovery over a 72 hour period though they are still significantly lower at 72 hours post-amphetamine. Correspondingly, dopamine levels were depleted by 80% at the 72 hour time point. We conclude that amphetamine's effects on glucose levels may play an important role in determining its toxicity in the brain.

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794 REDUCED VESICULAR STORAGE OF DOPAMINE CAUSES PROGRESSIVE NIGROSTRIATAL NEURODEGENERATION.

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The vesicular monoamine transporter 2 (VMAT2) is responsible for packaging dopamine into vesicles for subsequent release. VMAT2 has been suggested to serve a neuroprotective role in the dopamine system. Here we show that mice that express approximately 5% of normal VMAT2 (VMAT2 LO) display age-dependent nigrostriatal dopamine dysfunction. Over the course of 22 months, VMAT2 LO mice display elevated cysteinyl adducts to DOPA and DOPAC, and increased striatal protein carbonyl and 3-nitrotyrosine formation. These changes were associated with decreased striatal dopamine, decreased expression of the dopamine transporter (DAT) and tyrosine hydroxylase (TH). Furthermore, we observed an increase in alpha-synuclein immunoreactivity and aggregation, as well as evidence of neurodegeneration and cell loss in the substantia nigra pars compacta in aged VMAT2 LO mice. These data suggest that mishandling of dopamine via reduced VMAT2 expression is, in and of itself, sufficient to cause dopamine-mediated toxicity and neurodegeneration in the nigrostriatal dopamine system.

795 A MODEL FOR THE ANALYSIS OF NEUROTOXIC ACTION ON EMBRYONIC DEVELOPMENT BY THE ESTIMATION OF THE SOMATOSENSORY MATURATION IN MICE.

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The standard substance, cytosine arabinoside (Ara-C), was used for the creation of abnormality mice embryonic development model. This substance is an inhibitor of DNA synthesis. It has a potent cytotoxic property concerning the divisible cells. The dates of dosing were chosen in compliance with the critical periods of mice cortex development. The consequences of Ara-C administration at prenatal days 12,5-13,5 and 13,5-14,5 were studied with respect to mice soma-tosensory development in the postnatal period. For the testing of offspring, modified Fox battery was used. It was shown that such difference in substance administration date led to various neu-rotoxic damages of the posterity, which were detected in the range of tests (cliff avoidance response, geotropism response, grasping hind limb, hind limb placing response, fore limb coordination, hind limb coordination). The present model allows us to carry out a fine analysis of the neurotoxic action of new pharmacological substances on mice embryonic development.

796 DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL FOR PARAQUAT (PQ) IN C57BL/6 MICE AND ESTIMATION OF HUMAN BRAIN EXPOSURE TO PQ UNDER FIELD CONDITIONS.

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PQ and the combination of PQ and maneb (MB) have been investigated as possible risk factors for the development of Parkinson's Disease in humans (see Li et al., JOEM 47:1059-87, 2005). The first step toward determining whether laboratory

results obtained using i.p. injection of PQ in C57BL/6 mice are useful for human risk assessment is to compare i.p. exposure with the penetration of PQ into the body and brain using routes of exposure applicable to humans under realistic conditions. Adult mice were dosed by typical human routes of exposure: oral (10 mg/kg PQ $\pm$ 30 mg/kg MB), dermal (40 mg/kg PQ $\pm$ 120 mg/kg MB), and intranasal (to mimic inhalation of pesticide droplets) (3 mg/kg PQ $\pm$ 10 mg/kg MB), and 14C-PQ was quantified in blood, brain, carcass, and excreta over 24 hrs. Postnatal day 5 mice were dosed orally (0.3 mg/kg PQ $\pm$ 1 mg/kg MB) and similarly tracked for 24 hrs. Adult (10 mg/kg PQ $\pm$ 30 mg/kg MB) and juvenile (0.3 mg/kg PQ $\pm$ 1 mg/kg MB) mice were also dosed i.p. for comparison of time course and distribution of PQ. The doses were chosen to be high but below known toxicity to the lung and kidney in each route of exposure. Using separately determined tissue partition coefficients and the data from this study, the parameters for PQ entry into and exit from blood and brain were estimated from a PBPK model for PQ distribution in mice. Using literature-derived human absorption parameters for each route of exposure, and published descriptions of PQ exposure to humans during actual field conditions, a human PBPK model can be used to predict the human brain dose of PQ during field exposure conditions or through the general food and water supply. This information will allow calculation of human internal doses of PQ detected in food surveys like the Total Diet Study, PQ levels in pesticide applicator populations, and in research studies using doses applicable for risk assessment based on realistic human exposure conditions.

797 IMPROVED HUMANISED PXR AND CAR MOUSE MODELS.

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Nuclear receptors such as the pregnane X receptor (PXR) and the constitutive X receptor (CAR) regulate hepatomegaly and drug metabolising enzymes. To investigate the roles of PXR and CAR we have created improved mouse models in which the murine genes have been exchanged for their human counterparts in the same locus (huPXR, huCAR). Existing models require two independent genetic alterations (knockout of murine genes and insertion of the human transgenes) and encode unphysiological expression levels that are liver specific, due to constitutive expression from the albumin promoter. In our huPXR and huCAR mice, the expression of human CAR and PXR are under the control of their respective mouse promoters. This strategy ensured recapitulation of endogenous tissue expression patterns and levels of PXR and CAR. P450 inducers exhibiting differing affinities for the human and mouse receptors can differentially activate the human and murine receptors in Wild Type (WT) and humanised mouse liver. Treatment with the selective "human" CAR activator, CITCO (6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime 50 mg/Kg bwt., i.p.), resulted in the induction of the CAR-dependent gene, Cyp2b10, by 33- and 15-fold in the huCAR mice and WT mice respectively (pentoxifyresorufin-O-depentylation (PROD) activity). In contrast, PROD activity was increased 10-fold in WT mice and unchanged in huCAR mice following treatment with TCPOBOP (1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene, 1mg/Kg bwt., i.p.), a selective "murine" CAR activator. Debenzylation of 7-benzoyloxyquinoline was used as an indicator of Cyp3a expression, which is predominantly controlled by PXR. The "human" PXR activator, rifampicin (20 mg/Kg, i.p.) increased Cyp3a activity 5-fold in the huPXR mice and only 1.5-fold in the WT mice. Cyp protein expression (immunoblots) always correlated with enzyme activity data. Preliminary studies with the cross of the huPXR and huCAR mice suggest that chemical-selective induction is maintained in the double "humanised" mouse. Programme supported by ITI Life Sciences.

798 APPLICATION OF THE HEPATIC REDUCTASE NULL (HRN) MOUSE MODEL TO A LOW CLEARANCE COMPOUND.

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A diarylquinoline compound under development in our laboratories is extensively distributed and slowly cleared from the body. It is metabolized by the cytochrome P450 (CYP) system, mainly by CYP3A to the N-desmethyl derivative (M2) which is further metabolized via oxidative pathways. The effect of hepatic metabolism on the pharmacokinetics of both the parent and M2 was investigated using the Hepatic Reductase Null (HRN) mouse model (at CXR Biosciences Ltd, where it was developed). This HRN model has almost no liver CYP activity due to postnatal conditional deletion of hepatic cytochrome P450 reductase. Two single oral doses (10 and 60 mg/kg) of the drug substance were given to female HRN mice and one single oral dose (60 mg/kg) was given to female wild-type C57BL/6 mice. Blood samples were taken over 2 weeks after administration and the plasma concentrations of the parent compound and M2 were determined by LC/MS/MS. The

area under the plasma concentration-time curve of M2 calculated over two weeks ($AUC_{0-2weeks}$) was 2.4 times higher than that of the parent compound in the wild-type mice, whereas the $AUC_{0-2weeks}$ of M2 was similar to parent in the HRN mice. These results suggest that M2 may be partly formed by extrahepatic metabolism since the amount of circulating M2 was reduced in the HRN mice but still significant. These results demonstrate that the HRN model has the potential to elucidate certain *in vivo* metabolism characteristics of a compound or examine metabolite-associated toxicity. However, deletion of hepatic and extrahepatic CYP activity, eg in the gut, may be required to further decrease the systemic exposure to the M2 so that it becomes possible to understand the relative contribution of the parent compound and M2 to the toxicological profile of the diarylquinoline compound.

799 CYTOCHROME P450 ACTIVITY OF HUMAN HEPATOCYTES PROPAGATED IN UPA/SCID MICE.

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The metabolism of chemicals for medicines has been examined using rodents in laboratories. However, there are differences in drug metabolisms among animal species, indicating that fresh human hepatocytes are an ideal source for the test cell in investigations with such purposes. Thus, cryopreserved human hepatocytes have been used, but they are compromised upon thawing, resulting in decline and changes in their normal functions. We made "chimeric mice" by transplanting human hepatocytes into albumin enhancer/promoter-driven urokinase-type plasminogen activator transgenic/SCID mice whose liver showed normal expression patterns of human specific cytochrome P450 (CYP) genes and proteins as in the human body. Thus, these chimeric mice are utilized to predict metabolisms of drugs in human liver *per se*. In addition, when the mice were administered with ketoprofen, glucuronide conjugates were detected in their sera and bile. In the present study, we measured CYP activity of human hepatocytes isolated from the chimeric mice by the collagenase perfusion method. CYP activities of human hepatocytes from chimeric mice were higher/similar than/to cryopreserved donor cells. Importantly, ketoprofen was metabolized through glucuronide conjugates in these hepatocytes. Cryopreserved donor hepatocytes did not show such activities, demonstrating that frozen and thawed human hepatocytes regain their innate functions of drug metabolisms when propagated in mice liver. In conclusion, fresh human hepatocytes isolated from uPA/SCID mice humanized with cryopreserved hepatocytes are suitable for *in vitro* metabolic studies on drugs.

800 CHARACTERIZATION OF KNOCK-IN MICE EXPRESSING CYP1A1 PROTEIN ONLY IN MICROSOMES VERSUS ONLY IN MITOCHONDRIA.

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CYP1A1 participates in both the detoxication and metabolic activation of numerous polycyclic aromatic hydrocarbons. Although the CYP1A1 protein historically is known to localize to the endoplasmic reticulum (ER; microsomes), it is now clear that CYP1A1 is one of at least six members of the microsomal CYP1, CYP2 and CYP3 families that can also be targeted to the inner mitochondrial membrane. Mitochondrial import of CYP1A1 occurs after N-terminal processing exposes a cryptic targeting signal; mitochondrial (mt1A1) and microsomal (mc1A1) CYP1A1 exhibit both different substrate specificities and inducer profiles. To understand the physiological and toxicological variations between mt1A1 and mc1A1, we have generated three knock-in mouse lines, starting with the *Cyp1a1(-/-)* knockout genotype: *Cyp1a1(mtt/mtt)* mice encode a Truncated CYP1A1 protein that begins at residue 33, deleting the ER-targeting signal and exposing the cryptic mitochondrial-targeting signal; *Cyp1a1(mtp/mtp)* mice encode a CYP1A1 protein having Leu7Asn and Leu17Asn mutations so that it lacks the signal recognition particle (SRP)-binding and subsequent ER-targeting, but requires Proteolysis for mitochondrial import; *Cyp1a1(mc/mc)* mice encode a microsomes-only CYP1A1 protein having Arg34Asp and Lys39Ile mutations that abolish the mitochondrial targeting signal. Using different inducers, CYP1A1 has now been confirmed to target exclusively to mitochondria in the *Cyp1a1(mtt/mtt)* and *Cyp1a1(mtp/mtp)* mice and exclusively to the ER in *Cyp1a1(mc/mc)* mice. Preliminary data with oral benzo[a]pyrene (BaP; 125 mg/kg/day) for 30 days suggest that mc1A1, but not mt1A1, is required for protection against BaP that is seen in wild-type mice but not in *Cyp1a1(-/-)* knockout mice. Additional previous CYP1A1-mediated toxicity studies are being revisited, comparing these three knock-in lines with the wild-type mouse.—Supported in part by NIH grants R01 ES08147, R01 ES014403, and P30 ES06096.

801 OVARIAN MICROSOMAL METABOLISM OF BENZO(A)PYRENE IN VARIOUS MAMMALS.

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Knowledge of the ability of female reproductive system to metabolize polycyclic aromatic hydrocarbons (PAHs) is critical to the management of female infertility and for risk assessment purposes. The PAHs are a family of widespread pollutants that are released into the environment from automobile exhausts, cigarette smoke, burning of refuse, industrial emissions, and hazardous waste sites. In exposed animals, PAHs becomes activated to reactive metabolites that interfere with target organ function and as a consequence cause toxicity. The extent of susceptibility to PAH exposure may depend on the ability of animals to metabolize these chemicals. The present study has been undertaken to see whether any differences exist among mammals in the metabolism of benzo(a)pyrene (BaP), a prototypical PAH compound. Microsomes isolated from the liver and ovaries of rat, mouse, goat, sheep, boar, and cow were incubated with 5 μ M BaP. Post incubation, samples were extracted with ethyl acetate and analyzed for BaP/metabolites by reverse-phase HPLC with fluorescence detection. The metabolism of BaP in both liver and ovaries were in the order: cow > goat > sheep > boar > rat > mouse under conditions of the test system used. The rate of metabolism (pmol of metabolite/min/mg protein) was found to be more in liver than in ovary in all the species studied. The BaP metabolites identified were 4, 5-diol; 7,8-diol; 9,10-diol; 3-hydroxy BaP; 8-hydroxy BaP; and 9-hydroxy BaP. The rodent microsomes produced considerably higher proportion of BaP 4,5-diol and 9, 10-diol than did cow, boar, goat, and sheep. On the other hand, microsomes from higher mammals converted a greater proportion of BaP to 3-hydroxy and 9-hydroxy BaP, the detoxification products of BaP. Overall, our results revealed a great variation among species to metabolize BaP (supported by the NIH grants G12 RR03032, 1S11ES014156-01A1, 5T32HL007735-12, GM08037, 1U54HD0431501-09 and RO1 HD020419-19S1).

802 PRE-CLINICAL PHARMACOKINETICS: THE IMPORTANCE OF CHOOSING THE CORRECT ANIMAL MODEL.

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SCV-07 (D-glutamyl-L-tryptophan) is an immune-stimulating dipeptide being developed as a potential treatment for infectious diseases. The pharmacokinetic (PK) and bioequivalence (BE) profiles of SCV-07 were performed following subcutaneous (SC), oral, intraperitoneal (IP), or intravenous (IV) administration in rats and mice. In rats, a BE study following single oral, SC, and IV dosing of SCV-07 at 0.01, 1, or 100mg/kg produced both dose- and route of administration-dependent PK profiles. Mean plasma SCV-07 concentrations were below the limit of quantitation at all sampling times following all routes of administration of SCV-07 at 0.01mg/kg and following oral dosing at 1mg/kg. After oral administration at 100mg/kg, SCV-07 mean concentrations attained C_{max} (3.1ug/mL) at 4-hour postdose (T_{max}). C_{max} following SC administration of SCV 07 at 100mg/kg (176ug/mL) was observed at the first sampling time postdose (0.25h; T_{max}), indicating rapid absorption. C_{max} after IV dosing at 100mg/kg was 389ug/mL. The estimated bioavailability of SCV-07 after SC dosing was 82% at 1mg/kg and 94% at 100mg/kg. The estimated bioavailability following oral dosing at 100mg/kg was 7.5%. In contrast, a BE study following single oral, IP, and IV administration of SCV-07 at 10mg/kg in mice generated PK profiles that were significantly different from those of rats. In short, the PK data from the mouse study yielded an estimated bioavailability of SCV-07 after oral and IP dosing of 52% (vs. 7.5% in rats) and 66%, respectively. In conclusion, since it was unclear what the bioavailability of this dipeptide would be following oral administration in humans, a clinical development program for an oral formulation of SCV-07 based on the PK data obtained from either the mouse or the rat model would have led to widely different conclusions. This series of preclinical studies in comparative dosing of SCV-07 in rats and mice highlight the importance of using the appropriate animal model as a basis for a clinical drug development program.

803 SEMIQUANTITATIVE HISTOCHEMICAL ANALYSIS OF OLFACTORY EPITHELIAL CYTOCHROME OXIDASE ACTIVITY FOLLOWING ACUTE HYDROGEN SULFIDE EXPOSURE.

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Our laboratory has previously shown that hydrogen sulfide (H₂S)-induced inhibition of cytochrome oxidase may be associated with olfactory neuronal loss in rats. A positive correlation between H₂S flux to the exposed epithelium and olfactory neuronal loss has also been demonstrated using an anatomically correct computational

fluid dynamics model of the rat nose. The purpose of this pilot study was to develop enzyme histochemical methods to assess cytochrome oxidase activity in situ in the nasal epithelium and to evaluate whether cytochrome oxidase inhibition is correlated with the location and severity of H₂S-induced lesions. Male CD rats (n=3-4/exposure group) were exposed by nose-only inhalation for 2 hours at inspired concentrations of 0 or 200 ppm H₂S, an exposure level associated with olfactory neuronal loss in rats. Immediately following the exposure, animals were euthanized by pentobarbital overdose, perfused with paraformaldehyde, and the nose and brain were removed and placed in sucrose for cryoprotection. Frozen sections of brain (positive control) and three transverse levels of the nose containing olfactory epithelium were collected and processed for semiquantitative (densitometric) cytochrome oxidase enzyme histochemistry. The level of cytochrome oxidase activity in control nasal epithelium varied depending on location and exceeded the normal levels seen in some brain regions. Olfactory epithelial cytochrome oxidase activity was reduced in the dorsal meatus, septum, and certain turbinates of H₂S-exposed rats vs. air-exposed controls, sites that also exhibited olfactory neuronal loss. Ongoing efforts will complete production of epithelial mapping of cytochrome oxidase activity in treated and control animals with subsequent evaluation of site-specific flux to these sites.

804 REPEATED INTRANASAL EXPOSURES TO THE MACROCYCLIC TRICHOHECENE RORIDIN A EVOKES OLFACTORY SENSORY NEURON LOSS AND INFLAMMATION IN THE MURINE NOSE AND BRAIN.

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Macrocyclic trichothecene mycotoxins (MTMs) are produced by molds that may contaminate water-damaged buildings, like those along the Gulf Coast of the United States after Hurricanes Katrina and Rita. The purpose of the present study was to determine the adverse effects of repeated exposures to a representative MTM, roridin A (RA), on the nasal airways of laboratory mice. Female C57Bl/6 mice were exposed to a single daily, intranasal, instillation of RA (0.4, 2.0, 10.0, or 50.0 µg/kg bw) in saline (50 µl) or saline alone (controls), 5 days/wk, for 3 consecutive wks. Other mice were intranasally instilled with 250.0 µg/kg RA, once a day, 5 or 4 days/wk (9 days total), for 2 wks. All mice were sacrificed 24h after the last instillation and nasal airways were processed for histopathologic, immunohistochemical and morphometric analyses. Dose-response analyses revealed that the no-effect and lowest-effect levels were 10 and 50 µg/kg bw, respectively. RA exposure induced a dose-dependent neutrophilic inflammation and loss of olfactory sensory neurons (OSNs) in both the nose and olfactory lobe of the brain. Mice exposed to 50 and 250 µg/kg bw had approximately 70% and 90% less OSNs in their olfactory epithelium, respectively, compared to that in control mice. These results suggest that nasal inflammation and olfactory neural toxicity may be important adverse health effects associated with long-term inhalation exposures to RA or similar mycotoxins found in the indoor air of severely water-damaged buildings.

805 UPTAKE AND INTERNAL DOSIMETRY OF CHLORINE IN THE UPPER RESPIRATORY TRACT (URT) OF F344 RATS.

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Due to large-volume commercial use, chlorine (Cl₂) is an important hazardous air pollutant. Inhaled Cl₂ causes irritant effects in the respiratory tract. We conducted studies to characterize determinants of its mass transfer and tissue extraction in the URT. Extraction efficiency in the URT was determined as the percentage of inhaled Cl₂ retained in the surgically isolated URT of anesthetized F344 rats (both sexes) exposed by unidirectional delivery. Cl₂ analysis was done using a thermoionic analyzer previously used in human studies (Nodelman et al., 1998, Rev. Sci. Instrum. 69, 3978), modified to accommodate the lower flow rates and volumes in rodents. Exposure sets consisted of 5 animals exposed to a single concentration and flow rate. The flow rates (100, 200, or 400 ml/min) used correspond to the minute ventilation for rats of the size used, and one half and double that flow rate. The 3 concentrations (0.5, 1.0, or 2.5 ppm) correspond to a 2-year bioassay of Cl₂. The experimental design also compared uptake in live animals versus in animals killed 2 hours prior to exposure to evaluate the role of blood flow and mucus production. Internal tissue dose of Cl₂, specifically its hydrolysis product, hypochlorous acid (HOCl), that reacts with various tissue constituents, was measured by an assay of chlorotyrosines (3-chlorotyrosine and 3,5-dichlorotyrosine; Cl-Tyr). Cl-Tyr were measured in samples from 4 locations in the URT representing respiratory and olfactory tissues in the septal and lateral airstreams. High levels of extraction (>90%) were seen in all exposure sets, consistent with mice (Morris et al., 2005, Toxicol. Sci. 83, 380) and humans (Nodelman et al., 1998, J. Appl. Physiol. 87, 2073).

Flow- and concentration-dependencies were also apparent, but the responses as percent conversion to the biomarkers were complex. These data will be used to verify mass transfer coefficients and aid development of dosimetry models for Cl₂ for use in risk assessment. (This abstract does not reflect Agency policy).

806 RESPIRATORY RESPONSE AND INTERNAL TISSUE DOSE OF INHALED CHLORINE IN THE RESPIRATORY TRACT OF B6C3F1 MICE: SEX AND STRAIN COMPARISONS.

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Chlorine (Cl₂), a high-production volume air pollutant, is an irritant. Reactive airways disease or hyper-reactivity is reported after high-concentration occupational exposures. We studied sensory apnic responses and the respiratory dose associated with a 50% reduction in ventilation frequency (RD50) in both sexes of B6C3F1 mice, the strain used in a 2-year bioassay of Cl₂. Respiratory frequency and tidal volume were determined by whole-body and double-chamber plethysmography using a Buxco system (Buxco, Inc., Stamford, CT). The American Society for Testing and Materials protocol E981-84 (ASTM, 2000) was modified to extend the exposure to 120 minutes to ensure a maximal plateau in the response. Eight mice were exposed per concentration across a range from 0 to 15 ppm. Mice were sacrificed 1 hr after exposure to harvest tissues for use in biochemical assays to measure indicators of internal Cl₂ dose, including 3-chlorotyrosine and 3,5-dichlorotyrosine (Cl-Tyr), resulting from reactions of its hydrolysis product, hypochlorous acid (HOCl), with proteins. Soluble antioxidants and Cl-Tyr were measured from samples in 4 locations in the nose and 1 in the lower left lung lobe. The RD50 in these mice was lower than previously reported, which may be due to the longer exposure or differences in the plethysmography system employed. The differences between this strain and others tested (Morris et al., 2005, Toxicol. Sci. 83, 380; Gagnaire et al., 1994, J. Appl. Toxicol. 14 (6), 405) confirm differences postulated to be due to airway architecture (Moss and Oldham, 2006, J. Aerosol Med., In Press). Female mice have a slightly higher RD50 (~2-fold) than males, which may result in a greater internal delivery, providing insight on their increased response in various toxicological studies. The biochemical data on tissue biomarkers aid in verifying dosimetry model predictions of tissue reactions for use in dose-response assessment. (This abstract does not reflect Agency policy).

807 RESPIRATORY RESPONSE AND INTERNAL TISSUE DOSE OF INHALED CHLORINE IN THE RESPIRATORY TRACT OF F344 RATS: SEX AND SPECIES COMPARISONS.

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Inhaled chlorine (Cl₂) causes respiratory effects. Females show more severe effects than males in various toxicological studies. Females rats had decreased survival in a 2-year bioassay, requiring their exposure be reduced to 3 d / wk. We conducted a study to characterize the sensory apnic response and respiratory dose associated with a 50% decrease in ventilatory frequency (RD50) in both sexes of F344 rats, the strain used in the bioassay. Respiratory frequency and tidal volume were determined by whole-body and double-chamber plethysmography using a Buxco system (Buxco, Inc., Stamford, CT). The American Society for Testing and Materials protocol E981-84 (ASTM, 2000) was modified to extend the exposure to 120 minutes to ensure a maximal plateau in the ventilatory response. Eight rats were exposed per concentration across a range from 0 to 60 ppm. Rats were sacrificed 1 hr after exposure to harvest tissues for use in biochemical assays to measure indicators of internal Cl₂ dose, including 3-chlorotyrosine and 3,5-dichlorotyrosine (Cl-Tyr), resulting from reactions of its hydrolysis product, hypochlorous acid (HOCl), with proteins. Soluble antioxidants and Cl-Tyr were measured from samples in 4 locations in the nose and 1 in the lower left lung lobe. The internal dose measures will allow comparison of internal dose after in vivo cyclical breathing versus unidirectional steady-state exposures. Results show the RD50 is lower than previously reported and may be due to the longer period of exposure or differences in the plethysmography system employed. F344 rats have a higher RD50 (~20 ppm) than mice (~3 to 12 ppm, depending on the strain). Female rats have a higher RD50 than males which may result in an increased internal delivery, providing insight into their more severe effects. The biochemical data will help verify dosimetry model predictions of these reaction products for use in risk assessment. (This abstract does not reflect Agency policy).

808 SENSORY IRRITANT RESPONSIVENESS IN LPS-INDUCED LUNG INFLAMMATION.

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Our previous studies have shown that responsiveness to sensory irritants is enhanced in ovalbumin-induced eosinophil-rich allergic airway disease. The current study was performed to determine if a similar phenomenon occurred in neutrophil-rich airway inflammation. Toward this end, sensory irritant responsiveness was measured in female C57Bl/6J mice 1, 3 or 8 days after intranasal instillation of 20 µg lipopolysaccharide (LPS, Sigma 011:B4) or vehicle. Non-invasive plethysmography was used to assess irritant sensitivity using the induction of sensory irritation, characterized as a decreased breathing frequency due to a braking in the early phase of expiration, as a biomarker for respiratory tract sensory nerve activation. Acetic acid (80 ppm) and environmental tobacco smoke (1.7 mg/m³) were used as prototypical irritants. As assessed by lavage cellularity, neutrophilic airway inflammation was present 1 and 3 days post LPS, but had resolved by day 8. Tidal volume and peak expiratory flow to tidal volume ratio were significantly decreased from control levels on days 1 and 3 post LPS as would be expected with lung inflammation; both of these parameters had returned to control levels by day 8. At the concentrations used, both acetic acid and environmental tobacco smoke induced a mild sensory irritation response; similar irritant responsiveness was observed in both control and LPS treated mice at all time points. Thus, in contrast to ovalbumin-induced allergic airway disease, LPS-induced neutrophilic airway inflammation did not enhance irritant responsiveness, suggesting that sensory irritant hyperresponsiveness may be related to the presence of eosinophils in allergic airway disease.

809 DEPOSITION EFFICIENCY OF INHALED PARTICLES FROM 30 NM TO 10 µM IN DIFFERENT INDIVIDUAL HUMAN NASAL REPLICAS.

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Inhaled air that is filtered by the nose may prevent toxic particles from reaching the lungs. The deposition efficiency of the nose may be studied by using anatomically accurate nasal replicas. Nasal replicas were produced from magnetic resonance imaging (MRI) scans from four healthy individual humans, using a stereolithography process. In another individual with atrophic rhinitis, computed tomography (CT) scans were made pre- and post-reconstructive surgery that narrowed the nasal passages. Deposition efficiency measurements were made by producing a monodisperse aerosol and measuring particle concentration at the inlet and outlet to the nasal replica. A vibrating orifice aerosol generator was used to produce monodisperse particles from 1 to 10 µm in aerodynamic diameter. Nano-sized particles were produced using a nebulizer and differential mobility analyzer to select sizes from 30 to 100 nm. An aerodynamic particle sizer and scanning mobility particle sizer were used to count micrometer- and nanometer-sized particles, respectively. Airflow through the nasal replicas was in the inspiratory direction at a constant rate. Experimental results showed that nanoparticles around 100 nm were deposited with < 10% deposition efficiency that increased slightly as particle size decreased to 30 nm. Nanoparticle deposition efficiency did not differ significantly between the various replicas. At a particle size of 8 µm and flow rate of 20 L/min, deposition efficiency ranged from about 60% to 90% in the healthy nasal replicas. Particle deposition efficiency in the nasal replicas of pre- and post-surgical treatment for atrophic rhinitis differed significantly. Deposition in the pre-surgery nasal passages, which had a more open cross section, was much lower (about 20%) than in the narrowed post-surgery nasal passages. These studies suggest that nasal deposition efficiency and hence lung exposure may be less affected by variability in nasal passage geometry and airflow for nanoparticles than for larger-sized particles.

810 METHOD FOR EVALUATING PULMONARY DEPOSITION AND CLEARANCE OF DIFFERENT SIZED FINE-MODE PARTICLES IN A SINGLE ANIMAL.

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Over the last decade, great attention has been given to particles that are 2.5 µm in diameter or less (e.g., PM_{2.5}). While it is not uncommon for inhalation studies to involve the delivery of multiple sized particles, to trace the deposition and clearance pattern of particles of a specific size range, airborne particles generally have to be delivered in separate monodisperse aerosol applications. As a result, the deposition and clearance of different sized particles are evaluated in separate animal groups. This study describes a unique approach in which particles of varying sizes are delivered as a monodisperse aerosol and the deposition and clearance of these particles are evaluated within a single animal. Sprague Dawley rats were exposed to aerosolized 0.5, 1, and 3 µm microspheres (3hr/day, 3 consecutive days). Animals

were examined following the end of each exposure period, as well 24 and 48 hr following the last day of aerosolization. The mass concentrations of aerosolized 0.5, 1 and 3 µm microspheres were 0.3, 1.7 and 2.1 mg/m³, respectively. The deposition pattern for the different sized microspheres varied with airway pathlength, with the larger sized microspheres (3 µm) primarily depositing in the cranial lobe and the large airways. The deposition of 0.5 and 1 µm particles increased from the bronchus to the terminal bronchioles. Total particle burden (number) of 0.5 µm microspheres in the lung was observed to be 2- and 240-fold greater than the deposition of 1 and 3 µm microspheres, respectively. Significant clearance of 3 µm microspheres was noted (50% after 48 hr), whereas little clearance was found for 0.5 and 1 µm particles up to 4 days post-exposure. The retention pattern of the different sized microspheres corresponded well with known mechanisms of particle clearance. This study design could be used to assess how other air pollutants, which target different regions of the lung, may influence the total and regional pulmonary deposition and clearance patterns of different sized particles. USEPA R831714 and R832414

811 MURINE MODEL OF SO₂ EXPOSURE EFFECTS ON AIRWAY INFLAMMATORY PROCESSES.

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Oxidant air pollutants (ozone, oxides of sulfur and nitrogen, fine particulates, and aldehydes) are among the known risk factors contributing to the development of asthma and allergies. Sulfur dioxide (SO₂) is a known respiratory irritant and a major component of most PM 2.5 that induces bronchoconstriction in asthmatics, but its role as a modifier of allergic sensitization is less well understood. The generation of reactive oxygen species (ROS) may be responsible for the formation of an inflammatory profile leading to airway hypersensitivity and immunomodulation. C57/B6 mice and knock-out mice for the mast cell NADPH Oxidase (gp/91phox) gene and the epithelial NADPH oxidase gene (Duox1), were chamber exposed to both low (ambient exposure; 1 ppm) and high (accidental exposure: 50 ppm) dose SO₂. Mice were additionally sensitized to ovalbumin (Ova) by either IP injection or by nebulization. After Ova challenge, bronchoalveolar lavage (BAL) was collected for cell differentiation and cytokine profiling. Homogenized lungs were used for Bioplex cytokine measures and sulfite residue measures by fluorescent HPLC. Alternate lungs were removed and utilized for histopathology. Spleen T-cell proliferation was measured with H3-thymidine. Measurement of hypersensitivity to methacholine challenge was determined by the tracheal ring assay. High dose SO₂ induced an immunosuppressive effect, with evident damage to the epithelium, while exposure to ambient levels of SO₂ caused a Th2-type allergic response. Both exposures were dependent upon ROS production. ROS induced degranulation of mast cells appeared to be central to ambient level exposure responses, while signaling in epithelium appeared to be responsible for inflammation in the high level exposures. Ambient exposure via PM 2.5 may lead to asthma exacerbations through enhancement of allergic hypersensitivity. High concentration occupational exposure could lead to the syndrome known as occupational asthma via a chronic inflammatory state induced by ROS damage in the epithelium.

812 DIESEL PARTICLE INSTILLATION ENHANCES INFLAMMATORY AND NEUROTROPHIN RESPONSES IN THE LUNGS OF ALLERGIC BALB/C MICE.

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Neurotrophins, including nerve growth factor (NGF) mediate many features of allergic airways disease. Antibody blockade of NGF attenuates airways resistance associated with the allergen-specific airways responses in mice. Exposure to diesel exhaust particles (DEP) associated with the combustion of diesel fuel exacerbates allergic airways responses. We hypothesized that the intranasal instillation of DEP in allergic mice will increase the hallmark features of allergic airways disease including increased neurotrophin production. Ovalbumin (OVA)-sensitized and non-sensitized BALB/C mice were intranasally instilled six times over two weeks with 10 (DEP1) or 50 (DEP2) µg of the PM_{2.5} fraction of NIST SRM-2975 DEP or saline alone. The mice were then challenged with an OVA aerosol. Non-allergic mice instilled with DEP1 had a 2.7-fold increase in macrophages in the lung lining fluid one day after challenge, while those instilled with DEP2 had a 3-fold increase in macrophages and a 19-fold increase in eosinophils relative to saline instilled mice. OVA-allergic mice instilled with saline had increases in macrophages (2-fold) and eosinophils (7.1-fold) relative to non-allergic mice. DEP1 did not enhance OVA-induced responses. DEP2-exposed OVA-allergic mice had increases in macrophages (1.61-fold), eosinophils (1.45-fold), and neutrophils (1.78-fold) in the lung lining fluid one day after challenge relative to saline-instilled mice. DEP2 instillation also increased levels of the neurotrophin NT-3 (2-fold) in the lung lining fluid and serum IgE (1.4-fold) relative to saline-instilled mice. Three days after

OVA challenge, DEP2-exposed OVA-allergic mice had increased macrophages (1.4-fold), eosinophils (4.4-fold) and neutrophils (1.53-fold) in the lung lining fluid, but no change in NT-3 or IgE levels relative to saline-instilled mice. Neutrophin upregulation may trigger DEP-induced enhancement in serum IgE and pulmonary inflammation in allergic mice (This abstract does not reflect EPA policy).

813 RELEASE OF IL-8 AND IL-6 BY BEAS-2B CELLS FOLLOWING *IN VITRO* EXPOSURE TO BIODIESEL PM EXTRACTS.

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Biodiesel, an alkyl ester of plant oils that can be used in an unmodified diesel engine, is a renewable fuel alternative which show signs of becoming a commercially accepted part of our nation's energy infrastructure. Biodiesel exhaust has been physicochemically characterized, but little evaluation of the inflammatory potential of biodiesel emission Soluble Organic Fraction (SOF) has been performed. BEAS-2B cells, a transformed airway epithelial cell line, were exposed *in vitro* for 24 hr to the SOF emissions extracts from 2 petroleum diesel particulate matter (PM) samples [Standard Reference Material (SRM) 1975 and Phillips D2], hydrogenated soy ethyl ester (HySEE) PM, and soy methyl ester (SME) PM. Our data suggest HySEE and SME extracts are more potent inflammatory stimulants than SRM 1975 and D2 SOF on a mass PM equivalent per ml media ($\mu\text{g PM eq/ml}$) basis, an extracted material unit. The SOF extracts of biodiesel PM in an aqueous solution increased the release of pro-inflammatory cytokines IL-8 and IL-6 by BEAS cells relative to control (vehicle exposed) cells. The SOF of HySEE and SME biodiesel begins to elicit a cytokine response in BEAS cells at approximately 40 $\mu\text{g PM eq/ml}$, whereas SRM 1975 increased IL-8 and IL-6 at 922 $\mu\text{g PM eq/ml}$ (about 25 $\mu\text{g SOF extractable material/ml}$). The exposure range tested for all SOF was not cytotoxic. It was also noted that the solvent exchange method utilized may not be appropriate for the investigation of biodiesel extracts, though it has been used extensively in petroleum diesel PM research. More research is required to characterize the potency of the SOF of biodiesel PM from different sources (e.g., vegetation type) and run conditions (e.g., engine load) compared to petroleum diesel PM. This research was supported in part by the NHEERL-DESE Cooperative Training in Environmental Sciences Research, EPA CT826513. This abstract of a proposed presentation and does not necessarily reflect EPA policy.

814 *IN VITRO* RESPONSES OF AIRWAY CELLS EXPOSED TO ENVIRONMENTAL PARTICLES: SIGNALING PATHWAYS AND ARTIFACTS.

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Studying effects of inhalable particles by *in vitro* toxicology assays aids understanding of biochemical mechanisms and offers the potential to efficiently study complex mixtures found in ambient particulate matter. We have used these assays to assess soil-derived dusts, combustion ash, metal oxide nanoparticles, and laboratory surrogates. We have focused on transcription and secretion of the proinflammatory interleukins IL-6 and IL-8 in airway epithelial cells exposed to particles alone and in combination with pathway-specific inhibitors. Cell responses depend on the particle source and chemical composition in a complex manner suggesting that chemical speciation by elements, carbon fractions, and soluble ions is not measuring the toxicologically important parameters. Different cell types and cell culture conditions produce altered response to the same particle. Manufactured nanoparticles induce IL-6 and IL-8 secretion in human airway epithelial cells, but are not consistently more potent than an equal mass of micron-sized particles of the same nominal composition and the pure oxides are less potent than natural particles derived from soil dust. Different cell signaling pathways have been proposed to explain how dust components such as endotoxin, transition metals, and reactive oxygen species-generators induce release of cytokines by lung cells. We find that inflammatory soil dusts stimulate the p38 MAP kinase pathway. Use of specific inhibitors, however, indicate that Jnk and Erk1/2 as well as p38 MAP kinase pathway pathways are needed for full IL-6 response. Further work with specific signaling inhibitors indicates that the pro-inflammatory response of bronchial epithelial cells is mediated by several receptors located at the plasma membrane, including EGFR and IL-1R. In conclusion, bronchial epithelial response to soil dust is complex, involving many interacting molecules, and reliance on a single readout such as IL-6 release gives only a partial picture. Supported by the Southwest Consortium for Environmental Research & Policy, grant A-05-06 & NIEHS K25 ES11281.

815 DIFFERENTIAL GENE EXPRESSION BY SEX IN AIRWAYS OF MICE EXPOSED TO SIDESTREAM TOBACCO SMOKE (TS).

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Following birth, the lung continues to both grow and differentiate while interacting with the environment. Exposure to TS pre- and postnatally alters the conducting airways and has been correlated with airway diseases in both children and adults. Many of these airway diseases have sex-differences in prevalence and severity. We studied the effect of prenatal vs. pre+postnatal TS exposure on airway specific gene expression in adult male and female mice. Mice were exposed to 1 mg/m^3 total suspended particulate tobacco smoke (TS) or filtered air (FA) for 5 days per week, 6 hrs per day from d6 of gestation to adulthood. Exposure groups were: pre and postnatal FA (FA/FA), prenatal TS + postnatal FA (TS/FA), pre and postnatal TS (TS/TS). At 8 wks of age, lungs were removed and inflated with RNA laterTM. Airways were microdissected. Custom spotted glass slide 50 mer oligo microarrays containing 20,000 mouse genes were used. FA/FA RNA samples from males were combined; FA/FA females were combined in one estrus cycle stage. In males, the TS/FA group contained more unique upregulated (727) and down regulated genes (501) than the TS/TS (295; 172) male group. 1134 genes were up or down regulated in common. In females, the TS/FA group contained more unique up regulated (86) and down regulated genes (291) than the TS/TS (5; 30) female group. 126 genes were up or down regulated in common. In general the number of altered genes was less in females but was greater for mice exposed to TS solely during the prenatal period. In females, the tendency was for genes to be downregulated by TS but in males more genes were upregulated by smoke. This may be due to sex-differences in basal gene expression. We conclude that 1) there are sex-based differences in airway responses to tobacco smoke exposure, 2) exposure regime changes gene response patterns and 3) prenatal exposure to ETS is sufficient to cause persistent changes in gene expression in adult airways. Support: NIH ES013066 & ES05707

816 AIRWAY INFLAMMATION AND INJURY FOLLOWING IN-UTERO EXPOSURE OF RATS TO CIGARETTE SMOKE AND POSTNATAL EXPOSURE TO OZONE.

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An exposure to environmental pollutants during pregnancy raises the possibility of development related changes and increased sensitivity to subsequent pollutant exposures. In this study rats were exposed *in-utero* to environmental tobacco smoke (ETS), followed by exposure of the newborn to O₃. ETS was generated from research cigarettes (1R4F; Univ of Kentucky) in a Walton cigarette machine by mixing mainstream with side stream smoke. Timed pregnant S/D rats were exposed 3hr/day for 15 days to ETS (100 mg/m^3 TPM) or air, and the offspring received either a single 3 hrs exposure or 3 wks of repeated exposures to air or 0.6 ppm O₃ starting on day 13 after birth. The exposure groups were Air/Air (Control), Air/O₃, ETS/Air and ETS/O₃. The bronchoalveolar lavage (BAL) was analyzed for total protein, the cellular fraction for PMNs and tissues for cytokine gene expression using an inflammatory gene microarray (GEArray). A single postnatal exposure to O₃ resulted in a significant increase in total BAL protein and PMNs compared to controls. The results also showed an upregulation of genes for IL-1 α , IL-1 β , IL-8r β , IL-9 α , IL-13, IL-24, TGF β 1 and TNF α ; the increase was greatest for the ETS/O₃ group. Cellular expression of cytokines to detect the exposure-associated changes is currently in progress to complement the gene expression data. The differences in BAL protein and PMNs seen after a single postnatal exposure were not observed after repeated exposures. Tissue sections were also processed for immunocytochemical staining with an antibody against Podoplanin, a mucin-type glycoprotein expressed by lymphatic endothelial cells which appears to play a role in tumor progression. A lack of differential staining among exposure groups did not permit its utility as an early marker of injury. The results overall show a greater response after one day postnatal O₃ than after three weeks of postnatal exposure.

817 ASSESSING RESPIRATORY HEALTH IMPACTS OF GENERAL PURPOSE CLEANING PRODUCTS CONTAINING SOLVENTS WITH DIFFERENT REACTIVITIES.

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There is growing interest in switching from petroleum to biogenic-based cleaning products. States are bringing forth regulation to control product compositions based on reactivity, yet the health impacts from exposure to these cleaning products

cannot be described merely by assessing their reactivity. Several issues should be considered when assessing the impacts of consumer products. While determination of the ozone forming potential is crucial, the possible toxic effects from the components or their reaction products should not be ignored. To look more closely at the chemistry and relative health risks of consumer products, pure d-limonene, glycol ether, and two general purpose cleaning products, one containing d-limonene (GCP1) and one containing glycol ether (GCP2) were examined. Indoor use experiments included techniques that simulated spraying and mixing the cleaning products or the individual solvents with background levels of indoor ozone utilizing clean air found in the chamber. Human respiratory epithelial cells were exposed to both the gas and particle phases of the unreacted and reacted chamber mixtures using a modified *in vitro* exposure system and analyzed for cytotoxicity and inflammation using LDH and IL-8 protein mediators. Detailed chemical and particle composition analyses were performed both before and after oxidative aging. The analysis shows that glycol ether induces greater inflammation and cytotoxicity than d-limonene; GPC2 containing glycol ether also causes a greater response than GPC1 containing d-limonene. There was an increased cytotoxic and inflammatory response from exposure to particles from the glycol ether, GPC1 and GPC2 than from the exposure to gases alone. Assessing the indoor chemistry and the potential health effects of both primary and secondary toxics presents a more balanced approach for evaluating the environmental and health impacts of consumer products and their formulations.

818 MULTIPLE CHALLENGES IN A MOUSE MODEL OF CHEMICAL-INDUCED ASTHMA.

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To validate a mouse model of chemical-induced asthma we compared a single with a multiple challenge protocol.

On day 1 and 8 BALB/c mice were dermally sensitized with 0.3% TDI (20 µl/ear) or vehicle (acetone/olive oil). From day 15 on the mice received 1 to 6 intranasal instillations (challenges) with 0.1% TDI (10 µl/nostril) or vehicle, with 1 week interval between instillations. The airway responses were measured (Penh and pulmonary resistance) after the last challenge. Mice were sacrificed 24 hours later. Total and differential cell counts were performed in the broncho-alveolar lavage (BAL) fluid and TNF- α and MIP-2 concentrations were measured. Total serum IgE, IgG, and IgG_{2a} levels were measured. Lymphocyte subpopulations (CD4⁺, CD8⁺, CD19⁺) were determined in the auricular (ALN) and cervical (CLN) lymph nodes. IL-4 and IFN- γ release, *in vitro*, after concanavalin A (ConA) stimulation, was assessed.

After 1, 2 or 3 challenges with TDI, a significant increase in airway hyperreactivity was found in TDI sensitized mice. This response diminished with further challenges. An increase in the total cell count and percent neutrophils (15-20 %) was found in BAL fluid, but this decreased with increasing TDI challenges. There was a decrease in the percent of CD4⁺ and CD8⁺ cells and an increase in the percent of CD19⁺ cells in the ALN and CLN, which was restored to control values with increasing TDI challenges. Increased levels of IL-4 were found in CLN cells *in vitro*. The total serum IgE levels in TDI sensitized mice were always significantly increased compared to control mice.

Ventilatory responses and lung inflammation diminish after multiple challenges with 1 week interval between challenges. It is likely that this 'fading out' is due to the acquisition of tolerance. Nevertheless, some immunologic parameters are still increased after 6 challenges with TDI.

819 TIME COURSE OF VENTILATORY, INFLAMMATORY AND IMMUNOLOGIC PARAMETERS IN A MOUSE MODEL OF CHEMICAL INDUCED ASTHMA.

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To validate our mouse model of chemical-induced asthma we compared different time points of methacholine challenge and autopsy after the last intranasal instillation, in a multiple challenge protocol.

On day 1 and 8, BALB/C mice received 0.3% toluene diisocyanate (TDI; 20 µl/ear) or vehicle (acetone/olive oil) on each ear. On day 15, 16, 17, 22, 23 and 24 they received an intranasal instillation of 0.1% TDI (10 µl/nostril) or vehicle. Airway reactivity to methacholine (AHR) was measured (Penh and pulmonary resistance) 22 h and 46 h later. The autopsy was performed 2h later. Total and differential cell counts were assessed, along with levels of TNF- α and MIP-2, in bronchoalveolar lavage (BAL). Total serum IgE, IgG₁ and IgG_{2a} levels were measured. Relative lymphocyte subpopulations (CD4⁺, CD8⁺ and CD19⁺) in auricular (ALN) and superficial cervical lymph nodes (CLN) and release of IL-4 and IFN- γ by LN cells incubated with concanavalin A, were assessed.

Methacholine reactivity, was lower at 46 h than at 22 h after the last challenge. Pulmonary inflammation was characterized by neutrophils (15-20%) and eosinophils (2-3%), 24 h after the last intranasal instillation. Another 24h later, this proportion changed to 6-8% for neutrophils and eosinophils. MIP-2 in BAL was increased both at 24h and 48h after the last instillation. In both groups (24 and 48 h) an increase in CD19⁺ and a decrease in CD4⁺ and CD8⁺ lymphocytes in ALN and CLN was found. Increased levels of IL-4 in ALN and CLN cells could be detected in all treated groups. Increased levels of total serum IgE and decreased levels of total serum IgG_{2a} were found in TDI sensitized mice.

Ventilatory response decreased and pulmonary inflammation parameters change (less neutrophils, more eosinophils) with increasing time between the last intranasal instillation and the autopsy. Nevertheless, immunological parameters were not influenced by the time point of autopsy.

The time point of evaluation has an important influence on inflammatory parameters, but not on immunological parameters.

820 FETAL EXPOSURE TO ENVIRONMENTAL TOBACCO SMOKE (ETS) EXACERBATES SUBSEQUENT ADULT RESPONSES TO INITIAL ALLERGEN EXPOSURE.

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Epidemiology studies have associated fetal exposure to maternal smoking and post-natal exposure to environmental tobacco smoke (ETS) with increased asthma risk. We tested the hypothesis, in a mouse model of asthma, that in utero ETS exposure alters airway function and respiratory immune responses in adults. Pregnant Balb/c mice were exposed daily to ETS (10 mg/m³) or filtered air (AIR). Offspring inhaled aerosolized saline in weeks 7-8, and were sensitized to ovalbumin (OVA) via injections in weeks 11 and 13 and challenged via OVA aerosol in weeks 14 and 15. We identified eleven endpoints, including OVA-specific serum immunoglobins, bronchoalveolar lavage cell and cytokine levels, lung histopathology and airway hyperresponsiveness (AHR), that have a direct relationship to airway inflammation and/or airway Th2 responses (i.e., they increase as inflammation increases), and compared paired group means for these endpoint measures for both sets of mice in tests of trend. The Wilcoxon test on these paired sign data demonstrated that responses in ETS/OVA mice were consistently higher ($p = 0.001$) than in AIR/OVA mice across all data. This trend was further supported by a Pearson correlation value of 0.99 and a ranked regression yielding an adjusted R² of 0.98. These results support the conclusion that in utero ETS exposure exacerbates subsequent adult responses to initial allergen exposure. In mice, this sensitivity lasts at least 15 weeks after birth. If this heightened sensitivity extends beyond OVA to unrelated antigens, as seems likely mice exposed to ETS in utero and then to other antigens as adults (e.g., Aspergillus, cockroach antigen, bacterial lipopolysaccharide or respiratory viruses) would be expected to display more aggravated responses than mice not exposed in utero to ETS.

821 DEVELOPMENT OF A MURINE MODEL OF ASTHMA USING COCKROACH ALLERGEN AND DIESEL EXHAUST-RELATED AIR POLLUTANTS.

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Allergic asthma is a complex respiratory disease and its incidence is on the rise, particularly in children from urban areas. The underlying etiology of this upward trend is unclear, yet mounting evidence suggests that air pollution may play a significant role, specifically ambient urban particles like those found in diesel exhaust. Although ovalbumin (OVA) is the standard for asthmatic mouse models, indoor pollutants, such as cockroach antigen, are highly suspect in real world asthma cases. This study was conducted to develop a novel sensitization protocol using cockroach allergen Bla g 2 and to test the hypothesis that diesel exhaust acts as an immunological adjuvant in Bla g 2-sensitized mice. A model of allergic asthma was first established in adult female Balb/c mice by ip sensitization with 10 µg OVA followed by a 10 µg OVA ip boost and a 10µg OVA aspiration challenge. This model was later optimized for use with 1 µg of Bla g 2. Lavage and serum were recovered 24-48 h post-challenge and eosinophilia along with Th1:Th2 cytokine and antibody profiles were examined to characterize the degree of "asthmatic" response. Mice sensitized to either OVA or Bla g 2 were then co-exposed to increasing concentrations of diesel exhaust particles (DEPs) at the time of their final challenge. It was found that DEPs altered levels eosinophilia and IgE antibody production in a dose-dependent manner for both OVA- and Bla g 2-sensitized mice, suggesting that DEPs do act as an immunological adjuvant for these two allergens. Similar results were generated from a group of Bla g 2-sensitized mice co-exposed to 1 mg/m³ of

whole diesel exhaust (DE) at the time of Bla g 2 challenge. Further research will enable us to examine age-dependent differences in immune response, to delineate the adverse effects of DE inhalation on these responses, and to quantify the relative contribution of biological and environmental factors on the exacerbation of pediatric asthma.

822 SUPPRESSION AND ENHANCEMENT OF ALLERGIC AIRWAY RESPONSES BY PM_{2.5} IS DEPENDENT ON PHYSICOCHEMICAL CHARACTERISTICS.

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Increased concentrations of fine particulate matter (PM_{2.5}) have been associated with increased symptoms in asthmatic patients. Eosinophilic inflammation, excessive airway mucus and mucous cell metaplasia/hyperplasia (MCM) are common histopathologic features of asthmatic airways. We tested the hypothesis that exposure to concentrated fine airborne particles (CAPs) from two urban areas with qualitatively disparate PM atmospheres will exacerbate allergen-induced MCM and inflammation in airways of Brown Norway (BN) rats. Male BN rats were exposed in whole-body exposure chambers, located within a mobile laboratory located in either urban southwest Detroit, MI (DTW) with impact from local and transported PM sources, or Grand Rapids, MI (GRR), with transported and mobile PM sources. Ovalbumin (OVA)-sensitized BN rats were exposed to CAPs or filtered air (FA) for 1 day (7:00 am to 3:00 pm). A Harvard/EPA ambient fine particle concentrator was used to generate the CAPs from the urban air in DTW (542 µg/m³) and GRR (519 µg/m³). One hour before exposure, rats were intranasally challenged with 0.5% OVA in saline or with saline alone (SA). Rats were sacrificed 16h later and bronchoalveolar lavage fluid (BALF) was collected from right lung lobes. Left lung lobes were processed for light microscopy and morphometric analysis of intraepithelial mucosubstances (IM; measure of MCM) in pulmonary axial airways. In both DTW and GRR, SA/CAPs rats (controls) had no airway inflammation or MCM. In contrast, OVA/FA rats had more neutrophils (25-30-fold), eosinophils (8-10x), and total protein (3-4x) in BALF than controls. DTW CAPs enhanced all allergic endpoints by 30-100%, whereas GRR CAPs suppressed all allergic responses by 50%. GRR CAPs was derived primarily from motor vehicle sources. Conversely, DTW CAPs had high sulfate, smaller sized particles and originated from both coal and local combustion sources. These data demonstrate opposing effects of PM on allergic airway disease depending on source and characteristics of particles. (Supported HEI and MEDC)

823 ASSESSING THE RESPIRATORY SENSITIZATION POTENTIAL OF PROTEINS USING THE MOUSE INTRANASAL TEST (MINT).

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A need exists for a simple predictive animal model to identify proteins with the potential to induce respiratory sensitization in humans. The MINT has been successfully used to predict human sensitivity to inhaled industrial enzymes, however, its utility in predicting the allergenicity of other classes of proteins is untested. In this study, BDF1 mice were intranasally instilled on days 1, 3, 10, 17 and 24 with 0, 0.5, 5, or 25 µg Subtilisin Carlsberg (SUB; known respiratory sensitizer), ovalbumin (OVA; potent food allergen), β-lactoglobulin (BLG; moderately potent food allergen), or keyhole limpet hemocyanin (KLH; strong immunogen with no reports of respiratory sensitization). Pulmonary function (PF) data were collected for methacholine (MCh) or specific protein aerosol challenge using whole-body plethysmography (WBP). Bronchoalveolar lavage (BAL) was performed to evaluate cellular inflammation, and serum was collected to determine protein-specific IgG1/IgG2a levels (ELISA) and specific IgE by passive cutaneous anaphylaxis (PCA). All proteins induced significant eosinophilia (70-83%). BLG and KLH had no effect on PF parameters following MCh or protein challenge. Protein challenge using SUB and OVA altered minute volume and tidal volume responses, but only mice exposed to SUB demonstrated airflow restriction. Specific IgE PCA titers were ranked SUB>OVA>KLH>BLG. Based on IgG ELISA titers SUB, OVA and KLH were immunologically more potent than BLG, but only SUB and OVA had high IgG1/IgG2a ratios suggesting a Th2-oriented immune response. Acute phase airway response to inhaled protein in "sensitized" mice was not a better predictor of respiratory sensitization potential than MCh challenge. WBP did not appear to be a sensitive measure in comparison to antibody measurements. Using these endpoints the MINT can be used to identify allergenicity potential of potent protein allergens. These endpoints may not be effective for classification of proteins with weaker potential, and appear to be confounded by proteins with strong immunogenicity.

824 RELATIVE ORAL BIOAVAILABILITY OF POLYCHLORINATED DIBENZO-*P*-DIOXINS/DIBENZOFURANS IN SOIL.

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Bioavailability is a key consideration in quantitative assessments of the potential risk posed to humans exposed to polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs). Because PCDD/Fs are persistent in the environment, ingestion of soil may be an important route of exposure in some circumstances. The oral bioavailability of PCDD/Fs in soil samples collected from five representative locations at an industrial site was evaluated in female Sprague Dawley rats. The PCDD/F content of the soil was dominated by dibenzofurans; dibenzo-*p*-dioxins were responsible for less than 5% of the soil TEQ. Relative bioavailability determinations were based on comparisons of the concentration of PCDD/Fs in the liver of rats treated with an aqueous soil suspension to those in animals treated with a reference formulation. The reference formulations were prepared in corn oil and contained the seventeen 2,3,7,8-substituted PCDD/Fs in proportions similar to those in the soil. Each animal received a single oral gavage dose of a soil suspension or reference formulation, and liver samples were collected 24 hours after dosing. Eight to 16 PCDD/F congeners were detected at sufficient concentrations to be included in the bioavailability determinations for each soil. The bioavailability of individual PCDD/Fs was inversely correlated with the degree of chlorination, and the mean values ranged from 15% for 1,2,3,7,8,9-HxCDF to 90% for 1,2,3,7,8-PeCDD. The overall relative bioavailability of PCDD/Fs in the soil samples (on a TEQ basis using the 2005 WHO toxic equivalency factors) ranged from 17 to 51% with a mean of 38%. There was no correlation between overall bioavailability and the concentration of PCDD/Fs in the soil samples. These data indicate that the oral bioavailability of PCDD/Fs can vary by congener or location and suggest that site-specific evaluations should be utilized in quantitative risk assessments. The results of this study also indicate that reliance on a default assumption of 100% bioavailability may result in substantially overestimated values for the risk posed by PCDD/Fs in soil from this site.

825 ABSOLUTE ORAL BIOAVAILABILITY OF POLYCHLORINATED DIBENZO-*P*-DIOXINS/DIBENZOFURANS IN SOIL.

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The absolute oral bioavailability of polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs) in soil samples collected from five locations at an industrial site in the southwestern United States was evaluated in female Sprague Dawley rats. Bioavailability was calculated by comparing the concentration of PCDD/Fs in the liver of rats treated with an aqueous soil suspension by oral gavage to those treated with a reference formulation by via a 3-hour intravenous infusion. The reference formulations contained the seventeen 2,3,7,8-substituted PCDD/Fs in proportions similar to those present in the soil. Each animal received a single dose of the reference formulation or soil sample, and the liver was collected 24 hours after dosing. The overall absolute bioavailability of PCDD/Fs in the soil samples (on a TEQ basis using the 2005 WHO toxic equivalency factors) ranged from 11% to 38% with a mean of 27%. There was no correlation between the overall bioavailability of PCDD/Fs and the TEQ concentration of the soil samples. The absorption of individual PCDD/Fs in the soil samples was inversely correlated with the degree of chlorination, and the mean absolute bioavailability values ranged from 4% for OCDF to 110% for 2,3,7,8-TCDF. It was believed that the short initial half-life of 2,3,7,8-TCDF, 1,2,3,7,8-PeCDD, and 1,2,3,7,8-PeCDF in liver resulted in the calculation of erroneously high absolute bioavailability values for those congeners. The bioavailability of 2,3,7,8-TCDD could not be determined due to its low concentration in soil at this site. The bioavailability values for the remaining thirteen congeners, which accounted for 89-96% of the TEQ concentration of the soil samples, were considered to be reliable. These data are suitable for use in quantitative risk assessments of PCDD/Fs in soil from this site and may be applicable to other experimental or environmental scenarios in which PCDD/F exposure occurs by the oral route.

826 POLYCHLORINATED DIBENZO-*P*-DIOXINS, POLYCHLORINATED DIBENZOFURANS AND DIOXIN-LIKE PCBs IN HUMAN ADIPOSE TISSUE IN TURKEY.

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During the twentieth century, production and use of toxic chemicals has increased rapidly thousands of chemicals have been introduced into the environment. Persistent organic pollutants (POPs) are very important group of these chemicals.

Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are 3 of the 12 UNEP internationally recognized POPs and also they are most important, toxic members of POPs. There is no previous report from Turkey on chemically determined polychlorinated dibenzo-para-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs) in human tissues expressed as World Health Organization (WHO) toxic equivalents (TEQs). The objective of this study was to determine the occurrence of PCDDs/Fs, and dioxin-like PCBs in the general adult Turkish population. For this reason we measured adipose tissue concentrations of PCDDs/Fs and dioxin-like PCBs in 23 Turkish men living in Ankara, Turkey in 2004. PCDD/F concentrations ranged between 3.2 and 19.7 pg WHO-TEQ/g fat (5.34 and 42.7 pg WHO-TEQ/g fat, respectively, including dioxin-like PCBs). The mean concentrations of WHOPCDD/F-TEQ and WHOPCB-TEQ were 9.2 and 6.67 pg/g on a lipid basis, respectively. Samples were analyzed for PCDD/F and twelve dioxin-like PCB congeners using high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). This study is very important since it is the first report on PCDDs/Fs and dioxin-like PCB contamination in human adipose tissue from Turkey.

827 UP-REGULATION OF CYCLOOXYGENASE-2 EXPRESSION BY 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN IN THE HUMAN ENDOTHELIAL-LIKE ECV304 CELL LINE.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), a prototype of many halogenated aromatic hydrocarbons, is a ubiquitous, persistent environmental contaminant and the most powerful tumor promoter in rodent bioassays. In the present study, we investigated the effects of TCDD on the cyclooxygenase-2 (COX-2) gene expression in the human endothelial-like ECV304 cell line. TCDD significantly increased the prostaglandin E2 production and the expression of COX-2. To investigate the significant cis-acting regions which COX-2 promoter, transient transfection experiments were carried out using reporter vectors harboring deleted COX-2 promoters. The transcriptional factor binding sites for CRE and C/EBP between -372 and -51 in COX-2 promoter could be important for the induction of COX-2 by TCDD. The results of these studies suggest that induction of transcriptional activation of COX-2 by TCDD might be mediated through the CRE and C/EBP activation.

828 A PUTATIVE ROLE OF BLIMP-1 AND AP-1 IN TCDD-INDUCED SUPPRESSION OF THE IGM RESPONSE IN B CELLS.

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a potent and persistent environmental pollutant. Many toxic effects of TCDD are mediated through the aryl hydrocarbon receptor (AhR). The primary humoral (IgM) response to bacterial lipopolysaccharide (LPS) in CH12.LX cells, a mouse B cell line, is markedly suppressed by TCDD. Pax5, a major repressor of spontaneous B cell differentiation, is downregulated by LPS. In the presence of TCDD, Pax5 downregulation is attenuated, in correlation with the suppression of the IgM response. Our objective was to identify the upstream factors involved in the attenuation of Pax5 downregulation in activated B cells in the presence of TCDD. Four distinct Pax5 isoforms were shown to be downregulated in CH12.LX cells by LPS and attenuated by TCDD in a similar manner over a 72h culture period, suggesting a common regulatory mechanism for each one of the isoforms. AhR binding to a putative DRE site in Pax5 promoter was weak, suggesting that additional transcriptional factors are involved. Blimp-1, an important regulator of B cell differentiation acts as a transcriptional repressor of Pax5. Blimp-1 mRNA was induced by LPS and suppressed by TCDD during the 72h culture period. By comparison, Blimp-1 DNA binding activity in the Pax5 promoter was induced by LPS and suppressed by TCDD. Similarly to Pax5, two putative DREs in the Blimp-1 promoter showed weak AhR-binding, suggesting that additional transcription factors are involved in the suppression of Blimp-1 by TCDD. Gel shift assays analyzing three putative AP-1 binding sites in the mouse Blimp-1 promoter showed an increase in AP-1 binding in the presence of LPS, which was attenuated in the presence of TCDD during the 72h time course. In summary, these data link AP-1 to the suppression of the IgM response by TCDD through the regulatory axis involving transcription factors Blimp-1 and Pax5. (NIH grants R01 ES02520 and P42 ES04911)

829 QUANTITATIVE MICROARRAY ANALYSIS WITH "PERCELLOME" METHOD IN 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN-TREATED MURINE FETAL PALATE.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is well known as a potent teratogen, causing cleft palate. The mechanisms of the cleft palate induction by TCDD are not well understood. In this study, we have applied a newly developed quantitative microarray method (designated as "Percellome" method) for identifying genes associated with the teratogenic effect of TCDD. On gestation day (GD) 12.5 (plug day GD 0.5), pregnant mice (C57BL/6) were given single oral administration of TCDD (20µg/kg bw). On GD13.5, 14.5 and 15.5, the fetal palate were dissected and pooled. Grade-dosed spike cocktail (GSC) was added to the sample homogenates in proportion to its DNA concentration and processed by the Affymetrix Standard protocol. The GeneChips used were MG-U74v2A. By using the Percellome method, data was expressed as mRNA copy numbers per cell. We have already demonstrated that Affymetrix GeneChip microarrays using the Percellome method and quantitative PCR showed high concordance (up to 90%). Microarray data obtained from TCDD-treated group was compared to no-treated control group at GD13.5, 14.5 and 15.5. Cyp1a, Cyp1b1, AhRR and Tiparp (TCDD-inducible poly (ADP-ribose) polymerase), which were known to be induced by TCDD, were strongly up-regulated. Cyp1a1 was increased at all time points to a maximum of about 500-fold. Cyp1b1 induction was approximately 70-fold less than for Cyp1a1. AhRR induction was approximately 100-fold. We also detected changes of many other genes in expression associated with apoptosis, cell cycle, metabolism, differentiation etc. These quantitative microarray data will contribute the understanding of the molecular mechanisms of TCDD-induced cleft palate.

830 ALTERED EXPRESSIONS OF ARTHRITIS-ASSOCIATED GENES IN CHONDROCYTES IN CULTURE FOLLOWING DIOXIN EXPOSURE.

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While effects of dioxin on bone are relatively well documented, effects on cartilage tissues are rarely understood. Joint disease is common among "Yusho" patients and PAHs in the cigarette smoke has an association with rheumatoid arthritis (RA). RA is a chronic inflammatory condition with cartilage degradation. Cartilage tissue is solely composed of chondrocytes which play an essential role in keeping cartilage integrity. Since the effects of chondrocytes upon the exposure of pathogens are important in the etiology of RA, we attempted to look into possible effects of dioxin in chondrocytes to understand the mechanism of environmental toxicant-derived arthritis. Articular chondrocytes isolated from cartilage slices of 2-week-old New Zealand White rabbits were exposed with TCDD. TCDD exposure for 24hr increased levels of CYP1A1, CYP1A2, CYP1B1 mRNAs and EROD activity. The induction was both dose- and AhR-dependent. A considerable amount of AhR, ARNT and HIF-1α mRNAs were also expressed. Cartilage degradation is preceded by the production of proinflammatory cytokines, reactive oxygen species and angiogenesis. In the present study, dioxin induced increases of IL-1β, iNOS, NF-κB, COX-II and VEGF mRNA levels. Considering roles of these genes in arthritic pathogenesis, our results suggest that dioxin may interfere with the interdependent regulation of arthritis-associated genes in primary chondrocytes and lead to the pathogenesis of arthritis. Levels of PKC-α, -βII, and -ε proteins were altered at 10nM TCDD. TCDD also increased ROS production in a dose-dependent fashion. Altered expressions of PKC isozymes were blocked with NAC (10mM) or Trolox (100uM), indicating that ROS may play a role in activation of certain PKC isozymes. Our results strongly indicate that chondrocyte is a target cell type sensitive to the dioxin exposure and provide a scientific basis to understand the mechanism of joint diseases with respect to environmental exposures. This is the first report on the effects of dioxin in the primary chondrocytes. Supported by RIC, MOICE.

831 ALTERATIONS IN FEEDING BEHAVIOUR IN DIOXIN-EXPOSED RATS.

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TCDD is the most potent and toxic compound in the group of polychlorinated dioxins and related halogenated aromatic hydrocarbons. Despite numerous studies, mechanisms for the toxic effects of TCDD are still largely unknown.

We have utilized an exceptionally wide sensitivity difference between two rat strains to gain more insight into one of the hallmark effects of TCDD, the wasting syndrome. TCDD-sensitive Long-Evans (L-E, Turku A/B) rats were exposed to either 10 or 100 µg/kg TCDD (UFA-Oil institute, Russia, intragastric administration [ig]), and extremely resistant Han/Wistar (H/W, Kuopio) rats to 1000 µg/kg dose of TCDD. TCDD was dissolved in corn oil (Sigma, MO, USA), which was administered to controls (4 ml/kg [ig]). Only male rats were used. Behavioural changes in feeding (intake of dust-free Bio-Serv® food pellets) and drinking were monitored continuously with Coulbourn Habitest® system and Graphic State 3.02 software, the data-analysis was completed with SPSS 13.0.

Follow-up times varied between 5 and 14 days after the exposure. TCDD decreased dose-dependently feed consumption in L-E rats, and this was accompanied by weight loss. H/W rats decreased their feeding for a few days after exposure, but the suppression leveled off in appr. 10 days. The exposed H/W rats lost about 10 % of their initial body weight during the first week, and then sustained the new weight level throughout the study (14 days). In drinking, no marked dose-related effects were detected. A detailed analysis of feeding behaviour revealed distinct differences between the rat strains. Especially after the high dose of TCDD (100 µg/kg), L-E rats exhibited reduced meal sizes and a decelerated eating rate coupled with a compensatory increase in the number of both feeding and drinking episodes. These findings suggest that TCDD treatment does not abolish the sensation of hunger but rather enhances the satiating properties of feed in rats.

832 DOSE RESPONSE OF TCDD-INDUCED HEPATIC BILIVERDIN ACCUMULATION IN FEMALE RATS.

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We have previously reported a new 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced liver toxicity syndrome characterized by hepatic accumulation of biliverdin, an intermediate product of heme degradation, and its derivatives. The syndrome displays clear genetic variability as most cases have been recorded in one rat line (line B) originating from the crossbreeding of the TCDD-sensitive and the TCDD-resistant rat strains Long-Evans (Turku/AB) and Han/Wistar (Kuopio; H/W), respectively. Line B rats express normal aryl hydrocarbon receptor but are moderately resistant to acute toxicity of TCDD due to H/W-type alleles of another, yet unidentified gene contributing to TCDD sensitivity ("gene B"). Current studies aim at elucidating the mechanism of biliverdin accumulation as this might have implications for the toxic mechanisms of TCDD. Using HPLC with UV-VIS detection we measured the hepatic concentration (nmol/g) of biliverdin and biliverdin derivatives in line B female rats 28 days after intragastric exposure to 3, 10, 30, 100, 200 or 300 µg/kg of TCDD in order to define a dose-response relationship for the TCDD exposure and biliverdin accumulation. In addition, bilirubin, the end product of heme degradation, and its derivatives were quantified. Mean biliverdin concentration in control group was 1 nmol/g; other analytes were undetectable. 3-30 µg/kg of TCDD did not induce significant increases in any of the analytes. When TCDD dose was 100 µg/kg or more, the concentrations of biliverdin, bilirubin and their derivatives were dose-dependently elevated, except that the maximal response of biliverdin derivatives (49 nmol/g) was reached already when the dose was 200 µg/kg. Biliverdin derivatives were the main accumulating form of biliverdin as their amount was three- to tenfold relative to free biliverdin in groups dosed with 100-300 µg/kg of TCDD. Interestingly, the individual-level correlation between biliverdin and bilirubin concentrations was poor. Further research is needed to reveal the immediate precursor of the accumulating biliverdin.

833 EFFECTS OF TCDD ON DIFFERENTIATION OF BONE CELLS.

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Environmental contaminant TCDD has been shown to affect bone growth, modeling and mechanical strength in our earlier experiments *in vivo*. These TCDD-induced effects seem to be mediated by AhR receptor, but the detailed mechanisms are still unknown. Bone remodelling is a constant process involving resorption of bone by osteoclasts and formation of equal package of new bone by osteoblasts. In this study, we utilized differentiation of bone marrow stem cells to osteoblasts and osteoclasts as a model system to study the effects of TCDD on bones. Stem cells isolated from bone marrow of femurs and tibias of Sprague-Dawley rats or C57BL/6 mice were differentiated to both osteoclasts and osteoblasts. Progress of osteoblastic differentiation was monitored by measuring mRNA expression levels of differentiation markers from control and TCDD-treated cells using quantitative RT-PCR. TCDD significantly decreased the mRNA levels of all three measured markers of

osteoblastic differentiation, of which transcription factor RUNX2 is expressed in a period of active proliferation, alkaline phosphatase in matrix maturation and osteocalcin in the onset of mineralization. In addition, TCDD decreased significantly the amount of calcium depositions. Cell proliferation, however, was not affected. In the case of differentiating osteoclasts, the number of tartrate-resistant acid phosphatase (TRAPC) -cells and resorbing activity were used as differentiation markers. TCDD decreased the number of TRAPC-positive multinucleated cells and also the area of bone resorption. Both osteoblastic and osteoclastic effects took place at very low doses of TCDD; even 10 pM TCDD was enough to significantly affect the differentiation markers. Therefore, differentiation of osteoblasts and osteoclasts from bone marrow stem cells seems to be a very sensitive target for TCDD. Disturbed osteoclastogenesis, in addition to disrupting effects in osteoblastic cells, may play a role in the weakening of bone quality in TCDD exposed animals.

834 9-CIS-4-OXO-13, 14-DIHYDRO-RETINOIC ACID (9C-4O-DH-RA) – A NEW SENSITIVE BIOMARKER FOR TCDD TOXICITY.

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Dioxins are known to alter endogenous retinoid contents in various tissues. One toxicological endpoint after TCDD exposure is the decrease of hepatic retinyl-ester content. Here we show that the retinoic acid metabolite – 9c-4o-dh-RA – which was characterized as an endogenous retinoid occurring in the livers of mice, rats and humans, is especially sensitive to TCDD and that this TCDD/retinoid interaction is AhR-receptor (AhR) dependent. We analyzed the retinoid contents in rat and mouse liver from four TCDD exposure studies. These studies include a time-course experiment with adult SD-rats (1), a long-term low-dose study with Long-Evans rats (2), an *in utero*/lactational exposure study with SD-rats (3) and an experiment with AhR-KO mice (4). (1) 3 days after a single oral dose (10 µg/kg) the liver concentration of the metabolite decreased to 10% of the initial concentration, remained at this level until day 28 and recovered to the initial level on day 112. (2) The rats were dosed by sc injection once a week at calculated daily doses ranging from 0 to 100 ng/kg for 20 weeks. The liver concentration of the metabolite decreased to 36% of the control value at the low dose (1 ng/kg) and further under the detection limit at 10 ng/kg. (3) Pregnant SD rats were given a single dose (0.1 µg/kg) on GD 11. A dose dependent decrease in the liver concentration of the metabolite was shown in pups at PND 7 and PND 35 starting from the low dose (0.03 µg/kg). (4) AhR-KO and WT mice were given a total of 0 or 200 µg/kg over a period of 10 weeks. In TCDD exposed WT mice the metabolite's concentration decreased under the detection level whereas in AhR-KO mice it remained at the same level as in non-exposed animals. These results show that 9c-4o-dh-RA is a very sensitive marker of TCDD exposure. The retinoid's decrease correlates with increased TCDD levels. This interrelationship between the novel retinoid and TCDD is AhR dependent.

835 INDUCTION OF EROD ACTIVITY *IN VITRO* BY 2, 3, 7, 8 TETRACHLORINATED N-METHYL AND SULFOXO PHENOTHIAZINES.

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The structural dioxin analogue 2,3,7,8-tetrachlorophenothiazine (TCPT) is a known high affinity AhR ligand and an agonist regarding the induction of CYP1A1 activity *in vitro*. The expected main metabolites of TCPT are its two sulfoxo derivatives and its hydroxylamine derivative. Ring hydroxylation is likely to play only a minor role, if any, since TCPT lacks the required vacant vicinal aryl positions. In order to investigate the role (potency, efficacy) of TCPT-metabolites regarding EROD induction via the AhR-mediated pathway, TCPT and five derivatives were studied *in vitro* in the rat hepatoma cell line H4IIEC/T3. The following phenothiazine derivatives were compared to the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) after induction for 12, 24, 48, and 72 h: TCPT, 5-oxo-TCPT (TCPT-O), 5,5-dioxo-TCPT (TCPT-O2), N-methyl-TCPT (Me-TCPT), N-methyl-5-oxo-TCPT (Me-TCPT-O), and N-methyl-5,5-dioxo-TCPT (Me-TCPT-O2). N-methylated derivatives of TCPT were used as models for inhibited hydroxylation of the heterocyclic amine.

It was discovered that potency decreases with increasing degree of oxidation, which is associated with greater molecular bulk and changed sterics of the respective molecules compared to each parent compound. The only derivative with almost constant, though low, potency was Me-TCPT-O2, which provides very little access to metabolic transformation. The potency of the other TCPT derivatives was deter-

mined to be time-dependent under the experimental conditions used. This reduction in potency over time is most likely the result of a combination of biotransformation and its eventual saturation in this *in vitro* system. Relative to TCDD, all phenothiazine derivatives retained high induction efficacy at high concentrations, and at later time points.

836 GESTATIONAL EXPOSURE TO 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN ADVERSELY AFFECTS LUNG DEVELOPMENT.

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In mature laboratory animals, adverse effects on the lung have been observed after chronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and include lesions such as alveolar-bronchiolar metaplasia, hyperplasia of the bronchiolar epithelium, adenomatous hyperplasia and keratinizing squamous cell carcinoma. We hypothesized that the developing lung may also be adversely affected by TCDD which may represent a significant factor contributing to the high rate of perinatal mortality observed after gestational exposure. Pregnant Holtzman rats received a single dose of TCDD (1.5 or 6 µg/kg, po) on gestation day (GD) 10 or a vehicle control with fetal and neonatal analysis occurring on GD20 or post natal day (PND) 7. High rates of mortality were observed on GD20 (18% and 30%) and PND7 (40% and 100%) in the TCDD exposure groups (1.5 and 6 µg/kg TCDD, respectively). Significant decreases were observed in relative lung weight and the normalized lung to body weight ratio in the 6 µg/kg exposure group on GD20 and in the 1.5 µg/kg exposure group on PND7. Real time PCR was used to assess the gene expression of several genes including Ahr, Arnt, Ahrr and Cyp1a1 in the fetal lung on GD20. While the expression levels of Ahr and Arnt were unaltered between the control and treated animals, Cyp1a1 and Ahrr were both dramatically induced by TCDD exposure giving light to a responsive AhR-signaling pathway within the developing lung. Pups that were gestationally exposed to TCDD appeared to have retarded lung development which was manifested as a significant decrease in lung sacular area when compared to age-matched controls when morphology was assessed through histological examination of H&E stained slides. Together, these results identify the developing lung as target for the toxicity of TCDD and support the need for further mechanistic studies.

837 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) REVERSES HYPERGLYCEMIA IN A TYPE II DIABETES RAT MODEL.

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Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been linked to increased risk for diabetes in humans. The key clinical sign of human type II diabetes is hyperglycemia resulting from insulin resistance. A suitable animal model for the induction of diabetes by TCDD has not yet been found. An established diabetic rat model was used for the study of the effects of TCDD on this disease as a pre-existing condition. Type II diabetes was induced by a high fat diet and the administration of streptozotocin. After the complete manifestation of diabetes, monitored by measuring serum glucose levels, male Sprague Dawley rats received a loading dose rate of up to 12.8 µg/kg p.o. TCDD followed by weekly maintenance dose rates to replace the AUC lost during the week. Serum parameters measured before the first administration of TCDD (day 0) marked the clinical picture of type II diabetes in rats (412 ± 58.5 mg/dL glucose). Healthy negative controls were fed the same high fat diet as treated animals, without receiving streptozotocin or TCDD, but they were administered the respective vehicles. By day 2 after the loading dose rate of TCDD, serum glucose in streptozotocin treated high-dose animals was already significantly lower (202.6 ± 23.8 mg/dL glucose) than in diabetic rats, but still above control levels (160.1 ± 4.0 mg/dL glucose). By day 8, diabetic rats treated with TCDD had reached serum glucose levels of healthy controls, which they maintained throughout the remainder of the study (32 days after a loading dose rate of TCDD). These results demonstrate that TCDD effectively counteracts hyperglycemia as a result of type II diabetes in this rat model. It is assumed that the mechanism of action is sensitization of the animals to the action of insulin as suggested by earlier work (Gorski et al. 1987).

838 CPLA2 IS AN EARLY COMPONENT OF TCDD'S INFLAMMATORY SIGNALING.

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The main focus of this project has been to understand and characterize 2,3,7,8-tetrachlorodibenzo-p-dioxin's (TCDD's) inflammatory signaling pathway in human macrophage cells. TCDD is a ubiquitous environmental pollutant that ex-

hibits complex signaling and diverse end-point toxicities. This study focuses on the highly potent inflammatory cascade evoked by TCDD following its subsequent AhR activation. Using RT-PCR we have discovered a novel pro-inflammatory gene (cPLA2) that is activated following treatment with TCDD. We then performed inhibitor studies and used RT-PCR to evaluate any possible effects on well-known up-regulated inflammatory genes downstream. Reporter assays were then performed using the enzyme luciferase for cPLA2's promoter region. Inhibitor studies were performed to confirm AhR dependency and to test the specific actions of the inhibitors tested. Radiolabeled arachidonic acid was used to show a functional activation of cPLA2 following treatment by TCDD. TCDD exposure has been shown to result in increased levels of lipid peroxidation in a number of liver models. Using our human macrophage model, we tested for lipid peroxidation following TCDD treatment as well as diagnostic inhibitors specific for cPLA2. Our results indicate that TCDD treatment stimulates the up-regulation of cPLA2 mRNA in human macrophage cells as early as 3 hours. We found that by treating cells with AACOCF3, a specific blocker for cPLA2, a significant decrease was seen on well-known inflammatory genes induced by TCDD. In addition, we could show that TCDD stimulated cPLA2 at the promoter level, and that this effect was AhR-dependent. In addition, we could show that TCDD stimulates the release of arachidonic acid further supporting a functional activation of cPLA2. We could also show an increase in lipid peroxidation in human macrophage cells following TCDD treatment that was cPLA2-dependent. In summary, the data indicate that TCDD treatment results in a rapid activation of cPLA2 that is a necessary component of its inflammatory signaling in human macrophage cells.

839 CYTOCHROME P450 MEDIATED BIOTRANSFORMATION OF 2, 2', 6, 6'-TETRACHLOROBIPHENYL.

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The objective of this study was to identify the cytochrome P450 (CYP) enzymes involved in the biotransformation of 2,2',6,6'-tetrachlorobiphenyl (PCB 54). PCB 54 is a tetra-ortho substituted, non-coplanar polychlorinated biphenyl (PCB). Little is known regarding the metabolism of highly ortho-substituted PCBs. PCB 54 was incubated with liver microsomes prepared from Long-Evans rats treated with phenobarbital (PB, 80 mg/kg/day x 3 days), dexamethasone (DEX, 100 mg/kg/day x 3 days), 3-methylcholanthrene (25 mg/kg/day x 3 days) and corn oil. A validated and optimized method to analyze PCB 54 biotransformation was developed using liquid chromatography-negative electrospray mass spectrometry. The sole metabolite produced was a monohydroxyl metabolite. Based upon a difference in retention times, the hydroxylated metabolite was not 2,2',6,6'-tetrachloro-4-biphenylol (4-OH-CB54). Due to a lack of standards, the structure of the metabolite could not be determined. In order to quantify metabolite production, 4-OH-CB54 was used as a surrogate standard. No metabolite was detectable when PCB 54 was incubated with hepatic microsomes from MC- or corn oil-treated rats. The rate of product formation was greater in assays using hepatic microsomes of PB-treated rats (7200 pmol/min/mg protein) than assays using hepatic microsomes of DEX-treated rats (550 pmol/min/mg protein). The involvement of CYP enzymes was investigated further using a panel of recombinant rat CYP enzymes. Only recombinant CYP2B1 (5.9 pmol/min/pmol CYP) and CYP3A1 (0.03 pmol/min/pmol CYP) hydroxylated PCB 54 to the monohydroxyl metabolite. No metabolites were detectable when PCB 54 was incubated SUPERSOMES expressing CYP1A2, CYP2A2, CYP2C6, CYP2C11, CYP2C13, CYP3A2 or CYP2D1. In summary, these results demonstrate the involvement of the CYP2B enzymes and CYP3A enzymes in the biotransformation of PCB 54.

840 CHANGES IN PROTEOMIC PROFILES OF CEREBELLUM AND HIPPOCAMPUS FOLLOWING EXPOSURE TO A DEVELOPMENTAL NEUROTOXICANT.

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The vast literature on polychlorinated biphenyls (PCBs) makes it a unique model to understand major issues related to persistent chemicals. At background exposure levels, PCBs have been shown to affect human health, including learning and memory. Although the molecular basis is still unclear, a series of *in vitro* and *in vivo* studies have revealed that in addition to the effects on thyroid hormones, the disruption of Ca²⁺-mediated signal transduction play significant roles in PCB-induced developmental neurotoxicity. The culminating event in a variety of signal transduction pathways is the regulation of gene and protein expression, which ultimately affect the growth and function of the nervous system. Our previous results showed changes in gene expression profiles related to signal transduction and neuronal growth. In this study, the protein expression profiles in cerebellum and hippocampus following PCB developmental exposure were examined. Pregnant rats (Long

Evans) were dosed perinatally with 0 or 6 mg/kg/day of Aroclor 1254 (AccuStandard Inc., Lot # 124-191) from gestation day 6 through postnatal day (PND) 21, and the cerebellum and the hippocampus from PND14 animals were isolated by two-dimensional differential gel electrophoresis using fluorescent cyanine dyes and identified by mass spectrometry (MS). Two proteins were found to be differentially expressed in the cerebellum following chemical exposure while 18 proteins were differentially expressed in the hippocampus. MS identification revealed that these proteins are related to energy metabolism in mitochondria (creatine kinase and malate dehydrogenase), calcium signaling (endoplasmic reticulum ATPase and Ryanodine receptor type II) or growth of the nervous system (collapsing response mediator protein 3 (CRMP-3)). These results suggest that changes in energy metabolism and intracellular signaling may be involved in the developmental neurotoxicity of persistent chemicals. (This abstract does not necessarily reflect USEPA policy).

841 POLYCHLORINATED BIPHENYLS ALTER TIGHT JUNCTION PROTEIN DISTRIBUTION AND EXPRESSION IN HUMAN BRAIN MICROVESSEL ENDOTHELIAL CELLS.

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Polychlorinated biphenyl (PCB) congeners and related compounds exhibit a broad range of adverse biological effects from carcinogenesis, tumor-promoting effects to neurotoxicity. The mechanisms by which PCBs cause neurotoxic effects are still not completely understood. The blood-brain barrier (BBB) is a physical and metabolic barrier separating the microenvironment of the central nervous system (CNS) from the peripheral circulation and is mainly composed of endothelial cells connected by tight junctions. Tight junctions consist of the integral membrane proteins such as occludin, claudins, and junction adhesion molecules (JAMs) as well as the cytosolic accessory proteins such as zonula occludens (ZO) or AF6 that form a scaffold to link tight junction proteins to the cytoskeleton. In the present study, we examined the effects of exposure to different PCB congeners on expression of tight junction proteins in human brain endothelial cells. Several highly-chlorinated PCBs, such as 3,3',4,4'-tetrachlorobiphenyl (PCB77), 2,2',4,5,5'-pentachlorobiphenyl (PCB104), 2,3',4,4',5-pentachlorobiphenyl (PCB118), 3,3',4,4',5-pentachlorobiphenyl (PCB126), and 2,2',4,4',5,5'-hexachloro-biphenyl (PCB153) were employed in these experiments. Treatment for 24 h with specific PCB congeners at the concentration of 5 µM resulted in a disrupted pattern of plasma membrane expression of the tight junction accessory proteins AF6, ZO-1 and ZO-2. In contrast, PCB exposure did not alter JAM-A and claudin-1 expression. These data suggest that PCB-mediated alterations of tight junction protein expression may disrupt the BBB integrity and thus contribute to the development of the neurotoxic effects in the CNS. Supported by NIH/NIEHS (P42 ES 07380).

842 CROSS-TALK BETWEEN GLUCOCORTICOID AND AH RECEPTORS: IMPLICATIONS FOR CALUX/CALFUX DIOXIN BIOASSAY ANALYSIS.

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Halogenated aromatic hydrocarbons (HAHs), including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin) and related dioxin-like chemicals, are highly toxic environmental contaminants. Since at present the instrumental quantitation of HAHs is costly and time consuming, there is the need for validated alternative assay methods that are able to reliably estimate the overall relative TCDD-like activity in samples. For a scientifically sound use of the data generated, it is necessary to identify and characterize factors that can impact the outcome of these assays. Activation of the glucocorticoid receptor (GR) has been shown to alter HAH-induced gene expression, consequently we hypothesized that the presence of the GR activating compounds such as dexamethasone (Dex) in samples could have an impact on our Ah receptor (AhR)-based cell bioassays, CALUX and CALFUX. Co-exposure to Dex significantly increased in a concentration-dependent manner both luciferase activity and green fluorescent protein expression induced by TCDD in rat cells and this enhancement was significantly inhibited by the GR antagonist RU486. In fact, exposure to Dex induced reporter gene activity even in the absence of TCDD. Using different CALUX cell bioassays we found that the Dex induction or enhancement effects were species-specific, occurring in rat and human but not mouse and Guinea pig cells, and dependent upon the dioxin-responsive domain from the upstream region of the mouse CYP1A1. While identity of the specific regulatory element(s) are still unknown, these results clearly demonstrated that the use of rat or human cells for dioxin bioassay analysis could be problematic and produce false positives if GR activating chemicals are present in the sample extract. Our results highlight the importance of using the correct cell line for the screening of dioxin-like compounds in various matrices and of the purification of the sample extracts as an integral part of the bioassays. NIEHS ES04699, ES5707

843 EXPOSURE TO PBDES AND BTBPE IN THE INDOOR ENVIRONMENT.

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Polybrominated diphenyl ethers (PBDEs) are used as fire retardants in foam-containing furniture and electronics. 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) is being produced as a replacement for octaBDE. We previously showed a strong association, independent of diet, between penta-like PBDE concentrations in breast milk and dust sampled from participants' homes (collected using a standard protocol), implying exposure from the indoor environment, possibly via dust ingestion. We found no relationship between PBDE levels in breast milk or dust and home characteristics, but dust had been composited from more than one room per home. In the current study, we therefore collected three different dust samples from each of 20 homes: one from the participants' vacuum cleaner bag, and two from the bedroom and living room using our protocol (vacuuming measured areas using a Eureka Mighty-Mite with a cellulose extraction thimble inserted in the crevice tool). Dust samples were sieved, extracted and analyzed by GC/MS. We collected information by room on foam furniture, electronics and carpeting. Concentrations of PBDEs and BTBPE in house dust were lognormally distributed. Geometric mean (GM) concentrations of total PBDEs were highest in the living room (13900 ng/g, 33% BDE 209), followed by the bedroom and vacuum cleaner bag. Correlations between the three types of samples ranged from 0.1 to 0.6. The GM concentrations of BTBPE were much lower, 8.2 ng/g overall, and not correlated with PBDEs. We found no associations between dust concentrations by room and room contents. The BDE 197/BDE 201 ratio of a number samples was consistent with possible breakdown of decaBDE.

Our results suggest: 1) at least for PBDEs, vacuum cleaner bags and the crevice tool method are not particularly good surrogates for each other; 2) PBDE concentrations are not uniform within homes. BTBPE has not been previously found in house dust. Predicting PBDE concentrations in dust based on room characteristics remains an outstanding issue.

844 FREE RADICAL FORMATION POTENTIAL OF DECA-BROMODIPHENYL ETHER (DECA-BDE).

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Recent studies report increasing levels of polybrominated diphenyl ethers (PBDEs) in humans and our environment. A deca-BDE mixture is still widely manufactured and used. Deca-BDE absorbs UV light and is degraded to lower BDEs by solar or artificial irradiation. We hypothesized that this UV degradation is accompanied by free radical formation, which will result in synergistic toxicity of UV plus deca-BDE. As first step to test this hypothesis we examined UV-induced radical formation. For this purpose a commercial deca-BDE mixture (DE-83R, Great Lakes, USA) was dissolved in tetrahydrofuran (THF). Electron spin resonance (ESR) was used to monitor the generation of free radicals using 5,5-dimethyl-1-pyrroline N-oxide (DMPO) as a spin trap. Samples were purged with argon to remove oxygen and irradiated with a xenon lamp. A 307 nm cut-off filter was used to avoid absorption by DMPO at shorter wavelengths. Irradiation of deca-BDE under these conditions resulted in ESR spectra that were distinctly different from pure THF. Radical formation was positively correlated with both, the deca-BDE concentration and irradiation time. The hyperfine splittings suggest that with deca-BDE and light, carbon-centered radicals are dominant, whereas oxygen-centered radicals dominate in pure THF. The spectral pattern from deca-BDE changed and was similar to the pattern of THF alone, if a 400 nm cut-off filter was used with the BDE, indicating that the activating wavelength is between 307 and 400 nm, i.e. in the UVA to high UVB range. The spectrum from non-brominated diphenyl ether was different from that of deca-BDE, but the same as from pure THF, indicating the involvement of bromine. Our findings that UV irradiation of deca-BDE in THF leads to free radical formation indicates that synergism in toxicity between PBDEs and UV light should be examined. (Supported by ES013661 from NIEHS, DAMD17-02-1-0241 from DOD, and R-82902102-0 from EPA)

845 LACK OF ALTERATIONS IN THYROID HORMONES FOLLOWING A SINGLE POSTNATAL EXPOSURE TO POLYBROMINATED DIPHENYL ETHER 47.

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Polybrominated diphenyl ethers (PBDEs) are commonly used as commercial flame retardants in a variety of products including plastics and textiles. There has been a rapid accumulation of these chemicals in the environment and high levels of

PBDEs have been found in the adipose tissue, blood, and breast milk of humans. This is especially true for the congener, 2,2',4,4'-tetrabromodiphenyl ether (BDE 47), which has shown an accelerated accumulation in humans in recent years. Concern over BDE 47 exposure has also stemmed from the similarity of its molecular structure with that of polychlorinated biphenyls (PCBs) which are known to disrupt thyroid hormone homeostasis. Furthermore, there are reports of thyroid alterations following perinatal exposure to PBDE mixtures. Disruption of thyroid hormone levels has been shown to be important in development of the brain due to its role in neurite outgrowth, synapse formation, and neuronal guidance. Thus disruptions in thyroid hormone levels may underlie certain behavior deficits observed in animals following PBDE exposure, such as reduced habituation and learning and memory abnormalities. The aim of this study was to determine whether a single exposure to BDE 47 in C57BL/6 mice would alter thyroid hormone levels during a phase of rapid brain development. Male mice were dosed with either 0 (corn oil vehicle), 1, 10, or 30 mg/kg on postnatal day 10, and sacrificed for collection of serum at 1, 5, or 10 days after the dose (n=5-6 litters/dose/time point). Body weight gain was not altered. Total thyroxine (T4) and triiodothyronine (T3) were measured by RIA. No effects of BDE 47 were observed on the T4 levels at any age examined; T3 assays on remaining sera (n=2-5/dose/time point) also showed no changes. This indicates that alterations in thyroid hormone levels may not contribute to the purported behavior abnormalities seen in mice following acute neonatal exposure to BDE 47. This is an abstract of a proposed presentation and does not reflect US EPA policy.

846 EVALUATION OF THE DEVELOPMENTAL TOXICITY OF A POLYBROMINATED DIPHENYL ETHER MIXTURE (DE-71) IN THE ANURAN, *XENOPUS* (SILURANA) TROPICALIS.

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The commercial polybrominated diphenyl ether (PBDE) flame retardant, DE-71 was selected for further examination of developmental toxicity because of its ubiquity in the environment and potential to disturb endocrine activity. DE-71 was previously found to inhibit progesterone, and to a lesser extent androgen-induced, in vitro oocyte maturation in *Xenopus*. Therefore, a partial lifecycle study exposing *X. (Silurana) tropicalis* to 0.0, 0.43, 0.9, 2.1, and 3.8 mg/L DE-71 from early embryo through the completion of metamorphosis was conducted. Endpoints measured included the rate of metamorphosis, whole body weight, external malformation, internal abnormalities, gender ratio, and whole body PBDE residue. A concentration-dependent increase in whole body DE-71 residues was measured ranging from 43.4 mg/Kg to 203.3 mg/Kg in the 0.43 mg/L and 3.8 mg/L DE-71 treatments. The rate of metamorphosis decreased slightly with increasing concentration from 39 (37-41) d in control organisms to 44 (42-46) d in 3.8 mg/L DE-71 treatment. However, no marked effect on thyroid gland histology was found in specimens from any of the PBDE treatments. Whole body weight in newly metamorphosed organisms increased with increasing DE-71 exposure concentration. Gender ratios (male:female) were similar to the control in the 0.43 mg/L and 0.90 mg/L DE-71 exposure treatments. However, an increase in the proportion of females was observed in the 2.1 and 3.8 mg/L DE-71 treatments. No evidence of induction of external abnormalities was noted as the result of DE-71 exposure. However, a concentration-related increase in liver necrosis (males and females), and abnormal ovary development characterized by previtellogenic oocyte necrosis and arrested development in pools of vitellogenic oocytes in females were observed. No apparent effect on testis morphology was observed, although further examination of spermatogenesis is warranted. Results suggest that PBDEs are capable of interfering with *X. (Silurana) tropicalis* development.

847 THE POLYBROMINATED BYPHENYL ETHER MIXTURE DE-71 IS MILDLY ESTROGENIC.

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Our goal is to determine if the polybrominated diphenyl ether (PBDE) mixture DE-71 has estrogenic activity. METHODS: Ovariectomized BALB/c, C57BL/6, and ER α knockout (ER α KO) mice were treated for 5 weeks with vehicle, DE-71, estradiol benzoate (EB), or both DE-71 and EB. MCF-7 cell proliferation was assessed after 10-day incubation with either estradiol (E2), DE-71, antiestrogen, or combinations of DE-71 and E2 or antiestrogen. To test if metabolic activation occurred, DE-71 was incubated with female rat microsomes in a NADPH regenerating buffer. Extracted incubations and controls were tested for estrogenic activity

using an Estrogen Response Element (ERE) - luciferase reporter gene construct, and 3H-estradiol displacement from recombinant estrogen receptor alpha (ER α). RESULTS: DE-71 increased uterine weight in C57BL/6 mice over control, but not in BALB/c or ER α KO. DE-71 slightly increased uterine epithelial height (UEH) and vaginal epithelial thickness over control, and attenuated the EB-induced increase in UEH in BALB/c mice, but not in C57BL/6 or ER α KO. Both DE-71 and EB increased liver weight in BALB/c mice and their effects were additive. DE-71 but not EB increased liver size in C57BL/6 mice. DE-71 increased MCF-7 cell proliferation after 10-day incubation and this increase was prevented by co-treatment with the antiestrogen fulvestrant. When cells were co-treated with E2 and DE-71, there was a decrease in cell proliferation compared with E2 treatment alone. While DE-71 by itself weakly activates estrogen-responsive luciferase reporter constructs, this activation increased significantly when DE-71 was pre-incubated with liver microsomes. Ongoing experiments are aimed towards determining the effect of metabolic hydroxylation on the estrogenic activity of DE-71. Supported by NIEHS Grant # 1 F31 ES013341

848 DEVELOPMENTAL TOXICITY OF POLYBROMINATED DIPHENYL ETHER 47 IN THE AMPHIBIAN MODEL SPECIES *XENOPUS LAEVIS*.

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Polybrominated diphenyl ethers (PBDEs), used as flame retardants, are found ubiquitously in the environment. PBDEs have been shown to affect thyroid axis homeostasis in mammal, avian, and fish species. Effects on the thyroid axis in those species were observed as decreased circulating T4 levels. Recently, we have developed an amphibian screening assay for evaluating the effects of xenobiotics on the thyroid axis. In this study, we used this assay to determine if PBDE-47, the major component of DE-71, a specific flame retardant formulation, has antithyroid effects in *X. laevis* similar to those observed in other species. Stage 51 tadpoles were fed a control sera micron diet or a diet spiked with PBDE-47 concentrations of 1.58, 4.57, 9.29, and 19.6 mg/g. After 21 days of dietary exposure, all tadpoles were weighed and evaluated for developmental stage. Blood was collected from a subset for the analysis of serum T4 concentrations. All remaining animals were frozen for subsequent analysis of whole body PBDE-47 concentrations. Treatment had little or no effect on growth at concentrations of ≤ 9.29 . However, there were obvious signs of toxicity, as determined by a significant decrease in weight at 19.6 mg/g. PBDE-47 treatment also resulted in a dose dependent developmental delay at concentrations of 4.57 mg/g and above. As a result T4 measurements were made only in the 1.58 and 4.57 mg/g treatment groups, where no effects on serum T4 levels were observed. These results indicate that PBDE-47 is developmentally toxic to *X. laevis*, resulting in decreased growth at higher dietary concentrations and retarded development at all but the lowest dietary concentrations. Since these effects were observed in the absence of altered serum T4 concentrations, the mechanism of PBDE-47-mediated developmental toxicity remains unclear for this species. This abstract does not necessarily reflect EPA policy

849 PERINATAL EXPOSURE TO DE-71 ALTERS EXPRESSION OF HEPATIC GENES INVOLVED IN THYROID HORMONE DISRUPTION IN MALE RATS.

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DE-71, commercial mixture containing mostly tetra- and penta-bromodiphenyl ethers, has been commonly used as a flame retardant in polyurethane foam. These PBDE congeners are of concern because they are ubiquitous in our environment and increasing in human tissue. Developmental studies show exposure to PBDEs may affect thyroid hormone levels by inducing hepatic uridine diphosphate-glucuronosyltransferases (UGTs) and transthyretin (Ttr) binding. This study examines effects of DE-71 on genes related to thyroid hormone disruption at different developmental time points in male rats. Pregnant Long-Evans rats were orally gavaged at 0, 1.7 (low), 10.2 (mid) or 30.6 (high) mg/kg/day of DE-71 in corn oil from gestational day 6 to postnatal day (PND) 21. For pups at PND4 and PND22, serum was collected for thyroid hormone levels and livers were analyzed for mRNA expression by RT-PCR. Serum total T4 concentrations were unchanged at PND4 while they decreased 50% (mid) and 80% (high) at PND22. Ugt1a1 mRNA remained unchanged at PND4 while a 30% decrease was observed in the high dose at PND22. Ugt1a6 increased 2 (low), 8 (mid) and 21 (high)-fold at PND4 while there was no change observed at PND22. At PND4, Ugt1a7 increased 2-fold (mid) and 3-fold (high) while there was a 3-fold increase at PND22 (high). Ugt2b increased 5-fold (high) at PND4 and 58-fold (high) at PND22. At PND4, deiodinase I mRNA was reduced 40% (mid) and 50% (high) while at PND22 decreased 30% (high). Ttr mRNA was unchanged at PND4 and reduced 30% (high) at

PND22. This study demonstrates perinatal exposure to DE-71 alters the expression of genes involved in thyroid hormone homeostasis although total T4 concentration were not significantly decreased at PND4. Thus, the alterations in gene expression are dose- and time-dependent but may not necessarily correlate with significant decreases in circulating thyroid hormone. (This abstract does not necessarily reflect USEPA policy. Supported in part by NIH T32 ES007126)

850 POLYBROMINATED DIPHENYL ETHERS AND THYROID HORMONE METABOLISM IN CARP (CYPRINUS CARPIO).

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The debromination of polybrominated diphenyl ether (BDEs) congeners has been demonstrated in whale, rat, rainbow trout and carp microsomes. This research investigated a potential mechanism of BDE debromination and endocrine associated impacts of BDE exposure in carp. Fish were fed 2,2',4,4',5 pentabromodiphenyl ether (BDE-99) spiked food for 62 days and allowed to depurate for 5 days. Total plasma thyronine (T3) and thyroxine (T4) concentrations were assessed by radioimmunoassay. Liver and intestinal deiodination activity was assessed by radioactive deiodination assays. Iodothyronine products were analyzed by high performance liquid chromatography. Results indicated that total plasma T3 levels were depressed in dosed fish as compared with controls. On day 30, intestinal and hepatic microsomes from exposed fish exhibited lower T4 outer ring deiodination activity than controls. Further, intestinal microsomes from BDE-99 exposed fish exhibited lower T3 inner ring deiodination than controls on day 10 through day 62. Additional in vitro deiodination studies assessed the impacts of BDEs on deiodinase (DI) enzyme activity. Four sets of carp intestinal and hepatic microsomes were incubated with radioactive thyroid hormones, 2,4,4',6-tetrabromodiphenyl ether (BDE-75), BDE-99, or decabromodiphenyl ether (BDE-209), a DI inhibitor (iodoacetic acid), and/or competitor (reverse thyronine). Results indicated that BDE-75 inhibited DI activity whereas BDE-99 and BDE-209 had no effect. Together the carp in vivo and in vitro BDE studies suggest that: 1) BDE-99 and BDE-209 debromination are not likely occurring by deiodinase enzymes, 2) a BDE-99 metabolite may be responsible for the lower plasma thyroid hormone levels and deiodination activities and 3) tetra brominated BDEs may have the ability to interrupt thyroid hormone metabolism at the enzymatic level.

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851 EFFECT OF BDE-47 ON HEPATIC DRUG METABOLIZING ENZYMES IN ISOGENIC RAINBOW TROUT.

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Polybrominated diphenyl ethers have been used extensively as flame retardants over the past two decades. BDE-47 is the dominant congener in water systems and is found in fish from many parts of the world. There are increasing concerns about the effects of this contaminant on aquatic and upstream species, particularly if there is bioaccumulation. BDE-47 alters gene expression in trout, induces activity of the major hepatic drug metabolizing enzyme CYP1A, at low concentrations and inhibits CYP1A activity at higher concentrations, potentially decreasing detoxification capacity and increasing the risk of bioaccumulation. The effect of BDE-47 on the other major drug metabolism pathways has not been reported. In this study, sexually mature male rainbow trout (*Oncorhynchus mykiss*) were orally dosed with BDE-47 (50 µg/Kg/day) for 21 days. Semen from these and control trout was used to fertilize eggs obtained from a single, unexposed female. The resulting offspring were separated as per lineage and treated with BDE-47 for 21 days at 6 months in age. Livers from unexposed and exposed F1 control and F1 BDE treated fish were harvested and S9 fractions were prepared using standard methods. Activities for CYP2E, CYP1A, CYP2B, CYP3A, CYP2C and UGT were measured for each treatment group to evaluate 1) changes in enzyme activity following BDE 47 exposure and 2) transgenerational effects due to exposure. These results will aid in understanding of bioaccumulation of environmental contaminants such as polybrominated diphenyl ethers and potential effects on fish and other aquatic organisms.

852 EFFECTS ON HEPATIC GENE EXPRESSION IN MALE RATS ADMINISTERED PBDES IN HOUSEHOLD DUST.

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Studies show that polybrominated diphenyl ethers (PBDEs) decrease thyroid hormone concentrations via induction of hepatic uridinediphosphate-glucuronosyl-transferase (UGTs) and transthyretin (Ttr) binding. Because PBDEs exhibit en-

docrine disrupting properties and are present in human tissue, sources of human exposure are of increasing interest. Our laboratory has found that PBDEs fed to adult male rats in dust (NIST Standard Reference Material 2585) or an oil vehicle are equally bioavailable. This study examines the effects of PBDEs in household dust through their ability to induce genes involved in thyroid hormone disruption. Adult male Sprague-Dawley rats were fed either household dust at 319 or 1851 ng PBDEs/day or peanut oil containing 20 PBDEs at 181 or 906 ng PBDEs/day for 21 days. On day 22, livers were taken for gene expression analysis using real-time RT-PCR. Hepatic Cyp1a1 mRNA was unchanged in rats fed PBDEs in oil, but was increased 2 and 3-fold in the 319 and 1851 ng PBDEs/day dust treatment groups, respectively. Exposures to 181 ng PBDEs/day in the oil vehicle had no effect on hepatic Cyp2b1 mRNA. Increases in hepatic Cyp2b1 mRNA were seen with 906 ng PBDEs/day in oil, and in both dose levels in dust (319 and 1851 ng PBDEs/day), by 4, 2, and 3-fold, respectively. Hepatic Cyp2b2 mRNA was unchanged in both dust and oil treatment groups of 319 and 181 ng PBDEs/day, respectively; whereas, Cyp2b2 mRNA increased 5 and 3-fold in the 1851 and 906 ng PBDEs/day of the dust and oil treatment groups, respectively. There was no change in hepatic Ugt1a1, Ugt1a7, Ugt2b, or Ttr mRNA. This study shows that household dust is equally as effective as a PBDE oil mixture through its induction of CYP2b2 mRNA, but the administered concentrations may not be sufficient for thyroid hormone disruption. The observed effects on CYP1a1 mRNA are likely due to other polyhalogenated aromatic compounds found in the household dust. (This does not necessarily reflect USEPA policy).

853 PET CATS IN THE U.S. HAVE HIGH POLYBROMINATED DIPHENYL ETHER (PBDE) SERUM LEVELS.

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Coincident with the introduction of brominated flame-retardants in consumer products nearly 30 years ago, hyperthyroidism in cats has increased substantially. We hypothesize that increasing exposure to PBDEs has, in some manner, contributed to the abrupt increase in and now common occurrence of hyperthyroidism in older pet cats. In this pilot study, we assessed the feasibility of detecting PBDEs in small serum volumes that could be safely collected from geriatric cats. We evaluated 14 cats [4 young, 4 older, non-hyperthyroid (non-HT), and 6 hyperthyroid (HT) cats]. Results demonstrated that even in 1–2 mL of serum, a variety of congeners were readily detected (i.e., BDE-47, -99, -100, -153 and/or -154, -183, -207, -208-, and -209). Other congeners (i.e., BDE-66, -85, -196, -197, and -201) were present in some cats, in lesser quantities. Owing to insensitivity of the gravimetric lipid assay used, we were not able to accurately determine sample lipid content. Hence, results are expressed as the concentration of ΣPBDEs (ng/mL) present. The mean ± SE (and median) ΣPBDE serum concentrations in young, non-HT, and HT cats were 4.4 ± 2.0 (2.7), 7.20 ± 3.4 (4.76), and 15.0 ± 6.7 (5.55) ng/mL, respectively. We conclude that pet cats — with exposures likely paralleling that of their owners — are useful sentinels to assess potential endocrine health outcome related to cumulative, low-level PBDE exposure. (This abstract does not reflect USEPA policy).

854 PROTEOMIC ANALYSIS OF A [¹⁴C]-CHOLESTEROL ENRICHED FRACTION FROM HEPATIC CYTOSOL OF CHLORDEKONE PRETREATED MICE USING AMINE-SPECIFIC ISOTOPIC LABELING.

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Previous work has shown that the pretreatment of male C57BL/6 mice with low doses of the organochloride insecticide chlordane (CD) stimulated hepatic trafficking of [¹⁴C]-cholesterol. The intracellular transport of cholesterol is complex and both vesicular and non-vesicular transport mechanisms are involved. Their relative roles have not been fully elucidated and the non-vesicular transport mechanisms are not well understood. The present study was undertaken to see if a proteomic approach could be used to identify and monitor relative changes in hepatic cytosolic proteins that were initially isolated using [¹⁴C]-cholesterol challenged mice after pretreatment with CD.

Prospective protein fractions for analysis were isolated from CD (15 mg/kg) pretreated mice that had been challenged three days later with [¹⁴C]-CH. Animals were sacrificed 4 hours after the challenge dose was administered. The labeled protein fraction was isolated from hepatic cytosol with either ammonium sulfate or streptomycin precipitation.

Samples from CD (15 mg/kg) pretreated mice that were sacrificed three days later with no challenge were analyzed on a matrix assisted laser desorption ionization time of flight mass spectrometer. Isolated protein fractions were first di-

gested with trypsin and then the control and treatment groups were labeled with different isobaric mass tags (iTRAQ). Peptides were separated with strong cation exchange followed by C18 reverse phase high performance liquid chromatography. Tandem mass spectra were used for protein identification and iTRAQ mass tag ratios were used for relative quantification.

Initial results indicated that about 70 proteins could be identified with this protocol. Levels of glutathione s-transferase and liver fatty acid-binding protein increased about two fold in the CD pretreated group when compared to controls. The majority of proteins detected appeared to be unaffected by CD pretreatment, including sterol carrier protein 2.

855 A PILOT STUDY ON THE CONCENTRATIONS OF VARIOUS PERFLUORINATED ORGANIC COMPOUNDS IN THE BLOOD OF RESIDENTS IN CATALONIA, SPAIN.

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Fluorinated organic compounds (FOCs) are a group of chemicals widely used as surfactants, lubricants, polymers, and fire-fighting foams. Until recently, these chemicals were perceived as more biologically inert than chlorinated and brominated organic compounds and therefore, less likely to have an impact on human health or the environment. However, a number of recent studies have shown the ubiquitous distribution of FOCs in the environment, wildlife, and humans. We here report the results of a pilot study conducted to provide preliminary data on the levels of 13 FOCs in the blood of 48 residents in Catalonia, Spain, in relation to gender and age (25±5 and 55±5 years). The highest mean concentration was obtained for perfluorooctane sulfonate (PFOS, 7.64 ng/ml), followed by perfluorohexane sulfonate (PFHxS, 3.57 ng/ml) and perfluorooctanoic acid (PFOA, 1.80 ng/ml). Four other FOCs showed mean levels between 0.30 and 0.44 ng/ml, whereas those of the remaining 6 compounds were below the detection limit. Regarding gender, only the blood levels of PFHxS and PFOA were significantly higher ($p < 0.05$) in men than in women, while differences according to age were only noted for PFHxS ($p < 0.05$) and perfluorooctane sulfonamide (PFOSA) ($p < 0.001$), for which the levels were higher in the younger (25±5 years) group of subjects. A significant correlation between PFOS levels and those of the remaining detected FOCs (except PFDA) was found. In general terms, the current FOC concentrations were lower than those found in recent studies concerning levels of these chemicals in human blood and serum of subjects from different countries.

856 ROLE OF CAVEOLIN-1 AND ENDOTHELIAL NITRIC OXIDE SYNTHASE (eNOS) IN ACETAMINOPHEN-INDUCED HEPATOTOXICITY.

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Acetaminophen (APAP) is a mild analgesic and antipyretic agent known to cause centrilobular hepatic necrosis in a CYP2E1-dependent manner. We have previously demonstrated that nitric oxide (NO) generated via inducible nitric oxide synthase (iNOS) is an important mediator of APAP-induced hepatotoxicity. Thus, both wild type (WT) mice treated with an iNOS inhibitor and iNOS^{-/-} mice are protected from APAP-induced hepatotoxicity. Recent studies have demonstrated that NO can be hepatoprotective against CYP2E1-dependent toxicity by preventing oxidative stress. We speculated that this may be mediated by NO generated via eNOS, which is an important regulator of blood flow and of microvascular permeability during inflammation and this was examined. Treatment of WT mice with APAP (300 mg/kg, i.p.) resulted in a rapid (within 3 hr) upregulation of eNOS mRNA in the liver. This was associated with a marked decrease in expression of Cav-1, a 21-24 kDa membrane scaffolding protein that functions to concentrate eNOS and negatively regulate its activation state. Pretreatment of WT mice with N^G-nitro-L-arginine methyl ester (L-NAME, 10 mg/kg), an inhibitor of eNOS, had no major effects on APAP-induced toxicity. In contrast, pretreatment of Cav-1^{-/-} mice, which are resistant to APAP with L-NAME prior to APAP, resulted in marked increases in serum transaminases levels and centrilobular hepatic necrosis. These data suggest that NO generated via eNOS can contribute to hepatoprotection during APAP-induced inflammation and tissue injury. This is supported by findings that production of mediators important in repair of APAP damaged livers including MCP-1, IL-1, and IL-6 are altered in Cav-1^{-/-} mice, when compared to WT mice and that expression of the anti-inflammatory cytokine, IL-10 is upregulated immediately after APAP administration. These data demonstrate that Cav-1 plays an important role in regulating inflammatory responses and tissue repair initiated by APAP. (NIH GM034310, ES004738, ES005022).

857 ROLE OF β -CATENIN IN LIVER REGENERATION FOLLOWING ACETAMINOPHEN-INDUCED ACUTE LIVER FAILURE.

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Overdose of acetaminophen (APAP) is the leading cause of acute liver failure (ALF). Understanding the mechanisms of liver regeneration following APAP-induced liver injury is thus of high therapeutic relevance. To investigate the role of Wnt/ β -catenin, an important pathway in liver biology, in liver regeneration after APAP-induced ALF, male CD-1 mice were treated with a non-lethal dose of APAP (500 mg/kg), which induced extensive liver injury and subsequent regeneration. Rapid induction in the total and active β -catenin was evident at 1-3 hr after APAP treatment, along with its nuclear translocation at 6 hr. Investigation into the mechanisms of β -catenin activation revealed involvement of canonical (GSK-3 β -dependent) and non-canonical (c-met-dependent) components. Following treatment with a lethal dose of APAP (700 mg/kg) known to inhibit liver regeneration, β -catenin activation was completely suppressed. Furthermore, suppression of β -catenin activation was observed in human APAP-induced ALF liver samples along with suppression of liver regeneration. Taken together, these data indicate that β -catenin activation is among the earliest events related to stimulation of liver regeneration following APAP overdose and might have therapeutic and prognostic implications in APAP ALF. (Supported by RSG-03-141-01-CNE and NIH - 1R01DK62277 to SPSM).

858 LEFLUNOMIDE RESCUES MICE FROM ACETAMINOPHEN-INDUCED LIVER INJURY BY INHIBITING JNK-MEDIATED MITOCHONDRIAL PERMEABILIZATION.

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We have recently demonstrated that leflunomide (LEF), a disease-modifying anti-rheumatic pro-drug, and its active metabolite A77 1726 protect from acetaminophen (APAP)-induced cell injury in human hepatocytes by inhibiting the JNK pathway. The aim of this study was to determine whether LEF afforded protection from APAP-induced hepatotoxicity in vivo. Male C57BL/6 mice were given a hepatotoxic dose (750 mg/kg, ip) of APAP, followed by a single dose of LEF (30 mg/kg, ip) 4 h or 8 h after APAP. LEF fully prevented the increase in serum ALT activity and greatly reduced the extent of centrilobular necrosis determined at 8 h or 24 h post-APAP. LEF still protected when given as late as 8 h after APAP. The hepatoprotection was not due to inhibition of APAP bioactivation because LEF was administered after maximal depletion of GSH by the thiol-reactive intermediate. Similarly, hepatoprotection by LEF was not mediated through inhibition of the innate immune system as judged from unchanged transcript levels of key cytokines/chemokines involved in APAP hepatotoxicity. Both the APAP-induced mitochondrial release of cytochrome c and the drop in the ATP-biosynthesizing capacity of ex vivo isolated mitochondria were prevented by LEF, indicating that the protection was upstream of the mitochondrial permeability transition (mPT). Furthermore, APAP-induced nitrotyrosine-positive immunostaining was reduced by LEF, indicating that mitochondrial peroxynitrite formation was inhibited. Because both iNOS and the mPT are regulated by JNK, we determined the effects of LEF on APAP-induced increased JNK activation (phosphorylation). Immunoreactive P-JNK1/2 levels (but not JNK1/2 expression) were increased after APAP but remained low after combined APAP+LEF, thus blocking phosphorylation of downstream regulators of the mPT. In conclusion, LEF protects from APAP-induced hepatotoxicity by preventing mPT and release of death proteins by inhibiting the JNK-signaling pathway in mice.

859 COMPARISON OF PROTECTIVE EFFECTS OF S-ADENOSYL-L-METHIONINE (SAME) AND N-ACETYL-CYSTEINE (NAC) GIVEN AFTER ACETAMINOPHEN (APAP) TREATMENT IN MICE.

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Acetaminophen (APAP), in excessive doses, is associated with hepatic toxicity. At the present time, one-third of all acute liver failure in the U.S. are attributed to APAP overdose. This study examined the protective effect of SAME when administered after APAP similar to what occurs in a human overdose. Male C57BL/6 (16-25 g) n= 5 animals/group $\pm$ SEM were used in the studies. The mice were randomly divided into 6 groups, vehicle (VEH), APAP, SAME, SAME plus APAP

(SAME+APAP), NAC and NAC plus APAP (NAC+APAP). The animals were injected intraperitoneal (ip) with 250mg/kg APAP or water. One hr later, SAME and SAME+APAP groups were injected (ip) with 1.25 mmol/kg SAME and the NAC and NAC+APAP groups were injected (ip) with 1.25 mmol/kg NAC. Toxicity was assessed 4 h after APAP injection by monitoring hepatic glutathione (GSH) levels, changes in ALT, lipid peroxidation levels, liver weight and histology as parameters of APAP toxicity. SAME protected the liver from APAP-induced damage by decreasing ALT and lipid peroxidation levels as well as preventing depletion of glutathione. NAC also reduced APAP hepatic toxicity. Further studies investigated the protective effects of SAME against APAP-induced hepatotoxicity on 4-hydroxynonenal (4HNE) adducted proteins and protein carbonyls. The results of this study have found that SAME protects the liver from production of protein carbonyls and 4HNE adducts. In summary, APAP hepatic toxicity occurs within 1 h after injection in mice. SAME administered 1 h after APAP was successful at reducing APAP hepatic toxicity. SAME administered 1 h after APAP also reduced the extent of oxidative stress associated with APAP.

860 HEPATOTOXIC SENSITIVITY OF NONALCOHOLIC STEATOHEPATITIC (NASH) MICE TO ACETAMINOPHEN IS DUE TO LOW PPAR α

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The objective was to determine if the hepatotoxic sensitivity in NASH mice to acetaminophen (APAP) is due to downregulation of nuclear receptor PPAR α via lower cell division and tissue repair. Male SW mice fed methionine and choline deficient diet for 31 days exhibited NASH. On 32nd day, exposure to a marginally toxic dose of APAP (350 mg/kg, ip) yielded 70% mortality in NASH mice 8 to 24 h later, while all non NASH mice receiving the same dose survived. Covalent binding of ¹⁴C-APAP-derived radiolabel to the liver macromolecules, CYP2E1 protein, and enzyme activity did not differ from the controls, obviating increased APAP bioactivation as the cause of amplified APAP hepatotoxicity. Liver injury as assessed by liver histopathology and plasma ALT progressed only in NASH livers between 6 to 24 h. Cell division and tissue repair assessed by 3H-thymidine incorporation and PCNA were inhibited only in the NASH mice given APAP. These results strongly suggested that higher sensitivity of NASH liver to APAP-induced hepatotoxicity was due to lower tissue repair. Because PPAR α induction is known to promote tissue repair, the hypothesis that impeded liver tissue repair in NASH mice was due to downregulation of PPAR α was tested. PPAR α was downregulated in NASH evidenced by lower expression of CYP4A protein, consistent with the compromised liver tissue repair seen in NASH mice exposed to APAP. To investigate that downregulation of PPAR α in NASH is the critical mechanism of compromised liver tissue repair, PPAR α was induced in NASH mice with clofibrate (250 mg/kg for 3 days, ip) before injecting APAP. All clofibrate pretreated NASH mice receiving APAP exhibited lower injury (ALT and histopathology), which did not progress and the mice survived. The protection was not due to lower bioactivation of APAP as assessed by ¹⁴C-APAP covalent binding, CYP2E1 protein, and enzyme activity, but due to higher liver tissue repair as assessed by an increased DNA synthesis and PCNA. These findings suggest that inadequate PPAR α expression sensitizes NASH mice to APAP hepatotoxicity.

861 ROLE OF THE INNATE IMMUNE SYSTEM IN THE INCREASED SUSCEPTIBILITY OF INTERLEUKIN-13 DEFICIENT MICE TO ACETAMINOPHEN-INDUCED LIVER DISEASE.

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Recently, we have shown that endogenous interleukin (IL)-13 protected mice from acetaminophen (APAP)-induced liver disease (AILD). Several proinflammatory cytokines and chemokines downstream of reactive metabolite formation appear to be regulated by IL-13, as their serum expression was found to be higher in APAP-treated IL-13 knockout (KO) than wild-type (WT) mice. In the present study, we evaluated the pathologic role of proinflammatory factors in IL-13 KO mice following challenge with 200 mg APAP/kg. When interferon (IFN)- γ neutralizing antibody (NAb) was administered to APAP-treated IL-13 KO mice, IFN- γ levels decreased significantly and AILD was attenuated. Since natural killer (NK) cells and NK cells with T-cell receptors (NKT cells) are major sources for IFN- γ , the importance of these innate immune cells was investigated. Administration of NK 1.1 NAb to APAP-treated IL-13 KO mice depleted both NK and NKT cells, attenuated liver injury and abrogated IFN- γ levels, suggesting these cells play a critical role by secreting injurious IFN- γ . Additionally, based on observations of elevated neutrophil (polymorphonuclear leukocyte, PMN) chemoattractants, along with immunohistochemically-determined greater hepatic PMN accumulation in APAP-treated IL-13 KO mice compared to WT, the role of PMNs was also investigated.

Administration of PMN NAb to APAP-treated IL-13 KO mice greatly reduced hepatic PMN accumulation and attenuated liver injury, suggesting PMNs contribute to AILD in this susceptibility model. Collectively, these results indicate IL-13 modulates the pathologic role of the innate immune system in AILD and suggests IL-13 is an important hepatoprotective cytokine in drug-induced liver disease. *This research was supported by the Intramural Research Program of the NIH and NHLBI.*

862 HEPATOPROTECTIVE EFFECTS OF THE SAPONINS ISOLATED FROM ROOTS OF PLATYCODON GRANDIFLORUM AGAINST CARBON TETRACHLORIDE-INDUCED TOXICITY.

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The protective effects of the saponins isolated from the roots of *Platycodon grandiflorum* A. DC (Campanulaceae), Changkil saponins (CKS), on carbon tetrachloride induced hepatotoxicity and the possible mechanisms involved in this protection were investigated. Pretreatment with CKS prior to the administration of carbon tetrachloride significantly prevented the increased serum enzymatic activities of alanine and aspartate aminotransferase and hepatic lipid peroxidation in a dose-dependent manner. The effects of CKS on the cytochrome P450 (P450) 2E1, the major isozyme involved in carbon tetrachloride bioactivation were also investigated. Treatment of mice with CKS resulted in a significant decrease of P450 2E1-dependent p-nitrophenol and aniline hydroxylation in a dose-dependent manner. CKS showed anti oxidant effects in FeCl₂ ascorbate induced lipid peroxidation in mice liver homogenate and in superoxide radical scavenging activity. Our results suggest that the protective effects of CKS against carbon tetrachloride-induced hepatotoxicity possibly involve mechanisms related to its ability to block P450 mediated carbon tetrachloride bioactivation and free radical scavenging effects.

863 RETROSPECTIVE ANALYSIS OF ALANINE AMINOTRANSFERASE ELEVATIONS WITH THE MAXIMUM RECOMMENDED DOSE OF ACETAMINOPHEN SHOWS ELEVATIONS ARE LOW, TRANSIENT, AND CLINICALLY INSIGNIFICANT.

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In some osteoarthritis (OA) studies where acetaminophen (APAP) was given over multiple days, low-level alanine aminotransferase (ALT) elevations have occasionally been reported. No comparable animal model of this phenomenon is available, so careful evaluation in clinical trials is necessary. We retrospectively analyzed ALT data from McNeil studies to assess the frequency and magnitude of ALT elevations and the patterns of onset and resolution while on the maximum recommended daily dose (MRDD) of APAP. Nine controlled OA trials were identified in which APAP alone was administered in at least 1 treatment group. Baseline and on-treatment ALT values were measured in 7 studies. Patients receiving the MRDD of APAP (3900 or 4000mg/day) for 4 weeks to up to 12 months were included. In the 7 studies, 892 patients had baseline transaminase activity at or below the upper limit of the reference range (ULRR), had an on-treatment ALT measurement, and received the MRDD of APAP. In this analysis, 733 (82.2%) of 892 patients never had an ALT above ULRR. Of the 892 patients, 159 (17.8%) had an ALT >ULRR, but only 42 (4.7%) had an on-treatment ALT >1.5X ULRR. A subsequent ALT value following the elevation was available for 29 of these 42 patients. Documented resolution or a decreasing ALT while continuing APAP treatment was noted for 27 patients (93.1%). No patient had an on-treatment ALT >3 times ULRR in conjunction with hyperbilirubinemia. An ALT above ULRR was not associated with an increased frequency of symptoms potentially related to a hepatic origin. No patient treated with the MRDD of APAP developed hepatotoxicity or hepatic failure. This analysis involving 892 patients treated with 3900–4000mg/day of APAP shows that low-level ALT elevations may occur but usually resolve or decrease with continued APAP treatment, are unaccompanied by signs or symptoms of liver injury, and appear to be clinically insignificant. The maximum recommended daily dose of APAP did not cause liver failure or dysfunction.

864 REGULATION OF THE MEVALONATE PATHWAY INFLUENCES PROLIFERATIVE EFFECTS OF WY-14,643 IN MOUSE LIVER.

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Peroxisome proliferators (PP), such as WY-14,643, are a widely studied class of non-genotoxic rodent liver carcinogens. Presence of active peroxisome proliferator activated receptor (PPAR) α is required for the effects of PPs on cell proliferation,

induction of peroxisomes and liver carcinogenesis. In addition, a number of studies have suggested that PPAR α plays a central role in the synergistic effects of statins and PPs. Here, we explored the consequences of blocking the mevalonate pathway with lovastatin (20 mg/kg/day, i.g.) or increasing the availability of isoprenoids by adding exogenous farnesol (250 mg/kg/day, i.g.) on liver effects of WY-14,643 (WY, 0.1% diet for 3 days) in C57BL/6J male mice. Lovastatin alone had no appreciable effect on WY-induced liver enlargement, cell proliferation, induction of acyl CoA oxidase (ACO) activity or other liver-related endpoints with the exception of its anticipated effect on blood cholesterol levels. Interestingly, while farnesol had no effect on WY-induced ACO activity (i.e., peroxisomal proliferation), it blocked the increase in cell proliferation. In animals that received WY, lovastatin and farnesol, relative liver weight and ACO activity were significantly higher than in the WY-only group; however, cell proliferation rates in liver were lower than in the WY-only group, but higher than in the WY+farnesol-treated mice. Collectively, these results indicate that dysregulation of the mevalonate pathway affects the promotional ability of PPs in liver, but not their ability to cause induction of peroxisomes. Thus, the perturbations of lipid metabolism in liver that are caused by PPs may be linked to the ability of these agents to induce cell proliferation.

865 INCREASED ACYL COA OXIDASE ACTIVITY IN RATS AFTER FIVE CONSECUTIVE DAILY DOSES OF PERFLUOROBUTANESULFONATE, PERFLUOROHXANESULFONATE, AND PERFLUOROOCTANESULFONATE.

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Recent scientific and regulatory interest in perfluoroalkyl sulfonates (PFAS) led us to study liver peroxisome proliferation potential with a series of PFAS that included perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHS), and perfluorooctanesulfonate (PFOS).

Male SD rats (N=6/group) were given 5 consecutive daily doses of either vehicle control (1% carboxymethyl cellulose), or K⁺ salts of PFBS (4.44 mmole/kg-d), PFHS (0.11 mmole/kg-d), or PFOS (0.56 mmole/kg-d). Rats were sacrificed via CO₂ 4 hrs after the final dose, immediately after which liver weight (LW) was obtained and sections of livers were: (1) taken for histology (formalin fixation, paraffin embedding, H&E); and (2) snap frozen for acyl CoA oxidase (ACOX) activity and determination of test material concentrations. Serum was also obtained. Concentrations of test materials in liver and sera were determined by LC/MS/MS. Body weight (BW) was measured daily and at term.

Only PFOS affected BW (mean loss of ~ 13% vs. control). PFBS and PFHS increased mean LW over control by 15 and 43%, respectively. Mean LW/BW % was increased over control with statistical significance with all 3 test compounds, with mean values of 4.2, 4.8, 5.8, and 4.8% for control, PFBS, PFHS, and PFOS, respectively. Compared to control, ACOX activity was also significantly increased by 271, 303, and 207% by PFBS, PFHS, and PFOS, respectively. For PFBS, PFHS, and PFOS, respectively, mean $\pm$ SD liver concentrations were 428 $\pm$ 102, 576 $\pm$ 54, and 563 $\pm$ 62 μ g/g, and mean $\pm$ SD serum concentrations were 268 $\pm$ 38, 409 $\pm$ 65, and 261 $\pm$ 19 μ g/mL. No changes suggestive of toxicity occurred on histomorphologic examination of fixed liver sections. With PFOS, liver cells were more eosinophilic, possibly due to less stored glycogen.

PFBS doses in mmole/kg $\geq$ 50 times higher than either PFOS or PFHS were required to produce similar increases in ACOX activity; however, liver concentrations achieved were similar.

866 PERFLUOROOCTANOIC ACID AND WY 14, 643 TREATMENT INDUCED PEROXISOME PROLIFERATION IN LIVERS OF WILD-TYPE BUT NOT PPAR α -NULL MICE.

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Perfluorooctanoic acid (PFOA) is a fluorinated organic chemical widely used in consumer and industrial products. Its persistence in the environment and presence in humans and wildlife have raised considerable concerns. PFOA induces liver tumors in rodents, which is thought to be mediated by PPAR α activation. The extent of PPAR α 's contribution to the PFOA-induced liver tumors became questionable when liver enlargement, a response associated with PPAR α agonists, was detected in PPAR α -null mice exposed to PFOA. The current study evaluated hepatocyte proliferation, light microscopic and ultrastructural liver alterations in CD-1, 129S1 wildtype (WT) or PPAR α knock-out (KO) mice after 7 daily PFOA-NH₄⁺ (1, 3, or 10 mg/kg, p.o.) treatments, and the results were compared to mice treated with the

prototype PPAR α -agonist WY 14,643 (WY, 50 mg/kg). PFOA treatment induced a dose-dependent increase in hepatocyte hypertrophy and hepatocyte labeling index (LI) in WT mice. In these animals, liver alterations were similar between WY and 10 mg/kg PFOA. The ultrastructural alterations were essentially the same between WY- and PFOA-treated WT mice, and consisted of peroxisome proliferation. KO mice had a dose-dependent increase in hepatocyte vacuolation and increased LI only at the high dose of PFOA. In KO mice, only PFOA treatment produced ultrastructural changes, which consisted of accumulation of large clear vacuoles. These data suggest that PPAR α is required for WY- and PFOA-induced cellular alterations in the mouse liver. The origin and pathological significance of the vacuoles seen in the PFOA-treated KO mice are not clear and remain to be determined. This abstract does not necessarily reflect US EPA policy.

867 CHARACTERIZATION OF THE HEPATOMEGALY INDUCED BY AMMONIUM PERFLUOROOCTANOIC ACID (APFO) IN RATS.

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Ammonium perfluorooctanoic acid is non-genotoxic but has induced liver tumours when administered to rats. The objective of this study was to characterise the APFO-induced hepatomegaly previously reported in rats. APFO (300ppm, equivalent to a mean ingested dose of 19.57 $\pm$ 0.14 mg/Kg body weight) was administered in the diet to male Sprague Dawley rats for 1, 7 and 28 days. Animals were implanted with osmotic pumps containing bromodeoxyuridine (BrdU) 5 days before termination. Animals sacrificed after exposure to diet for 1 day were administered BrdU by subcutaneous injection 2 hours prior to sacrifice. Relative liver weights were increased to 150% of control values after 7 and 28 days. Hepatic peroxisomal β -oxidation (CN-insensitive palmitoyl CoA oxidation) was increased 2-, 6- and 10- fold after 1, 7 and 28 days respectively. Western blotting of liver microsomes demonstrated the marked induction of CYP2B2, CYP3A4 and CYP4A1 by APFO. Histopathology (H and E) of liver sections revealed that APFO caused dose-related hepatocellular glycogen loss, hypertrophy and hyperplasia. The nuclear incorporation of BrdU was determined as a measure of cell proliferation. APFO increased the hepatocellular labelling index (approximately 4-fold) 1 and 7 days after commencement of treatment. After 28 days the hepatocellular labelling index of livers from APFO-treated animals appeared to return to control levels. In conclusion, the administration of APFO to rats leads to hepatomegaly characterised by hypertrophy and hyperplasia. These data clearly demonstrate that APFO exhibits the prototypical properties of a peroxisome proliferator, but in addition possesses the properties of a mixed type of enzyme-inducing agent. The profile of CYP induction suggests that APFO interacts with multiple members of the of the nuclear hormone super family, particularly PPAR α , CAR and PXR.

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868 NEUTROPHIL INTERACTION WITH THE HEMOSTATIC SYSTEM CONTRIBUTES TO LIVER INJURY IN RATS COTREATED WITH LIPOPOLYSACCHARIDE AND RANITIDINE.

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Cotreatment of rats with nontoxic doses of ranitidine (RAN) and lipopolysaccharide (LPS) causes liver injury in rats, and this drug-inflammation interaction might be a model for idiosyncratic adverse drug response in humans. Both neutrophils (PMNs) and the hemostatic system have been shown to be important in the liver injury. We tested the hypothesis that PMNs cause liver injury by interacting with the hemostatic system and producing subsequent hypoxia. In rats cotreated with LPS/RAN, PMN depletion by anti-PMN serum reduced fibrin deposition and hypoxia in the liver. PMN depletion also reduced active plasminogen activator inhibitor-1 (PAI-1), an important downregulator of the fibrinolytic system. This suggests that PMNs cause fibrin deposition by increasing PAI-1. PMN activation as measured by HOCl-modified protein staining occurred in the livers of LPS/RAN-cotreated rats. Antiserum against PMN adhesion molecule CD18 protected against LPS/RAN-induced liver injury. Since CD18 is important for PMN transmigration and activation, this suggests that PMN activation is required for the liver injury. The selective upregulation of the chemokine MIP-2 after LPS/RAN treatment raises the possibility that chemokines participate in the PMN response. In summary, PMNs are activated in LPS/RAN-cotreated rats and appear to participate in the liver injury in part by contributing to hemostasis. (Supported by NIH grant DK061315).

869 SIGNIFICANCE OF CARBONYL GROUPS IN 3-(3,5-DICHLOROPHENYL)-2,4-THIAZOLIDINEDIONE (DCPT)-INDUCED HEPATOTOXICITY IN RATS.

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DCPT, which contains a 2,4-thiazolidinedione (TZD) ring, is hepatotoxic in Fischer 344 (F344) rats. A TZD ring is also found in the glitazones, which are insulin-sensitizing drugs used in the treatment of type II diabetes. These drugs have been associated with liver damage in some patients. To demonstrate that DCPT may be useful for investigating the role of the TZD ring in hepatotoxicity, we have been conducting studies with TZD ring-modified DCPT analogues. The importance of the carbonyl group in the 4-position of the TZD ring was therefore investigated by synthesizing and testing 3-(3,5-dichlorophenyl)-2-thiazolidinone (2-DCTD) in male F344 rats at 0.2, 0.4, 0.6 and 1.0 mmol/kg doses. 2-DCTD retains the carbonyl group in the 2-position between the sulfur and imide nitrogen atoms. Other rats received DCPT (0.6 mmol/kg) or corn oil (4 ml/kg, vehicle controls). Liver and kidney function and morphology were assessed 24 h post-dosing. Compared to the corn oil controls, serum alanine aminotransferase (ALT) levels were unaltered by 2-DCTD. In contrast, DCPT produced significant ALT elevations. Blood urea nitrogen levels were not altered in any of the treatment groups. Histological results from 2-DCTD-treated rats showed no alterations in hepatic morphology at any of the doses examined, compared to the corn oil controls. However, pockets of liver necrosis were observed in liver sections from the DCPT-treated rats. None of the treatments produced significant changes in kidney morphology. Our results suggest that replacement of the TZD ring of DCPT with the 2-thiazolidinone ring of 2-DCTD abolishes hepatotoxicity. This is consistent with our previous finding that 3-(3,5-dichlorophenyl)-4-thiazolidinone (4-DCTD), an isomer of 2-DCTD, was also non-toxic. We therefore conclude that DCPT-induced hepatotoxicity is critically dependent on the presence of both carbonyl groups in the TZD ring. Furthermore, metabolism of 2-DCTD to DCPT does not occur in rat liver. Supported by PHS grant ES012499.

870 EFFECTS OF LXR AGONIST (TO901317) ON GENE EXPRESSIONS AND LIPID DEPOSITION IN HUMAN HEPATOCYTES OF CHIMERIC MOUSE LIVER.

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Chimeric mice were produced by transplanting human hepatocytes into immunodeficient and liver-diseased mice [albumin enhancer/promoter-driven urokinase-type plasminogen activator-transgenic/SCID (uPA/SCID) mice]. Because the humanized liver expressed human specific cytochrome P450 genes at levels comparable to human liver, these animals are useful to predict metabolisms of chemicals and drugs in human liver *in vivo*. A variety of drugs targeted for nuclear receptors have been and will be designed. The microarray analysis of human hepatocytes isolated from chimeric liver showed that most of human nuclear receptors were expressed in the chimeric mouse liver at similar levels to human liver.

In this study, the responsiveness of human nuclear receptors in the chimeric mouse liver to TO901317, a well-characterized LXR agonist, was investigated. TO901317 is also known to be a strong liver steatotic inducer in rodents. The chimeric mice were made by transplanting cryopreserved human hepatocytes (7.5×10^5 cells) from a 4-year-old-Caucasian girl into the liver of uPA/SCID mice. These mice were orally administered with TO901317 for 3 days at 50 mg/kg body weight/day and sacrificed at 24 hours after the last injection. Liver cryosections were prepared and treated with Oil red O for lipid staining. mRNA expression levels were determined by real time RT-PCR using total RNAs isolated from the liver tissues as templates. TO901317 induced severe steatosis in human hepatocytes in the mice and elevated the expression of the targeted genes such as ABCG1 and FASN. In conclusion, the chimeric mouse was proved to be a useful animal model to predict effects of chemicals and drugs targeted for nuclear receptors on gene expressions and toxicity in human liver *in vivo*.

871 EFFECT OF SUBCHRONIC EXPOSURE ON TOXICITY OF PERCHLOROETHYLENE IN SWISS WEBSTER MICE.

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The aim was to study the subchronic toxicity of perchloroethylene (Perc) by measuring injury and repair in liver and kidney in relation to disposition of Perc and its major metabolites. Male SW mice (25-29 g) were given three dose levels of Perc (150, 500, and 1000 mg/kg/d) via aqueous gavage for 30 days. Tissue injury was

measured during the dosing regimen (0, 1, 7, 14, and 30 d) and over a time course of 24 to 96 h after the last dose (30 d). Perc produced significant liver injury (ALT) after single day exposure to all three doses. Liver injury was mild and regressed following repeated exposures, except after the high dose showing sustained injury until 7 d of exposure. Liver tissue repair peaked on 7 d, with all three doses preventing progression of injury. While hepatic tissue repair was sustained after medium and high doses for 14 d, it regressed to basal levels with low dose exposure. Hepatic CYP2E1 expression and activity were unchanged indicating unaltered bioactivation after subchronic Perc exposure. Hepatic CYP4A expression was transiently higher after d 7 with the high dose, but was unchanged at later time points. Kidney function was unaffected as evidenced by unchanged BUN levels during the entire time course and lack of kidney injury was confirmed by histopathology. Kidney tissue repair, as evidenced by replicative DNA synthesis and PCNA immunohistochemistry, was moderate. TCA was the major metabolite detected in blood, liver, and kidney. Traces of DCA were also detected in blood at initial time points after single day exposure. AUC/dose for Perc was significantly higher and for TCA was lower in liver and kidney, following repeated exposure indicating saturation of Perc metabolism. These data indicate that subchronic Perc exposure via aqueous gavage does not induce nephrotoxicity or persistent hepatotoxicity in SW mice suggesting adaptation (ATSDR U61/ATD 681482).

872 PBDE CONGENERS ARE PXR ACTIVATORS.

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Polybrominated diphenyl ether (PBDE) congeners are used as flame retardants and they are universally present in the environment. Researchers have detected an exponential increase of PBDE congeners in the US population over the last two decades, especially with unexpected high levels in breast milk from women. We are most commonly exposed to three PBDE congeners, BDE47 (tetraBDE), 99 (pentaBDE), and 209 (decaBDE). PBDE congeners induce cytochrome P450s (CYPs), disrupt thyroid hormone homeostasis, act as neurotoxins, and are labeled as potential carcinogens. We have little understanding of the mechanism by which PBDE congeners exert their toxicities. Our current research goal is to clarify the mechanism by which PBDE congeners induce P450 enzymes. We hypothesize that PBDEs are activators for xenobiotic nuclear receptor, pregnane X receptor (PXR), a ligand-activated nuclear receptor that mediates the induction of P450 enzymes by many xenobiotics. Treatment with PBDE congeners, BDE47, 99, and 209 induced cyp3a11 and cyp2b10 in a dose-dependent manner in C57BL6 mice, which was not shown in the PXR knockout mice. In addition, BDE47, 99, and 209 activated both mouse and human PXR as revealed by the luciferase reporter gene assay. These data strongly suggest that PBDE congeners are activators for xenobiotic nuclear receptors, PXR.

873 THE EFFECTS OF T-2 TOXIN ON HEPATIC DRUG METABOLIZING ENZYMES IN PIGLETS.

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T-2 toxin is one of trichothecenes, which are a structurally diverse group of toxic secondary metabolites produced by *Fusarium* and related species of fungi, which usually contaminate cereal grains throughout the world. Although the pig is increasingly being used in pharmacological and toxicological studies, there is not enough information about the effects of T-2 toxin on drug-metabolizing enzymes in pigs. The purpose of this study is to investigate the effects of T-2 toxin on the activities of hepatic Phase I and Phase II metabolizing enzymes in piglet liver. Piglets were administered 0.3 mg T-2 toxin/Kg Bwt dissolved in DMSO by single subcutaneous (s.c.) injection. Control animals received only vehicle (DMSO). The activities of Phase I and Phase II enzymes were determined at 24h and 48h after the last s.c. injection. The activities of cytochrome P450 (CYP) 1A2 and 2E1 increased slightly at 24h and 48h ($P < 0.05$). The CYP3A4 activity increased at 24h ($P < 0.01$), and tended to decrease at 48h, but not significantly ($P > 0.05$). The glutathione S-transferase activity toward cumene hydroperoxide increased slightly at 24h ($P < 0.05$), but decreased slightly at 48h ($P < 0.05$). No significant changes were observed in the glutathione S-transferase activity toward CDNB either at 24h or 48h. Western blot analyses of the liver fractions revealed increased levels of CYP1A2, 2E1, 3A4, GST alpha and GST M1-1 at 24h, and that of CYP2E1 at 48h. RT-PCR showed changes of the mRNA expression of those 5 kinds of enzymes in liver. The results suggest that T-2 toxin causes modulation of Phase I and Phase II drug metabolizing enzymes in piglets.

874 COX-2 INHIBITION AGGRAVATES sPLA₂-MEDIATED PROGRESSION OF ACUTE LIVER INJURY.

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Previous studies have shown calpain's involvement in CCl₄- and acetaminophen-induced progression of acute liver injury (PLI). Our present work identifies secretory phospholipase A₂ (sPLA₂) as another mediator of PLI. Upon toxicant challenge, perinecrotic cells secrete large amounts of sPLA₂, which along with COX-2 rid the tissue of necrotic cell debris. Action of sPLA₂ releases arachidonic acid, a precursor for protective prostaglandins (PGE₂ and PGF₂). Hence, co-localization of sufficient COX-2 with sPLA₂ is critical in tissue recovery. The objective of the present work was to test the hypothesis that sPLA₂ spreads hepatocyte injury through hydrolysis of plasma membrane phospholipids in the absence of sufficient COX-2. Two groups of male SD rats were administered two different doses of CCl₄ (2 and 3 ml/kg, ip). Liver injury was assessed by plasma ALT and histopathology. Plasma and liver sPLA₂ and COX-2 activities in rats challenged with a non lethal dose of CCl₄ (2 ml/kg) rose with liver injury. Immunohistochemical co-localization of these proteins was noted in the perinecrotic areas, suggesting a protective role of sPLA₂ when co-localized with COX-2. In contrast, the lethal dose (3 ml/kg) treated rats exhibited very high sPLA₂ activity, but minimal COX-2 activity, leading to progression of injury, liver failure, and 70% mortality between 12 to 36 h. Immunohistochemistry showed high staining for sPLA₂, but minimal COX-2 staining throughout the time course suggesting a destructive role of sPLA₂ in the absence of sufficient COX-2. Time- and dose-dependent increase in cell injury and death (LDH and MTT) of freshly isolated hepatocytes when incubated with sPLA₂ suggested sPLA₂'s ability to damage hepatocytes and contribute to PLI when present extracellularly. COX-2 inhibition using NS398 in Fisher rats challenged with CCl₄ (1 ml/kg) led to 50% increase in mortality due to progression of liver injury. These results support sPLA₂'s destructive role in PLI by hydrolysis of plasma membrane phospholipids of perinecrotic hepatocytes unless mitigated by simultaneous presence of sufficient COX-2.

875 DRUG CHALLENGES TO TEST THE FUNCTIONAL INTEGRITY OF NEURAL SYSTEMS INVOLVED WITH MEMORY AFTER EARLY DIAZINON EXPOSURE IN RATS.

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Developmental organophosphate pesticide exposure can cause persisting effects on cognitive function. We have found that developmental exposure of rats to chlorpyrifos can cause persisting memory impairment. Drug challenges helped elucidate the neural systems involved in the impairment. Rats with a history of chlorpyrifos exposure were under-responsive to the amnesic effect of scopolamine, suggesting that they were not relying on muscarinic acetylcholine neural systems to perform the memory task. Chlorpyrifos-exposed rats were hyper-responsive to amnesic effects of ketanserin, a serotonin type2 receptor antagonist, suggesting that instead they had increased reliance on these serotonergic systems for memory function. In the current study, rats were exposed to diazinon during early postnatal development (0, 0.5 or 2 mg/kg/day, PND 1-4). When trained in adulthood on the radial-arm maze, the 0.5 mg/kg diazinon group showed a significant ($p < 0.025$) impairment of working memory function. This dose group was hyper-responsive to scopolamine showing a significantly ($p < 0.01$) greater amnesic effect with a low scopolamine dose (0.04 mg/kg), which in unexposed animals was subthreshold for causing impairment. This suggested that these diazinon-exposed rats had less functional reserve in their muscarinic acetylcholine systems than controls. No differential effect of ketanserin was seen. An inverted dose-effect function in response to the GABA-A agonist muscimol was seen in the diazinon 2 mg/kg/day exposure group relative to control, demonstrating differential involvement of GABA systems in memory after early diazinon exposure. Studies with the NMDA glutamate antagonist dizocilpine are being conducted. Using selective ligands for transmitter systems involved in cognitive function can help determine how neural systems are functionally disrupted by toxicant exposure.

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876 MINNOW BIOGENIC AMINE TRANSPORTER BINDING AND RESPONSE TO PESTICIDES AND PHARMACEUTICALS.

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Model systems are needed to assess neurotoxicological effects of chronic pesticide exposures. Mammalian serotonin (SERT) and norepinephrine transporters (NET) are target sites for antidepressants, and are also affected by pesticide exposure.

Following chronic administration of certain antidepressants to rats, SERT or NET binding sites are reduced. Similarly, exposure to some pesticides causes SERT binding site reductions. Our research goal is to determine if minnow SERT and NET respond to pharmaceuticals and pesticides comparably to mammalian SERT and NET. If fish transporters are sensitive to chronic, low-dose exposures, then their SERT and NET can be used to model and monitor exposures. We examined central SERT and NET binding sites of the golden shiner minnow, and compared their pharmacological profile to those of rats. Homogenate binding with the radioligand [3H] citalopram indicated that the golden shiner SERT has a KD of 7 ± 3 nM and a Bmax of 226 ± 46 fmol/mg protein. These values are similar to those of rat SERT (KD 1.4 ± 0.1 nM and Bmax 240 ± 48 fmol/mg protein). The minnow NET (measured using [3H] nisoxetine) has a KD of 12 ± 5 nM and a Bmax 187 ± 49 fmol/mg protein, comparable to rat NET (KD 5 ± 2 nM and Bmax 93 ± 8 fmol/mg protein). Minnow SERT and NET binding is displaceable by antidepressants. We also examined brain SERT binding properties of fathead minnow and found them to be similar to golden shiner SERT. Finally, we exposed zebrafish to the SSRI sertraline at 1 or 10 ug/meal, or to the organophosphate chlorpyrifos at 10 ug/meal, for 21 days with 2 days of physiological washout. After either treatment, SERT binding in brain homogenates was reduced by 50% ($N=3-6$, $p < 0.05$). In summary, minnow SERT and NET express lower affinity for ligands and antidepressants than rats. However, since magnitudes of affinity are similar, and because SERT binding declines after chronic sertraline or chlorpyrifos administration, minnow biogenic amine transporters may be useful indicators of exposure.

877 ACUTE DELIBERATE ORGANOPHOSPHATE (COUMAPHOS) POISONING WITH INTERMEDIATE SYNDROME IN A ONE YEAR OLD CHILD.

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Objective: To report a case of acute organophosphate poisoning in a one year old child and development of intermediate syndrome in just eight hours after exposure. Case Report: This is a case of a one year old child who came in the emergency room because of sudden onset of difficulty of breathing, cyanosis, excessive oral secretions, diarrhea and weakness noted three hours earlier after ingesting allegedly contaminated powdered milk formula. Vital signs showed a blood pressure of 90/60, cardiac rate of 112 per minute, respiratory rate of 12 cycles per minute and axillary temperature of 35.4 degrees Celsius. Arterial blood gas analysis showed respiratory acidosis. Endotracheal intubation was immediately done and was hooked on mechanical ventilation. The toxidrome of the patient is compatible with acute cholinergic excess; hence a trial dose of atropine was given. Eight hours later, neurological examination showed absence of deep tendon reflexes, no spontaneous respiration, no response to pain, flaccid muscle tone, no neck rigidity and lateralizing signs but with spontaneous eye opening. Referral to a Pediatric Neurologist ruled out the possibility of CNS infection or trauma. Atropine was given at 0.02 milligrams per kilogram intravenously until full atropinization was achieved. Twenty-four hours later, patient was noted to have response to painful stimuli and spontaneous respiration. Atropine was continued until the patient regained full consciousness and restoration of full motor and sensory function. Test was done on the allegedly contaminated milk using GC-MS, positive for Coumaphos. The patient was extubated on the fourth hospital day. The rest of hospital stay was unremarkable and was discharge after seven days. Atropine was continued for six weeks. Conclusion: Intermediate syndrome usually develops within 48-96 hours after acute cholinergic crisis due to prolonged inhibition of cholinesterases(1). There is probably a difference with regards to time of occurrence of Intermediate Syndrome or maybe a difference in Acetylcholinesterase function between a very young child and adult.

878 ELUCIDATION OF THE ROLE OF ADULT DETOXICATION ENZYMES IN THE AGE-RELATED TOXICITY DIFFERENCES OF ORGANOPHOSPHATE INSECTICIDES.

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It is well documented that organophosphate (OP) insecticides are more toxic to juvenile animals than they are to adults. While it is common to refer to the enhanced vulnerability of juveniles to OP insecticides, it is may be more appropriate to refer to the reduced vulnerability of adults which is mediated primarily by the higher mature levels of detoxication enzymes such as the carboxylesterases and non-target cholinesterases. It is thought that if these enzymes were not present in the adult, the toxicity level of an OP would be similar in adults and juveniles. To test this, adult rats and juvenile rats at postnatal day 1 (PND1) and PND12 were treated with similar dosages of the prototypical OP compound paraoxon (P=O) and brain

cholinesterase (ChE) inhibition was determined. Additional adult animals were then pre-treated with specific inhibitors of the carboxylesterases and non-target cholinesterases prior to exposure to the same dosages of P=O and brain ChE inhibition was compared to the inhibition obtained without the pre-treatment. In adult animals, 0.25, 0.3, and 0.75 mg/kg resulted in 18%, 41%, and 89% inhibition, respectively, while at PND12, 0.25 and 0.3 mg/kg resulted in 90% and 92% inhibition respectively. The level of inhibition at PND1 was similar to PND12 with 0.25 yielding 96% inhibition. However, when the activity of the carboxylesterases and non-target cholinesterases was eliminated in adult animals prior to treatment with 0.25 mg/kg, the level of ChE inhibition increased to 86% which is comparable to that observed in the juvenile animals. These data suggest that the greater vulnerability of juvenile animals to OP insecticides is due to, for the most part, the lack of protective esterases which are present in the adult. (Supported by NIH R01 ES011287).

879 *IN VIVO* STRIATAL ACETYLCHOLINE ACCUMULATION IN ADULT AND AGED RATS FOLLOWING PARATHION EXPOSURE.

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Aged rats are more sensitive than adults to the acute toxicity of the organophosphorus insecticide parathion. We studied the effects of parathion on striatal cholinesterase activity and acetylcholine accumulation in 3- and 18-month old Sprague-Dawley rats. Adult and aged rats were treated with either vehicle (peanut oil, 2 ml/kg) or equitoxic dosages of parathion (adult, 27 mg/kg, sc; aged, 9 mg/kg, sc). Body weight, functional signs of toxicity, and motor activity were recorded for seven days. On day 3 and 7 after parathion, striatal microdialysates were collected and rats were then sacrificed for biochemical measurements. Both age groups showed severe signs of cholinergic toxicity (involuntary movements and SLUD signs) with peak effect 3-4 days after parathion. In general, there was an increase in diurnal (1000-1900 hrs) motor activity (ambulation and rearing) following parathion while nocturnal (1900-0700 hrs) activity was significantly reduced in both age groups. Similar extensive striatal cholinesterase inhibition (91-95 %) was noted in both age-groups at both time-points. In contrast, age-related differences in acetylcholine accumulation were noted, with aged rats showing about 2-fold lower extracellular acetylcholine levels compared to adult rats. When neostigmine (1 μ M) was subsequently infused to completely block local acetylcholinesterase activity, no age-related differences in acetylcholine accumulation were noted. Comparison of *in vitro* striatal acetylcholine synthesis in synaptosomes from adult and aged rats also suggested no age-related differences in synthetic capacity. The results suggest that equitoxic dosages of parathion can cause differential effects on acetylcholine levels in adult and aged rats and that the higher sensitivity to parathion noted in aged rats is not related to greater acetylcholine accumulation. Supported by NIEHS R01ES009119 and Oklahoma State University Board of Regents.

880 AGE-RELATED DIFFERENCES IN DETOXICATION AND ACUTE TOXICITY LEVELS OF TWO ORGANOPHOSPHATES IN RATS.

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The organophosphorus (OP) insecticides, chlorpyrifos (CPS) and methyl parathion (MPS), which cause acetylcholinesterase (AChE) inhibition, display greater acute toxicity in juveniles than in adults. The roles of carboxylesterases (CbxE) in stoichiometric detoxication and A-esterases (paraoxonases) in catalytic detoxication were investigated in liver and serum from adult (70 days of age) and juvenile (1 and 12 days of age) rats. The detoxication ability of both compounds by both esterases increased with age. CbxE were extremely sensitive (IC₅₀) to inhibition by chlorpyrifos-oxon (CPO $\leq$ 1nM) and much less sensitive to methyl paraoxon (MPO $\geq$ 300nM). CPO, but not MPO, was readily detoxified by A-esterases. Therefore, detoxication potential for CPO (both CbxE and A-esterases) was much greater than for MPO (neither esterase). To validate *in vitro* results, rats of the same 3 ages were exposed IP to each of the oxons to determine the dosages required to inhibit brain AChE at 3 levels (80-90%, 40-50%, and 10-20%). Subsequently, rats of the same 3 ages were exposed IP to each of the parent OPs to determine the dosages required to inhibit brain AChE at same 3 levels. The dosages that resulted in 40-50% inhibition were much higher for the parent insecticides than the oxons for both compounds. There was a much greater differential between the dosages yielding the same level of brain AChE inhibition between adults and juveniles for CPO than MPO, and for CPS than MPS. For example, the fold-differences in dosages yielding 40-50% inhibition between the ages were: CPO, 6; MPO, 1; CPS, 7; and MPS, 2. These results support the concept that the greater detoxication of OPs in the adult is an important factor in the greater tolerance of OPs by adults, and that

the extent of esterase-mediated detoxication along with differences in P450 metabolism among compounds is a critical determinant in toxicity levels. (Supported by NIH R01 ES11287).

881 SARIN CAUSES DIFFERENTIAL REGULATION OF mRNA EXPRESSION OF MUSCARINIC RECEPTOR SUBTYPES 24 HRS POST TREATMENT IN RATS.

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Sarin, isopropyl methyl phosphonofluoridate, is an organophosphate ester neurotoxin that inhibits acetylcholinesterase (AChE) in the central and peripheral nervous systems, and has been implicated in the Gulf War Syndrome. Recently, our published global gene expression data from sarin treated rats at different time points (both early and late) had shown the important role of muscarinic receptors and their down-stream molecules in exposure related abnormal clinical manifestations. In order to further investigate the role of different members of muscarinic receptor family members, we studied the expression pattern of m1, m2, m3, m4, and m5 muscarinic receptor subtypes in different regions of the rat brain exposed to 50 μ g / kg / ml dose of sarin at 24 hr post treatment. Semi-Quantitative reverse transcriptase polymerase chain reaction (RT-PCR) was used to study the mRNA expression levels. Both β -actin and 18S RNA levels were also estimated. 18S RNA levels were used for normalization, as β -actin levels were induced to 1.60 fold in cerebellum and midbrain, while 18S RNA levels remained unchanged in all regions. Differential regulation of mRNA expression levels was noted for m1, m2, m3, m4, and m5. In cerebellum, transcript levels of m3, m4, and m5 were decreased by 2.03, 1.70, and 1.20 fold respectively. In midbrain, transcript levels of m3, m4 and m5 were increased by 3.53, 1.45 and 2.09 fold respectively. The spatial differences in the expression of different members of the muscarinic receptor family members further support the complexity of the toxicity induced by sarin exposure, which may involve both cholinergic and non-cholinergic pathways in a region-specific manner. This work was supported by DAMD-17-98-8027 grant

882 EFFECTS OF SUBCHRONIC, LOW-LEVEL CHLORPYRIPHOS EXPOSURE ON FAST AXONAL TRANSPORT.

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Exposure to chlorpyrifos (CPF) compromises spatial learning and reduces presynaptic cholinergic markers. One possible mechanism for producing these effects is reduced fast transport of vital macromolecules. To determine if transport compromise is correlated with these changes, adult, male rats were given subcutaneous injections of CPF @ 18 mg/kg/d in peanut oil every other day for 30 days (n=5), or the same treatment plus 14 days of no injections (washout; n=5), or single injections @ 2, 12, 24 or 48 hrs (n=3/group) before sacrifice. Controls received equal volume of peanut oil only. The motor nerve to the quadriceps femoris was removed under anesthesia and dissected down to single fascicles for visualization of vesicle transport in single axons using video-enhanced DIC microscopy. The flux of vesicles in a standard volume of axoplasm over ten minutes of time was determined by an observer blind to experimental group. Thirty days of CPF exposure reduced fast anterograde transport (faAXT) from 428 $\pm$ 15 vesicles to 333 $\pm$ 27 and retrograde transport (rAXT) from 209 $\pm$ 19 to 171 $\pm$ 25, a 22 and 18% reduction, respectively. After 14 days of washout, the faAXT remained reduced by 20% from control while the rAXT was reduced by 28%. Single injections of CPF had no effect at 2 hours but did show a significant and progressive effect with time. At 12 to 24 hours, faAXT was reduced 10 to 20% from control, respectively and rAXT was reduced 7.5 to 32% from control, respectively. Forty-eight hours after a single injection the quantity of faAXT was reduced 14.5% from control while rAXT was still reduced 25.8%. We conclude that low levels of CPF exposure produces a significant reduction in both faAXT and rAXT. The lipid soluble CPF takes longer to produce a reduction compared to other water soluble toxicants observed to inhibit transport. The effect is more prolonged, consistent with continual release of CPF from lipid stores. Inhibition of fast transport may be responsible for altered delivery of presynaptic cholinergic markers and compromised spatial memory. Supported by R01ES012241 and R01ES011223.

883 INHIBITION OF GROWTH OF NEURAL PROCESSES OF PRIMARY RAT DORSAL ROOT GANGLIA (DRG) CULTURES AS A MODEL FOR PROMOTION OF AXONOPATHIES.

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Certain esterase inhibitors, such as phenylmethane sulfonyl fluoride (PMSF), cause clinical and morphological exacerbation (promotion) of toxic or traumatic axonal lesions. The hypothesis was made that promotion was due to interference with the

mechanisms of axonal repair/regeneration. Degeneration of neural processes of cultured rat DRGs caused by neuropathic organophosphates (OP) has been already described as possible model for studying promotion. Since the dissection of the DRGs is a traumatic axonal lesion, we tested this lesion as a possible model for studying promotion as defined by inhibition of process outgrowth. Desheathed DRGs from 13-16 day old rats were dissected and plated on poly-L-lysine pre-coated dishes. Since in untreated control DRGs processes appeared at about 48 hours after plating, inhibitors were incubated at 24 hours. PMSF (0.5-1.5 mM) caused a dose dependent impairment of process outgrowth as determined 4 and 6 days after plating. Control inhibitors were the neuropathic non-promoter dibutylchlorovinyl phosphate (DBDCVP; 0.01 or 0.1 mM), and paraoxon (0.01 or 0.1 mM) that is neither neuropathic nor promoter, but is a potent inhibitor of acetylcholinesterase (AChE). DBDCVP caused significant reduction in the number and length of the processes at 0.1 mM, whereas at 0.01 mM the number of processes was comparable to controls. However, their morphological characteristics was different from control because at day 6 a beading-like degeneration was observed as already described with another neuropathic OP. Paraoxon also caused significant inhibition at 0.1 mM, possibly associated with cell death. At 0.01 mM no effect on the number of processes was observed and the processes were morphologically undistinguishable from controls. It should be noted that DBDCVP concentration was more than 2 orders of magnitude higher than that necessary to critically inhibit the target of the neuropathy (i.e. neuropathy target esterase), and that paraoxon concentrations were more than 2 orders of magnitude higher than those required to completely inhibit AChE.

884 LONG-TERM EXPOSURE TO TWO NEUROTOXIC ORGANOPHOSPHATES AND CORTICOSTERONE IN RATS ALTERS SURAL NERVE G RATIO.

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We conducted an investigation of the effects of long-term, multiple dose exposure to two neurotoxic organophosphates in rats, in the presence of a chronic stress model. Details of the experimental design have been published (Jortner et al., *Toxicol. Pathol.* 33:378). In summary, young adult male Long-Evans rats were administered two 60 mg/kg subcutaneous chlorpyrifos exposures and/or 14 tri-orthotolyl phosphate (TOTP) gavage doses at 75, 150 or 300 mg/kg, over a 63-day period. Corticosterone was given at 400 micrograms/ml in the drinking water (to model chronic stress) for 90 days. Sacrifice was on days 63 and 90. Portions of this study already reported (Jortner et al.) showed organophosphate induced delayed neuropathy manifest by TOTP dose-related diminished activity of brain neurotoxic esterase on day 63, and distal gracile fasciculus and peripheral nerve Wallerian-like myelinated fiber degeneration. The lesions progressed in the 63 to 90 day period, although exposure to the organophosphates had ceased. Because of spinal somatosensory fasciculus gracilis involvement, we extended studies to sural nerve peripheral sensory myelinated fibers. Lesions were most evident on day 90, such as axonopathy progressing to fiber degeneration. Neurons in dorsal root ganglia, a source of gracile and sural fibers, showed chromatolytic changes. Morphometric alterations were seen in residual sural nerve fibers, with the g ratio (axon diameter/fiber diameter) most sensitive in differentiating treatment effects. This was reduced in rats receiving 300 mg/kg TOTP dosages plus chlorpyrifos and/or corticosterone ($p < 0.05$, ANOVA followed by LSD, $n = 3-6/\text{group}$), most marked in rats dosed with all three compounds. G ratios of rats dosed with TOTP alone were not different ($p > 0.05$) from the controls. In our dosing regimens, combining TOTP with chlorpyrifos and/or corticosterone diminished the g ratio in the sural nerve, likely related to axonal atrophy. Supported by USAMRMC DAMD17-99-1-9489.

885 MULTIPLE NEUROTRANSMITTER RECEPTORS ARE INVOLVED IN THE CHANGES IN BDNF GENE EXPRESSION INDUCED IN HIPPOCAMPAL ORGANOTYPIC SLICES EXPOSED TO CHLORPYRIFOS AND CHLORPYRIFOS-OXON.

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The organophosphate (OP) insecticides exert their toxicity by the inhibition of acetylcholinesterase leading to the accumulation of the acetylcholine which causes overstimulation of the cholinergic receptors. We have reported that both in vivo and in vitro exposure to chlorpyrifos (CPS) leads to increased expression of the brain derived neurotrophic factor (BDNF). To further investigate the basis for this alteration of gene expression, hippocampal organotypic slice cultures were prepared, maintained in culture for 14 days, incubated with either 10 μM CPS or 1 μM chlorpyrifos-oxon (CPO) for 32 hrs, harvested, and mRNA levels were determined. The concentrations of CPS and CPO produced 63% and 73% inhibition of cholinesterase in the slice cultures but did not alter their viability. Similar to pre-

vious studies both CPS and CPO induced BDNF expression. Inclusion of the muscarinic receptor antagonist atropine (5 μM) in the incubation eliminated the induction of BDNF expression. However, inclusion of the NMDA receptor antagonist MK-801 (20 μM) also eliminated the induction of BDNF expression. These data suggest that the observed increased BDNF gene expression involves indirect activation of glutamatergic receptors which occurs as a result of OP-induced muscarinic receptor activation. Direct receptor activation can induce BDNF expression as it displays an immediate early gene nature. However, increased reactive oxygen species (ROS) can also increase BDNF expression and CPS has been shown to induce ROS both in vivo and in vitro in cell culture. In our exposure paradigm, heme oxygenase-1, a marker for ROS, is increased in hippocampal slices by CPS and CPO exposure at levels similar to those obtained by exposure to glutamate alone. Thus, the basis for the increased BDNF expression is not clear but it is possible that the indirect activation of the glutamatergic receptor may be linked to both ROS production as well as the increased expression of BDNF. (Supported by NIH 1P20-RR17661)

886 CHLORPYRIFOS INDUCES REDOX-SENSITIVE GENE EXPRESSION IN OLIGODENDROCYTES.

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There are increasing concerns regarding the relative safety of chlorpyrifos (CPF) to various facets of the environment. Although, literature suggests that CPF is relatively safe in adult animals, recent evidence indicates that juveniles, both animals and humans may be more sensitive to CPF toxicity than adults. In young animals, CPF is neurotoxic and mechanistically interferes with cellular replication and cellular differentiation, which culminates in the alteration of synaptic neurotransmission in neurons. Our laboratory has previously demonstrated that CPF and its metabolites are also toxic to oligodendrocyte progenitors. To further characterize these toxic effects, targeted microarrays were utilized to specifically identify genes which may be involved in CPF-induced toxicity in glial cell progenitors. Results reveal that sustained exposure for eight (8) days to CPF at non-lethal concentrations (10 μM) up-regulated redox-sensitive transcription factors *egr-1* (krox-24), *c-jun*, *myc* and *c-fos* and down-regulated the anti-apoptotic gene *Birc3*. Real-time PCR analysis confirmed that CPF produced 3.637 ± 1.250 (SEM) -fold induction of the early response gene-1 (*Egr1* or *krox-24*) and 8.787 ± 3.610 (SEM) -fold induction and 2.320 ± 0.8401 -fold induction of *c-fos* and *c-myc*, respectively. In acute studies, at higher concentrations (30-60 μM), CPF exposure resulted in the up-regulation of *NF-k-B* as well as the heme oxygenase-1 gene. It should be noted the heme oxygenase-1 gene expression is induced under oxidative stress and is tightly controlled by redox sensitive transcription factor *Nrf2* (nuclear factor erythroid-2 related factor). Collectively, this data suggests that CPF induces toxicity by the generation of reactive oxygen/nitrogen species which, in turn, activates cellular responses at the transcriptional level.

887 INCREASE OF NEUROPATHY TARGET ESTERASE ACTIVITY IN BOVINE CHROMAFFIN CELLS IN CULTURE USING AN ADENOVIRAL VECTOR.

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Neuropathy target esterase (NTE) is present in many of tissues included the bovine chromaffin cell cultures which show the highest level of NTE of all the studied tissues. It is well known that inhibition and specific modification of NTE by some organophosphorous (OPs) compounds induce a neurodegenerative disorder. It has been suggested that NTE shows a lysophospholipase activity involved in the phosphatidylcholine homeostasis but its role in the neuropathy induction is not completely clear. The cDNA of human NTE (4.4 kb) has been inserted into an adenoviral vector. Bovine chromaffin cells cultured at 50,000 cells/well were incubated with the vector for 2 hours and after that cells were kept in the incubator. NTE activity measured after 24 hours was 6.8 ± 0.5 mU/106 cells in untreated cells and 14.8 ± 1.5 mU/106 cells, 19.3 ± 2.9 mU/106 cells, 24.8 ± 0.9 mU/106 cells and 30.9 ± 1.0 mU/106 cells in cells incubated with 2, 4, 8 and 16 μl of vector, respectively. Kinetic inhibition curves with mipafox of NTE activity were performed in cells treated and untreated with the vector. After 60 minutes of inhibition with the inhibitor the calculated IC_{50} values were 5.5, 6.2 and 6.6 μM for cells incubated with 0, 2 and 10 μl of vector. Results present in this work confirm that the adenoviral vector containing the human NTE gene is active in bovine chromaffin cells in culture and the NTE activity expressed by the vector show the same inhibition pattern by the neuropathic OP mipafox than NTE activity from bovine chromaffin cells.

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888 MOLECULAR MODELING PREDICTS CONFORMATIONAL CHANGES IN THE NEUROPATHY TARGET ESTERASE (NTE) CATALYTIC DOMAIN (PNTE) AFTER INHIBITION AND AGING.

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Inhibition and aging of NTE by organophosphorus compounds (OPs) triggers axonopathy. Identifying OP-induced structural changes will help illuminate NTE's pathogenic role. Lacking experimental structural data, molecular modeling can be used. We used docking with simulated inhibition (SI) and aging (SA) to test the hypothesis that these chemical alterations produce conformational changes in modeled PNTE. We did docking with Fujitsu CAChe using diisopropylphosphorofluoridate (DFP) as the ligand. We did SI using DFP-inhibited PNTE. We built the structure of diisopropylphosphoryl-serine (DPSer) from that of monoisopropylphosphoro-Ser (MPSer) downloaded from HIC-UP and substituted DPSer for Ser966 in PNTE using 'O' ver 10.1. We used the lego loop procedure in O to fix chain breaks arising from the substitution and subjected the resulting structure to 300 rounds of minimization using the MM3 protocol in CAChe. We did SA of DPSer-PNTE by deleting an isopropyl group from DPSer and minimizing the negatively charged adduct with MM3/CAChe. Docking showed that the P-atom of DFP was 4.7 Å from Ser966 OG and 3.7 Å from Asp1086 OD2, indicating that DFP can be accommodated in the active site of PNTE oriented suitably for inhibition. DFP's binding energy was -36.1 kcal/mol, indicating favorable energetics. SI resulted in a significant conformational change: the Ser966 OG to Asp1086 OD2 distance increased from 2.9 Å in PNTE to 5.2 Å in DPSer-PNTE. SA of DPSer PNTE showed a relaxation of the structure: the Ser966 OG to Asp1086 OD2 distance decreased to 3.7 Å in MPSer-PNTE. These results support the hypothesis that inhibition of NTE produces a conformational change in the tertiary structure of PNTE and that aging of the NTE-OP adduct results in another conformational change. These models provide templates to guide research in solving the crystal structures of native, inhibited, and aged NTE (Funded by ARO DAAD19-02-1-0388, MEDC, and NIH ES07062).

889 COMPUTATIONAL DOCKING OF CYCLIC NUCLEOTIDES INTO THE PUTATIVE REGULATORY DOMAINS OF NEUROPATHY TARGET ESTERASE (NTE).

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Specific chemical modification NTE leads to axonopathy. NTE's structure is unknown, but molecular modeling can give structural insight. Although little is known about regulation of NTE, we previously showed that NTE has 3 putative regulatory cyclic nucleotide (CNP) binding domains: CNP1, CNP2, and CNP3. We developed homology models for these structures using TM1171 (PDB ID 1o5l; a homolog of catabolite activator protein (CAP)) as the template. The present study used docking to test the hypothesis that CNPs form suitable regulatory-type complexes with NTE's CNP domains. The CAP-cAMP crystal structure indicated Gly72, Arg83, Ser84 and Thr128 were involved in binding cAMP. Sequence alignments of CAP, TM1171, and NTE's CNP domains showed that these residues correspond to Ser64, Arg80 and Thr81 in CNP1; Gly64, Leu74 and Ile75 in CNP2; and Gly63, Ala74 and Thr75 in CNP3. We docked cAMP and cGMP into each CNP domain using the automated docking algorithm in Fujitsu CAChe. Docking revealed cAMP's binding energies (kcal/mol) to be -33.3 (CNP1), -27.1 (CNP2) and -52.3 (CNP3). Binding energies for cGMP (kcal/mol) were -31.1 (CNP1), -32.4 (CNP2) and -50.2 (CNP3). This shows favorable binding of cAMP and cGMP to NTE's CNP domains. CNP1 and CNP2 had higher binding affinities for cAMP; CNP2 had a higher affinity for cGMP. Analysis of the docked structures showed cAMP and cGMP bind NTE's CNP domains in a hydrophilic pocket on the surface of the proteins. Ser64 and Thr81 of CNP1; Leu74, Phe76 and Ile75 of CNP2; and Thr75 and Gln24 of CNP 3 made ionic contact with cAMP. Similarly, Ser64, Thr81 and Lys54 of CNP1; Leu74 of CNP2; and Leu74 and Glu66 of CNP3 mediated ionic contact with cGMP. These results support the hypothesis that CNPs form regulatory-type complexes with NTE's putative regulatory domains and provide templates to guide research in studying regulation of NTE and solving its crystal structure. (Funded by ARO DAAD19-02-1-0388, MEDC and NIH ES07062).

890 DIFFERENTIALLY EXPRESSED PROTEINS AND CARBONYL CONTENT IN N, N-DIETHYLDITHIOCARBAMATE (DEDIC) MYELINOPATHY.

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The neurotoxicity of dithiocarbamates has been observed in both humans and experimental animals. Previous studies have shown that the neurotoxicity of certain dithiocarbamates can manifest as a primary myelinopathy of peripheral nerve. Because increased levels of copper and lipid peroxidation products in peripheral nerve accompany DEDC induced lesions it has been suggested that disruption of copper homeostasis and increased oxidative stress may contribute to myelin injury. To assess the potential of DEDC to promote oxidative stress in peripheral nerve; changes in protein expression and protein carbonyl content resulting from DEDC exposure were determined in nerve using 2D-DIGE and immunohistochemistry, respectively. Rats were exposed to DEDC by intraperitoneal osmotic pumps for 8 weeks; and sciatic nerve proteins were extracted from DEDC exposed and non-exposed animals. Fifty-six gel plugs with an increase or decrease change greater than 1.3 fold (p<0.05) were picked from the gel, excised for trypsin digestion and identified by MALDI-TOF/MS. Twenty-six spots were up-regulated and 18 down-regulated. Among the up-regulated proteins were immunoglobulins (IgG and IgG-2a), and three isoforms of glutathione transferase important for detoxification of reactive α,β -unsaturated aldehydes generated from lipid peroxidation, including glutathione transferase pi which was localized to the cytoplasm of Schwann cells. Protein carbonyl levels from the DEDC exposed rats were significant higher by approximately 2 fold. These data support the ability of DEDC to promote protein oxidative injury in peripheral nerve and to produce lipid peroxidation in either myelin or another component of the Schwann cell, accompanied by a protective cellular response to oxidative stress. (Supported by NIH grant ES06387)

Keywords: oxidative stress; diethyldithiocarbamate; protein carbonyls; copper; sciatic nerve, demyelination; myelinopathy

891 TIME-COURSE OF ACUTE NEUROTOXICITY PRODUCED BY N-METHYL CARBAMATES IN PREWEANLING RATS.

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N-Methyl carbamate insecticides are reversible inhibitors of central and peripheral acetylcholinesterase (ChE). Despite their widespread and long-term use, we could find no studies of a systematic comparison of neurotoxicity in young animals across this group of chemicals. To learn more about potential age-differential responses, we conducted time-course studies of seven carbamates in 17-day old Long-Evans (LE) male rats. Rats receive a single oral dose of either carbaryl (30 mg/kg), carbofuran (1 mg/kg), formetanate (3 mg/kg), methiocarb (12 mg/kg), methomyl (2.5 mg/kg), oxamyl (0.5 mg/kg), or propoxur (10 mg/kg). These doses were chosen based on data from range-finding studies. Treated rats were euthanized and blood and brain were collected 15, 45, 90, 180 min, or 24 hr after dosing; control tissues from rats dosed with the appropriate vehicles were collected at 45, 180 min, or 24 hr. A radiometric assay was used to obtain ChE levels for brain and erythrocytes (RBC). Almost all carbamates showed maximal ChE inhibition encompassing the 45-min time point. Methomyl was the only carbamate tested to show maximal inhibition only at 15 min, and at 45 min the enzyme inhibition was significantly less. At 24 hr, all effects had fully recovered except for carbaryl-inhibited RBC (87% of control) and methomyl-inhibited brain (94% of control). The RBC inhibition was generally 10-30% greater (the least for formetanate, the greatest for carbaryl) than the brain inhibition with all carbamates except for propoxur, which showed similar levels of inhibition. The time-course of recovery of enzyme inhibition was similar for RBC and brain for all carbamates. These data determined the time of maximal effect to be used in dose-response studies for each chemical. Compared to previous work with these carbamates in adult LE male rats, the preweanling rats are somewhat more sensitive and slower to fully recover. This is an abstract of a proposed presentation and does not necessarily reflect US EPA policy.

892 BRAIN CHOLINESTERASE INHIBITION PRODUCED BY PROPOXUR AND DEPRESSION OF THE PHOTIC AFTER DISCHARGE OF FLASH EVOKED POTENTIALS IN LONG EVANS RATS.

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Propoxur is a widely used N-methyl carbamate pesticide that acts by inhibiting cholinesterases (ChE), which may lead to cholinergic toxicity. Flash evoked potentials (FEPs) are a neurophysiological response following stimulation of the visual

system with flashes of light. They are often used to detect central nervous system (CNS) changes following exposure to neurotoxic compounds. Disruption of CNS function by ChE inhibitors produced decreases in the photic after discharge (PhAD) of the FEP. The PhAD is related to higher cortical processing of visual information. To determine the dose-response for propoxur on the PhAD, FEPs were recorded in adult male Long-Evans rats after oral gavage with propoxur 0, 0.3, 3, 10, 20, 30 or 40 mg/kg (corn oil vehicle). One week before testing, rats were implanted with electrodes over the visual cortex and allowed to recover. FEPs were recorded for 2 consecutive days to familiarize the rats with the test procedure and to develop the PhAD. On the 3rd day, pupils were dilated (40 min) and animals dosed (30 min) before testing. Immediately after testing, rats were sacrificed, and the brains removed and stored at -80°C, until determination of brain ChE levels by a radiometric assay. All doses greater than 0.3 mg/kg of propoxur significantly inhibited brain ChE. Three-40 mg/kg propoxur inhibited 38-63% of brain ChE. The duration of the PhAD was significantly decreased at 30 mg/kg by 54%. A correlation was obtained between brain ChE levels and the PhAD duration. A significant decrease in colonic temperature was observed at 10, 20 and 40 mg/kg propoxur. Treatment with 40 mg/kg propoxur increased the latency of peak N₁₆₆. No effect on the latency and amplitude of early components of the FEP was observed. These results indicate that ChE inhibition produced by propoxur disrupts the cortical processing related to the later portion of the FEP.¹ Supported by a NRC Fellowship. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

893 CHANGES IN ELECTROENCEPHALOGRAMS (EEG) OF LONG-EVANS RATS PRODUCED BY CARBARYL AND PROPOXUR.

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N-methyl carbamate (NMC) pesticides are widely used agriculturally and residentially and act by inhibiting cholinesterases (ChE), which may lead to cholinergic toxicity. We investigated the utility of EEG recording to assess changes in central neurophysiology post treatment with NMC pesticides. Long Evans rats were implanted with epidural screw electrodes. Following recovery, restrained animals were tested for 2 days to allow acclimation to the test procedures. On day 3, the rats' pupils were dilated and 10 min later they were dosed (po) with 0, 1, 10, 30, or 50 mg/kg carbaryl or 0, 10 or 30 mg/kg propoxur (corn oil vehicle). EEG recording occurred 5, 15, and 30 min later. Thirty EEG segments (2 s) were collected at each time point. The EEG was transformed using a FFT and the spectra averaged. Immediately after testing rats were sacrificed and the brains removed and stored at -80°C until determination of brain ChE levels by radiometric assay. Carbaryl produced significant reductions in ChE activity at 10, 30 and 50 mg/kg, and propoxur decreased ChE activity at 10 and 30 mg/kg. Fifty mg/kg carbaryl inhibited ChE activity to 42% of control and 30 mg/kg propoxur reduced ChE activity to 27% of control. Thirty mg/kg propoxur increased the peak amplitude in the Theta band when recorded between the visual cortex and the cerebellum. Carbaryl treatment resulted in a similar trend, but did not reach statistical significance. When considering animals with >20% brain ChE inhibition, regression of ChE activity vs. peak Theta amplitude showed a marginally significant (p=0.04) relationship after carbaryl, but not after propoxur (p=0.06; due to the small sample size). These studies show that treatment with NMCs may result in increased activity in the Theta range of the EEG.² Supported by a NRC Fellowship. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

894 NEUROTOXICITY OF CARBARYL IN THE AGING BROWN NORWAY (BN) RAT: EFFECTS ON CORE TEMPERATURE (TC) AND MOTOR ACTIVITY (MA).

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The US EPA is pursuing a variety of research efforts to assess the susceptibility of the aged to neurotoxicants. The BN strain is a popular animal model for aging studies but there is a need for improved methods of monitoring their physiological responses to neurotoxicants over the animal's life span. This study used radiotelemetry to follow longitudinal changes in Tc and MA, and sensitivity to carbaryl in 16 male rats surgically implanted with radiotransmitters (Data Sciences Int.). Tc and MA were recorded at 5 min intervals for 7 days in undisturbed animals. Monitoring was initiated at 90 days of age and was repeated every other month. Following the baseline monitoring, rats were dosed with carbaryl (25 mg/kg; po) or the corn oil vehicle. Tc and MA data in the undisturbed animals were ensemble into a single 24 hr period for statistical analysis. At the time of writing, data have been collected in animals at ages of 90, 150, and 210 days. Aging has quantitative and qualitative effects on the circadian rhythm of Tc and MA and on intra-subject variation. Aging has been associated with a slight reduction in Tc; rats at 90, 150, and 210 days had a mean Tc of 37.23, 37.15, and 37.11 °C. MA

has not paralleled the changes in Tc. Mean MA was 1.88 and 1.57 cts/min in 90 and 150 day old rats but increased to 1.64 cts/min in the 210 day old animals. The thermoregulatory response to carbaryl has also changed with aging. The first dosing with carbaryl led to a prolonged hypothermic response and little effect on MA that recovered within 24 hr. The peak decrease in Tc following carbaryl was -1.9, -1.9, and -0.7 °C at ages of 90, 150, and 210 days of age, respectively. Baseline Tc and MA and their response to carbaryl will be followed at 60 day intervals for at least two years. The data will provide a framework to develop methods for using the BN rat as a model of susceptibility to neurotoxic agents in the aged. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

895 MECHANISM OF SELECTIVE TOXICITY OF ENDOSULFAN IN INSECTS AND MAMMALS.

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Endosulfan is a broad spectrum insecticide and acaricide. It exhibits acute oral and inhalation toxicity to mammals, but is neither mutagenic nor carcinogenic. The acute toxicity is known to be due to overstimulation of the CNS, and the GABAergic system is suspected to be a major target site. Since the GABAergic system is found in both mammals and insects, a question arises as to whether there is another target site that confers a selective toxicity in insects over mammals. In vitro experiments were performed by the whole-cell patch clamp technique using the rat dorsal root ganglion (DRG) neurons and cockroach ganglion neurons. α -Endosulfan had a dual effect on the GABA-induced current in rat DRG neurons, the initial fast inhibition followed by a slow recovery in the continuous presence of the drug in a concentration-dependent manner. The IC₅₀ for the initial block was ~10 nM. α -Endosulfan also blocked cockroach GABA currents with an IC₅₀ of 5 nM (n = 3). However, unlike DRG neurons, no recovery was observed during application of α -endosulfan. In addition, the glutamate-activated chloride channels (GluCl_s), which are present in insects but not in mammals, were found to be potentially blocked by α -endosulfan. The GluCl had two components, a desensitizing and a non-desensitizing current. We found easy ways to separately record the two components. Trypsin inhibited the non-desensitizing current, leaving the desensitizing current intact. Soybean Trypsin Inhibitor and polyvinylpyrrolidone (PVP) inhibited the desensitizing current, leaving the non-desensitizing current intact. α -Endosulfan inhibited GluCl_s slowly, reducing the non-desensitizing current more potently and efficaciously than the desensitizing current. In conclusion, the selective toxicity of endosulfan in insects over mammals is due largely to its potent blocking action on GluCl_s and due partly to its higher potency to block the cockroach GABA receptor than the rat DRG GABA receptor. Supported by NIH grant NS014143.

896 β SUBUNITS INCREASE SENSITIVITY OF SODIUM CHANNEL Na_v1.3, BUT NOT Na_v1.2, TO TYPE II PYRETHROIDS.

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Voltage-sensitive sodium channels (VSSCs) are targets of pyrethroid insecticides and are comprised of a pore-forming α and auxiliary β subunits. Multiple isoforms of both subunit types exist. The pyrethroid sensitivity of different isoform combinations has not been well characterized, yet differences in VSSC sensitivity to pyrethroids exist in intact neurons. The present experiments examined the influence of the β subunit on pyrethroid sensitivity of Na_v1.2 and Na_v1.3 channels. *X. laevis* oocytes were transfected with rat α (Na_v1.2 or Na_v1.3) with or without co-transfection with β_3 or β_1 subunit mRNA and sodium currents were recorded using two-electrode voltage clamp techniques. Tetrodotoxin (1 μ M) blocked peak inward current, and minimal tail current was present upon repolarization. The %pyrethroid-modified channels was determined based on tail current induced by DLT. In the absence of any β subunit, DLT (10 μ M) modified 1.25 and 3.0% of Na_v1.2 or Na_v1.3 channels, respectively. Upon co-transfection with β_1 , DLT (10 μ M) modified 2.17% and 8.31% of Na_v1.2 and Na_v1.3 channels, respectively; while co-transfection with β_3 did not alter DLT modification of Na_v1.2 channels (1.92%) but increased modification of Na_v1.3 channels (11.71%). Expression of Na_v1.2/ β_3 subunits predominate in adult central neurons, while expression of Na_v1.3/ β_3 subunits predominates in embryonic neurons. Thus, differential sensitivity of these subunit combinations was examined for several pyrethroids (10 μ M each; n=3-21 oocytes/compound). Type II pyrethroids differentially modified Na_v1.3/ β_3 and Na_v1.2/ β_1 respectively: β -cyfluthrin (19.75% vs. 1.54%); esfenvalerate (28.36% vs. 2.67%) and fenvalerate (14.55% vs. 1.89%). These results demonstrate that the β subunit has a significant impact on the sensitivity of Na_v1.3, but not Na_v1.2 channels, and that Na_v1.3/ β_3 channels are more sensitive than Na_v1.2/ β_1 rat sodium channel to Type II pyrethroids. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy).

897 EFFECTS OF ACUTE PYRETHROID EXPOSURE ON THERMOREGULATION IN RATS.

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Pyrethroid insecticides produce acute neurotoxicity in mammals. According to the FQPA mandate, the USEPA is required to consider the risk of cumulative toxicity posed to humans through exposure to pyrethroid mixtures. Thermoregulatory response (TR) is being used to determine if the dose-additive action for motor function can be generalized to other endpoints in rats. Adult male Long Evans rats were monitored for 48 h for core temperature (Tc) and motor activity (MA) at 5 min intervals by surgically implanted radiotransmitters. Acute oral dose-response studies [n=6-13; 6 doses/compound] were carried out for a Type-I (Permethrin, PER, 5-220 mg/kg) and a Type-II (Deltamethrin, DLT, 0.5-12 mg/kg) pyrethroid. Both pyrethroids produced acute effects, including increased MA and compound-specific changes in TR that recovered within 12 hr after dosing. Area under the curve (AUC) from 1 to 7 hr post-dosing was determined for each pyrethroid dose and used for statistical analysis. PER elicited a hyperthermic response at 75-220 mg/kg. The TR dose-response to DLT was biphasic, with 1.5-3 mg/kg eliciting a significant elevation in Tc and higher doses eliciting a prolonged hypothermic response. In addition, animals dosed with 9-12 mg/kg DLT exhibited a slight elevation in Tc the day after exposure (0.16-0.22 °C above controls). In a preliminary mixture study, the TR response to 220 mg/kg PER and 12 mg/kg DLT resulted in an approximate algebraic summation of the TR responses to the individual compounds: the AUCs for PER, DLT and the mixture were 12.4, -8.8, and 4.65 °C.hr-1, respectively. Future work will include testing other pyrethroids and an environment relevant mixture of them using TR as a sensitive endpoint. This abstract does not necessarily reflect the policy of the US EPA.

898 DOSE-RESPONSE MODELING OF THE TRANSCRIPTIONAL RESPONSE OF RAT CEREBROCORTICAL TISSUE AFTER ACUTE PYRETHROID EXPOSURE IN VIVO.

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Pyrethroid insecticides produce neurotoxicity in mammals by disrupting ion channel function in excitable nerve membranes. Pyrethroid use has increased as regulatory guidelines have restricted the use of other pesticide classes. Currently, a sensitive, specific, dose-responsive biomarker of effect that correlates with pyrethroid-induced disruption of nervous system function is not available. This study used a novel combination of statistical methods to identify dose-responsive transcripts from a microarray data set. Our goal was to characterize potential biochemical markers of pyrethroid effects. Long-Evans rats (n = 8-12/group) were orally dosed with permethrin (1, 10, 100 mg/kg), deltamethrin (0.3, 1.0, 3.0 mg/kg) or corn-oil vehicle and cerebrocortical tissue was collected at 6 hours. Affymetrix Rat 230 2.0 GeneChip® microarrays were used to obtain transcriptional profiles. Dose-responsive transcripts were identified using a standard Significance Analysis of Microarrays (SAM) approach and an isotonic regression model that assumes a monotonic dose-response relationship, but otherwise makes no assumptions about the precise form of the dose-response curve (M-score, Hu et al. 2005). Permutation-based calculation of false discovery rates were used to provide multiple comparison error control. These analyses revealed several candidate transcripts commonly regulated by both compounds. qRT-PCR confirms dose-responsive relationships for: increased expression of Camk1γ and decreased expression of Abcb1a, prominin 1 and dopa decarboxylase, for both deltamethrin and permethrin. Future work will validate the use of these transcripts, their corresponding proteins, or components of cellular signaling pathways in which they are involved, as specific biomarkers of effect for pyrethroids. *This abstract does not necessarily reflect the policy of the US EPA.*

899 ROTENONE TOXICITY IN NURR1-NULL HETEROZYGOUS AND WILD-TYPE MICE.

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Parkinson's disease is the 2nd most common neurodegenerative disease effecting 1-2% of the population over 65 years of age. The hallmark of Parkinson's disease is selective nigrostriatal dopaminergic degeneration that is believed to be caused by a combination of environmental and genetic factors. One environmental factor implicated in Parkinson's disease is exposure to pesticides. A genetic factor may be the nuclear receptor Nurr1 as mutations in Nurr1 have been implicated in Parkinson's

disease and dopamine neurons in Nurr1-null heterozygous (+/-) mice are more susceptible to certain neurotoxins. The mechanism(s) for this increased susceptibility, however, has not been determined. Chronic exposure to the pesticide rotenone has been found to reproduce many features of Parkinson's disease in rodents. Rotenone is a common organic pesticide and acts as a complex I inhibitor of the mitochondrial respiratory chain. The purpose of the current experiment was to determine if the +/- genotype makes dopamine neurons more susceptible to the neurotoxic effects of rotenone than wild-type mice. In this experiment a subcutaneous osmotic pump was implanted into 26 male mice for 7 days. The subjects were split into two groups according to their genotype. Half of the subjects for each genotype received a pump containing rotenone (6-8 mg/kg/day), and the other half received a vehicle (containing a 1:1 ratio of DMSO and PEG). Rotenone treatment had no effect on the number of tyrosine hydroxylase immunoreactive neurons or on dopamine levels in the striatum. Roto-Rod performance, however, was impaired in +/- rotenone treated mice as compared to rotenone treated +/- mice. Wild-type mice were more sensitive to rotenone toxicity based on survival rates as 62.5% of these mice died from treatment while all +/- mice survived. Based on these data, the +/- mice appear more resistant to systemic rotenone toxicity. Furthermore, the toxic dose appears to be lower than the effective dose at destroying dopamine neurons in these mice.

900 SYNERGISTIC EFFECT ON NEURONAL CELL DEATH OF THE PESTICIDE ROTENONE IN THE PRESENCE OF EXCITOTOXINS ON ORGANOTYPIC RAT HIPPOCAMPAL SLICE CULTURES.

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Exposure to inhibitors of oxidative phosphorylation or activators of inotropic glutamate receptors is known to cause neuronal death associated with free radical production. We hypothesized that exposure to rotenone, an inhibitor of complex I of the mitochondrial electron transport chain, might exacerbate glutamate excitotoxicity, possibly by oxidative damage due to superoxide production. Using rat organotypic hippocampal cultures, we investigated the effects of rotenone (1 – 300 nM) given alone, or in combination with one of three excitotoxic agents, NMDA (1 – 10 μM), AMPA (1 – 10 μM) or TEA (0.5 – 2 mM). Low doses of rotenone (100 nM), NMDA (1 μM), AMPA (1 μM) or TEA (0.5 mM), caused neuronal death that was comparable to controls as measured by propidium fluorescence after 24 hr. However, exposure to rotenone combined with NMDA, AMPA or TEA, all at concentrations that individually were not neurotoxic, resulted in significant (p < 0.001) neuronal death. Combined exposure to rotenone and excitotoxin produced a synergistic effect on neuronal death at low doses, gradually becoming additive as the doses were increased. Stimulation of hydrogen peroxide production as measured by amplex red was also observed. These results suggest that sub-lethal exposure to rotenone can predispose neurons to excitotoxic injury.

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901 BFGF PROTECTS AGAINST ROTENONE-INDUCED DOPAMINERGIC APOPTOSIS THROUGH ACTIVATION OF ERK1/2 AND PI3-KINASE PATHWAYS.

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Administration of rotenone to rats reproduces many features of Parkinson's disease including dopaminergic neuron degeneration, and provides a useful model to study the pathogenesis of Parkinson's disease. However, the cell death mechanisms induced by rotenone and potential neuroprotective mechanisms against rotenone are not well defined. Here we report that rotenone-induced apoptosis in human dopaminergic SH-SY5Y cells is attenuated by pretreatment with basic fibroblast growth factor (bFGF). bFGF activated both extracellular signal-regulated kinase 1/2 (ERK1/2) and phosphatidylinositol-3 kinase (PI3-kinase) pathways in SH-SY5Y cells. Ectopic activation of ERK1/2 or PI3-kinase protected against rotenone, while inhibition of either pathway attenuated bFGF protection. bFGF induced a long-lasting phosphorylation of the pro-apoptotic protein BAD at serine 112, suggesting BAD inactivation through the ERK1/2 signaling pathway. Reducing the expression of BAD by siRNA rendered SH-SY5Y cells resistant to rotenone, implicating BAD in rotenone-induced apoptosis. Rotenone stimulated AIF release from the mitochondria and subsequent nuclear translocation, a process blocked by bFGF, further implicating mitochondrial dysfunction in rotenone-induced apoptosis. Moreover, the selective loss of dopaminergic neurons from primary mesencephalic cultures was attenuated by bFGF and was dependent on activation of ERK1/2 and PI3-kinase signaling. In addition, bFGF blocked rotenone-induced AIF nuclear translocation in primary dopaminergic neurons. These data suggest that rotenone-induced dopaminergic apoptosis requires BAD, and identify bFGF and its activation of ERK1/2 and PI3-kinase signaling pathways as novel intervention strategies to block cell death in the rotenone model of Parkinson's disease.

902 MECHANISMS OF MPP+, ROTENONE, AND PARAQUAT TOXICITY: THIOREDOXIN OXIDATION, AND ACTIVATION OF CELL DEATH PATHWAYS.

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The parkinsonian-inducing chemicals MPTP, rotenone and paraquat have been shown to reproduce several features of Parkinson's disease (PD) in animal and cell culture models. Although these chemicals are known to disrupt dopamine homeostasis and induce neuronal cell death, their molecular mechanisms of actions are not well delineated. Using human neuroblastoma cells stably expressing dopamine transporter (SK-DAT) as a model system, we have previously shown that paraquat does not require functional dopamine transporter (DAT) and does not inhibit mitochondrial complex I in order to mediate its toxic action (Richardson et al., 2005). In this study we show that paraquat specifically oxidized the cytosolic form of thioredoxin (Trx1) followed by a robust Jun N-terminal kinase (JNK) and mild p38 activation culminating in caspase-3 activation. Cells were almost completely protected from paraquat-induced toxicity by a cell permeable caspase-3 inhibitor. Conversely, MPP+ and rotenone preferentially oxidized the mitochondrial form of thioredoxin (Trx2), but did not activate JNK, p38 MAPK or caspase-3. These data suggest that, oxidative modification of cytosolic proteins is important to paraquat toxicity, while mitochondrial oxidative stress is crucial for MPP+ and rotenone mediated toxicity of SK-DAT cells (NIH U54 ES012068; F32 ES013457).

903 ENVIRONMENTAL NEUROTOXIC AGENTS INFLUENCE LRRK2, DJ-1, PARKIN AND PINK1 EXPRESSION IN DOPAMINERGIC NEURONAL CELLS: RELEVANCE TO GENE-ENVIRONMENT INTERACTION AND PROTEASOMAL DYSFUNCTION IN PATHOGENESIS OF PARKINSON'S DISEASE.

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Environmental exposure to metals and pesticides, genetic defects in LRRK2, PINK1, Parkin, DJ-1 and α -synuclein, proteasomal dysfunction and oxidative insults are implicated in Parkinson's disease (PD). However, interactions of environmental factors with PD genes and subsequent impacts on proteasomal dysfunction and oxidative damage are yet to be clarified. Herein, we examined the effect of environmental neurotoxicant exposure on LRRK2, PINK1, Parkin and DJ-1 levels in various cell models of PD. Acute exposure to 100 μ M dieldrin for 5-60 min induced a time-dependent decrease in protein levels of LRRK2, Parkin, PINK1 and DJ1 in the N27 mesencephalic cell line. LRRK2, Parkin, PINK1, and DJ1 expression was decreased by 35-75% as compared to vehicle-treated cells. In order to further understand dieldrin-induced alterations in PD gene expression and their role in proteasomal function and oxidative damage, we engineered N27 cells to stably express either human α -synuclein, caspase-cleavage resistant PKC δ D327A mutant, wild-type or mutant ubiquitin proteins Ub^{WT}, Ub^{K48R} and Ub^{K63R}. α -Synuclein overexpression attenuated dieldrin-induced decrease in Parkin protein but exacerbated DJ1 levels without any effect on LRRK2 and PINK1 levels. Overexpression of the oxidative stress insensitive kinase mutant PKC δ D327A significantly blocked dieldrin-induced decreases in Parkin and exacerbated PINK1, with no effect on LRRK2 and DJ-1. Furthermore, overexpression of wild-type ubiquitin protein Ub^{WT} exacerbated the dieldrin-induced reduction in LRRK2, Parkin, PINK1, and DJ1 levels. Conversely, overexpression of Ub^{K48R} and Ub^{K63R} mutants significantly attenuated dieldrin-induced reduction in Parkin, LRRK2, DJ-1 and PINK1 levels. Collectively, our data demonstrate a significant interplay between environmental exposure, PD gene expression and proteasomal function in cell culture models of PD. (supported by NIH grant ES10586).

904 EFFECTS OF THE ORGANOCHLORINE PESTICIDE METHOXYCHLOR ON DOPAMINE METABOLITES AND TRANSPORTERS IN THE MOUSE BRAIN.

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Organochlorine pesticides are implicated as environmental risk factors for neurodegenerative diseases, e.g., Parkinson's disease (PD). Inhibition of complex I of the mitochondrial electron transport chain has also been indicated as possibly playing a

role in the demise of vulnerable neuronal populations in PD. We previously demonstrated respiratory inhibition in non-synaptosomal mitochondria from rat brain mitochondria exposed to mxc in vitro, and from isolated mouse brain mitochondria following chronic in vivo mxc exposure. Further, stimulation of H₂O₂ production (in response to mitochondrial reactive oxygen species generation) and increased protein carbonylation, a marker of oxidative stress, were observed. In this study, female CD1 mice were chronically treated with mxc (16-64 mg/kg/day i.p. x 20 days) prior to striatal isolation to identify early markers of damage to the nigrostriatal dopamine system. HPLC measurements of dopamine and its metabolite DOPAC were significantly decreased ($p < 0.05$). Dopamine levels decreased ~ 15% at the lower doses and 31% at the highest mxc dose as compared to controls. Western immunoblots demonstrated a 48% decrease in dopamine transporter (DAT; $p < 0.01$) levels at the two higher doses, and a 35% decrease at the lowest dose compared to control. The vesicular monoamine transporter 2 levels were decreased (VMAT2, $p = 0.06$), and tyrosine hydroxylase levels were not affected. These results suggest that perturbation of dopamine metabolism and transport may be early indicators of damage to the dopaminergic system and is consistent with the possibility that mxc can act as an environmental neurotoxicant capable of promoting neurodegeneration.

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905 ELEVATED LEVELS OF CHLORINATED PESTICIDES IN THE BRAIN ARE ASSOCIATED WITH PARKINSON'S DISEASE.

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Parkinson's disease (PD) is a chronic, progressive neurological disorder that affects more than one million people in the United States each year. Numerous epidemiological studies have shown that pesticide exposure may be a significant risk factor for PD. Additionally, previous work from our lab has shown that the pesticide dieldrin, as well as polychlorinated biphenyls (PCBs), are capable of causing disruptions in the dopaminergic system that are consistent with PD pathophysiology and thus may be promoters of PD pathogenesis. Here, we report findings of a screen of 17 pesticides and PCB congeners in the brains of PD patients versus age-matched controls. Two, pesticides, dieldrin and DDE, the major metabolite of DDT, were three times higher in the brains of PD patients. In addition, we demonstrate that using occipital cortex tissue (non-precious tissue in PD cases) to analyze pesticide levels in humans is a valid approach by showing that the distribution of a single oral dose of dieldrin (11 mg/kg) in mice is consistent throughout a variety of brain regions over several time points. Furthermore, we show that the same 11 mg/kg cumulative dose of dieldrin given either orally or intraperitoneally to mice results in similar levels of pesticide in the brain at 30 days suggesting that the bulk of the dose is absorbed via both routes. Finally, the ratio of brain to blood levels of dieldrin (approximately 3.5:1, respectively) may be a useful clinical measure to identify patients with a higher brain burden of pesticide. These findings may help provide a strategy to identify other compounds that are associated with PD diagnosis as well as understanding the possible mechanisms involved in the association between pesticide exposure and increased risk of developing PD.

906 THE NEUROLOGICAL ASSESSMENT OF ACUTE AND SUBCHRONIC INHALATION EXPOSURE OF DIMETHYL DISULFIDE (DMDS) IN CRL:CD® RATS.

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Dimethyl disulfide (DMDS) is a naturally occurring methyl bromide replacement candidate. Acute (ANT) and 90-day neurotoxicity studies of DMDS were conducted using 6-hour, whole-body inhalation exposures either once (ANT) or daily for at least 90 days. For both studies, neurotoxicity was evaluated in 12 rats/sex/group using an FOB screening battery, motor activity and neuropathology. Exposure levels were 0, 100, 200 and 750 ppm (ANT) and 0, 5, 20 and 80 ppm (90-day). FOB and activity were evaluated at pretest, peak effect (Tmax) and 7 and 14 days post-exposure (ANT) and at pretest and during study weeks 3, 7 and 12 (90-day). One 750 ppm female died. Salivation was noted during exposure in the 100, 200 and 750 ppm groups. In the FOB at Tmax for 750 ppm animals, there was an increased incidence of drooping eyelids, soiled fur and red deposits around the mouth, increased frequencies of tremors, grooming and urination and decreased body temperatures; none of these differences were sustained. Soiling of the

fur, red deposits around the mouth and tremors were also noted at T_{max} in the 200 ppm group. Drooping eyelids, salivation, increased grooming, red deposits and soiled fur most likely resulted from the slightly irritating properties of DMDS and were not considered indicative of systemic toxicity. Motor activity at T_{max} was reduced at 750 ppm and at week 12 in 80 ppm males, although habituation was unaffected. Brain lengths were reduced in 80 ppm males after 13 weeks of exposure, although there was no evidence of neuropathy. Based on these results, the autonomic, CNS activity, CNS excitability and physiological functional domains described by Moser were affected by a single 6-hour exposure of DMDS while only the CNS activity and physiological functional domains were affected by repeated 6-hour exposures of DMDS. Therefore, the NOAEL for neurotoxicity after a single whole-body inhalation exposure to DMDS was 100 ppm and after repeated exposures to DMDS was 20 ppm.

907 ATRAZINE-INDUCED DISRUPTION OF THE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS: CENTRAL MONOAMINES AND BEHAVIOR.

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Our previous research shows that acute exposure to the widely used herbicide atrazine (ATR) increases levels of dopamine (DA) and its metabolites (DOPAC or HVA) in the striatum (STR), nucleus accumbens (NA), frontal cortex (FC) and midbrain (MB) of male rats and increases corticosterone (CORT) to levels observed in restraint stress in female mice, implicating it as a chemical stressor. This study sought to determine whether ATR concomitantly alters CORT and brain neurochemistry in both genders and the impact of such changes for behavior. Adult male and female mice were exposed to ATR (0, 10, 25 or 50mg/kg i.p.); blood was collected for serum CORT analysis and tissue from hypothalamus (HYPO), MB, STR and FC for monoamine determinations 1, 4 or 8 hrs later. Locomotor activity was measured for 45 min following ATR. In males, all three doses increased CORT at 1 and 4 hrs post-exposure, which returned to control levels at 8 hrs. In females, all doses of ATR produced a robust increase in CORT at all time-points. There was no effect of ATR on HYPO DA or DOPAC levels or DA activity in either gender. However, ATR increased HYPO HVA in both males and females. Similarly, ATR did not impact DA, DOPAC or DA activity in MB, STR or FC in either gender, but HVA was increased in FC of both genders. Interestingly, ATR increased HVA at the 1 hr time-point in male STR and female MB as well. ATR produced the greatest impact on CORT and HVA 1 hr post-exposure. In males, all ATR doses reduced horizontal and vertical activity; in females, reductions in horizontal activity were dose-dependent, while vertical activity was reduced only at 25 and 50 mg/kg. Thus ATR negatively impacts the HPA axis and increases HVA levels in several brain areas of both genders, suggesting that it may contribute to conditions involving abnormal CORT function or altered DA activity, such as depression, schizophrenia or Parkinson's disease. ES012712, ES007148.

908 ACUTE EFFECTS OF ATRAZINE EXPOSURE ON EXTRACELLULAR DOPAMINE LEVELS IN THE STRIATUM OF CONSCIOUS MICE.

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Recently, we demonstrated that 14-day oral exposure to atrazine (ATR), a widely used herbicide in the United States and the world, decreased striatal dopamine (DA) levels, as well as the number of tyrosine hydroxylase (TH)-positive dopaminergic neurons in substantia nigra pars compacta (SNpc) of male juvenile C57BL/6 mice. Similarly, other investigators have observed ATR-caused dopaminergic dysfunction in chronically exposed rats. In order to understand the mechanism of dopaminergic neurotoxicity, the acute effects of ATR exposure on extracellular levels of DA and its metabolites in the striatum of conscious mice were studied by an in vivo microdialysis. The microdialysis probe was implanted into the left striatum of adult male C57BL/6 mice and, following 3 days recovery from surgery, the mice were exposed to atrazine or corn oil (vehicle control), by oral gavage. Vehicle treatment had no pronounced effect on the efflux of DA. On the other hand, 250 mg/kg ATR inhibited the extracellular levels of DA to about 50% of baseline. In addition to the decrease in basal DA release, ATR also decreased the amount of amphetamine-stimulated DA release. The extracellular levels of the DA metabolites, DOPAC, and 3-MT were not affected by ATR, while the level of the other DA metabolite, HVA, was moderately decreased. In previous studies, we and others observed that the loss of TH-positive dopaminergic neurons caused by an exposure to ATR was not confined to the SNpc and it also occurred in the ventral tegmental area (VTA). To determine whether this neuronal loss in the VTA is also associated with DA alterations in a terminal field area, the nucleus accumbens (NA), besides

conducting experiments with lower doses of ATR and various pharmacological manipulations, we are currently investigating the consequences of ATR exposure of extracellular DA levels in the NA. (Supported by P20 RR017661 (NIH) and by the ACC/SOT Early Career in Neurotoxicology Award).

909 EFFECTS OF ACUTE ATRAZINE (ATR) EXPOSURE ON SERUM CORTICOSTERONE LEVELS AND HYPOTHALAMIC MONOAMINE CONTENT IN THE RAT.

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It has been reported that the herbicide atrazine is a putative neuroendocrine disruptor in the rat due to alterations in the levels of testosterone, luteinizing hormone, estradiol, prolactin, and thyroid hormones, among others. This study sought to further examine relationships between the endocrine and monoamine effects of ATR. Chronic ATR exposure in the rat causes alterations in hypothalamic monoamine content. In this study we acutely co-exposed rats to a dose of 100 mg ATR/kg of b.w. or 1% methyl cellulose (MC 1%) by intraperitoneal injections and 0.01 mg/kg of haloperidol (HAL) or saline (SAL) by subcutaneous injections. Rats were sacrificed 90 minutes or 4 hours later, blood was collected and the hypothalamus was dissected. Serum corticosterone levels were increased by 56 % and 65 % in the groups exposed to ATR-SAL and ATR-HAL respectively 90 minutes after exposure. However, 4 hours after exposure only the ATR-HAL group showed a sustained increase in corticosterone serum levels (96%). Significant alterations in hypothalamic monoamine content were observed 90 minutes after treatment. Dopamine content was decreased in the groups exposed to MC 1%-HAL and ATR-SAL whereas DOPAC was increased in the group exposed to ATR-HAL and HVA was increased in both ATR-SAL and ATR-HAL groups. Noradrenaline levels were decreased in the groups exposed to ATR-SAL and ATR-HAL. Four hours after treatment there was an increase in hypothalamic DOPAC content in the groups exposed to ATR-SAL and ATR-HAL. These increments in the corticosterone levels may be related to the hypothalamic alterations in the monoamine content in the groups exposed to ATR. Further studies to determine the effects of ATR exposure in stress related disorders are necessary. ES05903, 10791 and 012712.

910 ATRAZINE EXPOSURE CAUSES ALTERATIONS IN THE NIGROSTRIATAL AND MESOSTRIATAL SYSTEMS IN THE RAT.

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The herbicide atrazine (ATR) is widely used to control weeds and grass on a wide variety of crops. It has previously been shown that chronic ATR exposure in rats reduces striatal dopamine (DA) and hypothalamic serotonin levels, and produces a loss of dopaminergic and non-dopaminergic neurons in substantia nigra and ventral tegmental area. Microdialysis studies showed that acute ATR exposure decreases basal and high potassium-evoked striatal DA release in the striatum, an effect reversed by concurrent administration of a D2 receptor antagonist (haloperidol) with ATR. To further evaluate mechanisms of ATR effects on monoamine systems, rats were treated with either 1% methyl cellulose (vehicle) or 100 mg/kg ATR i.p. and sacrificed 90 minutes or 4 hr later. Striatum, ventral midbrain, nucleus accumbens, hypothalamus and prefrontal cortex were dissected and processed for monoamine concentrations and level of phosphorylated tyrosine hydroxylase (pTH) at sites 19 and 40. Acute ATR exposure significantly increased levels of DA and its metabolites (DOPAC and HVA) in the striatum, nucleus accumbens, prefrontal cortex and midbrain, while decreasing hypothalamic DA and NE levels. ATR exposure produced increases in the content of pTH at sites 19 and 40 in the striatum and nucleus accumbens. Taken together these results indicate that acute ATR exposure affects DA systems, decreasing DA release but increasing DA content and levels of pTH-19 and pTH-40. These findings suggest that ATR could act by increasing the activity of tyrosine hydroxylase and affecting vesicular DA dynamics in the dopaminergic systems. The impact of ATR on brain DA systems suggests it could contribute as a risk factor to diseases and dysfunctions that include learning disorders, attention deficit hyperactivity disorder and motor dysfunction, among others. ES05903, 10791 and 05017.

911 DECREASE OF 5-HT LEVELS AFTER GLYPHOSATE HERBICIDE TREATMENT IN RATS.

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Glyphosate (N-phosphonomethyl-glycine) is a synthetic amino acid with an empiric formula of C₃H₈NO₅P. The compound is used worldwide as a broad-spectrum systemic herbicide; it inhibits the shikimic acid pathway that is important for plant

protein synthesis. Glyphosate's mechanisms of action in mammals is still questioned, and it may have several enzymatic effects. Accidental ingestion of glyphosate is generally associated with mild to moderate effects such as spasms, panting, shaking, hind-leg ataxia and muscle spasms. Fatal cases in dogs have been reported and correlated with the agricultural use of glyphosate. Because of toxicological data are needed to further define the use safety of this herbicide, the objective of the present study was to investigate neurochemical effects following the administration of glyphosate (150 and 800 mg/kg/day, orally for 5 days) in male Wistar rats (n = 6/group). Animals were killed 24 hours after the last dose by cervical dislocation and decapitation and the brains removed. The frontal cortex, mid-brain and striatum were dissected and analyzed for content of 5-hydroxytryptamine (5-HT) and its metabolite 5-hydroxy-3-indole acetic acid (5-HIAA) using a HPLC method with electrochemical detection. The frontal cortex, midbrain and striatum were dissected and analyzed for content of 5-hydroxytryptamine (5-HT) and its metabolite 5-hydroxy-3-indole acetic acid (5-HIAA) using a HPLC method with electrochemical detection. A serotonin depleting effect was produced by this herbicide. Glyphosate decreased 5-HT levels in frontal cortex (32%; $P < 0.001$), midbrain (22%; $P < 0.01$) and striatum (49%; $P < 0.001$) and increased 5-HIAA levels in frontal cortex (54%, $P < 0.001$) and midbrain (33%; $P < 0.01$) but not in striatum. The data presented herein suggests that a lower activity of serotonergic system exists in the action of glyphosate. This work has been supported by project No. AGL2005-05320-C02-02 (MEC), Spain.

912 COMPARATIVE MICROARRAY ANALYSIS OF TOXIC AND NON-TOXIC ARYL HYDROCARBON RECEPTOR LIGANDS IN MURINE HEPA1C1C7 HEPATOMA CELLS.

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The aryl hydrocarbon receptor (AhR) mediates the response of many toxic and non-toxic ligands. In order to characterize and distinguish ligand-specific responses, gene expression effects elicited by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were compared to indolo[3,2-b]carbazole (ICZ) and β -naphthoflavone (β NF). Murine Hepa1c1c7 wild-type (WT) and aryl hydrocarbon nuclear translocator (ARNT) defective C4 mutant hepatoma cells were treated with either 10 nM TCDD, 1 μ M ICZ, 10 μ M β NF or vehicle (DMSO) for 1, 2, 4, 8, 12, 24 or 48 hrs. Dose-response studies at 12 hrs with WT cells exhibited EC50 values for Cyp1a1 induction of 19 pM, 21 nM and 1.6 μ M for TCDD, ICZ and β NF with varying levels of induction (293, 86, 53-fold, respectively) when compared to time-matched vehicle controls as determined using quantitative real-time PCR. Global gene expression profiles were monitored using custom cDNA microarrays containing 13,361 cDNA clones representing 8,194 unique genes. Empirical Bayes analysis of the microarray data identified 297 TCDD and 190 ICZ elicited gene expression responses differentially regulated at one or more time points. Further analysis identified 170 genes commonly regulated by both ligands, suggesting a conserved mechanism of action, while 127 gene responses were specific to TCDD and 20 to ICZ. These ligand-specific gene expression responses suggest selective AhR-mediated modulation of gene expression that may be important in the observed ligand-specific differences in toxicity. Supported by NIH T32 ES07255.

913 AN IMAGING-BASED ASSAY FOR ACTIVATION OF THE ARYL HYDROCARBON RECEPTOR: A MECHANISM-DEPENDENT, HIGH THROUGHPUT TOXICITY ASSAY FOR DRUG DISCOVERY.

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Early assessment of potential toxicity in drug discovery is now accepted as essential to the selection of candidate drugs with reduced risk of late-stage failure attributable to toxicological reasons. An important aspect of this is development of mechanism-based, high-throughput in vitro toxicity assays. Activation of the Aryl hydrocarbon receptor (AhR) can lead to wide array of toxicological responses including tumour promotion and endocrine disruption. We describe the development of a novel imaging assay able to monitor quantitatively ligand-induced receptor translocation in both immortalized cell lines and primary rat hepatocytes using specific antibodies. A confocal imaging plate reader, the IN Cell 3000 was used to image 96 well plates and algorithms were used to analyse both population data and individual cell responses. Endogenous AhR translocation and subsequent upregulation of the downstream target, CYP1A1 was monitored in mouse Hepa-1 cells, human HepG2 cells and rat primary hepatocytes in response to prototypical AhR ligands.

This assay was able to demonstrate the efficacy and potency of agonists and calculated EC50 values agreed closely with Kd values reported in the literature for the different receptor agonists tested. Furthermore, in line with published data, species-specific responses were observed. To conclude, we have established a robust, automated and quantifiable high throughput assay for the identification of AhR receptor activators in both cell lines and primary cells. We estimate this assay is able to screen 2000 compounds per week, allowing screening of compound collections for potential toxicity via activation of the AhR. This is consistent with the aim of front-loading toxicity assessment into drug discovery

914 NON-CLASSICAL ACTIVATION OF AHR BY OMEPRAZOLE STIMULATES THE EXPRESSION OF HIGFBP-1.

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It has been reported that Omeprazole (OME), a benzimidazole derived gastric proton pump inhibitor clinically used to alleviate gastric reflux disease; can stimulate the expression of CYP1A1 via activation of the Aryl Hydrocarbon Receptor (AhR) in a non-ligand binding fashion. We wished to test the specificity of OME-induced AhR activation with regard to gene expression. Human Insulin-like Growth Factor Binding Protein-1 (hIGFBP-1) is a multifunctional protein involved in regulating the bioavailability of mitogenic IGFs together with modulating integrin and other signaling cascades in its own right. Analysis of the hIGFBP-1 1Kb proximal promoter reveals the presence of a single consensus Dioxin Response Element (DRE). To test the functionality of this DRE with regard to OME induction, HepG2 cells were exposed to 0-300 μ M OME and hIGFBP-1 mRNA expression assessed by RTqPCR. OME treatment resulted in a dose-responsive stimulation of hIGFBP-1 achieving ~30 fold at the highest dose tested. Comparison with classical AhR ligands (10nM TCDD and 1 μ M β -NF) revealed OME to be a more potent inducer of hIGFBP-1 and not additive with TCDD. Antagonism of AhR with α -NF ablated TCDD-mediated hIGFBP-1 expression, however the effect on OME-mediated expression was much less pronounced perhaps suggestive of an AhR-independent effect of OME. To investigate this hypothesis, siRNA-mediated AhR "knockdown" studies were performed. Loss of AhR completely inhibited TCDD-mediated hIGFBP-1 induction, interestingly; OME-mediated induction was similarly ablated thus indicating an absolute requirement for AhR. Furthermore, reporter assays performed using the wild-type (wt) and mutant (Δ DRE) hIGFBP-1 promoter identified the requirement of an intact DRE to mediate both TCDD and OME-mediated stimulation of hIGFBP-1. These data demonstrate that non-classical i.e. non-ligand binding activation of AhR is a global phenomenon and not limited to CYP1A1. Additionally, we demonstrate that OME, acting via AhR is a potent activator of hIGFBP-1 expression and as such may impact IGF-dependent & independent signaling.

915 LIGAND-DEPENDENT INTERACTIONS OF THE AH RECEPTOR WITH COACTIVATORS IN A MAMMALIAN TWO-HYBRID ASSAY.

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Halogenated aromatic hydrocarbons (HAHs), such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 1,2,3,7,8-pentachlorodibenzo-p-dioxin (PeCDD), bind and activate the aryl hydrocarbon receptor (AhR). HAHs and many other structurally-diverse AhR agonists induce CYP1A1; however, induction of many other responses are ligand structure- and cell context-dependent. We hypothesize that HAHs may also exhibit selective AhR modulator (SAhRM)-like activity, and this was investigated by determining ligand structure-dependent differences in the interaction of AhR with a series of coactivators in mammalian two-hybrid assays. TCDD and PeCDD induced CYP1A1 in several Ah-responsive cancer cell lines including mouse Hepa-1, human Panc1, and human HEK293 cells. However, there was structure-dependent activation of luciferase activity in these same cells transfected with VP-AhR and GAL4-coactivator (SRC-1, SRC-2, SRC-3, CARM-1, PGC-1 and TRAP220) chimeras and a GAL4-response element-luciferase promoter construct. In Panc1 cells, TCDD and PeCDD induced transactivation in cells transfected with GAL4-TRAP220 and VP-AhR constructs, and TCDD, but not PeCDD, also induced activity in cells transfected with GAL4-CARM-1. In contrast, ligand-induced AhR-coactivator interactions were not observed for any other coactivators in a mammalian two-hybrid assay in Panc1 cells. The most striking difference in ligand-induced AhR-coactivator interactions in this assay was observed in HEK293 cells where TCDD induced activity only in cells transfected with the GAL4-CARM-1 chimera. In contrast, the other HAHs did not induce

AhR interactions with any of the coactivators. These results suggest that HAHs selectively interact with coactivators and these interactions are dependent on cell-context. (Supported by a grant from the Dow Chemical Co.)

916 THE ROLE OF COACTIVATORS IN THE DIOXIN-INDUCED DIFFERENTIAL ACTIVATION OF CYP1A1 AND CYP1B1.

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced activation of cytochrome P450 1A1 (CYP1A1) and cytochrome P450 1B1 (CYP1B1) transcription is mediated by the Aryl Hydrocarbon Receptor complex (AHRC). Transcriptional regulation by the AHRC requires the coordinated recruitment of transcriptional coactivator proteins and chromatin remodeling factors. The aim of this study was to further elucidate the role of coactivators in the differential induction of two AHRC target genes CYP1A1 and CYP1B1. We have identified dioxin-dependent recruitment of several coactivator proteins, as well as, histone modifications over the CYP1A1 and CYP1B1 genes. Specifically, by use of the Chromatin Immunoprecipitation (ChIP) assay, we demonstrate a dioxin-dependent recruitment of the p300, Sp1, Brg-1, Brahma (Brm) coactivator proteins, as well as, a dioxin- and time-dependent acetylation of lysine residues on histone H4. We show that dioxin-induced acetylation of histone H4 is very robust at the promoter regions of the genes when compared to the enhancer regions. We are able to inhibit the dioxin-dependent acetylation of histone H4 by knocking down the endogenous levels of the p300 coactivator protein by RNA interference. These data implicate p300 as a regulator of dioxin-induced acetylation of lysine residues on histone H4 on responsive genes. SiRNA has also allowed us to identify the chromatin remodeling factors Brg-1 and Brm as gene specific coactivators of dioxin induction of CYP1A1. Loss of Brg-1 and Brm has a negative effect on CYP1A1 dioxin-induction, while having no significant effect on CYP1B1 induction. This indicates that the Brg-1 and or Brm proteins may play a significant role in the differential regulation of CYP1A1 and CYP1B1 in these MCF-7 human breast cancer cell lines. Our studies have lead to significant insight into these cells transcriptional response to chemical insults.

917 INTERACTION BETWEEN THE ARYL HYDROCARBON RECEPTOR AND AHR REPRESSOR IN REGULATING 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)-RESPONSIVE GENES.

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The aryl hydrocarbon receptor (AHR), a member of the basic helix-loop-helix-Per-ARNT-Sim (bHLH-PAS) protein family, is a ligand-activated transcription factor that mediates transcriptional and toxic responses to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The AHR repressor (AHRR) is a closely related bHLH-PAS family member that forms a negative feedback loop to repress AHR signaling in vitro, but the function of AHRR in vivo is not well understood. As a result of a teleost-specific genome duplication, zebrafish (*Danio rerio*) have three AHR (*AHR1a*, *AHR1b*, *AHR2*) and two AHRR (*AHRRa*, *AHRRb*) genes. To further characterize the interactive role of zebrafish AHRs and AHRRs in the transcriptional control of endogenous TCDD-responsive genes, morpholino oligonucleotides (MO) were employed in zebrafish embryos and a liver cell line (ZF-L). Zebrafish embryos were exposed to DMSO or TCDD (2 nM and 8 nM) for one hour starting at 4 hours post fertilization (hpf) and quantitative PCR was used to assess gene expression at later time points. *CYP1A1* was significantly upregulated at 24, 48 and 72 hpf in a dose-dependent manner by TCDD, while *AHRRa* and *AHRRb* were significantly upregulated at 48 and 72 hpf. A MO targeted against *AHR2* decreased the TCDD-induced expression of *Cyp1A1*, *AHRRa* and *AHRRb* by 65%, 55% and 35%, respectively, in 48 hpf larvae, demonstrating a role for AHR2 in mediating AHRR transcription. Transfection of ZF-L cells with a MO targeted against *AHRRa* failed to yield any consistent effect on AHR target genes. However, co-transfection of MOs targeting both *AHRRa* and *AHRRb* significantly increased the basal expression of *CYP1A1* by 70% and enhanced induction of *CYP1A1* by TCDD in a dose-dependent manner by an average of 45%. Although additional genes (*AHR1b*, *CYP1B1*, *CYP1C1*, and *CYP1C2*) were induced by TCDD in ZF-L cells, the *AHRRa/AHRRb* knockdown did not enhance the basal or induced expression of these genes, suggesting that regulation by AHRRs is gene-specific. (R01ES006272)

918 CHARACTERIZATION OF AN ARYL HYDROCARBON RECEPTOR REPRESSOR FROM THE FROG *XENOPUS LAEVIS*

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Xenopus laevis and other frogs are extremely insensitive to the toxic effects of xenobiotic ligands of the aryl hydrocarbon receptor (AHR), including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Embryos and premetamorphic life stages are especially insensitive, and they are refractory to induction of *Cytochrome P4501A6* (*CYP1A6*), the most highly induced target gene in older tadpoles. The aryl hydrocarbon receptor repressor (AHRR) is a member of the AHR gene family. AHRR expression is ordinarily induced by TCDD in an AHR- and ARNT-dependent fashion. The AHRR protein then binds ARNT and represses AHR transcriptional activity in an apparent negative feedback loop. In this study, we sought to test the hypothesis that constitutive AHRR expression underlies the lack of *CYP1A6* induction and TCDD toxicity in early frog life stages. Using an RT-PCR approach, we determined the sequence of a single AHRR cDNA encoding a protein of 85.3 kDa sharing 52-55 percent identity with the aligned bHLH/PAS domains of AHRRs from mammals and fish. In transient transfection assays, *X. laevis* AHRR inhibited TCDD-induced reporter gene expression mediated by either *X. laevis* AHR paralog, AHR1alpha or AHR1beta. AHRR mRNA was minimally detectable by quantitative RT-PCR in *X. laevis* embryos (Nieuwkoop-Faber stage 33-38; approximately 52 h.p.f.), even following overnight exposure to ³H-TCDD (59.4 ng/g wet weight). In contrast, AHRR was induced 2- to 4-fold in whole tadpoles at stages 52-55 (prometamorphic; ~4 weeks post-fertilization) and in isolated viscera of stage 62 tadpoles (in the metamorphic climax; ~7 weeks post-fertilization), even at much smaller TCDD body burdens (3-8 ng/g). Although the magnitude of induction was much smaller, the temporal pattern of AHRR expression and inducibility resembled that previously observed for *CYP1A6*. Thus, attenuated transcriptional activation of AHR target genes and low TCDD toxicity in *X. laevis* embryos cannot be explained by constitutive, high-level expression of AHRR. [NIH R15 ES011130]

919 IDENTIFICATION AND FUNCTIONAL ANALYSIS OF A HUMAN AHR REPRESSOR.

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The aryl hydrocarbon receptor (AHR) repressor (AHRR) is a bHLH-PAS protein related to AHR, but acts as a transcriptional repressor of AHR function. Additionally, AHRR expression is regulated by the AHR signaling pathway. Previous studies have characterized the repressor function of AHRRs from mice and fish, but a human AHRR homolog identified in a large-scale cDNA screen appeared to be non-functional in vitro. We have identified a novel human AHRR cDNA that lacks an internal exon of the original AHRR clone. This new AHRR is expressed predominantly in the testis, spleen, and small intestine, whereas the original cDNA is not detected in these tissues. In transient transfection assays using COS-7 cells, the new human cDNA effectively repressed the transactivation function of the human AHR expressed from an AHR cDNA. Similarly, experiments in HepG2 cells showed that the AHRR repressed the ability of the endogenous AHR to activate transcription of a luciferase reporter gene. The repression was not relieved by overexpression of any of several nuclear receptor co-activators (SRC-1a, SRC-1e, GRIP1, CoCoA, GAC63, and p300), suggesting that squelching may not be involved in the mechanism of repression. Furthermore, co-transfection of human AHRR did not affect the ability of a mouse AHR-YFP fusion protein to translocate to the nucleus. Our previous studies showed that the mechanism of repression did not involve competition for ARNT or displacement of AHR from dioxin-responsive enhancer elements. Together, these results demonstrate that humans express a functional AHRR and that the mechanism of repression is more complex than previously indicated. [R01ES006272]

920 UP-REGULATION OF THE ARYL HYDROCARBON RECEPTOR AND ITS REPRESSOR (AHRR) DURING MAMMARY TUMORIGENESIS.

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Historically the aryl hydrocarbon receptor (AhR) has been studied in the context of its ability to bind to and be activated by ubiquitous environmental carcinogens such as polycyclic aromatic hydrocarbons (PAHs). DMBA (7,12-dimethylbenz[a]anthracene) is a prototypical PAH known to promote tumors by the indirect activation of AhR. Recently the focus of research has shifted to the endogenous role of the AhR, based on studies showing high levels of constitutive AhR being present in the absence of environmental ligands. The aryl hydrocarbon receptor repressor (AhRR)

has been shown to have profound suppression of AhR activity in mammary tumors suggesting that AhRR might be an effective approach to suppressing AhR-driven tumors growth and progression. Here, the repressive effects of constitutively active and environmental ligand induced AhRR on the AhR were investigated. Female FVB mice were given six weekly doses of 1 mg of DMBA by gavage and tumors arose with 75% of them being mammary tumors. A majority of these tumors showed higher levels of AhR and AhRR protein and mRNA compared to normal mouse mammary tissue. The endogenous expression of AhR and AhRR was studied by transducing an immortalized human mammary cell line, MCF-10F, with a lentivirus construct containing a GFP-expression vector or a construct encoding both GFP and the AhRR derived from *F. heteroclitus*. Cells transduced with the GFP-AhRR construct showed significantly slower growth by ³H-Thymidine incorporation when compared to the GFP control and wild type MCF-10F cells. A reduction of AhR activity was seen in the Fh-AhRR lentivirus transduced cells while the reporter activity and endogenous AhRR mRNA were found to be over expressed in the Fh-AhRR transduced line compared to the GFP controls. Based on this, the Fh-AhRR lentivirus transduced cell line will be a potent tool to understand how AhR is regulated by AhRR in relation to mammary tumorigenesis.

921 COMPARATIVE MICROARRAY ANALYSIS OF GENE EXPRESSION IN MOUSE HEPA-1C1C7 AND B MUTANT CELL LINES - THE EFFECT OF THE AROMATIC HYDROCARBON RECEPTOR ON THE PHENOTYPE OF THE CELLS IN THE ABSENCE OF EXOGENOUS LIGANDS.

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Studies on the Hepa-1c1c7 line, and B mutant derivatives that are deficient for expression of AhR, have helped to elucidate the mechanism of aromatic hydrocarbon receptor (AhR)-mediated gene expression. However, little is known about the other biological processes that may differ between the wild type Hepa-1c1c7 and the B mutant cells. In this study, we elucidated the profile of genes that are differentially expressed in these cells using DNA microarray analysis. We found that a large number of genes are expressed differentially between wild type Hepa-1c1c7 and B mutant cell lines. Further study of the profile of gene expression between c12, a B mutant cell line, and c12 cells containing re-introduced exogenous AhR, using DNA microarray analysis, showed that the gene expression changes between wild type Hepa-1c1c7 and c12 is not ascribed to the deficiency in AhR in c12 cells. Previous data from our laboratory indicated that the primary defect in the B mutants resides in a transcription factor or chromatin remodeling factor. Our current data suggest that the difference in gene expression between the Hepa-1c1c7 and B mutant cells is due to absence of this factor rather than AhR. The AhR has also been demonstrated to modulate the cell cycle, differentiated state, and morphology of Hepa-1c1c7 cells independently of exogenous ligands. In this study, we found that the expression of the albumin gene, previously reported to be upregulated by the unliganded AhR, was undetectable in c19 cells, another B mutant cell line, both before and after introduction of AhR. Consistent with these findings, we also found that in c12 cells, the expression of albumin, morphology, and cell cycle parameters did not undergo any changes in response to over-expression of exogenous AhR, indicating that the expression of albumin and changes in morphology may be dependent on the aforementioned transcription factor or chromatin remodeling factor.

922 COMPARATIVE ANALYSIS OF GLOBAL GENE EXPRESSION RESPONSES ELICITED BY TCDD IN HUMAN HEPG2, MOUSE HEPA1C1C7 AND RAT H4IIE CELL LINES.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a ubiquitous environmental contaminant that causes physiological and toxic effects in a species-dependent manner. In order to gain further insight into these differences, a comparative genomic approach was utilized to examine species-specific differential gene expression responses elicited by TCDD. Mouse Hepa1c1c7, rat H4IIE and human HepG2 cells were treated with 10 nM TCDD or vehicle control (DMSO) for 1, 2, 4, 8, 12, 24 or 48 hrs. Gene expression profiles were measured using custom cDNA microarrays representing 6,293 human, 8,194 mouse or 4,717 rat genes with cross-species analysis identifying 1,860 orthologous genes. Empirical Bayes analysis identified 308, 184 and 30 differentially expressed orthologs in the HepG2, Hepa1c1c7 and H4IIE cells, respectively. Pair-wise species comparisons identified 4 differentially expressed orthologs between the Hepa1c1c7 and H4IIE cells, while 32 differen-

tially expressed orthologs were identified between HepG2 and Hepa1c1c7 cells. The limited number of differentially expressed orthologs between Hepa1c1c7 and H4IIE cells is consistent with histopathology and *in vivo* microarray results. Furthermore, species-dependent gene expression responses are in agreement with computational dioxin response element searches, which report significant species differences in the promoter and enhancer regions of targeted genes that may also contribute to the differential responses to TCDD. Collectively, these results suggest that there are significant species-specific differences in the aryl hydrocarbon receptor regulon that increases uncertainty in the extrapolation of rodent data for use in human risk assessment. Supported by NIH P42 ES04911 and R21 GM075838.

923 STAGE-SPECIFIC EFFECTS OF TCDD ON GENE EXPRESSION PATTERNS DURING DEVELOPMENT OF THE FROG *XENOPUS LAEVIS*.

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Xenopus laevis embryos are insensitive to developmental toxicity associated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). TCDD insensitivity has been ascribed to species-specific properties, including aryl hydrocarbon receptors (AHRs) with low affinity for TCDD. However, stage-specific mechanisms may also limit toxicity during early development, since the primary transcriptional response, CYP1A6 induction, is much smaller in embryos than tadpoles. We sought to determine whether the attenuation of CYP1A6 induction in embryos was gene-specific or indicative of broad repression of AHR-mediated transcriptional regulation in early development by comparing global changes in gene expression in embryos and tadpoles following TCDD exposure. Embryos (stage 36-37; ~50 hpf) and tadpoles (stage 52-55; 4 weeks old) were treated with vehicle or ³H-TCDD for 24 hours (5 replicates/exposure group). Embryos accumulated mean TCDD body burdens of 32 ng/g; tadpoles had 20 ng/g. Total RNA was obtained from whole animals and gene expression patterns analyzed using Affymetrix GeneChips. Using two-way ANOVA, we identified 528 genes exhibiting significantly different expression after TCDD exposure. However, most changes were very small and of questionable biological relevance. In embryos, only 3 genes were induced more than 1.5 fold, including CYP1A7 (9-fold) and CYP1A6 (2.4-fold). 12 genes were repressed more than 1.5-fold, including amino acid synthesis enzymes and extracellular matrix components. In tadpoles only 6 genes were induced more than 1.5-fold, again including CYP1A6 (11.5-fold) and CYP1A7 (10.2-fold), while only five were repressed, most encoding unknown products. Notably, CYP1A6 was more strongly induced in tadpoles, while CYP1A7 was induced to a similar degree in both stages, suggesting a stage-specific regulatory mechanism for CYP1A6. The overall low responsiveness in both stages is consistent with previous observations of low binding affinity of *X. laevis* AHRs for TCDD. [NIH ES11130; HHMI Education Grant to Kenyon College]

924 IN VIVO GENOME-WIDE LOCATION ANALYSIS OF TCDD-MEDIATED PROMOTER BINDING OF THE ARYL HYDROCARBON RECEPTOR IN C57BL/6 HEPATIC TISSUE.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) exerts most, if not all of its toxicological effects through the aryl hydrocarbon receptor (AhR), a ligand activated transcription factor which binds to regulatory dioxin response elements (DREs). The *in vivo* genomic binding location of the activated AhR protein was investigated in liver samples from C57BL/6 mice treated with 30 ug/kg TCDD for 1, 2, and 4 hrs using a chromatin immunoprecipitation (chIP) assay for tissue samples. Formaldehyde cross-linked chromatin was isolated from TCDD treated and vehicle treated liver samples, and AhR bound genomic regions were antibody precipitated. PCR with primers designed against various known and putative DRE regions was performed to investigate the kinetics of AhR binding. These results show that activated AhR has a specific interaction with the previously identified DRE region of the Cyp1a1 promoter as well as the DRE-free region proximal to the transcriptional start site but not to other regions of the promoter. In addition, activated AhR interactions were characterized within regions of the Cyp1b1, Nqo1, Aldh3a1 and Por promoters containing high numbers of computationally identified putative DREs. These results complement *in vitro* studies examining AhR binding and correlate with *in vivo* hepatic gene expression studies, thus further elucidating AhR action through the identification of primary responses. Ongoing studies analyzing AhR chIP isolated DNA using microarray technology will allow for a more comprehensive identification of *in vivo* AhR interacting sites and will continue to refine the AhR regulon. Supported by grants NIH R21 GM75838 and T32 ES07255.

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COMPARATIVE ANALYSIS OF THE GENOMIC BINDING LOCATIONS OF THE ARYL HYDROCARBON RECEPTOR AND THE ARYL HYDROCARBON RECEPTOR NUCLEAR TRANSLOCATOR IN TCDD TREATED HEPA-1C1C7 CELLS.

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The aryl hydrocarbon receptor (AhR) is a ligand activated transcription factor that recognizes and binds a variety of environmental contaminants, including dioxins. Activated AhR heterodimerizes with the aryl hydrocarbon receptor nuclear translocator (ARNT), binds regulatory xenobiotic response elements (XREs) and activates the transcription of proximal genes. Global genomic binding of AhR:ARNT complexes was investigated in Hepa-1c1c7 cells following 2 hr treatment with 10 nM TCDD or DMSO using ARNT antibody chromatin immunoprecipitation (chIP) and mouse promoter tiling microarrays (chIP-chip). The AhR:ARNT-bound, enriched DNA was amplified and hybridized to a mouse promoter array, consisting of fifteen 50mer probes tiled across the regulatory region of 26,275 genes and putative coding regions, and was compared to vehicle (DMSO) treated sample. Genes associated with the regulatory regions putatively bound by AhR:ARNT complexes were identified by filtering for probe sets demonstrating a high density of enrichment. Comparisons to AhR antibody chIP-chip results showed a high degree of overlap, with 65% of the AhR-bound regions also showing an interaction with ARNT. PCR verification of chIP-isolated DNA demonstrated an ARNT interaction with the Cyp1a1, Cyp1b1, Nqo1 and Aldh3a1 promoter regions previously shown to interact with AhR. These results confirm the obligatory nature of the AhR-ARNT interaction in TCDD elicited gene expression and provide further evidence of activated AhR-ARNT binding to previously unknown XREs which warrant further investigation. Supported by grants NIH R21 GM75838 and T32 ES07255.

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CHARACTERIZATION OF TCDD INDUCED HISTONE MODIFICATIONS TO STUDY THE DIFFERENTIAL REGULATION OF THE CYP1A1 AND CYP1B1 GENES.

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The Aryl Hydrocarbon Receptor/ARNT heterodimer mediates carcinogenesis by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin) and certain polycyclic aromatic hydrocarbons (PAHs) by activating gene transcription. Metabolism of PAHs by CYP1A1 and CYP1B1 plays a major role in carcinogenesis mediated by these compounds. The overall goal of this study is to understand the mechanisms involved in differential regulation of the human CYP1A1 and CYP1B1 genes. We will study the regulation of these genes in three different cell lines (MCF-7, MDA-MB 231, and HepG2) as the endogenous expression and induction levels of these genes upon treatment with TCDD differ significantly between these cell lines. Studying the differential regulation of CYP1A1 and CYP1B1 genes in these cell lines will lend more insight into which specific coactivators and protein complexes are required for expression of each gene. To elucidate the mechanisms involved in the differential regulation of these genes, we characterized the different dioxin-inducible chromatin modifications (i.e. acetylation, methylation, and phosphorylation) that occur across the CYP1A1 and CYP1B1 enhancer and promoter regions using the Chromatin Immunoprecipitation (ChIP) assay. In accordance with our hypothesis, the results from the PCR and quantitative real-time PCR analysis of ChIP samples showed that differential chromatin modifications occur across the CYP1A1 and CYP1B1 enhancer and promoter regions. These modifications, at least in part are contributing to the differential induction of these genes upon treatment with TCDD. The future aim of this study is to characterize the different histone modifying proteins and transcription factors recruited to the regulatory regions. We hypothesize that different transcription factors and cofactors are recruited to the CYP1A1 and CYP1B1 enhancer and promoter regions leading to different covalent histone modifications.

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TOXICITY STUDIES OF THE DIPEPTIDE SCV-07 IN RATS AND DOGS.

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SCV-07 (D-glutamyl-L-tryptophan) is an immune-stimulating dipeptide that is being developed as a potential treatment for various infectious diseases and other indications. The repeat-dose toxicity of SCV-07 was evaluated in a 26-week study

in rats (20/sex/group) that were administered daily subcutaneous doses of 0, 2, 5, and 10 mg/kg/day and in a 39-week study in dogs (4/sex/group) that were administered daily subcutaneous doses of 0, 0.5, 0.5, and 5 mg/kg/day. There was no evidence of adverse systemic toxicity in either species; clinical pathology results in the rat study indicated minimally lower hepatic protein production and cholesterol metabolism but it was not considered to be adverse. Plasma toxicokinetic data indicated that SCV-07 was rapidly absorbed following administration; C_{max} was reached at 0.25 h in rats and from 0.5 to 1 h in dogs. The AUC indicated that exposure to SCV-07 was dose-dependent in both species with minimal accumulation with repeated dosing. The no-observed-adverse-effect-level for systemic toxicity was 10 and 5 mg/kg/day in rats and dogs, respectively, representing 1000- and 500-fold the anticipated human dose. The potential neurotoxicity of SCV-07 was assessed in rats (10/sex/group) that were administered subcutaneous doses of 0, 25, 50, and 100 mg/kg. The functional observational battery was conducted prior to dosing, and at approximately 0.5 and 24 hours following dosing; there were no test article-related effects. A cardiovascular safety pharmacology study was conducted in a group of 4 dogs at doses of 0, 1, 5, and 10 mg/kg, and respiratory safety was assessed in the same animals following a dose of 10 mg/kg. There were no test article-related effects on heart rate, blood pressure, electrocardiography (including QT interval and QTc), respiratory rate, Sp_{O_2} , or $ETCO_2$. These results, together with those obtained from in vitro safety pharmacology studies and genetic toxicology studies, indicate that SCV-07 has an appropriate toxicological profile in animals to support the conduct of human clinical safety trials.

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EVALUATION OF THE TOXICITY OF ALACRAMYN.

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About 250,000 people are stung by scorpions every year in México. Alacramyn® is an anti-venom that has cut yearly scorpion-related deaths to 100 from 800. In the present study we evaluated the in vitro cytotoxicity of Alacramyn® as well as its safety after topic and intradermic administration in humans. Primary cultures of hepatocytes and kidney cells and Chang liver and MDOK cell lines were used for cytotoxicity studies. Alacramyn® (Lote B-4D-10) was dissolved in 5 mL of diluting agent (isotonic NaCl solution) and further dilutions were done with culture medium. Twelve different concentrations were evaluated. Cells were cultured in 96-well plates (1 x 10⁴ cells/mL). Four wells were used for each concentration tested. Culture plates were incubated for 24 and 72 h. Appropriate controls wells were included in each tray. Cytotoxicity was determined by the MTT test. For clinical studies an experimental protocol was approved by the Human Subjects Ethical Committee of our institution. Written informed consent was obtained from 40 (18-25 years) healthy volunteers. Clinical history, physical examination, laboratory analyses (blood chemistry, hematology and urinalysis) and EKG were carried out in all the cases before the study. Twenty subjects received Alacramyn® by an intradermic injection (100 microliter of 1:1000 dilution) and the other 20 by dermic patches (100 microliter of 1:100 dilution). Physical examination and evaluation of dermic reaction (NOM-033-SSA1-1993) or systemic symptoms were made after 3, 6, 12 and 24-h. Laboratory analyses were made after the evaluation periods. Cytotoxicity: Alacramyn® was no cytotoxic to Chang liver, MDOK cell lines, primary liver and kidney cell cultures in any of the period tested. Clinical evaluation: No clinically significant drug-related symptoms were found in any of the subjects. All clinical laboratory results were normal in all the cases.

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THE SAFETY EVALUATION OF ITMN-191, A NOVEL ORALLY AVAILABLE HEPATITIS C SERINE PROTEASE INHIBITOR.

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ITMN-191 is a macrocyclic, orally available HCV serine protease inhibitor with an EC50 of <0.5 nM in biochemical protease assays and an EC50 of ~2 nM in HCV genotype 1 replicon assays. The preclinical toxicity studies described below support initial Phase 1 clinical trials with the molecule.

ITMN-191 passed the acute oral limit test in rats (2000 mg/kg). Neurobehavioral evaluations (FOB and MA) conducted in rats revealed no significant neurological impairment at doses ≤600 mg/kg. The Ames test and mouse lymphoma assays (each with and without rat metabolic activation factor S9) and rat micronucleus were negative at exposure levels of ≤5000 µg/plate, ≤175 µg/mL and ≤2000 mg/kg, respectively.

The hERG channel assay and the canine Purkinje fiber APD assay each suggested no potential for cardiotoxicity. The hERG produced only 18% inhibition at 100 µg/mL and no prolongation of repolarization was noted at ≤500 µg/mL in the APD assay. An acute telemetered-primate cardiopulmonary safety study revealed no changes in cardiac or pulmonary functions at oral doses ≤600 mg/kg. The 28-day repeat-dose oral toxicity studies in rats (≤1200 mg/kg/day) and in cynomolgus monkeys (≤300 mg/kg/day), revealed no significant, prolonged or adverse/life-threatening events associated with administration of ITMN-191. Standard hematology, serum chemistry, coagulation, and histopathologic evaluations revealed no adverse effects at ≤100 mg/kg/day in monkeys and ≤400 mg/kg/day in rats. The occasional recurrent emesis and liquid stool in the high dose group monkeys were readily controlled with standard veterinary supportive therapy (antiemetics and Pepto-Bismol®). ITMN-191 was determined to be safe and well tolerated in rats and monkeys over the dose ranges and durations of exposure examined.

930 SAFETY EVALUATION OF TRASTUZUMAB-DM1, AN ANTIBODY DRUG CONJUGATE TO HER2.

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Trastuzumab-DM1 (Tmab-DM1) is an antibody drug conjugate (ADC) composed of the humanized monoclonal antibody to HER2 conjugated to the maytansine derivative DM1. Safety studies conducted with Tmab-DM1 prior to initiation of clinical trials consisted of monkey and human tissue cross reactivity, single-dose (SD) toxicity studies in rats and monkeys, SD cardiovascular safety pharmacology and multiple-dose (MD) toxicity studies in monkeys, in vitro hemolytic potential and blood compatibility, and a SD study of DM1 in rats. ADC doses of 6, 20, 60 mg/kg in rats and 3, 10, 30 mg/kg in monkeys resulted in equivalent body surface area-based DM1 doses of 610, 2040, 6120 µg/m² in both species. The Highest-Non-Severely-Toxic-Dose (HNSTD) in SD studies was 20 and 30 mg/kg in rats and monkeys, respectively, and 10 mg/kg in the monkey MD study (dosing every 3 weeks x 4). The SD HNSTD produced increases in liver enzymes, decreases in platelets and an increased number of mitotic epithelial and/or phagocytic cells arrested in metaphase. More pronounced effects on liver enzymes and bone marrow hematopoietic cells occurred in the MD study. Microscopic findings of increased mitotic figures in various epithelial tissues were consistent with the pharmacologic activity of DM1 as a tubulin polymerization inhibitor. Hypertrophy and arrested mitoses in phagocytic cells were attributed to mobilization of scavenging mechanisms secondary to the presence of heterologous protein. Axonal degeneration of the dorsal funiculus of the spinal cord and the sciatic nerve associated with hypertrophy/hyperplasia of the Schwann cells were noted in the MD study at 10 and 30 mg/kg. With the exception of the axonal lesion, all findings were reversible or significantly reduced following the recovery period. Tmab-DM1 produced no evidence of cardiotoxicity, and was compatible with human and cynomolgus blood, serum and plasma. Tmab-DM1-related toxicities were consistent with those described for maytansine.

931 TWENTY-EIGHT-DAY ORAL TOXICITY STUDY OF PERFLUOROBUTYRATE IN RATS.

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Perfluorobutyrate (PFBA), has been detected in surface waters and in public and private wells up to low ng/mL levels. A 28-day oral toxicity study was performed with ammonium PFBA to support risk assessment. Ammonium perfluorooctanoate (PFOA, 30 mg/kg) was used as a positive control. PFBA was given to male (M) and female (F) Sprague Dawley rats (20/sex/group) at doses of 0, 6, 30 and 150 mg/kg-d for 28 days. Half of the rats in each group were allowed to recover for 21 days. Endpoints evaluated were: clinical chemistry; hematology; body and organ weights; food consumption; gross and microscopic histology; and functional observations. Primary treatment related effects for PFBA (M only) included increased liver weights and decreases in cholesterol (30 and 150 mg/kg-d), and absence of bilateral pupillary reflex and increased serum K⁺ and inorganic PO₄²⁺ (150 mg/kg-d). Effects in M rats receiving PFOA included absence of bilateral pupillary reflex, reductions in RBC parameters (slight), body weight, weight gain, absolute food consumption (first week), total serum protein, and physical activity and health, and increases in relative feed consumption (2nd week through recovery), albumin, ALT, BUN, ALP, liver weights, and platelets. Females receiving PFOA had increased ALT, albumin, and liver weight, and reduced physical activity and health. During recovery, liver weight and cholesterol effects in PFBA treated Ms resolved as did the liver weight effects for the PFOA treated Ms and Fs, and PFOA treated rats no longer showed signs of reduced health. The slight effects on RBCs in PFOA-treated

males persisted. Major differences between male rats given PFBA and PFOA were that PFBA did not affect body weight and did lower cholesterol; whereas, PFOA caused body-weight reduction, reduced activity and health, and slight reductions in RBC-related parameters (likely not adverse). Preliminary BMDL₁₀ estimates for PFBA-induced liver-weight increase and cholesterol decrease were 10.5 and 3.4 mg/kg-d, respectively.

932 NINETY DAY REPEATED DOSE ORAL (GAVAGE) TOXICITY STUDY WITH ALPHA-ISO-METHYLIONONE IN RATS.

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alpha-iso-Methylionone was evaluated for its toxicity in a ninety day repeated dose oral (gavage) study in rats (OECD #408). The test material or vehicle (corn oil) was administered by gavage to three groups, each of ten male and females Sprague-Dawley Crl:CD® (SD) IGS BR strain rats, for ninety consecutive days, at dose levels of 0, 5, 30 and 500 mg/kg/day. The study evaluation included: clinical observations, body weight, food and water consumption, haematology and blood chemistry, ophthalmoscopic, gross necropsy and histopathological evaluation. There were no unscheduled deaths and no clinical signs of toxicity observed. No adverse effect on bodyweight, dietary intake or food efficiency and treatment-related haematology changes was detected. A statistically significant increase in both absolute and relative liver and kidney weights were observed in animals treated with 500 mg/kg/day. Males treated with 500 mg/kg/day showed a statistically significant increase in spleen weight. A significant increase in plasma creatinine, total protein and cholesterol was observed in animals from 500 mg/kg/day group. Also, males from this group showed a significant increase in plasma albumin. No abnormalities were observed at necropsy examination. Histopathology revealed an enlargement of hepatocytes in the liver (regarded as adaptive in nature) of animals treated with 500 mg/kg/day, and a greater incidence of globular accumulations of eosinophilic material in the kidney's tubular epithelium of males treated with 30 and 500 mg/kg/day, a higher incidence of follicular cell hypertrophy in thyroid and adipose infiltration of the bone marrow (indicative of marrow hyperplasia) in males treated with 500 mg/kg/day. The No Observed Effect Level (NOEL) was considered to be 30 mg/kg/day for females and 5 mg/kg/day for males. Because the kidney changes were consistent with well documented changes that are peculiar to the male rat in response to treatment with some hydrocarbons, the No Observed Adverse Effect Level (NOAEL) for males was established as 30 mg/kg/day.

933 COMPARATIVE 13-WEEK CIGARETTE SMOKE INHALATION STUDY IN SPRAGUE-DAWLEY RATS: EVALUATION OF CIGARETTES WITH TWO BANDED CIGARETTE PAPER TECHNOLOGIES.

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This study compared the toxicological responses of Sprague-Dawley rats exposed nose-only to mainstream smoke (MS) from Test cigarettes (1, 2, and 3) with banded cigarette paper technologies (BCPT) to Control cigarettes without BCPT. Test cigarettes 1 and 2 used one technology (two band weights) while Test cigarette 3 used a different technology. Rats were exposed to humidified HEPA filtered air (Sham) or to MS at concentrations of 0.06, 0.20, or 0.80 mg wet total particulate matter/liter air. Each exposure group contained 30 animals/sex (sentinel had 20 animals/sex). The study had 2 phases (13 weeks each): MS exposure (1 hour/day, 5 days/week) and recovery without smoke exposure. Endpoints included clinical observations, respiratory physiology, hematology, serum chemistry, blood carboxyhemoglobin (COHb), serum nicotine, body/organ weights, gross pathology, and histopathology. Comparisons conducted were: Sham exposed vs. all cigarettes, Control cigarette vs. all Test cigarettes and Test 1 vs. Test 2. Control and Test MS had comparable effects on respiratory physiology, COHb, serum nicotine, serum chemistry, and hematology. While a few differences were observed, in general, Control and Test MS had comparable effects on clinical signs, body/organ weights, and gross pathology/histopathology. Consequently, exposure of rats to equivalent MS concentrations from the 4 cigarettes induced similar toxicological responses under the conditions of this study.

934 26-WEEK INHALATION TOXICOLOGY STUDY OF NEBULIZED DENUFOSOL TETRASODIUM IN SPRAGUE DAWLEY RATS.

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Denufosol tetrasodium (denufosol) is a P2Y₂ receptor agonist in Phase 3 clinical development as a potential pharmacological therapy for cystic fibrosis (CF). Denufosol was evaluated for local and systemic toxicity following daily inhalation

administration to Sprague Dawley rats for a minimum of 26 weeks at pulmonary-deposited dose levels of 0, 3.74, 11.7 and 17.9 mg/kg body weight/day (0, 0.943, 2.69 and 3.85 mg/g lung weight/day), followed by a 4-week recovery period. Evaluations included clinical observations, body weight, food consumption, ophthalmology, clinical pathology, toxicokinetics, macroscopic observations at necropsy, organ weight and histopathology. There were no treatment related deaths during the course of the study. Group mean body weight, body weight gain, and food consumption were reduced relative to controls in mid and high dose animals. Changes in body weight gain and food consumption were reversible. There were no changes in ophthalmology, hematology, clinical chemistry, organ weight or macroscopic observations attributed to denufosol exposure. Reversible squamous metaplasia of the ventrolateral larynx epithelium was observed in high dose animals. No other microscopic changes were attributed to denufosol exposure. Due to a lack of quantifiable denufosol in plasma, no toxicokinetic analysis was performed. In conclusion, the no observable-effect-level (NOEL) was considered to be the low dose of 3.74 mg/kg/day (0.943 mg/g lung weight) based on decreased body weight, body weight gain and food consumption at higher doses. Based on histopathological findings consistent with local irritation in the larynx at the high dose, and a general lack of systemic exposure and target organ toxicity at any dose, the no observed-adverse effect level (NOAEL) was considered to be the mid-dose of 2.69 mg/g lung weight/day. The NOAEL is at least 6.8- and 14.9-times greater than the highest proposed Phase 3 clinical dose of denufosol in children and adults with CF, respectively.

935 52-WEEK INHALATION TOXICOLOGY STUDY OF NEBULIZED DENUFOSOL TETRASODIUM IN BEAGLE DOGS.

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Denufosol tetrasodium (denufosol) is a P2Y₂ receptor agonist in Phase 3 clinical development as a potential pharmacological therapy for cystic fibrosis (CF). The objective of this study was to assess the potential toxicity and toxicokinetics of a nebulized aerosol formulation of denufosol to beagle dogs following daily oronasal administration for 52 weeks at pulmonary-deposited doses of 0 (vehicle), 29.9 and 58.5 mg/kg body weight/day (3.13 and 6.26 mg/g lung weight/day), followed by a 1-month recovery period. Evaluations included clinical observations, body weight, food consumption, ophthalmology, electrocardiography, respiratory minute volume, hematology, clinical chemistry, urinalysis, toxicokinetics, macroscopic observations at necropsy, organ weights, and histopathology of respiratory and over 40 other tissues. There were no deaths during the study. Coughing was observed sporadically during denufosol exposure. There were no changes attributed to denufosol exposure in any other parameter evaluated during the study, including lung histopathology. Toxicokinetic evaluation of plasma denufosol concentration indicated that systemic exposure to denufosol was maximal during or shortly after treatment. In conclusion, daily oronasal administration of nebulized denufosol to beagle dogs for 52 consecutive weeks at pulmonary-deposited doses of 29.9 and 58.5 mg/kg body weight/day (3.13 and 6.26 mg/g lung weight/day) produced no adverse changes in body weight, food consumption, ophthalmology, electrocardiography (including QTc interval), respiratory minute volume, clinical pathology, organ weights, or gross and histological pathology of any tissue examined. Based on a general lack of local or systemic toxicity, the no observed-adverse effect level (NOAEL) for this study was considered to be the high dose of 6.26 mg/g lung weight/day, or at least 15.7- and 34.8-times the highest proposed Phase 3 clinical dose of denufosol in adults and children with CF, respectively.

936 MAXIMUM TOLERATED DOSE AND PHARMACOKINETICS (PK) OF STARCH CONJUGATED DEFEROXAMINE (S-DFO OR 40SD02) FOLLOWING INTRAVENOUS INFUSION FOR 7 DAYS TO ADULT BEAGLE DOGS WITH 14 DAYS RECOVERY.

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Chelation therapy has been shown to reduce iron-related morbidity and improve the quality of life in patients with β -thalassemia (Cooley's anemia). Currently approved therapy utilizes deferoxamine mesylate (DFO) which has a short intravenous (IV) $t_{1/2}$ requiring frequent infusions for 8-10 hr/day. S-DFO, DFO covalently bound to a soluble starch polymer, has been shown to have low toxicity, extended plasma $t_{1/2}$ and stimulate dose-dependent urinary iron excretion in a single dose study with β -thalassemia patients. In the current study, S-DFO was evaluated for PK and toxicity following daily IV administrations (total dose = 7000 mpk), 4/week (4000 mpk), 3/week (3000 mpk), 2/week (2000 mpk) followed by 14 days observation. S-DFO was very well tolerated with few clinical signs and no

clinical pathology findings up to 15 mL/kg daily dosing. S-DFO was present in the plasma up to 120 hr. Dogs receiving two infusions per week had the same plasma levels on Days 1 and 7. All other treatment regimens had higher plasma concentrations on Day 7 suggesting accumulation. The $t_{1/2}$ (~20 hr) and mean residence time (40 hr or more) support a slow clearance of the drug from the circulation ($Cl \approx 5$ mL/hr/kg after daily dose administration). The volume of distribution (0.2 to 0.4 L/kg) indicates distribution only to the extracellular body fluids. S-DFO maintains the DFO moiety in the plasma ready for iron chelation. S-DFO appears to be relatively nontoxic even at high plasma concentrations potentially offering improved chelation therapy for both acute and chronically iron-overloaded patients. This work was supported by NIDDK contract N01-DK-3-2624.

937 COMPARATIVE PHARMACOKINETICS OF PERFLUOROBUTYRATE (PFBA) IN RATS, MICE, AND MONKEYS.

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Perfluorobutyrate (PFBA) is a 4-carbon fluorochemical acid salt formed by industrial synthesis and the metabolism and hydrolysis of certain chemicals. PFBA has been detected in surface waters and in public and private wells at up to low ng/mL concentrations. In support of dose-setting for toxicological studies in rats and mice, and pharmacokinetic (PK) modeling in primates, we undertook a series of PK studies with ammonium PFBA (SD rats and CD-1 mice) and potassium PFBA (cynomolgus monkeys). These included: (1) elimination studies over a wide range of single oral doses (6-300 mg/kg) in non-fasted male (M) and female (F) rats; (2) IV elimination studies at 30 mg/kg in M & F rats; (3) oral uptake and elimination studies at 30 mg/kg in fasted, jugular-cannulated M & F rats; (4) elimination studies following a wide range of single oral doses in M & F mice (10-100 mg/kg); and, (5) IV elimination in M & F cynomolgus monkeys (10 mg/kg). Group sizes per dose level and sex were 3 in all cases. PK analysis of rat, mouse, and monkey data was conducted using WinNonlin® software. PFBA was determined in sera (all species), liver (rats and mice), urine (rats and monkeys), and feces (rats) using LC/MS/MS techniques. The elimination of PFBA in M & F rats was rapid and almost entirely in urine. Serum half-life ($T_{1/2}$) values in rats were 7.0-9.2 and 1.6-1.8 hrs in M & F rats, respectively. In rats, liver PFBA concentration was 20-25% of serum PFBA concentration. The elimination of PFBA in mice appeared to be biphasic with the first phase occurring at serum PFBA concentrations above 0.1-1.0 μ g/mL and giving $T_{1/2}$ values of ~17 and ~3 hrs in M & F mice, respectively. By comparison, $T_{1/2}$ of PFBA in monkeys was biphasic, with first- and second-phase $T_{1/2}$ s of 1.6-2.4 hrs and 1.7 days, respectively, with the first phase also occurring at serum concentrations above 0.1 μ g/mL. The volumes of distribution suggested that PFBA was distributed primarily in extracellular space.

938 PK/RANGE-FINDING STUDY OF WOLINASE IN RATS.

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Lymphoblastic leukemia and lymphoma cells lack asparagine synthetase, thus they are unable to convert aspartic acid to asparagine (Asn) like normal mammalian cells. As such, these cells depend on the Asn from extracellular sources, making them particularly sensitive to asparaginase (Asp)-induced depletion of Asn in the blood. The Asp from *Wolinella succinogenes* (Wolinase) is currently in preclinical development, and like *E. coli* Asp and *Erwinia* Asp (both being used clinically), it shows strong activity in these disease models. Within 10 min of a single iv or im dose of 10,000, 30,000 or 60,000 U/m² Wolinase, circulating Asn levels in Sprague Dawley rats were depleted and remained so for 12-24 h. Dose dependent increases in Asp activities were achieved, suggesting that high concentrations of this Asp are not necessary for optimal activity. Mean Asp activities were 23 U/mL (10,000 U/m²), 78 U/mL (30,000 U/m²), and 216 U/mL (60,000 U/m²) 10 min after iv dosing, whereas mean peak Asp activities after im dosing (@90-120 min) were 0.45 U/mL, 1.89 U/mL and 2.59 U/mL for the respective doses. The iv doses of Wolinase produced the same undesirable effect on glutamine (Gln) as the other Asp, as Gln levels were completely depleted at 10 min with 30,000 and 60,000 U/m², and partially depleted with 10,000 U/m²; partial or full recovery occurred by 4 h. The im doses had no apparent effect on Gln levels. Modulation of Asn activity occurred without any treatment-related effect on clinical signs, body weight, or clinical pathology measurements. Similarly, microscopic evaluation of tissues from rats dosed with 60,000 U/m² iv and im did not reveal any treatment related effects relative to control rats. Additional studies will be required to determine if Wolinase shares the same adverse effects as the other Asp after repeat dosing, and whether it can be used as a substitute in the event of systemic allergic reactions to one of the other Asp. (N01-CM-42202 and N01-CO-12400)

939 MULTIPLE DOSE TOXICITY STUDY OF WOLINELLA ASPARAGINASE (WOLINASE) IN RATS.

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Due to their lack of asparagine synthetase, lymphoblastic leukemia and lymphoma cells are highly sensitive to depletion of plasma asparagine by asparaginases. To evaluate the biological activity and toxicity of Wolinase, an asparaginase derived from *Wolinella succinogenes*, Sprague-Dawley rats (15/sex + 9 male satellites/group) received iv injections of Wolinase (in saline) at doses of 0, 6,000, 18,000 or 60,000 U/m² three times/week for 3 weeks. Biomarkers were assayed on timed blood samples collected after the first and last doses of Wolinase. 5 rats/sex/group were necropsied for evaluations of toxicity on Day 6 (after dose 3), Day 20 (after dose 9), and after a 28-day recovery period. Wolinase induced dose-dependent increases in asparaginase activity. At five minutes after the first and last doses, mean asparaginase activities were 2, 67, and 153 IU/mL and 14, 11, and 149 IU/mL in the low, mid, and high dose groups, respectively. Plasma asparagine was completely depleted within 5 minutes at all Wolinase dose levels, and remained undetectable for at least 24 hours in the high dose group. The high dose of Wolinase also completely depleted plasma glutamine within 5 minutes, while partial depletion of glutamine was seen at the low and mid doses. Partial or full recovery of plasma glutamine levels was seen in all groups at 4 hours post-dosing. No treatment-related clinical signs or effects on body weight, food consumption or clinical pathology parameters were observed. Several deaths were observed in the mid and high dose groups after the 7th dose, but neither macroscopic nor microscopic evaluations identified a cause of death. No treatment-related microscopic changes were observed in any high-dose animals that were necropsied after the 3rd or 9th doses. Based on lack of histopathologic effects, the NO[A]EL for intravenous administration of Wolinase using a q.o.d. x 3 schedule was 60,000 U/m². (N01-CM-42202 and N01-CO-12400)

940 SAFETY EVALUATION OF A THAI TRADITIONAL ORAL HYPOGLYCEMIC FORMULA ORM1 RECIPE COMBINED WITH A HYPOGLYCEMIC DRUG TOLBUTAMIDE IN TYPE II DIABETES MELLITUS PATIENTS.

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At present diabetes mellitus (DM) is one of serious and chronic health diseases among the world population. Hypoglycemic drugs are needed to lower blood glucose concentration. Ignorance of health care and treatment in the DM patients can be leading to complications of many diseases such as cardiovascular diseases including atherosclerosis and hypertension, renal failure, blindness and gangrene. Oral hypoglycemic drugs can be used effectively in Type II DM or non-insulin dependent diabetes mellitus (NIDDM). Drugs resistance, hypoglycemia and renal insufficiency are some examples of adverse effects among other effects frequently observed in patients using hypoglycemic drugs. Therefore, an alternative treatment of DM has been developed as a combination with traditionally used to cure DM like symptoms in Thai medicine for some hundreds years ago. In the study, "ORM1 recipe" was given orally to 100 DM volunteers; 35 male and 65 female, aged 35-55, who have used hypoglycemic drugs both orally and injection administration for a period of 3-10 years. Monitoring of blood sugar was determined as fasting blood sugar (FBS) along the 6 months period of treatment with "ORM1 recipe". Decrease in mean FBS was found related to time and gradually lower after taking the formula for 6 months comparing to mean FBS before taking "ORM1 recipe" with statistically significant difference ($p < 0.05$). For safety evaluation of the recipe, it was found no toxicity to liver, pancreas and kidney as indicated by determining no difference between levels of AST, ALT, ALP, BUN and creatinine of the patients after taking the formula for 6 months comparing to the chemical data before taking the formula.

941 CHRONIC TOXICITY OF MURAGLITAZAR, A NOVEL PPAR ALPHA AND GAMMA DUAL AGONIST.

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Chronic toxicity of muraglitazar, an oxybenzylglycine, non-thiazolidinedione peroxisome proliferator activated receptor (PPAR α and γ) agonist was assessed in rats and monkeys. Rats were given 0.3, 3, 30 or 300 mg/kg for 6 months and monkeys were given 0.4, 2, or 5 mg/kg for 12 months. Comparable groups of 20 rats per sex and 4 monkeys per sex received vehicle alone and served as controls. At the highest

dose in rats, survival was significantly decreased with deaths or moribund sacrifices in greater than 50% of the animals. No additional muraglitazar-related deaths occurred in rats or monkeys. Similar to other PPAR gamma agonists, pharmacologically mediated effects including subcutaneous edema, hematologic and/or hematopoietic and serum chemistry changes and morphologic alterations in heart and adipose tissue were observed in rats and monkeys administered muraglitazar. In rats and monkeys, liver weights were increased but were not accompanied by increased serum transaminases or correlative histopathologic alterations. Mild to moderate elevations in serum thyroxine, unaccompanied by microscopic thyroid findings were noted in monkeys administered ≥ 2 mg/kg. In female monkeys, left ventricular wall thickening (diastole and systole) and increased calculated shortening fraction were observed at the highest dose of 5 mg/kg. No differences in systemic exposure were apparent between sexes in either species. Muraglitazar exposures [AUC (0-24)] at the clinically well tolerated doses of 30 mg/kg in rats and 2 mg/kg in monkeys were 53 to 59 and 9 to 14 times therapeutic exposure, respectively. Collectively these data demonstrate that chronic administration of muraglitazar resulted in predominantly pharmacologically mediated findings at significant systemic exposures in rats and monkeys.

942 EMBRYO-FETAL DEVELOPMENT STUDY OF ALL-TRANS-RETINOIC ACID IN SPRAGUE-DAWLEY RATS.

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The purpose of this study was to validate the rat model for teratology studies by studying the adverse embryo-fetal outcomes of *All-trans-retinoic acid* in pregnant Sprague-Dawley rats. Rats were single housed in polycarbonate, solid bottom cages under controlled environmental condition. Females were mated with males on a one to one basis, and the day which copulation was designated as gestation Day 0 (GD0). Fifty-nine control animals were dosed sterile water via oral gavage from GD6 to GD17. Twenty mg/kg of *All-trans-retinoic acid* (Sigma, dissolved by DMSO in corn oil) was administered orally to 20 SD rats from GD6 to GD12. All dams were euthanized on GD20, and fetuses were examined. In dams, no treatment related effects in clinical signs were observed. Reduced body weight gain due to high resorption rate was noted in *All-trans-retinoic acid* group (hereafter treated group). Percentage of body weight in treated group to the control group was 101, 98, 96, 87 and 80% on GDs 0, 6, 12, 18 and 20, respectively. Number of implantation (16.3/16.1 in the control/treated group) and implantation rate (83.6%/87.4%) were comparable between both groups. Number of live fetuses (48.9/2.1) and fetal viability rate (98.0%/13.0%) was markedly decreased in the treated group accompanied with increased number of dead embryo-fetus (0.5/14.0) and embryo-fetal death ratio (3.0%/87.0%). Fetal weight in the treated group (male/female = 2.61 g/2.56 g) was clearly lower than that of the control group (3.76 g/3.74 g). All live fetuses in the treated group had at least one malformation, including exencephaly (97.3%), micrognathia (72.9%), anal atresia (23.3%), spina bifida (12.7%), acardate (11.7%), anophthalmia (8.3%), curled tail (8.3%), cleft palate (6.7%), hydrocephalus (1.0%), agnathia (1.0%) and short tail (1.0%). These embryotoxicity and malformations observed in this study were comparable with those reported previously. Based on these results, it was concluded that the methods used in this study are suitable for embryo-fetal development assessment.

943 TOXICOLOGICAL EVALUATION OF DIMETHICONE 350 CST AS AN INACTIVE INGREDIENT IN DRUG PRODUCTS.

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The U.S. FDA requires that a safety evaluation be completed for inactive ingredients that are not fully qualified by existing safety data with respect to the currently used level of exposure, duration or route of administration. Dimethicone is a polydimethylsiloxane (PDMS) which is a mixture of fully methylated linear siloxane polymers and is widely used in medical devices as well as an inactive ingredient in drug products. A detailed toxicological evaluation of dimethicone 350 cSt was conducted. Currently, FDA's Inactive Ingredient Database indicates that dimethicone 350 cSt has a maximum use level 3.7 mg administered orally. Recently conducted toxicology studies with dimethicone 350 cSt administered in 28-day and 13-week studies in Fischer rats via dietary administration demonstrated no adverse effects or target organs. The highest doses tested in the 28-day and 13-week studies were 100,000 and 50,000 ppm, respectively, which were also established as NOEL doses in the respective studies. These exposures were equivalent to 10,615 mg/kg/day for males and 11,325 mg/kg/day for females in the 28-day investigation and 3,784 mg/kg/day for males and 4,275 mg/kg/day females in the 13 week study, respectively. PDMS has also been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) which established an acceptable daily intake (ADI) for chronic human exposure of 1.5 mg/kg based on a NOEL of 0.1% in

the diet (equivalent to 150 mg/kg body weight/day) in several chronic/carcinogenicity rodent studies. For a 60 kg individual, the total daily dose would be 90 mg/day. The use of the JECFA ADI would provide a conservative estimate of the maximum human level permitted via oral route. On the basis of the conducted 28-day and 13-week studies, together with the JECFA evaluation, the use of dimethicone 350 cSt in drug products is supported at levels greater than those currently employed.

944 TOXICOLOGICAL QUALIFICATION OF HYDRAZINE.

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Thresholds for genotoxic impurities have recently been developed by various groups including regulatory agencies (EMEA) and industry. However, if exposures exceed the threshold, possibly due to limitations in reducing the concentration in active ingredients, or if it is an excluded compound on the basis of high potency, it is necessary to characterize the risk of the impurity and develop chemical specific concentration limits. The results for hydrazine, a genotoxic compound, which is a known metabolite and degradant of isoniazid (a treatment for tuberculosis), is presented as a case study.

In determining concentration limits of hydrazine in isoniazid active pharmaceutical ingredient, the risk level considered should be in keeping with the risk benefit ratio as it relates to the intended patient group. Thus, while a risk level of 1-in-100,000 may be recommended for a generally healthy population, a higher risk level might be supported for a more serious indication where the medication is urgently needed, particularly if it is not possible to significantly reduce levels of the impurity in the medication. Based on maximum use levels of isoniazid, to be prescribed for a period of up to one year, hydrazine exposures that correspond to risk levels of 1-in-10,000 and 1-in-100,000 were determined to be 2.3 and 0.23 micrograms/kg/day, respectively. The corresponding concentration limits for hydrazine in isoniazid for the prevention of tuberculosis in adults (assuming 300 mg/day for a period of up to 12 months) that would correspond to exposures at each of the risk levels was calculated to be 460 ppm for 1-in-10,000 and 46 ppm for 1-in-100,000. For use in children (receiving 10 mg isoniazid/kg/day) the concentrations limits for hydrazine in isoniazid corresponding to exposures at each of the risk levels were 230 ppm and 23 ppm for 1-in-10,000 and 1-in-100,000, respectively. Concentration limits for other dosing regimens (other doses or different durations of treatment, etc.) can be determined using this same methodology.

945 COMPARATIVE BIOAVAILABILITY OF SOME XANTHENE DYES AFTER ORAL AND DERMAL EXPOSURE.

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Xanthene dyes are generally used as color additives in drugs and cosmetics. For the safety assessment of topically applied cosmetics, e.g. hair dyes, mainly NOAELs obtained from repeated dose oral rodent studies are required and the comparison of the systemic bioavailability after oral and dermal exposure is a key consideration. In our present study, we compared the systemic exposure of three Xanthene colors halogenated to different degrees: D&C Red no. 28 > Tetrabromophenol Blue > HC Blue No. 15.

Mass balance analysis of Tetrabromophenol Blue and HC Blue No. 15 demonstrated that the major route of elimination was fecal excretion after single intravenous (> 94%), oral (>95%), and dermal (10% and 2% respectively) exposure. The high rate of fecal elimination after intravenous exposure indicated an important role of biliary excretion for both dyes. The corresponding bioavailability calculated from the plasma levels after oral exposure was 7-16% and 33-35% for Tetrabromophenol Blue and HC Blue No. 15, respectively. These findings correspond to the published data for D&C Red No. 28 (Sweet et al., 2004), demonstrating that following oral and intravenous exposure the major route of elimination is fecal and biliary excretion, and that bioavailability after dermal exposure is moderate or low. A correlation between halogenation and systemic exposure, as suggested by Sweet et al., 2004, was found, since bioavailability after oral exposure of D&C Red no. 28 with 8 halogens was comparatively lower than of Tetrabromophenol Blue (7 halogens). The highest bioavailability after oral dosing was found for HC Blue No. 15 (2 halogens).

Bioavailability after dermal exposure of Tetrabromophenol Blue, HC Blue No. 15, and D&C Red No. 28 was analyzed by in vitro skin penetration studies, indicating a extremely low systemic exposure rate below 0.03 % of the applied dose. We conclude that the bioavailability of Xanthene dyes after oral dosing is 3-4 decimal orders of magnitude higher than after dermal exposure.

946 SAFETY PHARMACOLOGY: AUTOMATED ASSESSMENTS OF NEUROMUSCULAR DEFICITS USING ACRYLAMIDE IN SPRAGUE-DAWLEY RATS.

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Acrylamide, a classic peripheral neurotoxicant, was used to assess the sensitivity of an automated behavioral test system for neuropathy. Neuromuscular deficits were assessed in the rat following single intraperitoneal doses of acrylamide for 5 days. Thirty-two male Sprague-Dawley rats, assigned to 4 dose groups (N=8) were given acrylamide at 0 (saline), 5, 25 and 50 mg/kg. Evaluations for neurotoxicity were performed on test days-2, 5 and 8. As part of a "functional observational battery" testing regimen, animals were assessed using a 2-tiered approach. Tier I quantified sensorimotor activity in response to exercise procedures. Tests included Cage-side Observations, Open-field Assessments, Locomotor Activity, Body Weight, Forelimb Grip Strength and Hindlimb Footsplay. Tier II procedures measured overt changes in response to forced exercise endurance testing and included Forced Running Wheel and Treadmill. Greatest impact following acrylamide exposure was measured on Test Day 5 or after. Cage-side observations showed clinical signs associated with acrylamide were dose-dependent, increasing in incidence and severity with increases in dose. Changes in open field behavior were only detected at the high dose. Also at the high dose, locomotor activity tests showed a 64% decrease in Vertical Movement and a 35% decrease in Total Distance traveled¹. Body weight decreased 8% in the 50mg/kg dose group, compared to controls. There was no significant difference detected in dose-groups using forelimb grip strength, hindlimb footsplay and treadmill. Forced Running Wheel revealed a 19% decrease in duration on wheel and an increase in incidence of coasting¹. Results from this study indicate that the automated *in vivo* neuromuscular test battery was a sensitive and valid model for detecting drug-induced neuropathy; and that acrylamide is a suitable positive control for defining peripheral nerve loss of function.

¹ Results depict significant 50mg/kg acrylamide value differences as compared to vehicle control (p ≤ 0.05).

947 OCULAR EVALUATION OF POTENTIAL OCULAR TOXICITY IN REGULATORY NON CLINICAL SAFETY STUDIES.

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Regulatory non-clinical safety studies are required before EIH (Entry Into Human) in order to predict possible side-effects of administration of new pharmaceuticals. Such studies comprise ophthalmic examinations and are carried out in at least one rodent (generally rats, mice or hamsters) and one non-rodent species (generally dogs, non-human primates or minipigs). Risk assessments regularly identify visual system deficits in chronically exposed individuals.

After some considerations on ocular exposure, toxic mechanisms of action, guidelines and their consequences for regulatory testing visual function are discussed. Regulatory guidelines state that ocular investigations should be performed prior to administration of a test substance and at the termination of the non-clinical safety studies (end of treatment period and eventually end of treatment-free period) using appropriate equipment. The choice of this equipment is the responsibility of ophthalmologists and will be further detailed.

We describe then the normal ocular pattern of different non-rodent species alternative to dog used in ocular toxicology, as well as some ocular findings, in order to define the main variants and to compare them in different species used in toxicology studies.

There will be additional demand in future for less common animal models, notably for species whose metabolic assimilation of the drug under investigation is as close as possible to that of man.

948 N-METHYL-N-NITROSOUREA (MNU): A ROBUST POSITIVE CONTROL AGENT FOR P53+/- MOUSE CARCINOGENICITY STUDIES.

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The typical positive control agent for p53+/- mouse carcinogenicity studies, p-cresidine, has demonstrated inconsistent results that in some cases could invalidate a study. Treatment of p53+/- mice with a single intraperitoneal dose of N-methyl-N-nitrosourea (MNU), a DNA alkylating agent, produced malignant lymphoma (T-cell lymphoma) of the thymus in 80% of males and 90% of females. These tumors were identified at necropsy as thymic masses or enlargements in all affected animals, excepting 1 male. The high incidence of neoplasms in treated animals, cou-

pled with the close correlation of gross and microscopic findings, suggests that a single intraperitoneal dose of MNU at 75 mg/kg represents a suitable positive control model for 6-month p53+/- carcinogenicity studies. The high incidence of thymic malignant lymphoma in both sexes suggests that it should be possible to examine only the thymus in positive control p53+/- mice treated with MNU.

949 ARE DUAL CONTROL GROUPS NECESSARY FOR CARCINOGENICITY STUDIES? AN EVALUATION USING MOUSE DATA.

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Although current regulatory thinking allows for the use of single control groups for rodent carcinogenicity testing, it has been argued that dual control groups are vital in the identification of tumorigenic risk potential in these studies. However, whether this is indeed the case has had little coverage in the published literature? A recent evaluation of dual control carcinogenicity data in the rat (Baldrick, *Tox Path* 2005, 33: 283-291) showed that although no major differences in tumor incidences between the control groups were found, some inter-study variation occurred and in cases were a notable difference was seen, the use of 2 control groups, as well as robust, contemporary background data, allowed an easier interpretation of findings in drug-treated groups. As a follow-up to this investigation, the incidence of tumors from 10 mouse carcinogenicity studies, performed between 1991 and 2004, with 2 control groups, was examined. As in the rat, inter-study variation was seen and in some cases, the use of dual control groups assisted in the tumor risk assessment. Thus, the continued use of 2 control groups can have a vital role in mouse carcinogenicity studies.

950 MORTALITY AND IN-LIFE PATTERNS IN HAN WISTAR RAT TUMORIGENICITY STUDIES, COMPLETED OVER THE PERIOD OF 1994 TO 2006.

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Previous reviews of Han Wistar (HsdBrl:WH) rat tumorigenicity studies, completed over 1994 to 2000, indicated that the strain had a high survival rate, a satisfactory tumour profile not masked by geriatric lesions, and was a suitable strain for use in tumorigenicity studies. The purpose of this review of up to 35 studies, completed up to 2006, was to establish if the strain was still performing as expected in tumorigenicity studies by comparing possible differences over time and also between the dietary and oral gavage routes of administration, by evaluation of mortality, bodyweight and food consumption data. All these studies used low protein diet and the animals were gang-housed. The analyses have shown that the mortality values at termination (104 weeks) were low, with mean and standard deviation values of $24 \pm 6.5\%$ for males and $32 \pm 8.8\%$ for females. Statistical analyses of the terminal mortality against time (1994-2006) did not reveal any significant trends in either sex, for all studies or separately by route of administration. The terminal mortality values for oral gavage studies were slightly, but not statistically significantly, higher for both sexes when compared with dietary studies, with the mortality over 2 years showing a similar pattern for both routes. There were no major differences over time or between the routes of administration for bodyweight growth pattern, bodyweight gain and food consumption data. In conclusion, the results indicate that the Han Wistar rat continues to show a low mortality pattern with no indication of any major differences over time or between the routes of administration for mortality or in-life data, and therefore the strain continues to be suitable for use in tumorigenicity studies.

951 HISTOPATHOLOGY HISTORICAL CONTROL DATA FROM DOGS AND MONKEYS.

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Histopathology data constitute a critical part of the safety evaluation information submitted to regulatory agencies in support of the safety evaluation of new drugs or chemicals. Historical control pathology data have been shown to be valuable in the interpretation and evaluation of results from safety evaluation studies. The Registry of Industrial Toxicology Animal data (RITA) was developed to collect historical control neoplastic data in rodents. However, similar historical control data for non-neoplastic findings are generally unavailable in a single database for non-rodent species. Histopathology data from control dogs and monkeys were compiled and reviewed from safety evaluation studies conducted at Covance Laboratories, Virginia during the past 10 years (1996-2006). Most commonly occurring microscopic findings in control animals are presented and categorized based upon the length of dosing (4, 13, or 52 weeks) and the route of administration (oral gavage

or IV bolus). Some of the more common microscopic findings in the dog from the database included: renal mineralization characterized by the presence of basophilic concretions within the lumen of the tubules, primarily affecting the renal papilla; inflammation of the liver characterized by randomly distributed focal aggregates of lymphocytes and macrophages; and inflammation of the lung characterized by intra-alveolar macrophages and randomly distributed focal aggregates of mononuclear cells. Common microscopic findings in the monkey from the database included: renal mineralization similar to that observed in the dogs in addition to foci of inflammation characterized by infiltration of the cortical interstitium with lymphocytes and macrophages; inflammation of the liver and lungs similar to that observed in the dog; the presence of pigment (parasite origin) in the lungs; and inflammation of the heart characterized by randomly distributed aggregates of lymphocytes and macrophages. The use of historical control data in supporting interpretation of safety evaluation studies has its benefits. The data does however, have limitations and must be used prudently.

952 THE EFFECT OF PARTICLE SIZE ON THE LOCAL TISSUE CHANGES ASSOCIATED WITH THE INTRAMUSCULAR ADMINISTRATION OF DEPOT FORMULATIONS IN RABBITS.

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As part of an effort to identify and develop a long duration intramuscular (IM) injection of the atypical antipsychotic ziprasidone, the irritation potential of several proposed IM depot formulations at the injection site was evaluated in an in vivo rabbit model. Two of the formulations investigated were aqueous suspensions containing 210 mg/mL ziprasidone: one consisted of micron-sized particles of drug, while the other consisted of nanoparticles of drug with a mean particle size <500 nm. When administered intramuscularly in rabbits, both formulations were well tolerated; however, the gross and microscopic tissue changes at the injection sites demonstrated that the nanoparticle formulation was considerably less irritating. Microscopic changes, initially characterized by hemorrhage, myofiber degeneration, and mononuclear cell infiltrates with subsequent myofiber regeneration and fibroplasia, were produced with both formulations but were more severe and persisted longer with the micron-sized formulation. With both formulations, injected drug was still visible grossly within the muscle at study termination 4 weeks post-dose. Microscopically, there was a pronounced foreign body inflammatory cell reaction with the micron-sized formulation that was not observed with the nanoparticle formulation. Pharmacokinetic studies in dogs indicated that the nanoparticle formulation additionally increased bioavailability and led to greater and more prolonged depot-phase systemic exposure. These findings suggest that nanoparticle technology may warrant consideration as a formulation tool during the development of parenteral drugs.

953 CYTOKERATIN AND KI-67 EXPRESSION IN THE EPITHELIUM OF THE CANINE LARYNX.

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The larynx is a potential target organ in inhalation studies and is a standard tissue for histological examination on repeat dose toxicity studies (CPMP/SWP/1042/99). There is limited information on the microscopic anatomy of the normal canine larynx therefore a preliminary study was performed to map the epithelium.

Larynges from five, 18 month old, control Beagle dogs were collected at necropsy, fixed in formalin and processed routinely. Five cross sectional samples were cut through each larynx from the epiglottis to the first tracheal rings. Sections were stained with haematoxylin and eosin and by immunohistochemistry for a limited panel of cytokeratins (CK) and the proliferation marker Ki67. Twelve pre-selected areas of epithelium from each larynx were examined microscopically and by image analysis.

The epithelium was uniformly squamous through the main body of the larynx becoming pseudo-stratified abruptly at the cranial trachea. The mean number of layers in the squamous epithelium was generally 2 to 4 but it was significantly thicker (5 to 6 layers) at the ventral epiglottis and ventral floor of the glottis. There was a strong correlation between the number of layers of epithelium and the number of Ki67 positive nuclei/ μm ($r^2 = 0.4$; $P < 0.0001$). All epithelia stained positively with anti-pan-cytokeratin antibody. In addition the squamous epithelium was positive for CK4 and negative for CK18. The pseudo-stratified epithelium was positive for CK4 and CK18 and the submucosal glands were positive for CK18 and negative for CK4.

The results provide a baseline against which potential induced toxicological changes in the laryngeal epithelium can be assessed.

954 THE ROLE OF DOSING REGIMEN IN PPAR-INDUCED PHARMACOLOGIC EFFICACY AND SKELETAL MUSCLE DAMAGE IN RATS.

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Peroxisome proliferator-activated-receptor (PPAR) agonists are soluble nuclear hormone receptor agonists that bind to specific PPAR DNA response elements and activate a number of genes predominantly involved in lipid homeostasis. One untoward effect of PPAR agonism is skeletal muscle damage, which occurs in multiple species. Compound A is a PPAR agonist with dual beta/alpha agonist activity (PPAR β/α) designed to treat hyperlipidemia. Previously, Compound A was shown to produce skeletal muscle damage in male rats following 10 consecutive days of dosing at 50 mg/kg. The present study in rats was undertaken to determine the role of dosing frequency on pharmacologic efficacy and skeletal muscle toxicity. Efficacy was measured by effects on peroxisomal liver oxidation activities, and toxicity was evaluated by histologic changes in skeletal muscle architecture. In this study, animals were given 10 doses of Compound A either daily or every other day and necropsied. At termination, all animals given daily doses of Compound A had minimal to moderate skeletal muscle damage, and elevated creatinine kinase (CK) activities, relative to controls. In animals given Compound A every other day, the incidence and severity of skeletal muscle damage was similar as noted in their respective control group and mean CK activity was not elevated. Liver acyl CoA oxidase and fatty acid beta-oxidation activities were elevated in drug-treated groups above respective controls (up to 7-fold) and were comparable between the treated groups regardless of regimen. These data indicate that intermittent dosing of a PPAR β/α agonist can achieve similar pharmacologic activity as seen with daily dosing yet not produce untoward skeletal muscle injury.

955 COMPARISON OF TWO GASTRIC EMPTYING MODELS (CHARCOAL MEAL AND GLASS BEAD) IN RATS: EFFECTS OF MORPHINE AFTER ORAL AND SUBCUTANEOUS ADMINISTRATIONS.

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Gastric emptying studies are routinely used in safety assessment to ascertain the potential effects of new drugs on gastro-intestinal function. Charcoal meal model is very commonly utilized in GI studies, however, charcoal absorbs many substances. The present study compares the efficacy of morphine administered subcutaneously and orally to inhibit the gastric emptying in an effort to compare these models. Two groups of rats were administered with morphine orally or subcutaneously while another two groups were similarly administered with the vehicle. Fifteen minutes after the subcutaneous and 30 minutes after the oral doses of morphine or vehicle, rats were administered with a 10% suspension of activated charcoal at 3 mL/rat. Rats were euthanized 30 minutes after receiving the charcoal. The stomach (with contents) was excised, weighed and the "full weight" was recorded. The contents of the stomach were then removed, the stomach again weighed, and the "empty" weight recorded.

Two other groups of rats were administered with morphine orally or subcutaneously while another two groups were similarly administered with the vehicle. Fifteen minutes after the subcutaneous and 30 minutes after the oral doses of morphine or vehicle, rats were administered 60 glass microspheres (1000-1190 μ m in diameter, suspended in 1.0 mL of saline) by oral gavage. Rats were euthanized 30 minutes after receiving the glass microspheres, and the microspheres remaining inside the stomach counted from each rat.

Morphine (5mg/kg) after subcutaneous administration produced a 33% decrease in gastric emptying in the charcoal meal model, while in the glass bead model the inhibition was 47%. Similarly after oral administration, morphine (30 mg/kg) inhibited gastric emptying by 65% and 63% in charcoal and glass bead models respectively. The present study thus suggests that both charcoal meal and glass bead models are equally effective if the study design provides adequate contact time for the test article.

956 EVALUATION OF ADVERSE EVENT SAMPLING BIAS.

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As Americans consume increasingly complex combinations of prescription medications and dietary supplements, the concern for adverse events attributable to interactions between these agents increases accordingly. An emerging problem that will likely affect clinicians' perceptions and expert judgments about interactions stems from the use of anecdotal adverse event reports (AERs) to evaluate drug and dietary supplement safety. The only broad-based collection of information concerning

AERs from drugs and supplements is the FDA-sponsored voluntary reporting system, which does not provide a representative sample of adverse events in the population. We have evaluated the literature on AERs with respect to the statistical tests that might be used to estimate adverse event prevalence and incidence, and the inferences that can be drawn from those tests. We found at least two underlying sources of bias inherent in the statistical tests when using non-randomly sampled data: first, adverse event reports for specific outcomes are often recruited through advertising, producing a database that reflects trends in reported associations due to fluctuations in public awareness and worse, is vulnerable to reporting of associations influenced by special interests. A second type of bias results from over-reporting of disease or adverse events. It may seem that a well-studied population is very ill, but in reality, that perception can be created simply from an increased amount of information. The FDA is considering changes to the voluntary reporting system to minimize sampling bias. Until improvements are made, particularly regarding the use of the database in product liability litigation, reporting bias must be recognized and accounted for.

957 PREVENTION OF CROSS-CONTAMINATION IN CANINE NON-CLINICAL SAFETY STUDIES.

J. E. Burnett and S. Turner. *Covance Laboratories Ltd., Harrogate, United Kingdom.* Sponsor: *D. Everett.*

Rigorous management of non-clinical safety studies to prevent cross contamination of control animals, or the samples from control animals, with test substance is required by the regulatory authorities. This poster describes how cross contamination was successfully prevented during a non-clinical safety study conducted in the dog over a 52 week period.

Areas considered during the planning and in-life phases of the study were:

The pharmacology of the test substance

Formulation procedures

Housing and welfare of the animals

Blood sampling and processing procedures

As a result of the considerations made and subsequent preventative measures taken, interpretation of the toxicokinetic, in-life and histopathological data was straight forward allowing an adequate assessment of the risk to human safety and the submission of valid study to the regulatory authorities.

958 INTERACTIONS BETWEEN CHEMICALS AND DRUGS IN THE WORKPLACE. IS THERE A CAUSE FOR CONCERN?

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Workers are frequently exposed to mixtures of chemicals. Additionally, they intentionally use different prescription and over-the-counter drugs to treat various health conditions. Acetylsalicylic acid (AAS), acetaminophen and cimetidine are the most non-prescription drugs used. In respect of worker's life privacy and because of ignorance, the potential interactions between drugs and workplace chemicals are not usually considered. It appears that a better understanding of interactions between drugs and workplace chemicals may contribute to diminish the risk of toxic effects. The objective of this study was to identify binary interactions between drugs and regulated toxic substances found in workplaces (principally solvents and pesticides). Information was drawn from primary references available on TOXLINETM and PubMedTM databases. Human as well as animal studies were evaluated. Since at massive doses most chemicals would cause polysystemic effects, the interactions were evaluated only for realistic exposure concentrations of workplace chemicals up to the short-term exposure limit or ceiling value or five times the 8-h time-weighted-average (TWA) permissible exposure limit (PEL) for human data and up to 100 times the 8-h TWA PEL or ceiling value for animal studies. Only few reasonable human and animal studies were identified involving almost exclusively non-prescription drugs. Thus, 26 pairs of binary co-exposures of drugs and workplace chemicals from 20 studies were identified. From them 7 pairs presented potentiation effects, 7 presented kinetic inhibitions and 12 reported no interactions. For example, AAS potentiates ototoxic and developmental effects of toluene and phenobarbital potentiates hepatotoxic effects of trichloroethylene and carbon tetrachloride. This review highlights a lack of toxicological studies evaluating co-exposure of drugs and workplace chemicals for realistic exposure concentrations. An effort in this field might help scientists to understand changes in the metabolism and toxic effects of workplace chemicals due to co-exposure to drugs.

959 ELECTRORETINOGRAM (ERG) RECORDING IN THE DOGS, RABBITS, MONKEYS AND MINI-PIGS.

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Assessment of visual response can be evaluated through recording of electroretinograms (ERG) to provide indicators of the health and the physiological function of the retina, specifically rod and cone response. For compounds administered by in-

travertine injection, ERG evaluation is an important parameter, as the compound is in direct contact with the retina, and is especially important if there are prolonged effects on intraocular pressure (IOP) or if the compound is blood-retina barrier permeable. In our Laboratory, ERGs are obtained using an UTAS-E 3000 or UTAS-E 4000 ERG recorder and an automated Ganzfeld Dome. ERG Jet® contact lens electrodes are applied to each eye, along with an intradermal reference electrode and a ground electrode. Dogs, rabbits, monkeys and mini-pigs are anesthetized, a topical anesthetic is applied and for mini-pigs a small suture is used to maintain the eye position. Following at least 30 minutes dark adaptation, eyes are tested using a series of scotopic and photopic flash stimuli. Each ERG occasion consists of a series of 3 scotopic single flash ERG, from dim to bright light (-30 to 0 dB), with variable duration between each flash to assess rod activity alone and rod/cone activity combined. Following the scotopic ERG sequence, animals are adapted for 5 minutes to background light at 25-30 cd/m², followed by an average of 20 sweeps of photopic white flicker at 1 Hz, and 20 sweeps of photopic flicker at 29 Hz, to assess cone function. Parameters typically measured are a-wave and b-wave amplitude and implicit time.

Our studies show ERG data/trends are reproducible, however, individual variation should be considered since inter-eye variability in the same animal can be 30-35%. ERG data should also be evaluated in conjunction with ophthalmology, tonometry and histopathology, to provide perspective on possible changes. ERG can be a valuable parameter for determining early onset of retinal damage and consequently a vital marker in subsequent clinical trials.

960 *IN VITRO* HEPATIC METABOLISM OF N-ALKANES, NONANE, DECANE AND TETRADECANE.

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Exposure to jet propellant 8 (JP-8) jet fuel is a major public health concern. JP-8 is a complex mixture of aromatic and aliphatic hydrocarbons. Semi-volatile n-alkanes of C₉-C₁₄ make up significant proportion of JP-8. While toxicity of these alkanes has been reported, information on metabolism is very limited. The aim of this study was to determine Michaelis-Menten constants for the metabolism of nonane (C₉), decane (C₁₀) and tetradecane (C₁₄) by rat liver cytochrome P450s (CYPs) *in vitro*. The metabolism was assessed by measuring disappearance of parent compound. Various concentrations of n-alkanes were incubated with liver microsomes from adult male F344 rats in presence and absence of NADPH. The incubation conditions varied with compound; nonane - 1 mg microsomal protein and 30 min incubation; decane - 1 mg microsomal protein and 60 min incubation and tetradecane - 1-5 mg microsomal protein and 30-120 min incubation. The parent compounds were measured by gas chromatography. Nonlinear kinetic constants for nonane and decane were: V_{max} (nmol/mg protein/min) - 7.26±0.20 and 2.80±0.35, respectively and K_m (μM) - 294.83±68.67 and 398.70±42.70, respectively. Metabolic capacity of CYPs as assessed by intrinsic clearance (V_{max}/K_m) was 5-fold higher for nonane (0.03±0.005) than that of decane (0.007±0.001). There was no appreciable metabolism of tetradecane even with higher microsomal protein concentration (5 mg protein/ml) and longer incubation time (120 min). The results of the present study show a negative correlation between metabolic clearance and chain length of n-alkanes, suggesting that longer the chain, slower the metabolic clearance and higher the internal exposure. The metabolic rate constants reported in this investigation will be useful in the development of a physiologically-based pharmacokinetic model for JP-8 exposure and toxicity (Supported by AFOSR grant F49620-03-0157).

961 METABOLISM OF 1-FURAN-2-YL-3-PYRIDIN-2-YL-PROPENONE, AN ANTI-INFLAMMATORY PROPENONE, IN RAT BY LIQUID CHROMATOGRAPHY-ELECTROSPRAY IONIZATION TANDEM MASS SPECTROMETRY.

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1-Furan-2-yl-3-pyridin-2-yl-propenone (FPP-3) has been characterized to have an anti-inflammatory activity through the inhibition of the production of nitric oxide and tumor necrosis factor-α. In the present studies, *in vitro* and *in vivo* metabolisms of FPP-3 were investigated in male Sprague-Dawley rats. When FPP-3 was incubated with rat liver microsomes and cytosols in the presence of NADPH, 2 major peaks were detected on LC/ESI-MS. Two metabolites (i.e., M1 and M2) were characterized as reduced forms on propenone: M1 (1-furan-2-yl-3-pyridin-2-yl-propan-1-one) was the initial metabolite and M2 (1-furan-2-yl-3-pyridin-2-yl-propan-1-ol) was a secondary alcohol. Following a single intravenous injection of male Sprague-Dawley rats with 4 mg kg⁻¹ of FPP-3, three different metabolites of FPP-3 were identified as M1, M2 and M3 (a glucuronide conjugate of M2) in rat urine by LC/ESI-MS. Although all metabolites of FPP-3 were identified in rat

urine, M1 and M2 except M3 were observed in the bile and feces. Pharmacokinetic parameters for FPP-3 and its metabolites were determined at the same time by using high-performance liquid chromatography-ultraviolet spectrometry. (Supported by a grant from KOSEF and a BK 21 grant)

962 CHARACTERIZATION OF THE ENZYMES INVOLVED IN THE *IN VITRO* METABOLISM OF 1-FURAN-2-YL-3-PYRIDIN-2-YL-PROPENONE, AN ANTI-INFLAMMATORY PROPENONE COMPOUND.

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Carbonyl reduction is a significant step in the phase I biotransformation for a great variety of aromatic, alicyclic and aliphatic carbonyl compounds. 1-Furan-2-yl-3-pyridin-2-yl-propenone (FPP-3) has been characterized to have an anti-inflammatory activity through the inhibition of the production of nitric oxide and tumor necrosis factor-α. In the present studies, the metabolic fate and a possible involvement of 11β-hydroxysteroid dehydrogenase (11β-HSD) and carbonyl reductase (CBR) in the metabolism of FPP-3 were investigated in rat liver subcellular fractions. When FPP-3 was incubated with rat liver subcellular fractions in the presence of NADPH, two major peaks were detected by reduction on the propenone: M1 (1-furan-2-yl-3-pyridin-2-yl-propan-1-one) and M2 (1-furan-2-yl-3-pyridin-2-yl-propan-1-ol). The pH optimum for metabolism of FPP-3 was around 6.5. Selective inhibitors of CBR, such as quercitrin, ethacrynic acid and menadione, significantly increased the formation of M1, but effectively inhibited the formation of M2 in subcellular fractions. Moreover, 18β-glycyrrhetic acid, an 11β-HSD selective inhibitor, significantly inhibited the reduction of FPP-3 in microsomes. In correlation studies, good correlation was observed between the formation of M2 and CBR activity with 4-pyridine carboxaldehyde (r=0.72) and D,L-glyceraldehyde (r=0.63) as substrates in cytosol, respectively. The present results indicated that FPP-3 might be metabolized by microsomal 11β-HSD and cytosolic CBR in rats. (Supported by a grant from KOSEF and a BK 21 grant)

963 CYTOCHROME P450-CATALYZED INDOLINE DEHYDROGENATION IS A NOVEL AROMATIZATION PROCESS.

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Indoline derivatives possess therapeutic potential in a variety of drug candidates. In this study, we found indoline is aromatized by P450 enzymes to produce indole through a dehydrogenation pathway. Similarly, 3-substituted indoles like 3-methylindole and zafirlukast are dehydrogenated to form 3-methyleneindolenine electrophiles, which react with protein and/or DNA nucleophilic residues to cause toxicities. The aromatization of indolines is likely to change the therapeutic properties of these compounds. Indole was indeed efficiently produced by human liver microsomes (K_m = 194 μM, k_{cat} = 1.5 min⁻¹), and CYP3A4 had the highest "aromatase" activity (K_m = 135 μM, k_{cat} = 2.9 min⁻¹). Additionally, four other indoline metabolites (M1-M4) were characterized. M1-M4 were not metabolites of indole. M1, mainly produced by CYP2D6 and CYP3A4, appeared to be an unstable arene oxide, but its GSH adduct was characterized by LC/MS/MS. M2, M3 and M4 were produced by FMO3. Characterization of these metabolites showed that indoline is oxidized to N-hydroxyindoline (M3) and an unstable intermediate indoline nitron, which tautomerized to form N-hydroxyindole (M2) and then dimerized to produce a novel diindole (M4). UV/vis spectral and tandem mass fragmentation patterns, as well as ¹H NMR spectra, were consistent with the putative sequential indoline oxidation. No evidence was found for the P450-mediated production of an aliphatic alcohol from indoline that might dehydrate to produce indole. Taken together, these results demonstrate that several P450s catalyze the novel "aromatase" metabolism of indoline to produce indole. The mechanism of this process does not appear to occur through N-oxidation or dehydration of an alcohol, but rather through a formal dehydrogenation pathway. Supported by NIH grants HL13645 and GM074249.

964 NOVEL OXIDATIVE *IN VITRO* METABOLITES OF ZEARELENONE.

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Zearelenone is a macrocyclic resorcylic acid lactone produced by various fungi of the species *Fusarium* and frequently found in moldy corn. To date, the only known phase I metabolites of this mycotoxin are zearelanone and the alpha- and beta-isomers of zearelanol and zearelanol, which are formed from zearelenone via reductive pathways.

The aim of the present study was to clarify whether zearalenone is prone to oxidative biotransformation. When zearalenone was incubated with liver microsomes from male Sprague-Dawley rats in the presence of a NADPH-generating system, subsequent analysis by HPLC showed the formation of seventeen metabolites. In addition to alpha-zearalenol and small amounts of beta-zearalenol, seven monohydroxylation products of zearalenone and three monohydroxylated zearalenols were detected by gas chromatography-mass spectrometry after trimethylsilylation. Small amounts of three dihydroxylated zearalenones were also found. The two major metabolites of zearalenone were monohydroxylated at the aliphatic macrocycle, as tentatively concluded from the electron impact mass spectra of the trimethylsilyl derivatives. When these major metabolites were isolated by HPLC and again incubated with rat hepatic microsomes, metabolites were obtained that were also observed in the microsomal incubation of zearalenone.

Our study has shown for the first time that zearalenone not only forms reductive metabolites but also a large number of oxidative metabolites with rat hepatic microsomes. Further studies are now needed to unambiguously establish the exact chemical structures of the hydroxylated metabolites and to find out which of them are formed *in vivo*.

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965 IN VITRO METABOLISM OF ARYLAMINE HAIR DYES IN HUMAN HEPATIC MICROSOMES AND HUMAN HEPATOCYTES.

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Metabolism is a key consideration in the safety assessment of primary arylamines used in oxidative hair dyes, especially for the assessment of potential carcinogenicity, since N-hydroxylamine formation by CYPs is regarded as a key step in the activation of certain arylamines that have been identified as human bladder carcinogens (4-aminobiphenyl, 2-naphthylamine, benzidine). Previous studies have shown that *p*-phenylenediamine and 2,5-diaminotoluene, two widely used oxidative hair dye ingredients, do not form mono-oxygenated metabolites in cryopreserved human hepatocytes, hepatic microsomes or recombinant CYPs. Only the mono- and diacetylated metabolites were observed in human hepatocytes. The purpose of the present work was to screen additional arylamine hair dyes for oxidative metabolism in human hepatic microsomes and to conduct more in depth metabolism studies with any compounds exhibiting evidence of oxidative metabolism. Five primary arylamines that are widely used in oxidative hair dyes were screened and showed no evidence of oxidative metabolism. One compound, 4-amino-2-hydroxytoluene (AHT), showed evidence of formation of two hydroxylated metabolites by human, rat, and mouse hepatic microsomes. However, there was no evidence of covalent binding of ¹⁴C-radiolabeled AHT to human microsomal protein, suggesting that the monohydroxylated metabolites formed were not biologically reactive. One of these metabolites was confirmed to be hydroxylated on the methyl group and the other is likely to be ring hydroxylated. Further *in vitro* studies in human, rat, and mouse hepatocytes indicated that phase II reactions (sulfation, acetylation, glucuronidation) predominate in the intact hepatocyte and that mono-oxygenated metabolites are not detected. Our results indicate that none of the hair dyes tested show evidence of hepatic metabolism to potentially biologically reactive oxidative metabolites. As such, the metabolism of these compounds appears distinct from that exhibited by known human bladder carcinogens.

966 IN VITRO METABOLISM OF 8-2FLUOROTELOMER ALCOHOL IN MULTIPLE SPECIES.

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The identification of perfluorinated organic compounds in the environment has generated interest in understanding the biological and environmental fate of these chemicals. 8-2 Fluorotelomer alcohol (8-2 FTOH) is a raw material used in the manufacture of fluorotelomer-based products. This study investigated the *in vitro* metabolism of 8-2 FTOH and selected metabolites by rat, mouse, trout and human hepatocytes and by rat, mouse and human liver microsomes. Clearance of 8-2 FTOH in hepatocytes was rat > mouse > human > trout. The major metabolic pathway for 8-2 FTOH in hepatocytes was conjugation of parent 8-2 FTOH with glutathione or glucuronic acid with no chemical breakdown. Another metabolic pathway was formation of 8-2 FT aldehyde and saturated acid. These were consistent with previously reported metabolites. Metabolites not previously reported included 7-3 saturated and unsaturated aldehyde, 7-2 α -ketone, 7-3 β -ketoaldehyde, and the taurine conjugate of 7-3 saturated acid. No unique metabolites were observed in human hepatocytes. The relative ranking of perfluorooctanoate (PFO) production in hepatocytes was mouse > rat > human = trout. Incubation of 8-2

FTOH with liver microsomes resulted in formation of many of the same metabolites observed in hepatocytes including PFO with the exception of human microsomal incubations in which no PFO was detected. To further elucidate the metabolic pathways for 8-2 FTOH, hepatocytes and microsomes were incubated with various 8-2 and 7-3 acids. Hepatocyte incubations with 8-2 saturated acid metabolized to 8-2 unsaturated acid then 7-3 unsaturated and 7-3 saturated acid which was subsequently conjugated with taurine. In microsomes, no metabolism of the 8-2 or 7-3 acids was observed in any species. Hepatocytes from all species were capable of metabolizing the 8-2 and 7-3 acids but more slowly than 8-2 FTOH. Overall, the results suggest that 8-2 FTOH is rapidly metabolized in rats, mice and humans and to a lesser degree in trout and production of PFO may be mediated by microsomal enzymes.

967 HYDROXYLATED POLYBROMINATED DIPHENYL ETHERS (HO-PBDES) AND BROMOPHENOLS FOUND IN MICE PLASMA AFTER EXPOSURE TO A PENTABROMO DIPHENYL ETHER FLAME RE-TARDANT.

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Previous studies showed that *in vitro* microsomal metabolites of polybrominated diphenyl ethers might weakly activate estrogen-responsive luciferase reporter constructs. To de-terminine the metabolites, mice plasma samples were collected after continuous oral and subcutaneous exposure to the commercial pentabromo diphenyl ether product (DE-71) for 34 days. Samples were denatured, extracted, separated into neutral and phenolic fraction, and analyzed with GC/MS-ECNI. In the neutral fraction, the concentration of BDE-153 was nearly three times higher than that of BDE-99 for oral exposed samples, while BDE-153 was only 10% of BDE-99 in DE-71. This suggested that BDE-153 was resistant to enzymatic degradation in mice. For the phenolic fraction, two main bromophenols (2,4-dibromophenol and 2,4,5-tribromophenol) and seven HO-PBDEs were detected and quantitated. The presence of 2,4-bromophenol and 2,4,5-tribromophenol suggested cleavage of the ether bond of BDE-47 and BDE-99, which were the two main components of DE-71. The HO-PBDEs might come from hydroxylation or hydroxylation and debromination. For the HO-tetra-BDEs, which might come from the hydroxylation of BDE-47, the concentration of 4-HO-BDE-42, 3-HO-BDE-47, 6-HO-BDE-47, and 4'-HO-BDE-49 were 180, 53, 22, and 42 ng/g wet weight, respectively. The presence of 4-HO-BDE-42 and 4'-HO-BDE-49 suggested that there was a shift of a bromine during the hydroxylation process. The relatively high concentration of 4-HO-BDE-42 and 4'-HO-BDE-49 also suggested that para-HO-PBDE might be the most important hydroxylated metabolites. This was significant because para-HO-PBDE could be the most thyroxine like compounds.

968 CONGENER SPECIFIC PCB METABOLISM IN VITRO BY HUMAN LIVER MICROSOMES.

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Polychlorinated biphenyl congeners (PCBs) such as PCBs 101, 118, 138, 153, and 180 are common, persistent environmental contaminants. Information related to their metabolism in humans is lacking. PCB metabolism was investigated using an *in vitro* human liver microsome (HLM) system, which represents a mixture of human CYP 450 isoforms and their relative ability to metabolize PCBs. The PCBs were incubated with HLM's and evaluated for the presence of metabolites by GC. No or minimal metabolite peaks were observed for all the PCBs except for PCB 101. The human CYP isoform responsible for PCB 101 metabolism was identified as human CYP2A6 using specific recombinant human CYP isoforms. Study results indicated selective and extensive metabolism of PCB 101 to 4-hydroxy-PCB 101.

969 DEVELOPMENT OF A NEW UPLC/MS/MS METHOD TO STUDY THE METABOLISM OF BENZO[A]PYRENE BY CYP1S.

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Our goal is to compare benzo[a]pyrene (BaP) metabolism and catalytic efficiency of the newly identified CYP1B and CYP1C isoforms that have been identified in fish to mammalian CYP1s. In order to sensitively detect the BaP metabolites we have

developed an UPLC/MS method which is more rapid and sensitive than traditional HPLC methods. Two types of samples were extracted by liquid-liquid separation for BaP metabolite determination. *In vivo* metabolism was assessed using bile from BaP exposed fish, while *in vitro* metabolism was measured using human and fish recombinant CYP1s. UPLC separation was carried on an Acquity UPLC BEH C18 column (1.7 μ m, 2.1 $\times$ 50 mm) at a flow rate of 0.25 mL/min with an analysis time of 10 minutes. BaP and eight metabolites were separated with a gradient mobile phase (0.3% formic acid/acetonitrile). 6-Hydroxychrysene was used as the internal standard. Mass spectrometry detection was carried out with Waters Quattro micro API quadrupole mass spectrometer in selected ion recording (SIR) mode, equipped with an APCI+ interface. BaP and the following BaP metabolites: BaP-1, 6-dione, BaP-3, 6-dione, BaP-6, 12-dione, BaP-9, 10-dihydrodiol, BaP-7, 8-dihydrodiol, BaP-7, 8, 9, 10-tetrol, 9-hydroxyBaP, 3-hydroxyBaP and internal standard 6-hydroxychrysene were base line separated. The calibration curves of all compounds were linear between 0.2 ng/ μ L and 6 ng/ μ L ($r^2 = 0.97$ for BaP-7, 8, 9, 10-tetrol, $r^2 = 0.99$ for all other compounds). The intra- and inter-day assay precision were within $\pm 10\%$ CV and the accuracy were in the range of 85% to 115% for all the analytes. The extraction recoveries of all compounds were between 80%-115%. This method will be useful for further defining species differences of cytochrome P450-mediated BaP metabolism and genotoxicity. Supported by NIEHS R01ES012710 and CDC CA5 U01 CI00021.

970 EXTRA-HEPATIC METABOLISM OF DELTAMETHRIN.

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Deltamethrin (DLM) is a relatively potent neurotoxin and a widely used pyrethroid insecticide. DLM is metabolized by carboxylesterases (CaEs) and cytochrome P450s (CYPs). Although these enzymes are present predominantly in liver, they are also found in other tissues and are regulated differently in different tissues. While metabolism in intestine (oral exposure) and lung (inhalation) will contribute to first-pass metabolism, brain metabolism may contribute to target tissue levels and total body burden of the neurotoxic parent compound. Hence, extrahepatic metabolism, if any, may influence the internal dosimetry of DLM and other pyrethroid insecticides. The objective of this study was to evaluate metabolism of DLM in intestine, lung and brain relative to liver. Metabolism was quantified *in vitro* by monitoring the disappearance of DLM from S9 fractions of these tissues obtained from male SD rats. CYP-mediated metabolism was not observed in brain, intestine or lung. While intestinal CaEs did not metabolize DLM, intrinsic clearance (V_{max}/K_m) by CaEs in lung (0.9 ± 0.1) was 2-fold higher than in brain (0.4 ± 0.04). These values were ~25- to 50-fold lower than intrinsic clearance by liver CaEs (24.9 ± 2.1). The present results show that intestinal metabolism is apparently not a factor in the first-pass elimination of oral DLM exposure. Other than plasma CaE-mediated hydrolysis, extra-hepatic metabolism does not seem to contribute significantly to the clearance and toxicity of DLM (Supported by EPA STAR Grant R830800).

971 *IN VIVO* AND *IN VITRO* METABOLISM OF DIISOPROPYL ETHER (DIPE).

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Diisopropyl ether (DIPE) is a volatile solvent that can be added to gasoline to improve combustion efficiency and thus reduce toxic emissions. Due to the volatility of ethers, respiratory toxicity has been assessed using lavage fluid analyses after inhalation exposures. Our lab has shown an increase in toxicity markers after methyl-tert butyl ether (MTBE) and ethyl-tert butyl ether (ETBE) exposures to 250 - 500 ppm. DIPE produces minimal respiratory toxicity after inhalation exposures at concentrations as high as 5000 ppm. Other branched aliphatic ethers such as MTBE and ETBE are metabolized by the CYP450 system to produce potentially toxic metabolites (tert-butyl alcohol, formaldehyde and acetaldehyde). Currently, no data is available on the metabolism of DIPE although it has been suggested that metabolism produces isopropyl alcohol and acetone. The aim of this study was to determine the major metabolites of DIPE using rat nasal mucosa microsomes. *In vitro*, acetone and isopropyl alcohol were produced in a concentration-dependent manner. Organ specific variations in metabolism of DIPE in Sprague-Dawley rats indicate that nasal mucosa tissue has the highest rate of DIPE metabolism, while lung and the liver tissue produce much lower levels of metabolites, less than 0.01% of the nasal mucosa. To evaluate the role of P450 isoforms in DIPE metabolism, we used 8-methoxypsoralen (8-MOP; CYP2A3/6 inhibitor), 4-methylpyrazole (4-MP; CYP2E1 inhibitor) and ketoconazole (KTZ; CYP3A4 inhibitor). K_i values were 0.56, 7.7 and 32 μ M for 8-MOP, 4-MP and KTZ, respectively, implicating CYP2A3 and 2E1 as the major enzymes involved in DIPE metabolism. *In vivo* studies showed elimination of DIPE, acetone and isopropanol from the blood within 24 hours of inhalation exposures to 5000 ppm DIPE (1 day, 6 hours/day). Rapid systemic clearance of DIPE as well as the production of relatively less toxic metabolites may contribute to reduced toxicity compared to MTBE and ETBE.

972 COMPARATIVE METABOLISM AND DISPOSITION OF SINGLE AND MULTIPLE DOSES OF ACRYLAMIDE (AM) USING CYP2E1-/- (KO) AND WILD-TYPE (WT) MICE.

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AM is a reactive monomer that is used to synthesize polyacrylamide for use in chromatography gels, paper and textile industries, and cosmetics. AM is formed in starchy foods that are prepared at high temperatures including French fries, potato chips, and cereals. Because AM is neurotoxic, carcinogenic, and a reproductive toxicant, there is major concern about the consequences of exposure to AM on human health. AM causes dominant lethal mutations after daily administration to male mice for 5 consecutive days. Cytochrome P450 2E1 (CYP2E1) is a major enzyme involved in AM metabolism. Current studies were undertaken to compare the metabolism and disposition of 2,3- ^{14} C-AM after a single or 5 consecutive daily doses at 12.5 or 50 mg/kg/day using KO and WT mice. Renal excretion of AM-derived radioactivity was dose-dependent and was the major route of elimination in mice of both genotypes. Elimination of unmetabolized AM was less in the urine of WT vs. KO mice. AM-mercapturate was the main urinary metabolite and accounted for ~81-88% in KO vs. 44-57% of the urinary radioactivity in WT mice. In contrast to their levels in WT mice, glycidamide (GA) and GA-mercapturate (GAMA) were barely detectable in KO mice. However, while GA accounted for 3-13% of the AM-derived urinary radioactivity, GAMA constituted 8-14% in WT mice administered at 12.5 or 50 mg AM/kg/day. Elimination of AM-derived radioactivity in the feces and in the expired air as CO_2 and organic volatiles in both KO and WT mice was minimal. Blood and tissue concentrations of AM-derived radioactivity increased as a function of dose and the number of doses administered in mice of both genotypes. In conclusion, this work confirmed that CYP2E1 is the primary enzyme responsible for the formation of the proximate metabolite, GA. Further, retention of AM-derived radioactivity in blood and tissues increased as a function of the number of doses administered and points out to the potential accumulation of this chemical in association with long-term exposure.

973 COMPARATIVE METABOLISM AND DISPOSITION OF SINGLE AND MULTIPLE DOSES OF TRICHLOROETHYLENE (TCE) USING CYP2E1-/- (KO) AND WILD-TYPE (WT) MICE.

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TCE is classified as a rodent carcinogen and a "probable human carcinogen." It was reported that Metabolism of TCE occurs via cytochromes P450 (CYPs) and CYP2E1 is thought to play an important role. The objective of the present work is to assess the role of CYP2E1 in TCE metabolism using KO vs. WT mice. Animals were administered a single 250 or 1000 mg/kg dose of 1,2- ^{14} C-TCE or 250 mg/kg/day for 4 consecutive days. Mice were placed in glass metabolism cages that allowed for the collection of expired air, urine, and feces. At 24 hr after a single dose or after the last of the 4 daily doses, animals were euthanized and blood and tissues were collected. Exhalation of TCE-derived $^{14}CO_2$ was time- and dose-dependent. Further, while exhalation of TCE-derived $^{14}CO_2$ gradually increased in WT mice with continued daily dosing, it plateaued after the first dose in similarly treated KO mice. Elimination of organic volatiles, of which TCE is the only detectable chemical, in the expired air was significantly higher in KO vs. WT mice exposed to single or multiple doses of TCE. A significantly greater % of the dose was excreted in the urine of WT vs. KO mice. Three metabolites (trichloroethanol (TCOH)-glucuronide, trichloroacetic acid, and free TCOH) were identified with TCOH-glucuronide being the major metabolite in the urine of mice of both genotypes. Free urinary TCOH was significantly higher in KO vs. WT mice. Further, free TCOH in the urine of KO mice was significantly greater after 4 daily doses vs. a single dose. Levels of TCE-derived radioactivity in tissues were significantly higher after 4 daily doses vs. a single dose in mice of both genotypes with the highest levels found in the liver, forestomach, and kidneys. In conclusion, these data confirmed earlier finding that while CYP2E1 plays an essential role in the metabolism of TCE, other CYPs are also important. However, it remains to be established if CYP2E1 or other CYPs are involved in the metabolism of TCE metabolites such as trichloroacetic and dichloroacetic acids.

974 STUDY OF THE EFFECTS OF DICHLOROACETIC ACID ON THE METABOLISM AND DISPOSITION OF TRICHLOROETHYLENE IN CYP2E1-/- (KO) AND WILD-TYPE (WT) MICE.

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Dichloroacetic acid (DCA) is a chlorination by-product formed during water disinfection and it is one of the most abundant haloacetic acids in water supplies. Several studies showed that DCA causes peroxisome proliferation and is a liver carcinogen

in mice. DCA is a metabolite of trichloroethylene (TCE), which is also a water contaminant and liver carcinogen in mice. DCA was reported to be an inducer of hepatic cytochrome P450 2E1 (CYP2E1) and a suicidal substrate inhibitor of glutathione-S-transferase zeta (GSTz). Because these enzymes are involved in TCE metabolism, we hypothesized that DCA may influence TCE metabolism via its effects on CYP2E1 and GSTz. In order to test this hypothesis, KO and WT mice were administered by gavage a single dose of DCA at 500 mg/kg or at 50 mg/kg/day for 5 consecutive days followed 24 hr later by the administration of 1,2-¹⁴C-TCE at 250 mg/kg by gavage. Mice were immediately placed in glass metabolism cages that allowed for the collection of expired air, urine, and feces. Twenty-four hours after TCE administration, animals were euthanized and blood and tissues were collected and frozen for analysis. Pretreatment of mice with DCA resulted in a significant inhibition of the exhalation of TCE-derived CO₂ and significantly increased the exhalation of organic volatiles (primarily parent TCE) in mice of both genotypes. Further, DCA pretreatment caused a significant decrease in the elimination of TCE-derived radioactivity in the urine of mice of both genotypes. These results confirmed that DCA pretreatment causes inhibition of TCE metabolism. Subsequent studies using real time PCR showed that DCA pretreatment, similar to those used in the current metabolism studies, decreased the expression of CYP2E1 in WT mice and significantly inhibited the activity of GSTz in mice of both genotypes. In conclusion, inhibition of TCE metabolism by DCA may be mediated by its inhibitory effects on GSTz and CYP2E1.

975 DISPOSITION OF 1-BUTYL-3-METHYLIMIDAZOLIUM CHLORIDE (BMIM-CL) FOLLOWING IV, ORAL OR DERMAL ADMINISTRATION IN MALE F-344 RATS.

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These studies characterized the effect of dose and route of administration on the disposition and metabolism of the ionic liquid, 1-butyl-3-methylimidazolium chloride (Bmim-Cl). [14C] Bmim-Cl was administered intravenously (IV), dermally or orally to male Fischer-344 rats. Following IV administration of [14C] Bmim-Cl (5 mg/kg), it decreased rapidly from blood in a biphasic manner. The key calculated kinetic parameters were: $t_{1/2\beta} = 960$ min; $AUC = 150 \mu\text{g} \cdot \text{min}/\text{mL}$; $CL_b = 5.8$ mL/min. The major route of elimination was urinary (84±10% of dose in 12 h). Urine was also the major route of elimination (60-70% in 12 h) following a single oral administration of Bmim-Cl (50, 5 or 0.5 mg/kg). Systemic bioavailability was >70% at 48 h. Bmim-Cl (50 mg/kg) was absorbed following dermal application in an methanol vehicle (25-35%), but resulted in apparent irritation at the site of administration. At a 10X lower dose (5 mg/kg) irritation was not observed. It exhibited poor to moderate absorption following application in an aqueous, ethanol or dimethylformamide vehicle (approx. 1, 3, or 13%). Regardless of route of exposure, analysis of urine by UV/Vis-radio HPLC showed a single radiolabeled peak that co-eluted with the Bmim-Cl standard, indicating that Bmim-Cl is excreted unchanged. While metabolism of Bmim-Cl following oral administration is negligible, and systemic bioavailability is high, that which is absorbed is extensively extracted by the kidney and eliminated in the urine as parent compound. (This research was supported by the NIEHS NTP Grant No. N01-ES-45529 and NIEHS-sponsored Southwest Environmental Health Science Center Grant Number P30-ES-06694.)

976 DISPOSITION OF N-BUTYLPYRIDINIUM CHLORIDE (NBUPY-CL) BY MALE F-344 RATS FOLLOWING IV OR ORAL ADMINISTRATION.

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Studies were conducted to characterize the effect of dose and route of administration on the disposition and metabolism of the ionic liquid, N-Butylpyridinium Chloride (NBuPy-Cl). [14C] NBuPy-Cl was administered intravenously (IV) or orally to male Fischer-344 rats. Following IV administration of [14C] NBuPy-Cl (5 mg/kg), the radiolabel found in the blood decreased rapidly and could be described by a biexponential equation, consistent with a two compartment model. The key calculated kinetic parameters were: $t_{1/2\beta} = 860$ min; $AUC = 179 \mu\text{g} \cdot \text{min}/\text{mL}$; $CL_b = 5.4$ mL/min. Urine was the major route of elimination of [14C] equivalents following both oral (60-70% in 12 h at 50, 5 or 0.5 mg/kg) and IV (80±13% of dose in 12 h) dosing with 5 mg/kg. Systemic bioavailability as calculated from AUC_{oral}/AUC_{IV} was 70% at 48 h. Preliminary results suggest NBuPy-Cl is poorly absorbed after dermal application in an aqueous vehicle (5 mg/kg). Regardless of route of exposure, analysis of urine by UV/Vis-radio HPLC showed a major radiolabeled peak that co-eluted with the NBuPy-Cl standard and a smaller peak representing less than 1% of the administered dose. While metabo-

lism of NBuPy-Cl following oral administration is negligible, and systemic bioavailability is high, that which is absorbed is extensively extracted by the kidney and eliminated in the urine as parent compound. This research was supported by the NIEHS NTP Grant No. N01-ES-45529 and NIEHS-sponsored Southwest Environmental Health Science Center Grant Number P30-ES-06694.



977 SCIENTIFIC AND ETHICAL CONSIDERATIONS IN HUMAN EXPOSURE STUDIES.

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This symposium will review some of the issues surrounding the scientific and ethical considerations related to intentional human exposure studies and purely observational studies. The relevance and importance of these studies will also be discussed. In early 2006, EPA published a Human Studies Rule that banned all third-party intentional dosing research on pesticides involving children and pregnant women intended for submission to EPA, and required that EPA neither conduct nor support any intentional dosing studies that involve pregnant women or children for all substances EPA regulates. The final rule also adopted stringent enforceable ethical safeguards to protect adult individuals who volunteer to participate in third-party intentional dosing research with pesticides, and called for establishment of a Human Studies Review Board (HSRB) to obtain independent scientific and ethical review on whether to accept certain existing human studies and to review new study protocols. The HSRB then met to review a series of studies on pesticides undergoing re-registration. In a separate effort, EPA has also developed a set of best practices for the design and implementation of observational exposure measurement studies that involve human participants, including children, but do not involve intentional exposures. These activities define a new landscape in which human studies performed or supported by EPA, or performed by third parties to generate data that will be used by them under the pesticide laws, will be reviewed, evaluated, and conducted. We will discuss these new EPA efforts and consider more broadly some of the issues related to defining the scientific value and justification of such studies, and some of the scientific and ethical challenges of conducting them with pesticides.

978 PROTECTION AGAINST IMMUNE-MEDIATED HEPATITIS BY THE SYNTHETIC TRITERPENOID, CDDO-IM.

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The synthetic triterpenoid, 1-[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDO-Im), is a potent chemopreventive agent protecting against aflatoxin-mediated hepatocarcinogenesis in rodents in part through Nrf2-mediated induction of a cytoprotective response. Co-exposure to aflatoxin together with chronic inflammation from hepatitis B virus infection can drive liver cancer risk in humans. CDDO-Im has also been shown to have anti-inflammatory activity. The present study examined the anti-inflammatory efficacy of CDDO-Im using Concanavalin A (ConA), a mouse model of T-cell mediated hepatitis. Mice treated daily with CDDO-Im (30 $\mu\text{mole}/\text{kg}$, p.o.) for three days prior to administration of a single dose of ConA exhibited dramatically decreased hepatotoxicity and inflammation 24-hr following ConA administration. H&E stained liver sections from CDDO-Im treated mice display decreased manifestations of cell death. CDDO-Im completely inhibited increases in serum liver enzyme levels and blunted ConA-induced biomarkers of oxidative stress. Additionally, the number of inflammatory cells in liver was decreased by CDDO-Im treatment. Similarly, CDDO-Im markedly inhibited hepatic myeloperoxidase activity representing decreased infiltration of neutrophils. These results suggest that CDDO-Im treatment reduces ConA-induced hepatotoxicity by decreasing inflammation. The serum concentration of pro-inflammatory cytokines were measured 3-hr following ConA administration. CDDO-Im treatment strongly decreased the secretion of IL-6, IL-12, IFN- γ , MCP-1 and TNF- α suggesting that CDDO-Im treatment resulted in decreased T-cell activation and a dampened inflammatory response. This study clearly demonstrates the anti-inflammatory potential of CDDO-Im against immune-mediated hepatitis thereby illustrating an additional chemopreventive role of CDDO-Im. Supported by CA94076, ES06082, CA78814, T32ES07141, National Foundation for Cancer Research and Reata Pharmaceuticals.

979 RESTRAINT STRESS CAUSES PHOSPHORYLATION OF STAT3 IN LIVER OF C57BL/6J MICE THROUGH ACTIVATION OF ADRENERGIC RECEPTORS.

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Psychological stress elicits two classical responses: 1) it activates the hypothalamic-pituitary-adrenal axis, resulting in the release of glucocorticoids, and 2) it activates the sympathetic nervous system, which results in the release of norepinephrine and epinephrine. Although these two systems often work in tandem, our studies indicate that activation of the JAK-STAT signaling pathways in livers of mice subjected to restraint stress is solely controlled through the adrenergic stress response. Using a combination of focused-microwave irradiation to preserve protein phosphorylation state, and phospho-specific immunoblotting, we observed a 10-fold increase in PSTAT3^{tyr705} in the livers of mice restrained for two hours. This effect could be blocked by both alpha and beta adrenergic antagonists, but not by adrenalectomy. Additionally, alpha and beta adrenergic agonists (phenylephrine 10 mg/kg or isoproterenol 10 mg/kg s.c., respectively) mimic the stress response in vivo and trigger phosphorylation of liver STAT3. This activation appears to be controlled through the beta-adrenergic receptor, since blocking this receptor precludes PSTAT3 induction upon administration of phenylephrine, an alpha-adrenergic agonist. Histological analysis of livers from saline and phenylephrine treated mice showed a translocation of STAT3 from cytoplasm to the nucleus, respectively, and a blockade of translocation by propranolol pretreatment. Altogether, these data suggest that restraint stress triggers phosphorylation of liver STAT3 through a solely adrenergic pathway, which initially requires beta-adrenergic receptor activation. These findings support the idea the acute psychological stress can elicit STAT3 activation in the liver, and may therefore have ramifications on liver diseases or in modulating hepatotoxicity.

"The findings and conclusions in this report (abstract/presentation) have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy."

980 EFFECT OF AHR NULL ALLELES ON IRON-ENHANCED PORPHYRIA IN MICE.

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A consequence of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in mice is a hepatic dysfunction of heme synthesis (porphyria) similar to human porphyria cutanea tarda. Susceptibility to this type of porphyria in mice is associated with polymorphism of the *Ahr* gene, although not exclusively, and with a threshold for CYP1A2 expression but not always with the degree of induction. The porphyria is potentiated by iron and the heme precursor aminolevulinic acid (ALA) both of which may also cause porphyria. It is unknown whether *Ahr* expression is essential to the probable oxidative mechanisms of all hepatic porphyria developments. Male C57BL/6J *Ahr* ^{+/+}, ^{+/-} and ^{-/-} mice received TCDD (75 µg/kg orally) ± s.c. dosing of iron (800 mg/kg). After 5 weeks liver toxicity as shown by histology and plasma ALT and AST enzymes, occurred in ^{+/+} mice and to a lesser extent in ^{+/-}. Hepatic toxicity in both strains was markedly enhanced by iron. Porphyria, as judged by uroporphyrin levels, occurred in ^{+/+} and ^{+/-} mice especially with iron. Only mild toxic effects of TCDD were observed in ^{-/-} mice even with iron, and there was no influence whatsoever on uroporphyrin levels whatever the iron status. Even 5 weeks after TCDD CYP1A2 levels, as measured by MROD activity and by immunoblotting, were still massively induced in ^{+/+} and to the same degree in ^{+/-} mice, but not in ^{-/-}. However, low basal expression of CYP1A2 (25-40% of wild type) was observed in *Ahr* ^{-/-} mice by RT-PCR and by immunoblotting. When iron-treated *Ahr* null mice were administered ALA in the drinking water (2 mg/ml) for 8 weeks without TCDD, uroporphyrin levels were markedly elevated although less than the wild type. In contrast, C57BL/6J *Cyp1a2* null mice are known to be totally resistant to the development of porphyria with this regimen. The results confirm that a minimum level of CYP1A2 may be required for porphyria development and demonstrate that although expression of *Ahr* appears essential for TCDD-induced porphyria it is not an absolute requirement for every porphyric response of the liver.

981 UROPORPHOMETHENE, THE INHIBITOR OF UROPORPHYRINOGEN DECARBOXYLASE CAUSING PORPHYRIA CUTANEA TARDA IDENTIFIED.

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Porphyria cutanea tarda (PCT), the most common form of porphyria in humans, engaged the toxicology community when it was linked to human ingestion of hexachlorobenzene in Turkey in the 1950s. Porphyrin accumulation in the skin ac-

counts for the photodermatitis. However, the disease is due to reduced activity of uroporphyrinogen decarboxylase (URO-D) in the liver, often in association with increased hepatic iron stores. Protein levels of URO-D do not change when catalytic activity is reduced, suggesting the possible presence of an inhibitor of URO-D. An inhibitor of URO-D has been identified in deproteinized liver cytosolic extracts from two murine models of PCT: wild type mice treated with polychlorinated biphenyls, together with iron and delta-aminolevulinic acid, and mice with one null allele of *Uro-d* and two null alleles of the hemochromatosis gene (*Uro-d*^{-/-}, *Hfe*^{-/-}) which develop PCT with no treatment. The inhibitor presence in the latter model indicates the inhibitor is chemically unrelated to PCBs. The inhibitor has now been characterized by solid phase extraction, chromatography, UV-vis spectroscopy and mass spectrometry and has proven to be uroporphomethene, a compound in which a single bridge carbon in the uroporphyrinogen macrocycle is oxidized, forcing the two adjacent pyrrole rings into a planar conformation. Synthetic uroporphomethenes were prepared by photo-oxidation of enzymatically-generated uroporphyrinogen I or III. Both uroporphomethenes inhibited recombinant human URO-D but the III isomer porphomethene was the more potent (2-3 fold) inhibitor. An inhibitor of rhURO-D has also been detected in cytosolic extracts of liver biopsy samples of patients with PCT. These studies support the hypothesis that the mechanism underlying clinical expression of the PCT phenotype appears to be the oxidation of uroporphyrinogen to uroporphomethene, which acts as a competitive inhibitor of uroporphyrinogen decarboxylation by URO-D. Support:NIH DK20503

982 HEPATIC TRANSPORTER mRNA CHANGES ASSOCIATED WITH TREATMENT REGIMENS LEADING TO UROPORPHYRIA IN A MOUSE MODEL OF PORPHYRIA CUTANEA TARDA.

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Porphyria cutanea tarda (PCT) is characterized by increased body burdens of uroporphyrin, originating from hepatic accumulations as a result of severely reduced uroporphyrinogen decarboxylase (URO-D) activity. Active transport mechanisms have been implicated in the movement of porphyrins across cellular membranes and it was of interest to examine whether changes in the regulation of transporter transcription was occurring in the livers of animal models of the disease. We have investigated transporter mRNA changes following treatment of female mice possessing one null allele of *Uro-d* with a single dose of polychlorinated biphenyls (PCBs). The mice showed mean hepatic porphyrin levels of 6 nmol/g liver after 1 week and plateau levels of ~750 nmol/g at 2 and 3 weeks. When the mice also received delta-aminolevulinic acid (ALA) in their drinking water, the 1-week level was increased to 373 nmol/g and the 3-week level was increased to 1062 nmol/g if they also received an iron-dextran injection. *Abcc3* mRNA levels correlated with porphyrin levels, with values of 134% (1 week) and 240% (2-3 weeks) of control for the PCB-alone treatment, and 191 and 276% respectively for the two further supplemented regimens. *Slco1b2* showed an inverse correlation with porphyrin levels, reaching 67% depression with the treatment regimen that gave porphyrins of 1062 nmol/g. The only significant changes in *Abcb1a*, *Abcb1b* and *Abcg2* mRNAs were the 301, 490 and 146% elevations, respectively, seen with the 3-week PCB/ALA/iron-dextran treatment regimen giving mean porphyrin levels of 1062 nmol/g. No significant changes were observed in *Abcc2* from any treatment regimen. *Abcb11* was significantly depressed with the 2-week PCB (37%) and 1-week PCB/ALA (60%) regimens, and therefore in a manner unrelated to porphyrin levels. Thus transcript levels of numerous transporters appear responsive not only to PCBs, but stimuli which in many cases arise as the disease-state develops. Support:NIH DK20503

983 QUANTIFICATION OF PROLIFERATING LIVER SINUSOIDAL ENDOTHELIUM USING LASER-SCANNING CYTOMETRY.

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The liver has a major role in storage and metabolism of nutrients, and it is the main organ for drug biotransformation. Liver sinusoidal endothelial cells constitute the lining of the hepatic sinusoid and perform a wide range of important functions: exchange of blood components, endocytosis of xenobiotics, secretion of inflammatory mediators, and antigen presentation. Proliferation of sinusoidal endothelium in the liver has been related to drug toxicity, hemangiosarcoma formation and liver regeneration after acute liver injury.

Laser scanning-cytometry (LSC) analyzes fluorescently-labeled tissue sections in a fashion similar to flow cytometry. LSC collects tissue data by sequential scanning of microscopic fields, while preserving sample integrity and recording spatial localization of data within the tissue.

Here we describe a method to quantify proliferation of liver sinusoidal endothelium in whole sections of mouse liver using fluorescent immunolabeling and LSC analysis. Using VEGF-R2 immunofluorescence, we were able to specifically identify the sinusoidal endothelium in sections of mouse liver. In addition, using dual fluorescent immunolabeling, we labeled endothelium (VEGF-R2) and proliferating cells (Ki-67) concurrently. Using LSC analysis, we quantified co-localization of endothelial and Ki-67 staining across the entire tissue section, successfully obtaining data regarding total tissue area, endothelial sinusoidal area, and proliferating sinusoidal endothelial area.

This method can be used to quantify variations in endothelial sinusoidal area and sinusoidal proliferation due to drug toxicity and/or liver disease.

984 FXR NOT REQUIRED FOR INFLAMMATION DURING CHOLESTASIS.

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Cholestasis, a condition that occurs when hepatic bile acid excretion is interrupted, causes liver injury in humans and animals. Recent studies have shown that neutrophils accumulate in the liver and exacerbate injury during cholestasis. What remains unclear is the mechanism by which cholestasis stimulates production of proinflammatory mediators that promote neutrophils to accumulate in the liver and produce injury. Recent studies suggested that farnesoid X receptor (FXR), a bile acid nuclear receptor, regulates expression of proinflammatory mediators in bile acid-treated hepatocytes in vitro, suggesting that FXR is critical for neutrophil accumulation in liver during cholestasis. To test this hypothesis in vivo, wild-type and FXR knockout mice were subjected to bile duct ligation (BDL), a commonly used model of cholestasis. Three days later, levels of intercellular adhesion molecule-1 (ICAM-1) and macrophage inflammatory protein-2 (MIP-2) mRNA in liver, hepatic neutrophil accumulation, and liver injury were measured. ICAM-1 mRNA levels were increased to a similar extent in wild-type and FXR knockout mice subjected to BDL. MIP-2 mRNA was increased in liver after BDL, and was increased to a greater extent in FXR knockouts. Hepatic neutrophil numbers were increased in both wild-type and FXR knockout mice after BDL. Next, liver injury was evaluated by measuring serum levels of alanine aminotransferase (ALT), an indicator of hepatocyte injury. ALT levels were significantly elevated in wild-type mice subjected to BDL, and were increased further in FXR knockout mice after BDL. Results from these studies suggest that FXR is not critical for production of proinflammatory mediators and accumulation of neutrophils in liver during cholestasis.

985 DOSE-RESPONSE OF CHENODEOXYCHOLIC ACID AND LITHOCHOLIC ACID ON MOUSE LIVER PATHOLOGY.

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Bile acids not only play important physiological roles in facilitating the absorption of dietary fat and the elimination of cholesterol from the body, but also are important signaling molecules. High doses of bile acids, however, cause hepatotoxicity. Little information is available on the doses of endogenous bile acids that produce hepatotoxicity. Therefore, the purpose of this study was to determine the toxic dose and histopathological effects of chenodeoxycholic acid (CDCA) and lithocholic acid (LCA). Mice were fed CDCA or LCA at concentrations of 0.1, 0.5, 1.0, and 1.5% (W/W) in the diet for one week. Livers were collected for immunohistochemistry of Kupffer cells, neutrophils, and apoptotic cells, as well as for H&E staining. mRNA levels were quantified using the branched DNA assay. Concentrations of 1.0 and 1.5% in the diet of both CDCA and LCA caused lethality. Extensive hepatic necrosis was produced by 0.5% LCA. An increase in apoptosis was observed after 0.5% CDCA, as well as 0.1 and 0.5% LCA. CDCA at 0.5% and LCA at 0.1 and 0.5% caused hepatic inflammation, as indicated by neutrophil infiltration. The number of Kupffer cells was increased by 0.5% CDCA, whereas, they were decreased by 0.5% LCA. The expression of TNF α was increased by 0.1% LCA. Hepatic stellate cell (HSC) activation was evaluated by quantifying the mRNA of α -smooth muscle actin (α SMA) and collagen-1. CDCA did not alter the mRNA levels of either, whereas 0.5% LCA increased mRNA levels of both α SMA and collagen-1 in livers. In summary, CDCA at 0.5% in the diet results in neutrophil infiltration, apoptosis, and activation of Kupffer cells. LCA at 0.1% similarly causes neutrophil infiltration, apoptosis, and activation of Kupffer cells, whereas at 0.5% causes the same effects plus activation of stellate cells. In conclusion, CDCA and LCA appear to produce a similar pattern of hepatocellular injury, but LCA is more potent. (Supported by NIEH grants ES-09649 and ES-09716).

986 REGULATION OF GLUTATHIONE S-TRANSFERASES (GST) IN MOUSE LIVER BY CHEMICAL ACTIVATORS OF RECEPTOR-MEDIATED PATHWAYS.

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Induction of various phase-I and -II drug-metabolizing enzymes is regarded as a mechanism of protection against chemical stress. Gst enzymes are clinically relevant enzymes that conjugate electrophilic xenobiotics and by-products of oxidative stress with glutathione. High Gst levels have been correlated with lower incidences of cancer in rodents and humans. Classical microsomal enzyme inducers increase the expression of some biotransformation enzymes (phase-I and -II) and transporters through transcription factors, such as the aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), pregnane X receptor (PXR), peroxisome proliferator-activated receptor α (PPAR α), and NF-E2 related factor 2 (Nrf2). Because the effects of activators of these 5 transcription factors on the induction of hepatic Gst enzymes in mice have not been comprehensively characterized, the purpose of this study was to fill that knowledge gap. Adult male C57BL/6 mice were treated with different activators of the transcription factors AhR, CAR, PXR, PPAR α , and Nrf2. In general, most Gst isoforms are readily induced. All five transcription pathways appear to play a role in Gst induction, and all Gst enzymes are induced by some of these transcription pathways, except Gst π 1/2. The Nrf2 activators induced the most Gst isoforms, whereas AhR ligands induced the least. Additionally, clofibrate, a PPAR α agonist, was the single compound that induced the most Gst isoforms; however, all three PPAR α agonists decreased Gst π 1/2 mRNA. Of all members of the Gst mu class, Gst μ 3 mRNA expression had the largest increase by all three CAR ligands, PXR ligands, and Nrf2 activators (6- to 13-fold). In conclusion, Gst enzymes are readily inducible, however, not all Gst(s) are induced by all transcription activators. Thus the protection afforded by a ligand for one of these transcription factors will depend on the activator, as well as which Gst that detoxifies the chemical of interest. (Supported by NIH grants ES-09716, ES-013714 and ES-08156)

987 REGULATION OF HEPATIC BILE ACID TRANSPORTERS NTCP AND BSEP mRNA EXPRESSION IN MICE.

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REGULATION OF HEPATIC BILE ACID TRANSPORTERS Ntcp AND Bsep mRNA EXPRESSION IN MICE. XG Cheng, J Maher, D Buckley, and CD Klaassen. University of Kansas Medical Center, Kansas City, KS 66160.

Na⁺-taurocholate cotransporting polypeptide (Ntcp) and the bile salt export pump (Bsep) are key transporters for hepatic bile acid uptake and excretion. Alterations in Ntcp and Bsep expression have been reported in pathophysiological conditions such as cholestasis, but little is known regarding their regulation under other conditions. Thus in the present study, the effects of age, gender, and prototypical microsomal enzyme inducers on the regulation of these two transporters were determined in mouse livers. Both Ntcp and Bsep are exclusively expressed in liver. Ntcp and Bsep mRNA in mice were low in the fetus, but increased 10 fold to their highest levels at parturition. Mouse Ntcp and Bsep mRNA decreased by more than 50% by day 5 after birth, and then gradually increased to adult levels by day 30. Female-predominant Ntcp mRNA expression in mouse liver was observed in adulthood. In contrast, no gender difference exists in Bsep mRNA expression in mouse liver. To examine whether sex hormones or growth hormone secretion patterns are responsible for the observed Ntcp gender difference, hormone replacements were conducted in gonadectomized, hypophysectomized, and growth-hormone-releasing-hormone receptor mutant (lit/lit) mice. The data indicate that female-predominant Ntcp expression in mouse liver is due to the inhibitory effect of male-pattern GH secretion, but not the effects of sex hormones. A few microsomal enzyme inducers altered Ntcp or Bsep mRNA expression in mice. Specifically, dexamethasone and oltipraz induced Ntcp mRNA expression. Spirolactone induced Bsep mRNA expression. However, Ntcp and Bsep expression are resistant to induction by most of microsomal enzyme inducers. In conclusion, mouse Ntcp and Bsep are extensively regulated by tissue, age, gender, but only a few microsomal enzyme inducers. (Supported by NIH grants ES-09649, ES-09716, and ES-07079)

988 CONSTITUTIVE mRNA EXPRESSION OF VARIOUS GLUTATHIONE S-TRANSFERASE ISOFORMS IN DIFFERENT TISSUES OF MICE.

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Glutathione S-transferase (Gst) enzymes are instrumental in protecting cellular macromolecules against electrophiles and products of oxidative stress. The ability of these enzymes to detoxify xenobiotics is of special interest to toxicologists. Constitutive expression of Gst(s) might determine a tissue's ability to respond to certain forms of chemical stress. Therefore, from 13 tissues of adult male and female mice, the mRNA expression of 19 Gst enzymes were quantified by the branched DNA method. In all tissues examined, multiple isoforms of Gst were constitutively expressed, but some isoforms were expressed at higher levels than others. The tissue expression of different isoforms had no relationship to their class designation; members of the same class were often highly expressed in different tissues. Constitutive expression of many Gst isoforms showed gender difference in a few but not all tissues; in most such cases, females had higher expression than males. The majority of cytosolic Gst enzymes in mice were highly expressed in stomach, liver, and kidney. Several other isoforms, such as Gstk1, Gstm1, Gstm4, Gstm6, and Gstt1, were expressed in other parts of the gastrointestinal (GI) tract as well. Gstm1, Gstm6, and Gstt3 were highly expressed in testis. Interestingly, Gstm5 was the Gst isoform that had highest expression in brain, relative to other tissues. The microsomal Gst(s) had the highest constitutive expression in liver and stomach. Specifically, microsomal Gst1 (MGst1) had the highest expression in liver, whereas microsomal Gst2 and 3 (MGst2 and MGst3) had the highest expression in stomach, followed by various sections of the intestine. In summary, most Gst isoforms are highly expressed in the GI tract, liver, and kidney, which strongly suggests an important role of many Gst isoforms in detoxifying ingested xenobiotics. (Supported by NIH grants ES-09716, ES-013714 and ES-08156)

989 NRF2 PROMOTES LIVER INJURY IN CHOLESTATIC MICE.

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Recent studies have shown that nuclear factor E2 p45-related factor 2 (Nrf2) protects the liver from xenobiotic toxicity. Whether it protects the liver from cholestatic injury is not known. Accordingly, the hypothesis was tested that Nrf2 protects the liver from injury during cholestasis. To test this, wild-type and Nrf2-null mice were subjected to bile duct ligation (BDL), an animal model of cholestasis. The results show that liver injury and fibrosis were reduced in Nrf2-null mice suggesting that Nrf2 promotes cholestatic liver disease. Because inflammation is required for liver injury during cholestasis, it was next determined whether liver inflammation was reduced in Nrf2-null mice. The results show that upregulation of several proinflammatory mediators, as well as neutrophil accumulation and activation were reduced in Nrf2-null mice after BDL. Serum and liver bile acid concentrations were reduced in Nrf2-null mice after BDL when compared to wild-type mice, suggesting that Nrf2 promotes bile acid accumulation in liver and serum during cholestasis. Because transporters affect hepatic bile acid accumulation, bile acid transporters were quantified in liver. Multidrug resistance associated protein (Mrp) 3 and Mrp4 mRNA were increased in liver of wild-type but not in Nrf2-null mice after BDL. Organic solute transporter (Ost)β mRNA levels were increased in wild-type mice after BDL and were increased even further in Nrf2-null mice. These results indicate that Nrf2 negatively regulates Ostβ during cholestasis causing bile acid accumulation, which promotes liver inflammation, injury, and fibrosis.

990 ROLE OF ESTRADIOL IN SECRETED PHOSPHOPROTEIN 1 (OSTEOPONTIN) INDUCTION IN A FEMALE RAT MODEL OF ALCOHOLIC STEATOHEPATITIS.

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Recent studies from our laboratory suggest that higher neutrophil infiltration in females significantly correlate with increased hepatobiliary expression of secreted phosphoprotein 1 [SPP1, commonly referred to as osteopontin (OPN)] in the rat Lieber DeCarli model of alcoholic steatohepatitis (ASH). Confirmation experiments with neutralizing antibody against SPP1 revealed the contributing role of SPP1 in higher hepatic neutrophil infiltration and liver injury. The objective of this study was to first examine the effect of ethanol (EtOH) on estrous cycle and then investigate the potential relationship between estradiol (E2) and hepatobiliary OPN mRNA and protein expression in the female ASH model. When normally cycling adult female Sprague Dawley rats were administered EtOH for a period of six weeks, there was prolongation of diestrus stage of the estrous cycle. Ovariectomized (OVX) and E2-implanted OVX rats (E2; 0.18, 0.36mg, 0.72mg and 1.7mg) in the ASH group (EtOH+LPS), evaluated for OPN mRNA and protein expression by in situ hybridization, RT-PCR and Western Blotting revealed a biphasic pattern. Low

doses (0.18 and 0.36mg) of E2 resulted in significant down-regulation of OPN protein and mRNA as compared to the OVX group. However, with the increasing doses of E2 (0.72 and 1.7mg) there was up-regulation of both OPN mRNA and protein. OPN mRNA and protein was localized mostly to the biliary epithelium and occasionally within mononuclear cells and hepatocytes. Liver injury assessed by serum ALT, AST and histopathology also revealed a biphasic pattern similar to OPN expression. In all the groups, hepatic neutrophilic infiltration correlated positively with OPN protein and mRNA expression. Based on these data, we can conclude that in our ASH model, low doses of E2 are hepatoprotective while the protective effect appears to be negated with increasing doses of E2, both of which are SPP1-mediated.

991 NON-ALCOHOLIC FATTY LIVER DISEASE ALTERS EXPRESSION AND ACTIVITY OF DRUG METABOLIZING ENZYMES.

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Non-alcoholic fatty liver disorders (NAFLD) comprise a spectrum of pathologies known to progress from simple fatty liver (SFL) to the severe disease of non-alcoholic steatohepatitis (NASH). Importantly, a number of medical conditions (e.g. type 2 diabetes, dyslipidemia, heart disease, hypertension) are commonly associated with NAFLD. Therefore, a significant portion of NAFLD patients currently takes prescription medications. Of the 2 million adverse drug reactions (ADRs) that occur annually in the US, < 20% are attributable to genetics. Thus a considerable proportion of ADRs are thought to be the result of environmental factors like disease state and nutritional status. Phase I and II drug metabolizing enzymes (DMEs) are essential for the conversion of drugs into hydrophilic, more readily-excretable metabolites. The purpose of this study was to determine the effect of NAFLD on the expression and activity of a number of the major DMEs in rat liver, including cytochrome P450s and UDP-glucuronosyl transferases (UGTs). Male Sprague-Dawley rats were fed a high-fat (SFL model) or a methionine-choline-deficient diet (NASH model) for 8 weeks. H&E staining revealed steatosis in both models, while fibrosis was found only in NASH livers. In SFL, there was a 38% decrease in Cyp2E1 protein expression. In NASH livers, branched DNA analysis of hepatic RNA revealed increased Cyp2E1 (1.4-fold) and Cyp4A2/3 (2.9-fold) mRNA levels, while Cyp3A1/23 and Cyp2C11 mRNA levels decreased by 55% and 74%, respectively. Western blot analysis of NASH livers revealed increased protein expression of Cyp2C11 (~11-fold) and Cyp4A1 (2.7-fold), while Cyp3A1 expression was decreased by 75%. UGT activity increased 1.4-fold in NASH livers. These data reveal significant changes in the expression and activity of several DMEs during NASH. Such alterations are likely to influence the metabolism, efficacy and safety of various clinically relevant drugs in NASH patients. (DK-068039, ES-07091, ES-011646)

992 HEPATIC DRUG METABOLISM ENZYME AND TRANSPORTER EXPRESSION IN HUMANS WITH NON-ALCOHOLIC FATTY LIVER DISEASE.

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Severe adverse drug reactions among patients in the USA number as high as two million per year. Less than 20% of these cases are due to genetic polymorphisms, whereas the majority of adverse drug reactions are due to environmental factors such as age, nutrition, or disease state. Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of diagnoses ranging from simple fatty liver (SFL) to non-alcoholic steatohepatitis (NASH). NASH can progress to cirrhosis which is irreversible and requires liver transplant. The purpose of the current study was to determine whether human livers diagnosed with mild to severe NAFLD show altered expression of hepatic drug metabolizing enzymes and transporters. Human liver samples diagnosed with normal liver, SFL, NASH with fatty liver (early stage) or NASH without fatty liver (end stage, with cirrhosis) were provided by the Liver Tissue Procurement and Distribution System. Total RNA was isolated from all liver samples and mRNA levels of hepatic Phase I drug metabolizing enzymes and transporters were quantified using branched DNA analysis. While no significant changes were observed in SFL samples, the expression of several hepatic transporters and cytochrome P450s were significantly altered in human NASH livers. In human livers diagnosed with early stage NASH, BCRP, MRP1, MRP4 and MRP5 hepatic transporter mRNA levels were significantly increased relative to normal human liver samples. Similarly, livers diagnosed with end stage NASH displayed significant increases in MRP1, MRP4 and MRP5 expression. CYP2E1 mRNA levels were significantly decreased in human livers diagnosed with early stage and end stage NASH, respectively, while CYP4A mRNA levels were increased only in early

stage NASH livers. These data suggest that the expression of hepatic transporters and drug metabolizing enzymes is altered during the more severe stages of NAFLD, and may result in impairment of hepatic metabolism and disposition of clinically relevant drugs administered to these patients. (DK068039, ES07091, ES011646)

993 FMO MEDIATED METABOLISM AND THE PHARMACOKINETICS OF IN-1130 IS A NOVEL ALK5 INHIBITOR, AN ANTIFIBROTIC AGENT.

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The transforming growth factor- β (TGF- β) plays a central role in the progression of renal fibrosis. TGF- β transduces its signal through the ALK5 serine/threonine kinase receptor. IN-1130 (2-pyridinyl-[1,2,3]triazoles) is a novel small molecule ALK5 kinase inhibitor. We investigated the metabolism, toxicity and pharmacokinetics of IN-1130 in rodents, dog and monkey.

In vitro enzyme assay with human, monkey, dog, rat and mouse microsomes did not show any metabolite from IN-1130 based on the HPLC, and LC-MS analysis (detection limit = 10 nM). Ex vivo enzyme assay with the isolated perfused rat liver system, no metabolite was detected based on the HPLC, and LC-MS analysis (detection limit = 10 nM). These results from the both in vitro and ex vivo studies, indicated that besides CYP system, other enzyme system might be involved in the metabolism of IN-1130. We have carried out the FMO experiment with microsomes of human, rat and mouse livers, and rat kidney, and with human FMO1 cDNA expressed supesosome. The results from the experiments showed that IN-1130 was metabolized by FMO. At the moment, we are not certain which isozyme is responsible for the IN-1130 metabolism, although we could exclude FMO1. Most likely FMO3 and/or FMO5 would be involved in the metabolism of IN-1130. IN-1130 was assayed for mutation in two histidine-requiring strains of *Salmonella typhimurium* (TA1535, TA1538) and IN-1130 did not induce mutation in two histidine-requiring strains of *Salmonella typhimurium* (TA1535, TA1538) when tested under the conditions of this study. The approximate minimum lethal intravenous dose levels of IN-1130 in the male and female rats were found to be 250 mg/kg and 300 mg/kg, respectively. The approximate minimum lethal intravenous dose level of SB-505124 in the male and female rat was found to be between 15 and 30 mg/kg. The approximate minimum lethal oral dose level of IN-1130 in both male and female rat was found to be 2000 mg/kg.

994 IN VITRO EXPOSURE OF BILIARY EPITHELIAL CELLS (BEC) TO BILE FROM RATS TREATED WITH CDNB (1-CHLORO, 2,4-DINITROBENZENE) INDUCES DNP ADDUCTS ON PROTEINS.

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Background. Idiosyncratic drug-induced biliary disease is believed to occur because reactive drugs/ metabolites covalently bind (adduct) endogenous proteins to form abnormal proteins that are recognized as "foreign" by the immune system.

Objectives. Characterize BEC proteins adducted by biliary metabolites of the model compound CDNB using an in vitro system where BEC are exposed to bile collected from CDNB-treated rats. **Methods.** Male SD rats were given 250 mg CDNB/kg po (corn oil); bile was collected for 3 h. BEC were exposed to CDNB-Bile for 5 h at 37°C, washed, fragmented by freeze-thaw + sonication, and separated into cell fractions. Proteins were separated on parallel 2D gels; adducts were identified by Western blot on one gel and protein spots were excised on the other gel for digestion and analysis by MALDI-TOF spectrometry for protein identification.

Results. Adducts were observed by immunohistochemistry in BEC in the livers of rats exposed to CDNB, and by immunocytochemistry and confocal microscopy in BEC exposed to CDNB-Bile in vitro. Nine protein spots that showed adduction by Western blot were identified by peptide mass fingerprints (MS + MS/MS) using NCBI and Swiss-Prot/TrEMBL databases: [Cytokeratin 8] [type II keratin, gene 7] [selenium-binding protein] [ezrin (microvillar protein)] [carbonic anhydrase] [glutathione S-transferase, pi] [dihydrolypoamide acetyltransferase] [prohibitin (mitochondrial chaperone protein)] [MAWDBP (binding protein to MAWD)]. The first 7 proteins are known human or rodent BEC proteins. Moreover, dihydrolypoamide acetyltransferase is the E2 subunit of the pyruvate dehydrogenase complex in mitochondria and the most prominent autoantigen in primary biliary cirrhosis.

Conclusion. An in vitro BEC/ bile-exposure system is an applicable approach to investigate mechanisms of drug-induced bile duct disease. (Supported by John Sealy Memorial Endowment Fund for Biomedical Research, UTMB)

995 EVALUATION OF CHANGES IN MITOCHONDRIAL FUNCTION IN RAT HEPATOCYTES USING FLOW CYTOMETRY AND TWO-PHOTON MICROSCOPY.

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Xenobiotic-induced changes in mitochondrial function are recurring causes of toxicity in organs such as the liver. There is a significant need to identify in vitro and in vivo imaging technologies that assess mitochondrial morphology and function. In vitro experiments using isolated rat hepatocytes were conducted to identify a set of responsive fluorescent probes that would be used for in vivo two-photon imaging. For in vitro experiments, rat hepatocytes were isolated by enzymatic perfusion; plated in tissue culture plates or seeded in suspension cultures; treated with the mitochondrial toxicants CCCP, DNP, oligomycin, or valinomycin; incubated with fluorescent probes; and analyzed by flow cytometry. We observed compound-induced mitochondrial membrane depolarization that preceded loss of cell viability in cultures of isolated rat hepatocytes. For in vivo experiments, anesthetized rats were prepared surgically to expose the liver and to perform blood vessel cannulation. Fluorescent probes were administered intravenously as a bolus and images were acquired over a period of 1 hour using a Zeiss LSM-510 Meta Confocal / Multiphoton Microscope system. Two-photon in vivo imaging of the liver was possible a few minutes after probe delivery and mitochondrial membrane potential depolarization was detected immediately after subsequent administration of valinomycin. In summary, a method for in vivo imaging of mitochondrial functional changes in the rat liver using two-photon microscopy was developed. Evaluating mitochondrial function and morphology in the physiological context of the living animal is a benefit when assessing compound-induced toxicity and supplements traditional labor intensive ex-vivo evaluation techniques.

996 HEPATOCYTE GROWTH FACTOR PROTECTS AGAINST OXIDATIVE STRESS INDUCED BY ETHANOL METABOLISM.

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Ethanol (EtOH) metabolism-induced cellular damage is a process which involves both generation of reactive oxygen species (ROS) and a decrease in the antioxidant system generating oxidative stress. EtOH is metabolized in chronic intake by cytochrome P450 2E1 which produces ROS and acetaldehyde. Oxidative stress can damage lipids, DNA and proteins which lead an impaired cellular function and eventually cell death. Hepatocyte growth factor (HGF) is a potent mitogenic factor which exerts a protective role inducing activation of NF- κ B survival pathway. Although HGF-induced apoptosis protection is well characterized there is little evidence in protection against EtOH metabolism-induced cellular damage.

HepG2 cells transfected with cytochrome P450 2E1 gene (VL-17A) were seeded and pretreated or not with 50 ng/ml HGF for 12 hr and then treated or not with 100 mM EtOH for 24 and 48 hr. Catalase and superoxide dismutase enzymes were assayed by Western Blot. Protein oxidation was determined using OxyBlotTM kit, ROS production was quantified by FACS flow using DCF-DA and lipid peroxidation was determined by malondialdehyde production. ATP was measured by ApoGlowTM bioassay kit.

EtOH treatment stimulated ROS production in VL-17A cells and induced cellular damage judged by protein oxidation, lipid peroxidation and peroxides production determined by FACS flow. HGF prevented EtOH damage. In order to explore the mechanism of HGF protection we determined the expression of some antioxidants enzymes. We observed that HGF increased SOD1 and catalase expression which is correlated with a decrease in protein oxidation. ATP content was increased as a result of HGF pretreatment.

These data present clear evidence that HGF can modulate redox status in cells subjected to oxidative stress suggesting that HGF-induced protection is mediated by an increment in the antioxidant system.

CONACYT: 45921.

997 ALPHA GST IS A SENSITIVE, TRANSPORTABLE, HEPATOTOXICITY BIOMARKER IN HUMAN HEPATOCYTE CULTURE.

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The expectations for new toxicity biomarkers are high. They must be predictive, specific, and early indicators of toxicity. Ideally, they should also be transportable – similar measurements and data interpretations for the same biomarker whether it is

measured in the clinic, in preclinical animal species, or during *in vitro* experiments. There is strong evidence that alpha GST (glutathione S-transferase) leakage into the blood is a sensitive and specific hepatotoxicity biomarker in the clinic and in animal studies. However, little experimental data has been published measuring this biomarker *in vitro* in cell culture media and no data has been published using human hepatocytes. We have examined the use of an alpha GST EIA method to measure the release of alpha GST in human hepatocyte cell culture. We compared alpha GST leakage to the release of LDH (lactate dehydrogenase) under the same experimental conditions. A panel of drugs (including many known hepatotoxins; i.e. tamoxifen) was tested. We found that alpha GST leakage could be detected earlier and was more a more sensitive assay (significant leakage could be detected at 5x lower concentrations) compared to LDH leakage in both cryopreserved and fresh human hepatocyte cultures. We conclude that the measurement of alpha GST leakage in hepatocyte cell culture supernatants is a useful and more sensitive assay for hepatotoxicity assessment *in vitro* than LDH leakage. These data, combined with published data showing GST's utility for measuring hepatotoxicity *in vivo*, indicate that alpha GST leakage is a useful, transportable, hepatotoxicity biomarker.

998 PATHWAYS FOR CHELATABLE IRON MOVEMENT INTO MITOCHONDRIA FROM LYSOSOMES AFTER BAFILOMYCIN INHIBITION OF THE VACUOLAR ATPASE.

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Background: Lysosomes sequester iron, and iron augments alcoholic and other liver diseases in which oxidative stress plays a role. Iron catalyzes reactive oxygen species (ROS) formation and induces the mitochondrial permeability transition. Bafilomycin (Baf), a vacuolar proton pump inhibitor, enhances oxidative injury to hepatocytes (Uchiyama et al., *Hepatology* 42, 636A). Our Aim was to investigate pathways of BAF-induced lysosomal iron release and uptake into mitochondria. Methods: Hepatocytes were isolated from fasted male C3Heb mice and Sprague-Dawley rats. Calcein whose fluorescence is quenched by Fe²⁺ was loaded into the cytosol by warm ester loading (1 μ M for 20 min) or into mitochondria by cold ester loading (1 μ M for 60 min at 4°C) and warm incubation. Some hepatocytes were co-loaded with 70 kDa rhodamine-dextran (50 mg/kg i.p. prior to isolation) to visualize lysosomes and/or tetramethylrhodamine methyl ester (100 nM) to determine mitochondrial polarization. Results: When hepatocytes were treated with 50 nM Baf, cytosolic calcein fluorescence declined by $47.8 \pm 0.7\%$ after 120 min. Desferal and starch-desferal (2 mM) suppressed BAF-induced cytosolic calcein quenching nearly completely, indicating that lysosomes were releasing chelatable iron into the cytosol. Baf also induced quenching of mitochondrial calcein that was blocked by desferal, starch-desferal and Ru360 (20 μ M), the last an inhibitor of mitochondrial Ca²⁺ uptake. After Baf, lysosomes did not release 70 kDa rhodamine-dextran, and mitochondria remained polarized. Conclusions: Lysosomal retention of iron depends on lysosomal Δ pH. Collapse of lysosomal Δ pH with Baf releases chelatable iron into the cytosol, which is taken up into mitochondria via the Ca²⁺ uniporter. Mitochondrial iron may then contribute to ROS formation after oxidative stress.

999 IDENTIFICATION OF LIPID MODIFICATIONS OCCURRING IN 1C1C7 HEPATOMA CULTURES FOLLOWING 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN EXPOSURE.

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Recent studies suggest that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure can profoundly influence both cholesterol and triglyceride content in murine liver and hepatoma cell lines. However, the effects of TCDD on specific classes of lipids, particularly those constituting membranes, are not known. Treatment of the murine hepatoma cell line 1c1c7 with 5 nM TCDD for 72 hr resulted in a modest cytostatic effect, characterized by increased and decreased percentages of cells in G1 and S, respectively. Analysis of cholesterol using HPLC in tandem with atmospheric pressure chemical ionization mass spectrometry (LC-APCI-MS) demonstrated that TCDD increased the abundance of hydroxy-cholesterols and total cholesterol. Fluorescence microscopy of filipin stained cells indicated that TCDD also increased markedly non-esterified cholesterol content. Electrospray ionization-mass spectrometry (ESI-MS) analyses demonstrated that 1c1c7 cells express at least 5 different types of sphingomyelin species, none of which were significantly altered by TCDD. ESI-MS analyses of phosphatidylcholines (PtdCho) identified 17 species, and demonstrated that 16:0-20:4, 18:1-20:4 and 18:0-20:4 PtdCho were all signif-

icantly reduced (25-55%) in TCDD-treated cultures. This pattern of reduction in specific PtdCho species is similar to what occurs in several cell lines following pharmacological inhibition, or siRNA-mediated reduction, of cytosolic calcium-independent phospholipase A₂ beta (iPLA₂β), and has been associated with reduced proliferation. These data demonstrate for the first time that TCDD exposure affects the cellular composition of specific phospholipids, which may influence cell cycle progression/duration. This work was partially supported by RO1 ES009392 and the Georgia Cancer Coalition.

1000 INHIBITION OF PHOSPHOLIPASE C BY A CATIONIC AMPHIPHILIC ANTI-INFLAMMATORY COMPOUND AS A MECHANISM OF PHOSPHOLIPIDOSIS.

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Many marketed drugs induce phospholipidosis in preclinical animal toxicity studies and in many cases the compounds belong to a family of molecules referred to as cationic amphiphilic drugs (CAD). This family includes agents like fluoxetine, amiodarone and perhexiline. Possible mechanisms of drug-induced phospholipidosis include modification of lipid handling either by a) modulating the lipid structure (by covalent binding or altered physiochemical environment) or b) competitive or allosteric inhibition of the phospholipase enzymes involved in lipid catabolic processing.

We investigated one of the AZ molecules from a series of anti-inflammatory agents that possesses CAD-like properties of which some have been shown to induce phospholipidosis *in vivo*. We have demonstrated that the myelin bodies, diagnostic by EM of phospholipidosis *in vivo*, can be successfully modelled *in vitro* in rat hepatocytes. We have studied the effects of the AZ compound plus chlorpromazine and perhexiline on the activity of phospholipase C using a modification of the Invitrogen™ Phosphatidylcholine-Specific Phospholipase C Assay Kit. Compounds were tested over a range of concentrations between 10 and 500 μ M and assessed for inhibition of phospholipase C. The AZ compound, fluoxetine and perhexiline produced concentration-dependant inhibition of phospholipase C activity with IC₅₀ values of 91.4 μ M, 49.2 μ M and 51.1 μ M respectively. These concentrations are comparable with those that can produce phospholipidosis in rat hepatocytes *in vitro* suggesting that inhibition of phospholipase C plays a role in phospholipidosis induced by this series of anti-inflammatory agents.

1001 ISONIAZID INDUCES APOPTOSIS AND INHIBITS MMP SECRETION IN HUMAN HEPATOMA CELL LINE SK-HEP-1.

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Introduction:

The incidence of tuberculosis (TB), a chronic recurrent infection, caused by *Mycobacterium tuberculosis*, has increased alarmingly among persons infected with HIV. Case rates vary by country, age, race, sex and socioeconomic status. Isoniazid (INH), one of the most widely used drugs for the treatment of TB, has proven to be very effective with relatively low toxicity. However, therapeutic doses of INH lead to liver injury in some patients.

Objective:

The aim of the present *in vitro* investigation was to study mechanisms of toxicity, evaluating the effect of INH on viability, apoptosis, MMP secretion and morphology of human hepatoma cell line SK-Hep-1.

Methods:

Human hepatoma cell line SK-Hep-1 (ATCC) was grown in MEM medium with 10% FBS, penicillin and streptomycin in 24-well tissue culture plates. At near confluence, cells were treated with INH at 0, 1, 5, 10, 15 and 20 mM in triplicate at each concentration. Viability was assayed by MTT assay, apoptosis using live green caspase detection kit (Molecular Probe), MMP secretion by zymography and morphology by H&E staining.

Results:

INH inhibited the growth of SK-Hep-1 cells in a dose response fashion, 10% at 5 mM, 20% at 10 mM and 25% at 15 and 20 mM concentration. INH induced slight apoptosis at 5 mM, moderate at 10 and 15 mM and significant at 20 mM. INH also showed a dose-dependent inhibition of MMP-9 activity, 5%, 10%, 20%, 30%, and 40 % at 1, 5, 10, 15 and 20 mM concentration. H&E staining showed no morphological changes even at highest concentration.

Conclusions:

The present study demonstrates that INH causes toxicity by inducing apoptosis and inhibiting MMP secretion.

1002 GINKGOLIDE A CONTRIBUTES TO THE POTENTIATION OF ACETAMINOPHEN TOXICITY BY GINKGO BILOBA EXTRACT IN PRIMARY CULTURES OF RAT HEPATOCYTES.

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The present cell culture study investigated the effect of *Ginkgo biloba* extract pretreatment on acetaminophen toxicity and assessed the role of ginkgolide A and cytochrome P450 3A (CYP3A) in hepatocytes isolated from adult male Long-Evans rats provided ad libitum with a standard diet for rodents. Acetaminophen (7.5-25 mM for 24 h) conferred hepatocyte toxicity, as determined by the lactate dehydrogenase (LDH) assay. *G. biloba* extract alone increased LDH leakage in hepatocytes at concentrations $\geq 75 \mu\text{g/ml}$ and $\geq 750 \mu\text{g/ml}$ after a 72 h and 24 h treatment period, respectively. *G. biloba* extract (25 or 50 $\mu\text{g/ml}$ once every 24 h for 72 h) potentiated LDH leakage by acetaminophen (10 mM for 24 h; added at 48 h after initiation of extract pretreatment). The effect was confirmed by a decrease in [^3H]-leucine incorporation. At the level present in a modulating concentration (50 $\mu\text{g/ml}$) of the extract, ginkgolide A (0.55 $\mu\text{g/ml}$), which increased CYP3A23 mRNA levels and CYP3A-mediated enzyme activity, accounted for part but not all of the potentiating effect of the extract on acetaminophen toxicity. This occurred as a result of CYP3A induction by ginkgolide A because triacetyloleandomycin (TAO), a specific inhibitor of CYP3A catalytic activity, completely blocked the effect of ginkgolide A. Ginkgolide B, ginkgolide C, ginkgolide J, quercetin, kaempferol, isorhamnetin, and isorhamnetin-3-O-rutinoside did not alter the extent of LDH leakage by acetaminophen. In summary, *G. biloba* pretreatment potentiated acetaminophen toxicity in cultured rat hepatocytes and ginkgolide A contributed to this novel effect of the extract by inducing CYP3A.

1003 EFFECTS OF 18 β -GLYCYRRHETINIC ACID ON THE HEPATIC METALLOTHIONEIN EXPRESSION IN HEPA-1C1C7 CELLS.

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Glycyrrhizin, a triterpenoid saponin fraction of licorice, is reported to have inducing activity of hepatic metallothionein (MT) and is metabolized to 18 β -glycyrrhetic acid (GA) in the intestine by intestinal bacteria. However, the mechanism underlying its effects is not known. This study investigated the effects of GA on the metallothionein (MT) expression in the murine hepatoma cell line Hepa-1c1c7 cells. Treatment with GA caused increase of MT expression in a dose-dependent manner. This induction was inhibited by addition of AG490, an inhibitor of signal transducer and activator of transcription (STAT) 3. Furthermore, transient transfection experiments showed that MT promoter activities were increased by GA. Taken together, these results demonstrate that MT expression by GA could mediate through STAT3 signal pathway.

1004 NEW THERAPY TO TREATING ACUTE INTOXICATIONS WITH FLUOROACETATE AND OTHER POISONS.

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Fluoroacetate (FA) and other monofluorides are highly toxic compounds, which action characteristically involves a latent period. We have undertaken large-scale experiments which embraced electrophysiological, biochemical, biophysical and morphological studies mostly with rats, but mice and rabbits were used too. It was found that apart from the classic increase of citrate level, glutamate and glutamine concentrations were sharply decreased in blood and tissues of rats under acute intoxication with FA (1/2LD50). Electron microscopic studies revealed drastic changes of capillary endothelium and the blood-brain barrier. The level of NAD(P)H can affect intracellular calcium homeostasis, together with citrate as calcium chelating agent. In its turn, intracellular calcium disbalance and a primary hypersensitivity of platelets may cause the ischemic disturbances in heart and brain.

As a result of our integrated research, two principal ways of developing effective therapies for FA intoxication have been highlighted. Firstly, competitive inhibition of FA interaction with CoA and fluorocitrate interaction with aconitase, and secondly, channeling the alternative metabolic pathways in order to replenish the Krebs cycle and regulate the cellular NAD(P)H level. An effective therapeutic means called METIS has been developed for treating acute intoxications with FA, the coefficient of efficiency (ratio of LD50 of treated and non-treated rats) being 2.5 and more. Further still, METIS turned out to be a non-specific therapy and

could be applied in some other cases of poisoning (e.g. with ethylene glycol and cyanide), as well as ischemic and coma states, enhancing the efficacy of traditional therapy (e.g. 4-methyl-pyrazol for ethylene glycol).

The work was supported by the BioIndustry Initiative of the U.S. Department of State (Grant# BII-ISTC-2629).

1005 IS THE FARNESOID X RECEPTOR IN JAPANESE MEDAKA (ORYZIAS LATIPES) A TARGET FOR EXOGENOUS COMPOUNDS?

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The farnesoid X receptor (FXR), a nuclear receptor activated by bile acids, results in altered expression of genes involved in bile acid metabolism and transport such as CYP7A1, CYP8B1, and the bile salt export pump (BSEP). In comparative research involving FXRs, it was found that the receptor can also be activated by other compounds such as retinoids, and via synthetic agonists such as GW4064. Co-administration of GW4064 with α -naphthylisothiocyanate (ANIT), a cholestasis-inducing compound widely studied in rodent models, results in an alleviation of liver injury. This finding has opened an avenue of research involving FXR that focuses on exploiting its function for the treatment of cholestasis. Recently, our lab has shown that a single administration of ANIT induces bile preductular epithelial cell injury in medaka liver, resulting in decreased clearance of the bile pigment, biliverdin. Resumption of clearance returns at 72 hours post-exposure, as indicated by a significant change in gallbladder color. However, changes in bile acid levels as a result of ANIT exposure have not been described in this model. Preliminary research shows that exposure to ANIT at 40 mg/kg induces a 2.6 fold increase in taurocholic acid at 72 hours post-exposure compared to 48 hours, indicative of an inability to clear bile constituents from the liver. It is believed that these changes in bile composition are concomitant with alterations in FXR activity. Herein we present the isolation and characterization of FXR alpha from medaka (medFXR α), and demonstrate its activation by bile acids. Experiments are ongoing to determine how ANIT, retinoids and GW4064 alter FXR activity in medaka. We hypothesize that effects of ANIT will be reduced by co-administration of retinoids or GW4064. Supported by the EPA STAR Fellows Program (FP916427); the NIH NCRR R01 RR18583, and Duke University's Cancer and the Environment Program.

1006 GENE EXPRESSION CHANGES IN HUMAN TRACHEAL HS680 CELLS EXPOSED TO FORMALDEHYDE.

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Formaldehyde is an occupational and environmental toxicant used extensively in the manufacturing of many household and personal use products. It is known to induce squamous cell carcinomas in the nasal turbinates of rats and mice and nasopharyngeal cancer in human. It is also associated with sick building syndrome, a set of diffuse and irritative symptoms predominantly involving the eyes and the respiratory tract. To identify biomarkers for formaldehyde exposure that might contribute to pathological changes, human trachea fibroblast Hs 680 cells were exposed to formaldehyde at 10 or 100 μM for 4 h and total RNA was isolated. Microarray hybridization was performed using Sentrix Human-6 Expression BeadChip from Illumina. Data were extracted using the software provided by the manufacturer (BeadStudio v. 1.0.0.5) and normalized by quantile normalization. One-way analysis of variance (ANOVA) and Tukey's HSD test were applied to determine differentially expressed sets of genes across three experimental groups. Statistical significances were adjusted by Benjamini-Hochberg multiple-testing correction. Microarray analysis identified 44 genes as being formaldehyde-responsive, in that their expression levels differed more than 2-fold and significantly from the controls ($p < 0.05$). We confirmed 11 genes induced by formaldehyde using quantitative real time PCR. The changes of these genes in SD rats and human exposed to formaldehyde were analyzed. Our results suggest that the global changes in gene expressions following formaldehyde exposure can be used as biomarkers for formaldehyde toxicity.

1007 GLOBAL GENETIC ANALYSIS OF THE CARDIOVASCULAR EFFECTS OF MAINSTREAM TOBACCO SMOKE.

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Mainstream cigarette smoke (MCS) is an important risk factor for diverse cardiovascular ailments. Although the impact of smoking on human health is well defined, little is known about the mechanisms by which MCS mediates its cata-

strophic effects. In the present study, we investigated the effects of MCS on gene expression in heart tissues of mice. Male C57B1/CBA mice were exposed to MCS from two cigarettes daily, 5 days/week for 6 or 12 weeks or to ambient air in restrainers. Mice were sacrificed immediately or 6 weeks following the last MCS exposure. RNA from heart tissue was hybridized against universal mouse reference RNA to Agilent Oligo DNA microarrays containing 22,000 transcripts.

A James-Stein shrinkage test (MAANOVA 2.0) was used to identify 120 genes that were significantly differentially expressed (1.5-14 fold up or down regulated). We have confirmed the altered expression of a select number of genes using real-time quantitative RT-PCR.

Our results revealed up-regulation of Cyp1A1 (14-fold), a key player in the xenobiotic metabolism pathway. A number of genes involved in matrix remodeling, wound healing and fibrinolysis were down regulated (1.5-3 fold). The roles of a subset of these genes involved in MCS response are analyzed in detail including: A disintegrin and metalloprotease 9 (ADAM9), matrix metalloproteinase 25 (MMP25), cysteine-rich angiogenic inducer 61 (Cyr61), the chemokine CXCL-1 and the plasminogen activator inhibitor (PAI-1). Changes in gene expression were transient and were mostly reversed when smoking was discontinued.

Gene expression profiling of heart tissue provides a novel approach to study the molecular mechanisms operating in cardiovascular response to MCS. The present work identifies several novel candidate biomarkers of exposure and potential adverse effect.

1008 CYTOTOXICITY AND GENE EXPRESSION PROFILES IN CELL CULTURES EXPOSED TO WHOLE SMOKE FROM THREE TYPES OF CIGARETTES.

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The purpose of this study was to evaluate and compare the cytotoxicity and gene expression profiles in cell cultures exposed to whole smoke generated from a full flavor cigarette (Test 1), a low tar cigarette (Test 2) and an ultra-low tar cigarette (Test 3). In addition, a reference cigarette 2R4F was evaluated for cytotoxicity. Neutral Red assay was performed to determine relative cell death. LC50 was generated using wet total particulate matter (WTPM), cigarette number, or nicotine concentrations. The overall order of cytotoxicity was Test 1 >> 2R4F ≈ Test 2 > Test 3. Cell samples were collected for RNA extraction at WTPM concentrations of each cigarette that gave similar nicotine concentrations. Affymetrix mouse whole genome 430 2.0 array was used to characterize the gene expression profiles for each cigarette. A total of 598 genes in Test 1, 176 genes in Test 2, and 234 genes in Test 3 samples were differentially expressed compared to sham controls. The major biological processes associated with the changed genes in Test 1 samples were down-regulated DNA replication and cell proliferation; the same biological processes were much less affected in Test 2 and Test 3 samples. The common findings in all three cigarettes were increased glutathione biosynthesis/consumption and inflammatory response, which are known biological effects of smoke exposure. The most significantly up-regulated genes were CYP 1A1, GSTs, Hmox1, and Procr in smoke-exposed samples, which are either related to well-studied mechanisms of smoke exposure-related diseases, or potential new biomarkers for assessing and monitoring biological effects of smoke exposure in vivo and in smokers. In summary, both the neutral red cytotoxicity assay and gene expression profiling were able to differentiate the three types of test cigarettes, and the results demonstrated reduced biological effects for the Test 2 and Test 3 cigarettes compared to the Test 1 cigarette in BALB/c-3T3 Cells.

1009 PULMONARY EFFECTS OF JP-8 JET FUEL EXPOSURE - LABEL-FREE QUANTITATIVE ANALYSIS OF PROTEIN EXPRESSION IN ALVEOLAR TYPE II EPITHELIAL CELLS USING LC/MS.

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Lung epithelial cells are vulnerable to the primary effects of inhaled chemicals. This is true of JP-8 jet fuel, whose untoward effects have been documented in both *in vivo* and *in vitro* exposure systems. The present study was undertaken to assess the effect of JP-8 exposure on rat alveolar type II epithelial cells, at sub lethal levels that are occupationally relevant. An equally important aim was to assess the utility of a novel gel-free/label-free proteomics approach to quantify JP-8-mediated alterations in cellular protein expression. Alveolar type II cells (1×10^6 cells/well) were cultured in 6-well plates and exposed to JP-8 jet fuel (0.1 µg/mL, 0.4 µg/mL, 2.0 µg/mL, vehicle control, and media control) for 24 h. Cells from each of 5 wells

per treatment group (n=5) were solubilized *in situ* and proteins trypsinized. Tryptic peptides from each sample were analyzed serially via linear ion-trap LC-MS/MS. Proteins were identified using SEQUEST and X!Tandem while protein quantification was carried out based on total ion chromatograms using the proprietary technology of Indiana Centers for Applied Protein Sciences (INCAPS). In this approach, the integral volume under each selected peptide peak was measured, normalized, and compared for relative abundance. In this study, 1766 proteins were identified and quantified. Of these, 1135 were identified with high confidence. The identities of the significantly altered proteins will be presented in terms of the functional significance of their JP-8 mediated up- or down-regulation. Supported by AFOSR Grants FA9550-06-1-0083 & FA9550-05-1-0216 to FAW and FA9550-04-1-0231 to MLW.

1010 MICROARRAY ANALYSIS OF CALCINEURIN INHIBITOR IMMUNOSUPPRESSANT-MEDIATED NEPHROTOXICITY IN THE RAT.

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The use of calcineurin inhibitor immunosuppressants has revolutionized the clinical practice of organ transplantation. However, chronic nephrotoxicity limits their long-term utility. In order to understand the molecular mechanisms involved in the injury process, a study was designed to investigate changes in gene expression that underlie the development of calcineurin inhibitor immunosuppressant-mediated nephrotoxicity. Male SD rats were dosed daily with cyclosporine A (CsA) at 2.5 and 25 mg/kg/day i.p. or FK506 at 0.6 and 6 mg/kg/day i.p. for 1 to 28 days. Rats were also dosed with rapamycin, a novel immunosuppressant that does not elicit nephrotoxicity at therapeutic levels, at 1 and 10 mg/kg/day i.p. to serve as a negative control. Gene expression profiling was performed on RNA isolated from kidneys 24 hours after 1, 7, 14 and 28 daily doses using the Affymetrix GeneChip Rat Genome 230 2.0 array. Principal components analysis of differentially expressed genes from each treatment group clearly demonstrates a distinction of the rats treated with CsA and FK506 from the rats treated with rapamycin. Examination of the differentially expressed genes across time suggests that CsA and FK506, but not rapamycin, disrupt calcium homeostasis in the kidney as early as the first dose. By 7 doses, gene expression changes consistent with perturbation of the kallikrein-kinin and renin-angiotensin systems are observed only in rats treated with the high doses of CsA and FK506, coinciding with the onset of histopathologically verified renal injury. At the peak of kidney damage (14 to 28 daily doses), kidneys from rats treated with high dose CsA and FK506 exhibit changes in expression for genes involved in cell death and/or apoptosis, response to cellular damage and the development of a fibrogenic response. Thus, using microarrays to assess changes in global gene expression has provided us with insights into mechanisms underlying the development of and cellular responses to nephrotoxicity elicited by calcineurin inhibitor immunosuppressants.

1011 GENE EXPRESSION CHANGES IN KIDNEY FROM RATS EXPOSED TO D-SERINE.

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Renal toxicity commonly occurs after exposure to xenobiotics/pharmaceutical agents or environmental pollutants. Changes in the gene expression in the kidney cells are probably critical events that accompany renal injury. We investigated gene expression changes in kidney after exposure to the nephrotoxin D-serine, which targets the proximal tubule of the kidney. Male F-344 rats (Charles River Laboratories, 222 – 258 g) were injected i. p. with a single dose of D-serine (0, 5, 20, 50, 200 or 500 mg/Kg). Rats were sacrificed 96 hours after the treatment and kidney tissue was collected. Gene expression profiles were determined using DNA microarrays. Gene expression changes including 1158 up-regulated genes and 749 down-regulated genes were identified based on the combined results of t-test ($p < 0.01$ in two consecutive doses) and ANOVA (with Bonferroni correction for multiple testing). Self organized map (SOM) clustering analysis using the expression profiles of the altered (1907) genes as input data showed 2 tight clusters, one of 1158 up-regulated genes and another of 749 down-regulated genes, respectively. Interestingly, there appears to be minimal changes in the expression level of these genes at doses less than 200 mg/Kg, while the most prominent changes were observed at 200 and 500 mg/Kg in both cases. Pathway analysis of the differentially expressed genes showed that at least 27 biochemical pathways are effected including apoptosis, cell cycle, G protein signaling, TGF β signaling pathway, and transcription initiation. The results of our microarray study showed alterations in the expression of a large number of genes after exposure to D-serine. Further analysis of the biological pathways and processes involved should lead to better understanding of the mechanisms of D-serine toxicity. It would also be of interest to investigate if these genes may serve as organ- and/or region-specific biomarkers for acute renal injury.

1012 KIDNEY TOXICOGENOMICS OF 2-WEEK SODIUM AND POTASSIUM BROMATE EXPOSURE IN F344 MALE RAT.

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Bromate, used in both the food and cosmetics industry, is a drinking water disinfection by-product that is nephrotoxic and carcinogenic to rodents. To gain insight into the carcinogenic mechanism of action, identify possible biomarkers of exposure, and determine if the cation, potassium, significantly alters gene expression, kidney gene expression was examined from male F344 rats exposed to NaBrO₃ and KBrO₃ in drinking water for 2-weeks. Total RNA was isolated from kidney samples and gene expression analyzed using Affymetrix Rat 230A 2.0 GeneChips. Gene expression patterns from two non-carcinogenic (1, and 20 ppm) and one carcinogenic dose (400 ppm) at 2-wk, were compared to each other and to previous gene expression from a 52 wk, 400 ppm KBrO₃ study. Statistical analysis revealed 1, 167, and 229 genes in the NaBrO₃ exposure group and 17, 195, and 139 differentially expressed genes (DEGs) in the KBrO₃ exposure group at the low, medium, and high doses, respectively. Gene ontology classification of the Na- and KBrO₃ groups showed similar expression alterations of genes involved in oxidative stress, lipid metabolism, kidney function/ion transport, and cellular function. Of these, all were equally affected except for an increase in kidney function/ion transport genes observed in the Na- versus KBrO₃ (34 vs. 9 DEGs). In comparing DEGs from 2-week 400 ppm Na- (139 DEGs) and KBrO₃ (229 DEGs) to the chronic 400 ppm KBrO₃ (224 DEGs), there were 14 and 32 shared DEGs respectively that also matched in direction of change. Thus, this study identifies the potential for 2-week bromate gene expression profiles to forecast gene expression changes at chronic time points. However, an important finding was that sodium and potassium bromate treated kidneys yield similar kidney gene expression profiles at two weeks exposure. [This abstract does not necessarily represent EPA policy]

1013 DIFFERENTIAL GENE EXPRESSION BETWEEN TWO SEXES IN HEART OF RAT STRAIN FISHER 344.

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Various gender differences in cardiovascular disease and heart failure have been noted in humans, including age of onset, initial manifestations, and drug response. This variability may be reflective of differential gene and protein expression in the two sexes. To understand these sex-associated differences, gene expression profiles in the heart were examined in both males and females at three different ages in a rat model system. In this study, hearts from both sexes of young (8 weeks), middle age (21 weeks) and old age (78 weeks) Fisher 344 rats were ground into powder under liquid nitrogen and aliquots were used for RNA extraction and other analyses. Home-made long-oligonucleotide microarrays of 10,000 rat genes (MWG, Inc.) were used in two-color hybridizations using a reference design experiment. The data were analyzed using ArrayTrack software that was developed at the NCTR/FDA. The raw data were normalized using Total Intensity Normalization method. Welch T-test was used for determining significant changes in gene expression between samples of the two sexes. The results from the microarray experiments showed that the overall gene expression patterns are very similar between the sexes at the three different ages. The data showed much greater gender (sex) difference at 21 weeks compared to 8 weeks and 78 weeks. There are about 218 differentially expressed genes between the male and female at 21 weeks. Among them, many genes are involved in heart structural protein, hormone metabolism and energy metabolism (ATP synthase). These results may lead to more insights of gender-related disease and drug response.

1014 THE ROLE OF THE GUT MICROFLORA IN HYDRAZINE METABOLISM AND TOXICITY: A TRANSCRIPTOMIC AND METABONOMIC APPROACH.

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Symbiotic gut microbes contribute to the health and digestive function of the host, but microfloral differences may also be a factor in idiosyncratic drug toxicity. We have used an integrated transcriptomic (using Affymetrix RG230 genechips) and metabonomic approach to explore the influence of gut microbiota on the toxicity and metabolism of hydrazine, a steatotic hepatotoxin, also known to disrupt the central nervous system. Conventional and germ-free Sprague Dawley rats were administered a single sub-toxic oral dose of hydrazine (60 mg/kg) for four days. Two out of three of the germ-free dosed group were moribund after 24 hours and were sacrificed prematurely; conventional rats were not obviously affected by dosing. ¹H

NMR spectroscopy of urine and plasma indicated that conventional animals excreted hydrazine metabolites more rapidly. Magic angle spinning ¹H NMR of liver showed increased lipid levels in germ-free dosed animals. This was accompanied by decreased expression of genes in sterol and fatty acid metabolism pathways, suggesting that steatosis was more pronounced in germ-free dosed rats. The absence of gut microflora did not influence the hepatic expression of genes associated with xenobiotic metabolism, and most modulated genes were associated with inflammatory pathways. However, the expression of genes for several aminotransferases (alanine aminotransferase, serine dehydratase) was markedly decreased in germ-free animals. The function of aminotransferases is reliant upon the microbial derived co-factor pyridoxal 5' phosphate (PLP). As PLP is sequestered during hydrazine toxicity, this may explain the enhanced susceptibility of germ-free rats to hydrazine. This highlights the potential of gut microbiota imbalances as a factor in drug toxicity.

1015 EFFECTS OF TCDD ON CRANIOFACIAL CARTILAGE GENE EXPRESSION IN DEVELOPING ZEBRAFISH.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) produces craniofacial malformation in developing vertebrates. Fish species are especially sensitive to this effect and we have used zebrafish as a model system for studying this process. It is thought that most if not all toxic endpoints caused by TCDD result from activation of the aryl hydrocarbon receptor/aryl hydrocarbon receptor nuclear translocator (AHR/ARNT) transcription factor. Thus, we hypothesized that activated AHR/ARNT misregulates critical genes controlling craniofacial development. We used Affymetrix microarrays to profile gene expression changes occurring in zebrafish jaws after exposure to TCDD at 96 hours post fertilization. Cartilage mRNA samples were collected at 1, 2, 4 and 12 hours after initial exposure to allow us to identify both immediate transcriptional changes as well as genes affected as jaw malformation begins. We identified 204 genes that were altered by at least 2 fold (p-value ≤ 0.05). We found immediate changes in xenobiotic metabolism genes, such as cytochrome p4501a1 (cyp1a1), cytochrome p4501b1 (cyp1b1) and cytochrome p4501c1 (cyp1c1). These are known to be AHR/ARNT targets, and this result is consistent with direct activation. Downregulation of cell replication transcripts, such as minichromosome deficient 2, 3 and 5 (mcm2, mcm3 and mcm5) at 4 hours post TCDD exposure suggests a possible mechanism for the stunted growth in jaw structures caused by TCDD. Finally, upregulation of chondrogenesis pathway genes endothelin 1 (edn1) and exostosin 1c (ext1c) and downregulation of sry-box containing gene 9b (sox9b), hyaluronan and proteoglycan link protein 1 (hapln1a) may indicate that TCDD alters terminal differentiation in the jaw, leading to malformation. Whether these gene expression changes are direct results from AHR/ARNT-mediated transcription remains to be elucidated. (Supported by NIH RO1 ES012716 and UW Sea Grants R/BT17, R/BT20 and R/BT22)

1016 TRANSCRIPTOME ANALYSES OF MULTIPLE ENDOCRINE TISSUES FOLLOWING AN ACUTE EXPOSURE OF MALE RATS TO THE ANTIANDROGEN FLUTAMIDE.

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Exposure of adult male rats to antiandrogens has been shown to affect the pituitary-gonadal axis and lead to toxicity in the male reproductive organ and tissues. However little is known about the profile of gene expression in multiple target tissues following exposure to antiandrogens. To better assess the coordinated molecular changes induced by antiandrogens in different target tissues, male Sprague Dawley rats were treated with a single dose of vehicle only (methylcellulose) or the androgen receptor antagonist flutamide (150 mg/kg) and were sacrificed 8, 24 or 48 hours after dosing. At necropsy, blood samples were collected for luteinizing hormone and testosterone measurements. Pituitary gland, testis and prostate samples were collected for full genome transcriptome analyses coupled with Gene Ontology and metabolic pathway analyses. Results indicate that testosterone levels were affected from 8 hours after treatment although statistical significance was only achieved at 24h and 48h. Gene expression analysis shows that the largest number of genes affected for the pituitary, the prostate and the testis was found at 8h, 24h and 48h respectively. Overall, lipid and steroid biosynthesis and cell proliferation were the main biological processes affected in the testis whereas cell to cell signaling, inflammatory response and cell proliferation were the main processes affected in the prostate. No histopathological change was observed at any time points. Overall,

these results show that dynamic molecular changes at different organ levels can be studied in short term kinetic studies, well before the presence of histopathological lesion.

1017 CROSS-SPECIES COMPARISON OF TAMOXIFEN & ESTROGEN INDUCED UTEROTROPY IN RODENTS.

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A ligand and species comparative analysis approach was used to systematically assess the physiological, morphological and transcriptional uterine alterations elicited by tamoxifen (TAM) and ethinyl estradiol (EE) in immature ovariectomized Sprague-Dawley rats and C57BL/6 mice. Temporal gene expression was evaluated for each compound in each species. Animals were dosed once or once daily for 3 consecutive days and uteri were harvested 2, 4, 8, 12, 18, 24, or 72 hrs after treatment. TAM responsive genes were filtered by the species-conserved EE genes to distinguish estrogenic and novel effects of TAM. 377 orthologous gene pairs (79%) which overlap with the EE-induced profiles were identified, suggesting conserved gene expression events important in uterine proliferation. In addition, 50 genes with species-conserved TAM regulation that were not previously described as EE responsive were identified, suggesting species-conserved effects that were unique to TAM. Functional annotation for these novel TAM responses were associated with water and solute transport, proliferation, red-ox control, protein synthesis and transport, xenobiotic metabolism, and cell-cell communication which were similar EE regulated endpoints but used different signaling mechanisms. Moreover, several genes were identified which exhibited divergent TAM regulation when compared to EE. This multi-species approach provided insights into compound-specific differences in uterine architecture during uterotrophy. The identification of comparable temporal phenotypic responses linked to related gene expression profiles demonstrates that systematic comparative genomic assessments can elucidate important conserved and divergent uterine proliferation mechanisms in rodent estrogen signaling during proliferation. Funded by US EPA RD 83184701, R21 GM075838 and *NIEHS Training Grant ES07255-16

1018 17 α -ETHINYLESTRADIOL INDUCED GENE EXPRESSION CHANGES AND SUBSEQUENT TESTICULAR PATHOGENESIS AND DECREASED FERTILITY IN THE MEDAKA FISH.

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Currently, several toxicogenomic projects in our lab use microarrays for gene discovery, pathway analysis, and biomarker determination. Each utilizes aquatic vertebrate models for assessments of toxicity and associated phenotypes. Driving this research is the general assumption that there are evolutionarily conserved regulatory processes associated with observed toxicities. We hypothesize that most vertebrate species will activate transcription of a similar set of genes following drug or xenobiotic exposure. Moreover, discovery of this conserved set of responsive genes will focus attention on genes and pathways that have greater predictive relevance to human toxicities and pathology. In this study, changes in testis gene expression following exposure to 17 α -ethinylestradiol (EE2) were examined and linked to subsequent alterations in testis pathology and more apical end points including fertility. Medaka fish were exposed to 0.1, 1.0 or 10 ppb of EE2 and sacrificed at day 1, 3, 7 or 14 for gene expression and pathological analysis. In addition, fertility was assessed after 14 days of exposure to evaluate reproductive capability. Using a custom 277-gene cDNA membrane array, we found that changes in gene expression and histology were dose and time dependent. Putative estrogen response elements (EREs) were identified upstream of the transcriptional start site indicating that a component of the gene response is mediated by the classical ER/ERE mechanism. Several genes demonstrated a lack of ERE binding motifs, suggesting alternate pathways for estrogen mediated gene expression. The relation of gene changes, development of testicular pathologies and ultimate impact of reproductive capacity will be addressed. These data will help correlate the temporal and dose relationship between the transcriptional response to EE2 and associated cellular and reproductive alterations.

1019 TESTICULAR GENE EXPRESSION PROFILING FOLLOWING GLYCOL ETHER SOLVENT EXPOSURE IN MALE RATS.

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2-Methoxyethanol (2-ME) and 2-ethoxyethanol (2-EE) are glycol ether solvents that produce testicular toxicity characterized by spermatocyte degeneration and depletion. 2-EE is less potent than 2-ME, while a structurally similar glycol ether, 2-

butoxyethanol (2-BE), produces no testicular lesions. The goal of the current study was to assess the mechanism of glycol ether testicular toxicity using gene expression profiling. Male rats were administered daily oral doses of 2-ME (150 and 500 mg/kg/day), 2-EE (500 mg/kg/d), 2-BE (125 mg/kg/d), or vehicle for 3 days, after which testes were collected for histopathological and gene expression analysis. Histopathological changes were observed only in animals given 150 mg/kg/d 2-ME, consisting of degeneration and necrosis of spermatocytes with reductions in spermatocyte numbers. Microarray analysis was performed using an unpaired t-test following data normalization. There were 28, 1095, 945, and 1721 genes that were differentially expressed at >1.5-fold, $p < 0.05$ versus control in testes from animals exposed to 2-BE, 2-EE, 2-ME (50 mg/kg), and 2-ME (150 mg/kg), respectively. There was an overlap of 525 genes between the 2-EE and two 2-ME treatment groups. To assess what categories of genes were affected by 2-EE and 2-ME, expression data was analyzed using the Expression Analysis Systematic Explorer (EASE) application. This analysis demonstrated statistical enrichment ($p < 0.05$) in genes in categories including protein transport/endocytosis, protein kinase activity, cell cycle, meiosis, transcriptional regulation, and cell death. While enrichment of genes involved in cell death and meiosis are consistent with the morphological changes produced by these compounds, enrichment of genes such as those involved in protein transport represent possible novel targets of glycol ether solvents. Additional experiments will be performed to confirm these findings and to assess their relevance in the mechanism of glycol ether testicular toxicity.

1020 MOLECULAR PROFILING OF FORMALIN-FIXED PARAFFIN-EMBEDDED (FFPE) TISSUES: CONFIRMING DIFFERENTIAL GENE EXPRESSION IN THE RAT UTERUS AND CERVIX.

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Several approaches have been reported for the isolation of RNA from FFPE tissues for analysis by RT-PCR, microarrays, or other methods for measuring gene expression. However, many of these techniques for RNA isolation suffer from similar limitations, including recovery of degraded RNA, low yields, and time-consuming labor-intensive protocols. We compared several commercially available kits for RNA isolation from FFPE tissues and found the FFPE kit from Qiagen performed the best. To help resolve an issue identified in a microarray study (reported separately by Koza-Taylor et al.), RNA was isolated using the Qiagen kit from FFPE blocks containing uterus and cervix from rats treated with estrogen. The quality of this RNA was compared to RNA isolated from fresh-frozen tissue. Real-time PCR was then performed on the frozen RNA and the FFPE RNA using four different primer/probe sets that were chosen based on Affymetrix RAE230v2 GeneChip data. The gene matrix metalloproteinase 7 (MMP7) was used since microarray data showed that it was highly up regulated with estrogen. The gene Plasminogen activator inhibitor 2 type A (Pai2A) was chosen from microarray data and evaluated as a cervix specific marker present at moderate levels. The results of RT-PCR for all genes evaluated were comparable to the Affymetrix results. Also, the patterns of expression for the frozen and FFPE RNA were the same. Finally, a strong correlation between the presence of cervix, as demonstrated by H&E staining, and a positive signal for Pai2A expression was confirmed by RT-PCR. These results allowed the confirmation of the hypothesis that a few samples used to generate microarray data were contaminated with cervix tissue.

1021 GENE EXPRESSION OF TESTICULAR TOXICITY INDUCED BY EMODIN OR ACRYLAMIDE IN MALE MICE.

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Emodin is an active constituent isolated from the root of Chinese Rhubarb. Pharmacological studies have demonstrated that Emodin possesses anti-bacterial, anti-inflammatory, vasorelaxant, anti-ulcerogenic, and anti-cancer effects. Our unpublished data indicated that Emodin showed the testicular toxicity in mouse. In this study, we investigated the testicular toxicity of Emodin using a toxicogenomic method in mice, as compared with Acrylamide, known as a testicular toxicant. Emodin or Acrylamide was administered orally to male CD-1 mice for 5 days at a dose of 1000 and 50 mg/kg/day respectively. Twenty four hours after final dosing, testes of all mice were extracted for total RNA, and the gene expression profile in testis of chemical-treated mice were compared to the control mice using a cDNA microarray (Asahi Techno Glass Corporation), Testis-1 (172 genes: haploid germ cells-related) and Testis-2 (2704 genes: testis-related). In all treated groups, degenerative histopathological changes of the testes were observed and the number of TUNEL-positive germ cells, were increased. No changes in haploid germ cell-related gene expression were observed in microarray analysis using Testis-1. The majority of testis-related genes examined showed slight differences in expression between the treated and control groups using Testis-2. However, there were 34 and 30

genes, whose expression was more than 1.5 fold or less than 0.67-fold in the Emodin and Acrylamide treated mice, respectively, as compared with the control mice. Interestingly, it was found that expressions of the same 3 genes, MutS homolog 4, Chromobox homolog 5 and SMC-like 2 were decreased by both compounds. Furthermore, Emodin affected the gene expressions of calpain 3, Dynein, DEAH box polypeptide 8, Cyclin-dependent kinase inhibitor 1B and CD47 antigen. These results suggest that Emodin has a testicular toxicity and specifically affects the expression of some genes not relevant to the testicular toxicity of Acrylamide.

1022 COMPARATIVE ANALYSIS OF TEMPORAL GENE EXPRESSION, MORPHOMETRY AND UTEROTROPY IN O, P'-DDT AND ETHYNYLSTRADIOL TREATED RATS.

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Changes in uterine physiology, cell morphology and gene expression were evaluated in a comprehensive time course study after oral administration of 300 mg/kg o,p'-DDT (DDT) or 100 ug/kg bw ethynylestradiol (EE) to immature, ovariectomized Sprague-Dawley rats. Animals were dosed once or once daily for 3 consecutive days and uteri were harvested 2, 4, 8, 12, 18, 24, or 72 hrs after treatment. EE and DDT elicited uterotrophic EC50s of 16.2 ug/kg and >64.6 mg/kg, respectively, in dose response studies. DDT only induced a 3.3-fold increase in wet weight with a marked decrease in water imbibition compared to the 7-fold induction with EE. Temporal gene expression profiles were measured using custom rat cDNA microarrays with 8,567 features representing 3,042 genes. Genes differentially regulated by either DDT or EE were identified using a model-based t-statistic/Empirical Bayes approach and their temporal expression profiles were compared to identify conserved and divergently regulated genes. In order to phenotypically link these changes in gene expression to the physiological alterations observed in the uterotrophic response, complementary histological and morphometric assessment of uterine mid-horn sections stained with eosin and hematoxylin were performed. DDT treated histological samples were characterized by marked apoptosis increases in the luminal epithelia and stroma at 72 hr compared to EE. Quantitative real time PCR analysis confirmed increased efficacy of genes involved in apoptosis and water shuttling (Pycard, Aqp0/MIP) while classically estrogen regulated genes (C3, Calb3) exhibited equivalent or lower responses in response to DDT. These results suggest that DDT and EE elicit disparate differential gene expression sets responsible for differences in uterotrophic and pathological effects.

1023 USE OF TOXICOGENOMICS DATA IN RISK ASSESSMENT: A CASE STUDY ON DIBUTYL PHTHALATE AND MALE REPRODUCTIVE DEVELOPMENTAL EFFECTS.

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A case study to incorporate toxicogenomics data qualitatively into an EPA health assessment has been performed for dibutyl phthalate (DBP), focusing on the male reproductive developmental effects. Using EPA's IRIS external peer review draft DBP assessment as the starting point, the case study asked whether toxicogenomics data could further define the mode(s) or mechanism(s) of action and inform interspecies extrapolation. The modes of action that explain some of the male reproductive developmental effects observed after in utero DBP exposure in rodents are reduced fetal testicular testosterone production and insl3 gene expression. The male reproductive developmental toxicology dataset was assessed for low incidence findings that may indicate additional pathways. The toxicogenomics dataset is composed of eight published microarray or real-time reverse transcriptase-polymerase chain reaction (RT-PCR) rat studies. The microarray data were analyzed using pathway analysis and regulatory network modeling to determine whether additional pathways were affected by in utero DBP exposure. Taken together, the results of the toxicity and toxicogenomics data analyses corroborate the DBP modes of action of reductions in fetal testicular testosterone production and insl3 gene expression, and indicate that additional pathways were also affected. Additionally, research needs for designing gene expression studies for use in risk assessment were identified. The approach for utilizing toxicogenomics data in risk assessment defined in this study may be applied to other chemical assessments. Disclaimer: The views expressed are solely those of the authors and do not represent the policies and/or endorsement of the U.S. Environmental Protection Agency.

1024 HUMAN PBMCs AS AN *IN VITRO* MODEL FOR IDENTIFYING HUMAN BIOMARKERS TO PREDICT MECHANISMS OF COMPOUND INDUCED TOXICITY.

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Using an *in vitro* system to determine a chemical's propensity for inducing particular toxicities in humans would represent a significant advance in drug development. *In vitro* cell systems have the distinct advantage of requiring only milligram amounts of test compound compared to *in vivo* systems. Our library of Drug Signatures® or biomarkers derived from gene expression profiling of compound-treated rat primary hepatocytes *in vitro* has successfully been used to predict *in vivo* rat hepatotoxicity during drug development. To establish a human peripheral blood mononuclear cell (PBMC)-based system to predict mechanisms of drug action and toxicity relevant to humans, cells from normal donors are treated with reference compounds *ex vivo*. Human PBMCs are clinically accessible and therefore ideal to assess the global transcriptional effects of compounds on human primary cells. We have analyzed gene expression profiles of PBMC from multiple donors treated with >40 compounds with well studied toxicity and mechanisms of action. Our data indicate that gene expression changes in response to one compound or compound class are consistent among donors, suggesting that donor-to-donor variability is not a limiting factor in this system. Our data show that compound-induced gene expression responses are specific to different mechanistic classes of compounds and impact specific pathways as expected. Glucocorticoid receptor agonist treatments suppress genes involved in immune response pathways, and DNA alkylator compounds induce genes involved in DNA damage response pathways. We are now adding gene expression data from ~60 more compounds to our PBMC database in order to derive biomarkers for specific pharmacologic mechanisms of action and mechanisms of toxicity. Results to date support the potential of a human PBMC-based *in vitro* system to characterize the toxicological and pharmacological mode of action for experimental compounds in order to better anticipate human clinical responses.

1025 PRIMARY CELLS AS PRIME MODELS: TOWARDS AN EFFECTIVE MODEL SYSTEM FOR PREDICTIVE TOXICOGENOMICS.

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Predictive toxicogenomics using *in vitro* systems is emerging as an important tool in determining the toxicities of various chemicals and potential pharmaceutical compounds, with efforts to establish regulatory guidelines in the field. Primary human cells have the advantage of closer correlation to humans *in vivo* with possibilities to extrapolate *in vitro* toxicity signatures. Human peripheral blood mononuclear cells (PBMCs), one of the most clinically accessible primary human cells show promise as a model system to assess global transcriptional effects of compounds. Our main objective was to establish a quality-controlled database of compound signatures for predictive profiling by analyzing gene expression profiles of reference compounds across multiple mechanistic classes. Towards this goal, we performed all studies under GLP-compliance to establish human PBMCs as an effective model system for predictive toxicogenomics. We evaluated differences in human lymphocyte sources, culture conditions and donor variability. We conducted various cytotoxicity, apoptosis and genotoxicity assays. To correlate PBMCs *in vivo* and *in vitro*, we compared lymphocyte subpopulations by flow cytometry and gene expression profiles by transcriptomics. Towards initializing a toxicogenomics database of chemicals we exposed cultured human PBMCs to 6 genotoxic compounds that induce genotoxicity either by direct interaction with DNA (MMC, MMS, CIS) or by indirect secondary mechanisms (ETOP, HU, TAXOL). Using Affymetrix platform for transcriptomics we have shown that gene expression profiling could identify compound-specific signatures and distinguish mechanisms of action of the genotoxic compounds (direct or indirect, aneugenic or clastogenic). Donor variability or technical variations were not limiting factors. Our data support the potential of human PBMCs as a model system to characterize and predict the toxicities of chemical compounds by transcriptional profiling. Future studies will expand the database of expression profiles of a wide range of chemicals to predict toxic outcomes for *in vivo* and other human *in vitro* models.

1026 COMPARISON OF GENE EXPRESSION ANALYSIS METHODS FROM FORMALIN-FIXED PARAFFIN-EMBEDDED TISSUES.

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Microscopic evaluation of formalin-fixed paraffin-embedded (FFPE) tissues from preclinical animal studies is the gold standard for identifying safety signals of new chemical entities (NCEs). While formalin fixation is an excellent way to preserve

specimens for these evaluations, there are limitations to the use of these samples when molecular analyses are required. The fixation process itself as well as prolonged storage can cause modifications and degradation to RNA. Coupled with high temperatures used for embedding and the natural degradation of RNA over time, it is very difficult to isolate intact and unmodified RNA. Furthermore, reverse transcriptase is less efficient at converting modified RNA to cDNA. Numerous methods have been proposed to isolate RNA from FFPE tissues for gene expression analysis. This study was designed to compare QuantiGene's branched DNA technology, which does not require RNA purification and target RNA amplification to Ambion's RecoverAll quantitative PCR method for measuring gene expression in FFPE tissues. In addition, these isolation methods were also compared to standard TaqMan analysis when RNA was isolated from flash frozen tissue. Direct comparisons between TaqMan (flash frozen) to QuantiGene (flash frozen & FFPE) demonstrate that both methods yield similar trends in target mRNA induction. Gene expression results from the RecoverAll method were difficult to interpret, the reasons for which are still under investigation. This data clearly indicates it is possible to generate reliable gene expression data from FFPE tissues and could have usefulness in retrospective analyses.

1027 COMPARISON OF 1 COLOUR AND 2 COLOUR METHODS FOR MICROARRAY-BASED TRANSCRIPTION PROFILING ANALYSIS WITH LASER CAPTURE MICRODISSECTED SAMPLES.

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Understanding molecular mechanisms of toxicity as reflected by parallel changes in gene expression requires techniques that are capable of measuring alterations in cell types relevant to the toxicity. To determine the utility of applying whole genome microarray-based transcription profiling analysis to laser microdissected tissue sub-regions for mechanistic studies of rat fetal testes toxicity, we have compared data generated using 1 colour (1C) and 2 colour (2C) microarray protocols. Total RNA was isolated from the laser microdissected (PALM microbeamTM) interstitial-Leydig cell (INT) region of in utero dibutylphthalate (DBP)-exposed fetal rat testes at gestational day 19.5. Linear amplification of 500pg of RNA from control- and DBP-treated rats was performed using 2 cycles of cDNA synthesis and T7 polymerase-based RNA synthesis. RNA was fluorescently labelled using Kretech ULSTM cy-dye reagents and hybridised to Agilent whole rat genome oligo microarrays in a one or two colour analysis. GeneSpring software was used to generate signature lists of significantly regulated genes for each region. 1C and 2C analysis identified 511 and 107 genes that were significantly regulated ($p < 0.05$), respectively, in the INT region. Variability in the data between replicate arrays was higher in the 1C compared to the 2C data. Bioinformatic analysis of the 1C and 2C 'signature' gene lists using Ingenuity Pathways AnalysisR (IPA) software indicated that there was some overlap in the biological pathways that were identified as significantly affected. However the 2C data output was more consistent with the published literature for this class of compound than the 1C data. Corroboration of the data generated by the two methods using immunohistochemical staining is required to fully assess the biological relevance of the signature list differences observed.

1028 NEAR-INFRARED LIGHT THERAPY INCREASES CELL SURVIVAL IN A *IN VITRO* MODEL OF PARKINSON'S DISEASE.

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A large body of evidence supports a central role for mitochondrial dysfunction in the pathogenesis of Parkinson's Disease (PD). PD patients express systemic defects in mitochondrial complex I activity and complex I inhibitors have been shown to produce PD. There are also inherited forms of PD that result from mutations in genes that code for α -synuclein. The underlying pathological feature in both genetic and environmentally induced forms of PD is mitochondrial dysfunction resulting in increased production of reactive oxygen species followed by subsequent cell death of dopaminergic neurons in the substantia nigra. Photobiomodulation by far-red to near-infrared (630 to 1000 nm) light has been shown to enhance mitochondrial energy production and promote cellular survival following a mitochondrial insult. The present studies were undertaken to test the hypothesis that near-infrared (NIR) light emitting diode (LED) photobiomodulation will stimulate mitochondrial function, attenuate oxidative stress and improve cell survival in a cellular model of Parkinson's Disease. Experiments were conducted in human

dopaminergic cell lines (SH-SY5Y) engineered to stably over express mutant human α -synuclein (A30P). Cells were plated at a density of 400,000 cells/mL in 24 or 6 well plates and incubated at 37° C in DMEM supplemented with 10% FBS. At 107 hr cells were exposed to increasing concentrations of MPP+ (1, 2.5, or 5 mM) for 24 hrs. Duplicate cultures were treated with 670 nm NIR-LED light (8 joules/cm²) at 108, 120 and 132 hrs. Cells were harvested at 144 hr for analysis of mitochondrial dehydrogenase activity, hydrogen peroxide production and cellular proliferation. 670 nm light treatment produced a significant increase in mitochondrial dehydrogenase activity and cellular proliferation while hydrogen peroxide production was unchanged.

1029 GENDER SPECIFIC ALTERATIONS IN DOPAMINERGIC FUNCTION IN THE PARAQUAT AND MANEB EXPOSURE ANIMAL MODEL OF PARKINSON'S DISEASE.

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Epidemiological and clinical studies suggest a higher incidence of Parkinson's disease (PD) in males than females, suggesting the presence of some neuroprotective mechanisms in the female gender. To determine whether there is a differential susceptibility between genders to paraquat (PQ) and maneb (MB), male and female C57BL/6 mice were treated with saline or 10 mg/kg PQ + 30 mg/kg MB twice a week and sacrificed 24hrs after the 2nd dose. Although lipid peroxidation (LP) levels were elevated in midbrain and striatum of both males and females treated with PQ+MB, the males showed significantly higher LP levels than the females. Western blot analysis of key dopaminergic proteins indicated that tyrosine hydroxylase was unchanged in response to PQ+MB in either gender, however, dopamine transporter levels were significantly decreased in the striatum only in females and vesicular monoamine transporter levels were significantly increased in the striatum in both genders. Acute exposure to PQ+MB significantly increases dopamine levels in the striatum in both genders, which in turn can lead to an increase in dopamine mediated oxidative damage. Determination of concentration of key anti-oxidant proteins within the dopaminergic system revealed a significant increase in Nrf2 and superoxide dismutase (SOD) in females in both the striatum and midbrain in accordance with a protective function, whereas in the males the levels of these proteins were either decreased or unchanged. Cell survival and dopaminergic phenotypic proteins were decreased, especially in the midbrain, of both males and females, with a more pronounced decrease in the males. These observations are consistent with protection conferred in females by increased anti-oxidant capabilities, altered dopamine uptake and storage or increased trophic support. A further understanding of the mechanism of neuroprotection seen in females can lead to the development of possible therapeutic interventions for PD. Supported by APDA & ES10791

1030 INHIBITION OF 3, 4-DIHYDROXYPHENYLACETALDEHYDE METABOLISM LEADS TO PROTEIN MODIFICATION.

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Parkinson's disease is a neurodegenerative disorder characterized by cell death of dopaminergic neurons. Current studies have indicated that cell death may originate from increased oxidative stress and reactive dopamine (DA) metabolites. DA undergoes biotransformation through oxidative-deamination catalyzed by monoamine oxidase to 3,4-dihydroxyphenylacetaldehyde (DOPAL), a neurotoxic intermediate. DOPAL is then primarily oxidized to 3,4-dihydroxyphenylacetic acid (DOPAC) by mitochondrial aldehyde dehydrogenase (ALDH). ALDH can be inhibited by aldehydes derived from oxidative stress, e.g. 4-hydroxynonenal (4HNE), which might impair DOPAL metabolism yielding atypical levels of the dopamine metabolite. Based on this information, it is hypothesized that DOPAL, generated at aberrant levels due to impairment of dopamine metabolism, will cause protein modification. This work utilizes rat striatal synaptosomes to demonstrate that upon addition of 4HNE a decrease in DOPAC levels and a corresponding increase in DOPAL concentrations are observed. It was also established that DOPAL will modify proteins using a detection method involving nitro blue tetrazolium, indicating that the catechol-containing adducts undergo redox cycling. Furthermore, DOPAL-modified proteins demonstrate increased oxidative modification when in the presence of either 50 or 100 μ M CuCl₂-H₂O, which may be due to redox cycling of DOPAL. The data establish the ability of DOPAL to modify proteins thus, demonstrating its potential role in the pathogenesis of neurodegenerative diseases.

1031 REACTIVITY OF DOPAMINE OXIDATION PRODUCTS TOWARD PROTEINS.

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Parkinson's disease (PD) is a progressive neurodegenerative disorder that currently affects over a million Americans. Dopaminergic neuronal death is known to be involved in the disease pathology. The loss of such neurons may be due to the inherent toxicity of certain aldehyde intermediates created during neurotransmitter metabolism. For example, oxidative deamination of dopamine results in the formation of 3,4-dihydroxyphenylacetaldehyde (DOPAL). Recently, it has been hypothesized that a possible mechanism for DOPAL toxicity is protein adduct formation via reaction with primary amines such as those found on proteins. In order to test this hypothesis, antibodies were generated toward blue-carrier protein modified by DOPAL. Antibody assays indicated that adduct-formation occurred with bovine serum albumin treated with 1.0-100 μ M DOPAL. Dopamine can be oxidized to the o-quinone, which is known to be reactive with proteins. In order to test this scenario with DOPAL, NaIO₄ was used to oxidize the dopamine-derived aldehyde. Based on spectrophotometric assays this oxidation product rearranges; however it was found to be reactive towards 1.0 mM glutathione but not 1.0 mM N-acetylated-His or 1.0 mM N-acetylated-Lys. Therefore, these data demonstrate that DOPAL and its oxidized product are reactive towards proteins.

1032 GLIAL-NEURONAL INTERACTIONS: ASTROCYTES MODULATE TOXICANT-INDUCED NEURODEGENERATION VIA THE EXTRANEURONAL MONOAMINE TRANSPORTER, OCT3, IN MOUSE BRAIN.

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Astrocytes are increasingly being recognized as playing critical roles in maintaining normal physiological functions as well as in inducing and aggravating neuronal pathology. The neuroprotective and neurodegenerative roles of astrocytes depend largely on the molecules that they remove from or release into the extracellular microenvironment that is shared by astrocytes and neurons. Recently, the extraneuronal monoamine transporter, the organic cation transporter-3 (Oct3) in rodents, was shown to be expressed in astrocytes. The present study tested the hypothesis that astrocytes modulate neurodegeneration by regulating the levels of toxic cations in the extracellular space of neurons. Immunostaining in C57BL mice showed that Oct3 was selectively expressed in astrocytes in the nigrostriatal region but not in the astrocytes that reside in the cerebellum, hippocampus and cortex. Similar pattern of expression was also detected in post-mortem human brain sections. Functional transport studies in cultured primary astrocytes confirmed bi-directional transport of [³H]-MPP⁺ mediated by Oct3, thus supporting its ability to modulate the levels of certain organic cations. To assess the role of astrocyte Oct3 in regulating the extracellular levels of neurotoxic cations, C57BL mice were injected with MPTP, a parkinsonian neurotoxicant, to introduce the cation MPP⁺ (the active metabolite of MPTP) into astrocytes. Stereological cell counts of dopaminergic neurons in the nigra showed significantly less cell death in Oct3-knockout mice and in animals treated with an Oct3 inhibitor, as compared to their respective MPTP treated controls. This study provides insight into a novel mechanism that contributes to selective cell death, as observed in the MPTP animal model of Parkinson's disease.

1033 THE TRIGGER ROLE OF MITOCHONDRIAL PERMEABILITY TRANSITION IN THE DEVELOPMENT OF NEURODEGENERATIVE DISORDERS.

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Mitochondrial permeability transition pores represent a multiprotein complex, which includes components of both inner and outer membrane. The pores regulate transport of ions and peptides in and out of mitochondria, and their regulation is associated with a general mechanism of the maintaining Ca²⁺ homeostasis in the cell and apoptosis. Various pathological factors may induce a pathological activation of the permeability transition and an irreversible opening of mitochondria pores. It was supposed that this process is a key step in the development of neurotoxicity and neurodegeneration. The present report explores the effect of model neurotoxins, such as MPP⁺ and beta-amyloid fragment (25-35), which simulate the development of Parkinson's and Alzheimer's disease, on the mitochondrial pores

functioning. Both neurotoxins induced the opening of mitochondrial pores, which is prevented by cyclosporin A, a specific inhibitor of the permeability transition. The effect of MPP⁺ and beta-amyloid may be also prevented by N-acetylsertotonin (an endogenous precursor of melatonin), by natural neuroprotector Ginkgo biloba extract Egb 761, and some drugs (tacrine, memantine) that have been used for treatment of Alzheimer's disease and relative disorders. Our study illustrates that the effect on mitochondrial pores is an important aspect of the mechanism of neurotoxicity. Substances that may prevent the opening of mitochondrial pores induced by neurotoxins may preserve the mitochondrial function and, thus, may have a potential as neuroprotective agents. On the ground of this hypothesis the series of novel potential neuroprotectors have been designed and studied. The GLP preclinical trials of drug-candidate agent Dimebon was performed that approved its efficacy as protective agent against development of Alzheimer's type dementia.

1034 AMBIENT PARTICULATE MATTER STIMULATES OXIDATIVE STRESS IN BRAIN MICROGLIA AND DAMAGES NEURONS IN CULTURE.

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Ambient particulate matter (PM) damages biological targets through oxidative stress (OS) pathways. Several reports indicate that the brain is one of those targets. Since microglia (brain macrophage) are critical to OS-mediated neurodegeneration, their response to concentrated ambient particles (CAPs) collected from Tuxedo, NY was examined in culture. Immortalized, mouse microglial (BV2) were exposed to CAPs for various time points. Significant increases in pro-inflammatory cytokines and reductions of glutathione were measured in these cells. Up-regulation of genes coding for apoptotic and OS were recorded using Affymetrix microarrays. To assess neurotoxicity, immortalized rat, dopaminergic neurons (N27) were exposed to Fine (F=0.18-1 μ m) and Ultrafine (UF<0.18 μ m) PM also collected in Tuxedo, NY. Evidence of reduced nuclear materials, as measured by Hoechst 33258 stain occurred only in response to >12.5 μ g/ml UF and 50 μ g/ml F after 24 hr exposures. Primary cultures, dissociated from embryonic rat brain striatum were exposed to F (75 μ g/ml) and UF (7.5 μ g/ml) to determine if neurotoxicity was particle size-dependent in complex cultures. Equivalent increases of reactive nitrogen species (nitrite) were seen after 24hr in response to both size particles in spite of their 10-fold differences in concentration. A 39% and 47% reduction of immunohistochemically stained neurons relative to controls was measured in cultures treated with F and UF, respectively. Microscopic examination revealed that the majority of PM exposed neurons were apoptotic after 24hr exposure. Together, these data indicate that PM damages isolated neurons directly in the absence of microglia but also through microglia initiated apoptotic pathways in complex cultures. Disclaimer: This abstract has been reviewed by the NHEERL of the EPA and approved for publication.

1035 MPP⁺-INDUCED ALTERATIONS IN SELECTIVE GENE EXPRESSION CORRELATE WITH DOPAMINERGIC DEPLETION IN MN9D CELLS.

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Parkinson's disease (PD) is a common neurodegenerative disease characterized by progressive loss of midbrain dopaminergic neurons with unknown etiology. MPP⁺ (1-methyl-4-phenylpyridinium) is the toxic metabolite of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which can induce Parkinson's-like symptoms in humans and animals. MPTP/MPP⁺ can produce selective dopaminergic neuronal degeneration; therefore, these agents are commonly used to study the pathogenesis of PD. However, the mechanisms of their toxicity have not been completely elucidated. Recently, we reported in a microarray study using a murine midbrain-derived dopaminergic neuronal cell line, MN9D, that MPP⁺ induced significant changes in a number of genes associated with the dopaminergic system. In this study, we investigated the expression time-courses of six genes using real-time RT-PCR, and compared them with the progressive dopaminergic depletion caused by MPP⁺. Our data showed that the dopamine content was significantly decreased after 0.5 hr of MPP⁺ (200 μ M) exposure and was completely depleted after 40 hr. The expression of Gpr37, which is closely related to the pathogenesis of PD, was up-regulated after 0.5 hr, and remained up-regulated up to 48 hr. Txnip, which is critical to the adjustment of cellular redox status, was down-regulated after 1 hr and remained down-regulated up to 48 hr. Ldh1 and Cdo1, which are also involved in oxidative stress, were down-regulated after 16 hr and remained down-regulated up to 48 hr. Two pro-apoptotic genes, Egl3 and Bnip3, were down-regulated after 2 and 4 hr, and remained down-regulated up to 48 hr. These findings suggested that

multiple systems were involved in MPP⁺-induced dopaminergic neurodegeneration, and MPP⁺-induced neurotoxicity in MN9D cells could be used as a model to further explore the roles of these and other genes in the pathogenesis and possible treatment of PD.

1036 THE USE OF PRIMARY SUBSTANTIA NIGRA NEURONS GENERATED FROM VMAT2 HYPOMORPH MICE AS AN *IN VITRO* MODEL OF ASSESSING THE NEUROTOXICITY OF CYTOPLASMIC DOPAMINE.

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Neurodegenerative disorders such as Parkinson's disease (PD), is characterized by loss of tyrosine hydroxylase (TH) neurons in the substantia nigra. Although the precise mechanism of how dopaminergic neurons are lost remains unknown, it has been suggested that excess cytoplasmic dopamine (DA) may generate reactive oxygen species (ROS) and cause degeneration of DA neurons. It is widely accepted that the vesicular monoamine transporter (VMAT2), removes DA from the cytoplasm preventing its degradation, and subsequent production of ROS. In the present study, we generated primary substantia nigra chimeric cultures from wild type, heterozygote (expressing 50% VMAT2), and hypomorph (expressing 5% VMAT2) mice to evaluate the neurotoxicity of unsequestered DA. The nigra neurons were harvested from pups at P0-P6 and plated on a glia feeding layer one week before treatment with DA. The findings show that cultures treated with DA 10 μ M resulted in no loss of TH positive neurons in wild type and heterozygote mice, whereas there was a 33.4% loss of TH positive neurons in cultures generated from hypomorph mice. The preliminary results suggest that decreased sequestration of cytoplasmic DA promotes neurodegeneration of dopamine neurons. Studies are ongoing to assess the mechanism(s) of DA mediated neurotoxicity in this model.

1037 AGE-DEPENDENT HETEROGENEITY OF GENE EXPRESSION IN FISHER 344 RAT RETINA.

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Recent evidence suggests the elderly may be a sensitive subpopulation with regard to environmental exposure to toxic compounds. One source of this sensitivity within the aged subpopulation could be an enhanced variability in response to exposures. This variability, if sufficient, would require that a larger safety margin for risk management be applied to ensure protection of the entire population. Studies on phenotypic differences have suggested that variation increases with age. However, few reports address the question of variation in gene expression as an underlying cause for increased variability of phenotypic response in the aged. In this study, we compare variation in constitutive gene expression in the retinas of Fisher 344 rats at 3 different ages, 4, 11 and 23 mos (8-9 animals/age group). Global gene expression levels were determined on Affymetrix genomic rat 230A arrays. After removing trend effects of aging using an ANOVA (Analysis of Variance) model, the residuals were examined for evidence of increasing or decreasing variability with age using a variant of a standard approach to test for such trends in which the absolute values of the ANOVA residuals are regressed on age. This approach enabled application of Significance Analysis of Microarrays (SAM) procedure to identify genes for which there exists a significant age-dependent heterogeneity, as well as gene-specific estimations of false discovery rates (FDR) (q) values. Controlling the FDR at $\leq 10\%$, we found 269 genes in which variation in expression significantly increased with age, and 12 genes in which it decreased. These data support the hypothesis that gene expression levels can become heterogeneous with age, and serve as a proof-of-principle to identify genes for further study on the consequences of this variation. (This abstract does not necessarily reflect USEPA policy).

1038 VEGF DERIVED FROM RETINAL GLIAL (MULLER) CELLS IS AN IMPORTANT INITIATOR FOR DIABETIC RETINOPATHY.

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Yuehui Wang¹, Ying Leng², Ying Xin², Jiaxiang Wu², Lu Cai^{1,2}, University of Louisville School of Medicine, Louisville, KY and Jilin University, Changchun, China. Diabetic retinopathy (DR) is closely related to retinal neovascularization for which vascular endothelial growth factor (VEGF) plays a critical role. Muller cell as the principal glial cell of the retina is specialized radial glial cell spanning the entire

depth of the retina and may involve in both physiologic and pathologic angiogenesis. In the present study, we investigated whether Muller cells overproduce VEGF in response to high levels of glucose (HG) and whether the secreted VEGF can stimulate endothelial cells proliferation and migration. Muller cells were isolated from the retina of rabbits, and exposed to HG (30 mM) for 72 hr, and VEGF mRNA and protein expressions were found to be significantly increased. In the media of cultured Muller cells exposed to HG, there is a significant increase in VEGF contents, detected by ELISA assay. In order to define the role of VEGF derived from Muller cells, endothelial cells were cultured in the conditioned media that were harvested from HG-exposed Muller cells. These endothelial cells significantly proliferated and migrated in Boyden Migrating Chamber. Addition of anti-VEGF antibody attenuated the increased cell proliferation and migration of endothelial cells, specifying the role of VEGF. Furthermore, PI3K/Akt signaling pathways was found to involve in cell VEGF-stimulated endothelial cell proliferation and migration since endothelial cells cultured in the conditioned media from HG-exposed Muller cells with PI3K inhibitor wortmannin (50 nM), did not show any increase in cell proliferation and migration. These results suggest that VEGF derived from Muller cells under diabetic condition plays an important role in stimulating endothelial cells proliferation and migration through PI3K/Akt-dependent pathway, which is a critical step for the neovascularization in DR.

1039 MICROTUBULE-BASED AXONAL TRANSPORT IS IMPAIRED IN PRIMARY CULTURE OF HIPPOCAMPAL NEURONS FOLLOWING TREATMENT WITH THE NEUROTOXIC γ -DIKETONE 1, 2-DIACETYL BENZENE.

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The protein reactant 1,2-diacetylbenzene (1,2-DAB), but not the non-protein reactant isomer 1,3-DAB, induces proximal giant axonal swellings filled with 10 nm-neurofilaments and organelles in rodents. We asked whether changes in microtubule-based axonal transport contribute to the development of 1,2-DAB axonopathy. A model of rapid-onset aromatic γ -diketone axonopathy provided the substrate to address this question. Rat primary hippocampal neurons (7 days in vitro) were treated with 0.25 mM of either DAB isomer in HBSS imaging medium containing 0.3% DMSO (vehicle). Time-lapse recordings of moving mitochondria stained with MitoTracker Green FM (Molecular probes, Eugene) were taken every 10 min for up to 60 min. Transport events were measured using the kymograph function of the Metamorph software (Molecular Devices, PA). Both 1,2-DAB and 1,3-DAB induced early acceleration of mitochondrial transport; the average initial rate of moving mitochondria was 2.2 times higher (i.e. 0.24 μ m/sec) for 1,2-DAB treated samples ($p = 0.02$) and 1.6 times higher (i.e. 0.17 μ m/sec for 1,3-DAB ($p = 0.06$) relative to vehicle control. The rate decreased significantly over time (i.e. 11 % per 10 min) in 1,2-DAB- treated cultures ($p < 0.001$). Transport rates for vehicle- and 1,3-DAB treated samples remained steady ($p = 0.36$ and 0.85 for vehicle and 1,3-DAB, respectively). Changes in number of moving mitochondria revealed a pattern similar to the one observed for the transport rates. Studies are needed to elucidate susceptibilities of axon transport motor proteins (kinesin and dynein) to chemical attack by neurotoxic γ -diketones. Supported by NIEHS grants ES 10338 and ES 11384, NINDS NS 052183, and the Oregon Workers Benefit Fund.

1040 IMPAIRMENT OF CALCIUM HOMEOSTASIS: RELEVANCE TO THE PATHOGENESIS OF γ -DIKETONE AXONOPATHY.

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The neurotoxic protein reactant 1,2-diacetylbenzene (1,2-DAB), but not its isomer 1,3-DAB, induces an early segregation of cytoskeletal elements and mitochondria in rodent axons, suggesting that changes in axonal proteins may be the key to the pathogenesis of γ -diketone axonopathy. We used 7 day-old rat primary cultures of hippocampal neurons to assess whether disruption of intracellular Ca²⁺ homeostasis was temporally associated with the development of this type of neurotoxicity. Intracellular Ca²⁺ concentration was measured using fluorescence microscopy and the Ca²⁺-sensitive indicator Fura-2/AM at 0, 2, 4, 8, 24, and 48 hours following exposure to 1,2-DAB 250 μ M or vehicle (1% v/v of HBSS containing 0.3% DMSO). 1,2-DAB raised resting estimated intracellular Ca²⁺ concentration ([Ca²⁺]_i) in a time-dependent relationship requiring ~ 3.8 hours to reach half the maximal [Ca²⁺]_i response. At 24 hours, most 1,2-DAB-treated neurons had degraded; however, remaining neurons had a [Ca²⁺]_i $\pm$ SEM of 859 $\pm$ 184 nM ($n=6$), while vehicle-treated neurons remained at 102 $\pm$ 8 nM ($n=42$, $p \leq 0.0001$). [Ca²⁺]_i in vehicle-treated neurons at 24 hours did not differ significantly from controls 116 $\pm$ 5 nM ($n=104$). The number of neurons per low-power field (LPF) at the center

of each dish decreased over time in 1,2-DAB-treated cells. At 24 hours, there were only 2.0 ± 0.6 neurons/LPF in contrast to 14 ± 2.6 neurons for vehicle-treated cells ($P \leq 0.005$). Neuronal degradation was complete by 48 hours of 1,2-DAB treatment. 1,2-DAB markedly impaired calcium homeostasis, but several hours of treatment were required to alter resting $[Ca^{2+}]_i$ and membrane permeability significantly. This suggests the 1,2-DAB-induced effect on Ca^{2+} homeostasis may be a consequence of disruption of integral cellular processes rather than a direct effect of high intracellular calcium concentrations initiating cell death. Supported by NIH grants ES10338 and ES11384, NS052183, and the Oregon Workers Benefit Fund.

1041 EFFECTS OF MEMANTINE, GALANTAMINE AND DONEPEZIL IN THE HIPPOCAMPUS OF APP/PS1 MICE, AN ANIMAL MODEL OF ALZHEIMER'S DISEASE.

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Alzheimer's disease (AD) is a fatal neurodegenerative disorder characterized by progressive cognitive impairment. Although the cause of AD remains unknown, disease progression from mild to severe correlates well with incremental loss of nicotinic receptors in discrete brain areas. The pathophysiology of AD also includes increased brain levels of the amyloid β peptide 1-42 ($A\beta$ 1-42) that have been associated with mutations in the genes coding for presenilin 1 or 2 (PS1, PS2), and amyloid precursor protein (APP). Treatment of mild-to-moderate AD relies on the use of cholinesterase inhibitors, including galantamine and donepezil. More severe forms of the disease are treated with memantine, a non-competitive NMDA receptor antagonist. It has been recently suggested that patients with mild-to-moderate AD could benefit from treatment with both cholinesterase inhibitors and memantine. However, memantine at clinically relevant concentrations inhibits $\alpha 7$ nAChR activity. Thus, this study was designed to analyze in vivo how the association of memantine with galantamine or donepezil alters neuronal functions, structure and viability in mice overexpressing mutant forms of the human APP/PS1 mice. We are studying mice at two specific ages: 2 and 9 months. At 2 months of age, the transgenic mice compared to wild type mice show impairment in long-term potentiation (LTP). At 9 months of age, the transgenic mice show impairments of both LTP and basal synaptic transmission. We are using the patch-clamp technique to identify the effects of long-term treatment with clinically relevant doses of memantine, donepezil and galantamine (alone or in association) on basal synaptic transmission, tonic GABAergic and glutamatergic activities and LTP in the hippocampi of APP/PS1 mice and age-matched wild type mice. Results from this study should lay the groundwork for a better understanding of the benefits of specific drug associations for treatment of AD at different stages. Support: NIH NS25296 & Ortho-McNeil GAL-ALZ-0004

1042 INDUCTION OF APOPTOSIS IN NEURONAL PC-12 CELLS BY SATRATOXIN G FROM THE BLACK MOLD STACHYBOTRYS: ROLE OF DOUBLE-STRANDED RNA-ACTIVATED PROTEIN KINASE (PKR).

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Satratoxin G (SG) is a macrocyclic trichothecene mycotoxin produced by the black mold *Stachybotrys chartarum* which has been etiologically linked to illnesses associated with water-damaged buildings. Acute SG exposure specifically induced apoptosis of olfactory sensory neurons (OSN) in the olfactory epithelium (OE) of mouse nose. PC-12 neuronal cells were used here as a model to elucidate the mechanisms of SG-induced cell death. SG-induced apoptosis of PC-12 cell was verified by agarose gel electrophoresis, flow cytometry using Annexin V-FITC and propidium iodide uptake and by microscopic morphology. Kinetic studies revealed that apoptosis was maximum between 48 to 96 h after SG (10 ng/ml) treatment. Expression of the pro-apoptotic genes p53, PKR, BAX and CAD were significantly elevated 6 and 18 h after SG treatment. SG also induced significant caspase-3 activity from 18 to 48 h. Chemical inhibition of PKR completely inhibited SG induction of apoptosis and pro-apoptotic gene mRNA expression. Surprisingly, PKR inhibition did not inhibit SG-induced caspase-3 activation, nor did caspase-3 inhibition block SG-induced apoptosis. However, mRNA expression for two alternative nucleases associated with apoptosis, DNase I and DNase II, were also found significantly elevated in SG-treated PC-12 cells and these could be blocked by PKR inhibition. Taken together, PKR appears to play the critical role in SG-induced DNase induction and resultant apoptosis in PC-12 cells.

1043 CELLULAR PRION PROTEIN PREVENTS STAUROSPORINE-INDUCED CYTOTOXICITY VIA PI3K/AKT PATHWAY IN NERVE CELLS.

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Cellular prion protein (PrPc) plays a protective role in neuronal cells, but the function of PrPc is not yet understood. Our recent findings suggest that PrPc specifically prevents pathological protein aggregation in only neuronal cells, but not in non-neuronal cells. The purpose of this study was to elucidate the protective role of PrPc in neuron cells and non-neuronal cells during staurosporine (STP) induced cell death. In SN56 cells, prion depletion by RNAi treatment caused an increase in the number of apoptotic cells during treatment of STP. In contrast, when non-neuronal Caco2 cells that normally express prion, were depleted of prion, there was no effect. We found that in prion-depleted SN56 cells there was a decrease in PI3K/AKT phosphorylation, but there was no similar effect in prion-depleted Caco2 cells. Therefore, this data suggest that PrPc may specifically protect against STP-induced toxicity only in neuronal (SN56) cells but not in non-neuronal (Caco-2) cells. The enhanced apoptosis induced by STS was shown by increased p38 and JNK phosphorylation. Therefore, we checked p38 and JNK activity in both neuronal and non neuronal prion-depleted cells. As a result, activation of p38 was significantly increased in prion depleted STP-induced SN56 cells, compared to the control cells. But there was no similar effect in prion-depleted Caco2 cells. As p38 and JNK activation involves Bax mitochondrial translocation, cytochrome c release and caspase activation, we investigated which of these events was inhibited by PrP in SN56 cells and Caco-2 cells. PrP inhibits Bax mitochondrial translocation, cytochrome c release and cell death in SN56 cells but not Caco-2 cells. Moreover, PrPc in SN56 cells does not prevent AKT non-involved induced apoptosis, such as active caspase-mediated cell death or Bak induced apoptosis. Overall, we conclude that PrP protects against STP-mediated cell death via PI3K/AKT pathway that blocks initially in p38 and JNK activation in neuronal cells.

1044 NEUROTOXICITY OF METHYL MERCURY AND PCBs: AN IN VITRO APPROACH.

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Methyl mercury (MeHg) and polychlorinated biphenyls (PCBs) are known developmental neurotoxicants. Aim of this study was to evaluate the effect of MeHg (0.1-20 μ M) and PCB126 and 153 (3-200 μ M) in a battery of in vitro cell systems. Cell viability was assessed by measuring MTT reduction and Trypan blue exclusion. The studies were carried out in eight different rat or human cell lines chosen as models of neuronal cells, astrocytic cells and non-nervous-system cells. Primary rat astrocytes and neurons from different brain areas (hippocampus, cortex, cerebellum and brainstem), and cerebellar granule neurons from a mouse genetic model of glutathione deficiency were also utilized. The results indicate that: 1) MeHg is more cytotoxic than PCB126 and PCB153; 2) Nervous system-derived cell lines are in most cases more sensitive than non-nervous cell lines to all compounds; 3) Neuronal cells are more sensitive than astrocytic cells to the toxicity of MeHg, but not of PCBs; 4) Human CNS cells are slightly more sensitive to the toxicity of MeHg and PCBs than rat cells; 5) Primary neurons were the most sensitive to MeHg and PCBs toxicity; 6) Cerebellar neurons from mice with genetically determined low glutathione levels were more sensitive than wild-type neurons to the toxicity of all three compounds, supporting a role for oxidative stress in their cytotoxicity; 7) Thyroid cells (TT), non cancerous prostate cells (PZ-HPV-7), and cerebellar Purkinje neurons were highly sensitive to the toxicity of PCB126. PZ-HPV-7 cells were also highly sensitive to the toxicity of PCB153. Epidemiological and laboratory studies have also suggested that MeHg and PCBs may have synergistic effect on CNS functions; however, in vitro studies in various cell lines indicated an additive effect, at most. This study provides initial evidence that an in vitro screen may provide information useful for assessing neurotoxicity, as well as information on potential new targets of biological action or toxicity. [Supported in part by PRIN 2004 and DEVNERTOX-CONTRACT n°FOOD-CT-2003-506143]

1045 PCB-INDUCED ALTERATIONS IN THE SEROTONERGIC SYSTEM: A POTENTIAL MECHANISM OF PCB-INDUCED IMMUNOTOXICITY?

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Toxicological studies have shown the serotonergic system to be sensitive to polychlorinated biphenyl (PCB) exposure in both mammals and fish, and epidemiological studies have demonstrated similar findings for humans. Likewise, the sensitivity of the immune system to PCBs has been well established in numerous species.

Given the cross-talk between the immune and nervous systems, PCB-induced alterations in the serotonergic axis is a plausible mechanism by which PCBs may induce their immunotoxic effects. Previous investigations in this lab have demonstrated the sensitivity of the bluegill sunfish immune response to PCB exposure as well as to serotonin (5-HT) – induced alterations in immune function. In order to establish a link between PCB exposure and alterations in the serotonergic system in this species, the impact of PCBs on 5-HT levels, synthesis and metabolism were evaluated. Fish were exposed via i.p. injection to either coplanar PCB 126 (0.01 or 1.0 µg/g BW) or noncoplanar PCB 153 (5.0 or 50 µg/g BW) and sacrificed after 1, 3 or 7 d. Analysis by HPLC demonstrated that both PCB congeners (at all doses) decreased whole brain 5-HT and norepinephrine levels at 3 d post-injection. However, no effects were observed on whole brain tryptophan hydroxylase level (the rate-limiting enzyme in 5-HT synthesis) or on monoamine oxidase activity (the primary enzyme involved in the metabolism of 5-HT) in PCB-exposed fish at any post-injection time point. Results from this study show that while both coplanar and noncoplanar PCBs can alter whole brain levels of 5-HT, this effect does not appear to be due to alterations in neurotransmitter synthesis or metabolism. Taken together with results from previous studies demonstrating immune dysfunction in bluegill with depressed brain 5-HT levels, the PCB-induced decrease in brain 5-HT levels observed in the current study supports a role for 5-HT in PCB-induced immunotoxicity. Hudson River Foundation Graduate Fellowship and USACEHR No. DAMD 17-99-9011.

1046 POLYCHLORINATED BIPHENYL-MEDIATED LOSS OF STRIATAL DOPAMINE TERMINAL MARKERS: POSSIBLE ROLE OF VESICULAR MONOAMINE TRANSPORTER 2 INHIBITION.

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Epidemiological and laboratory studies have identified exposure to polychlorinated biphenyls (PCBs) as a potential risk factor for Parkinson's disease. Previously, our lab has shown that environmentally relevant exposures of PCBs (7.5 or 15 mg/kg/day Aroclor 1254:1260 for 30 days by oral gavage) cause a dramatic dose-dependent decrease in dopamine transporter (DAT) levels. These reductions were specific to striatal DAT, and no change in the level of dopamine (DA) or tyrosine hydroxylase (TH) was observed. To further investigate the potential mechanism of PCB-mediated disruption of the DA system, mice were given 50 mg/kg/day (for 30 days oral gavage) of the Aroclor 1254:1260 mixture. Analysis of dopaminergic brain regions revealed a 60% loss of DAT levels and function. Immunohistochemistry showed reduced staining in DAT and TH. We have hypothesized that these changes may be due to inhibition of the vesicular monoamine transporter 2 (VMAT2). Aroclor and its primary metabolites found in the brain were discovered to be direct inhibitors of VMAT2. Additionally, total DA levels are reduced in the striatum and cortex of the PCB animals. These data further support the suggestion that PCBs damage the nigrostriatal system, leading to the disruption of DA homeostasis and subsequent cell death.

1047 1 α , 25-DIHYDROXYVITAMIN D3 BLOCKS CYANIDE-INDUCED NEUROTOXICITY BY ALTERING PPAR α -MEDIATED UCP-2 EXPRESSION.

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1 α , 25-dihydroxyvitamin D3 (VD3) is an active metabolite of vitamin D. Recent studies have shown that VD3 is a neuroprotectant that can attenuate 6-hydroxydopamine and L-buthionine sulfoximine (BSO)-induced toxicity. At the mitochondrial level, VD3 inhibits uncoupling protein 2 (UCP-2) expression leading to increased mitochondrial membrane potential ($\Delta\psi$ m) and ATP production. We have shown that Wy14,643, a selective agonist of peroxisome proliferator activated receptor alpha (PPAR α), enhances cyanide neurotoxicity through induction of UCP-2 expression. In this study VD3-mediated neuroprotection was evaluated in cortical cells exposed to Wy14,643 to stimulate UCP-2 overexpression and enhance cyanide toxicity. Pretreatment with VD3 attenuated Wy14,643-mediated enhancement of cyanide-induced necrotic death. VD3 reversed cyanide-induced mitochondrial dysfunction as reflected by restoration of cellular ATP and $\Delta\psi$ m. To investigate the mechanism of the neuroprotection, UCP-2 expression was determined. VD3 blocked Wy14,643-mediated upregulation of UCP-2 at both mRNA and protein levels. This effect was the result of increased IkB expression, which inhibits

NF- κ B activity. Co-treatment with Wy14,643 and cyanide significantly reduced cellular GSH levels and enhanced ROS generation; these effects were also blocked by VD3. To determine the functional activity of UCP-2, the mitochondrial proton leak, as reflected by state 4 cellular oxygen consumption, was measured by a Clark oxygen electrode. Cyanide markedly increased state 4 oxygen consumption in Wy14,643 treated cells, possibly due to cyanide stimulating formation of superoxide and thus activating UCP-2. Pretreatment with VD3 or other antioxidants attenuated the enhancement of state 4 oxygen consumption. It is concluded that VD3 blocks Wy14,643-mediated enhancement of cyanide neurotoxicity by inhibiting UCP-2 upregulation and the subsequent activation of mitochondrial proton leak. (This study was supported by NIH grant ES04140).

1048 UP-REGULATION OF UNCOUPLING PROTEIN-2 BY WY14, 643 IN DOPAMINERGIC CELLS INVOLVES PPAR ALPHA-DEPENDENT AND INDEPENDENT MECHANISMS.

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Uncoupling protein 2 (UCP-2) is a member of the mitochondrial anion carrier superfamily expressed in the inner mitochondrial membrane. We have shown that up-regulation of UCP-2 expression by treatment with Wy14,643, a selective agonist of PPAR α , increased vulnerability of primary cortical cells to cyanide-induced cytotoxicity. In this study, the mechanism(s) by which Wy14,643 induces up-regulation of UCP-2 in an immortalized dopaminergic cell line (N27 cells) was determined. We have established that Wy14,643 potentiates cyanide-induced cell death through up-regulation of UCP-2. Western blot analysis showed that either treatment of cells with MK-886 (a selective antagonist of PPAR α) or RNAi-mediated knock-down of PPAR α only partially decreased the Wy14,643-mediated up-regulation of UCP-2, thus indicating the possible involvement of a non-PPAR α pathway. Wy14,643 treatment also decreased cellular glutathione (GSH) levels that was accompanied by accumulation of reactive oxygen species (ROS). Supplement of cellular GSH by loading with glutathione ethyl ester (GSH-EE) or pre-incubation with the antioxidant α -tocopherol (Vitamin E) attenuated the Wy14,643 mediated up-regulation of UCP-2. Knockdown of PPAR α by RNAi did not alter the Wy14,643-mediated ROS generation, indicating ROS generation was independent of PPAR α . Co-treatment with PPAR α -RNAi and GSH-EE blocked both Wy14,643-upregulated UCP-2 and potentiation of cyanide cytotoxicity. These findings demonstrate that both PPAR α -dependent and -independent mechanisms are involved in Wy14,643-mediated up-regulation of UCP-2 and the associated increased vulnerability of dopaminergic cells to cyanide. (This study was supported by NIH grant ES04140)

1049 MECHANISTIC ACTIONS OF THE HIV-1 NEUROTOXIN TAT ON NEURONAL MITOCHONDRIA.

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HIV Associated Dementia (HAD) is a progressive neurological disorder that results in motor, cognitive and social dysfunction. Recent epidemiologic studies suggest that HAD incidence has increased in prevalence since 2000, even in the era of highly active antiretroviral therapy. However, evidence suggests that some aspects of HAD may be reversible. In a previous study, we observed mitochondrial hyperpolarization in cortical neurons treated with the HIV-1 neurotoxin Tat. Mitochondrial hyperpolarization may have profound consequences for synaptic transmission and other energy-dependent processes. The present study investigates the mechanism(s) leading to mitochondrial hyperpolarization, focusing on several mitochondrial systems including: pH, electron transport chain (ETC) complex activity, ion homeostasis, and energy intermediates (NADH). We confirmed our earlier observation that rodent cortical neurons treated with Tat undergo mitochondrial hyperpolarization using the dye rhod123. Application of Tat to cortical neurons elicited a rapid drop in internal mitochondrial pH in concordance with hyperpolarization. The addition of Tat to purified mitochondrial protein not only demonstrated an inhibition of complex IV of the electron transport chain, but also decreased cortical neuron respiration, corroborating the physiological inhibition of complex IV. Using a mitochondrial targeted YFP-calmodulin construct, we observed a decreased in [Ca²⁺]_{mit}. Finally, we measured the energy intermediate NAD(P)H after Tat treatment, and found a 20% decrease in the auto-fluorescence. We speculate that the decrease in calcium and NADH contributes to mitochondrial hyperpolarization after exposure to Tat. Future studies will focus on a detailed examination of the molecular mechanisms involved in these observed mitochondrial deficits and novel treatments to attenuate the hyperpolarization. Support Contributed by: RO1 MH56838, PO1 MH64570, MH071176, PO1NS31492, ES07026

1050 EFFECTS ON DOPAMINE (DA) AND SEROTONIN (5-HT) METABOLISM FOLLOWING AN ESCALATING DOSE-BINGE REGIMEN OF METHAMPHETAMINE.

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Methamphetamine (METH) is an illicit drug that is experiencing an alarmingly high rate of abuse in the United States and Asia. Initially, METH use is associated with feelings of euphoria to low doses of the drug. Subsequently, users administer the drug at higher doses and at more frequent intervals in attempts to maintain these pleasurable effects. In the present study, we utilized an escalating dose (ED)-binge regimen of METH administration in rodents in order to mimic human drug use patterns. Male Sprague-Dawley rats were given either an ED of METH or an equivalent volume of saline. At the end of the pretreatment regimen, each group was divided again such that half of the animals received either a neurotoxic dose of METH (3x10 mg/kg every 2 hr) or saline. We have previously shown that animals receiving the neurotoxic dose of METH had decreased levels of DA and 5-HT in the striatum at 2 hr following the final treatment. Moreover, there was less depletion of DA in animals that were given the METH ED regimen relative to drug-naïve animals receiving only a METH challenge. Levels recovered significantly by 24 hr. For this study, we approximated the metabolism of DA and 5-HT by calculating ratios with regard to their respective metabolites (DOPAC+HVA and 5-HIAA). This approach would determine whether alterations in their metabolism might account for the protective effects of the repeated METH pre-exposure. At 2 hr following the challenge dose, DA and 5-HT turnover rates are significantly increased in the naïve-challenged animals versus the METH pretreated-challenged rats. However, at 24 hr post-challenge, only drug-naïve animals had significant decreases in both DA and 5-HT turnover rates following the METH challenge. These data suggest that changes in monoamine metabolism following a METH pretreatment may be responsible for the some of the neurochemical changes seen in DA and 5-HT following an ED-binge regimen of METH.

1051 ESTRADIOL AND PHYTOESTROGENS DO NOT ATTENUATE THE INCREASE IN INTRACELLULAR CALCIUM CAUSED BY AMYLOID-BETA PEPTIDE AND MPP+.

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Published reports demonstrated that estrogen can protect against amyloid-beta (AB) and 1-methyl-4-phenyl-pyridine (MPP+)-induced cellular death. We investigated MPP+ and AB toxicity in SH-SY5Y cells and their ability to disrupt intracellular Ca²⁺ regulation. Also investigated was possible neuroprotection resulting from pretreatment with estrogen and phytoestrogens. Phytoestrogens, compounds that resemble estrogens structurally, have attracted interest as potential drugs for the treatment of neurodegenerative diseases. MPP+ (2 uM for 48 hr) caused approximately 50% death of SH-SY5Y cells as measured by lactate dehydrogenase (LDH) release. Pretreatment (24 hr) with estradiol and the phytoestrogens apigenin, coumestrol, daidzein, equol, formononetin, genistein, quercetin and resveratrol was not protective. MPP+ (100 uM) caused a rapid 24% increase in Ca²⁺ as measured with the Fluo-4 NW calcium assay kit. Pretreatment (24 hr) with estradiol (0.1 to 10 uM) or the above phytoestrogens (up to 5 uM) did not reduce this Ca²⁺ influx. In fact, 5 uM formononetin increased Ca²⁺ influx significantly (p<0.01). AB at concentrations up to 200 uM did not induce increased LDH release. AB did not cause a rapid increase in Ca²⁺ but 3-4 hr pretreatment with AB (50 uM) increased the Ca²⁺ influx caused by carbachol (200 nM) by an additional 16%. Pretreatment with estradiol and the phytoestrogens had no effect on this Ca²⁺ influx. Pretreatment with the L-type Ca²⁺ channel blocker, nifedipine (100 uM), however, significantly reduced the Ca²⁺ influx caused by MPP+ and AB. We can conclude from these experiments that estradiol and the tested phytoestrogens are not protective against certain neurotoxic insults of MPP+ in the SH-SY5Y cell line, and also did not attenuate the increase in Ca²⁺ caused by exposure to AB and MPP+.

1052 CYTOTOXICITY OF CYCLOTTRIMETHYLENETRINITRAMINE (RDX) IN RAT AND HUMAN PRIMARY NEURONAL CELLS CULTURES.

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Cyclotrimethylenetrinitramine (RDX, an explosive and a contaminant at certain disposal facilities and training ranges, has been found to produce convulsions and tremors in animals and in human, suggesting potential neurotoxicity. We report

here the in vitro cytotoxicity of RDX towards primary rat and human astrocyte cultures. Primary rat and human astrocytes were plated at approximately 5000 cells/well and cultured for 24-hours before treatment with RDX. The treatment period was 24-hours. Cellular ATP content was used as a cytotoxicity endpoint. In the first experiment, primary rat and human astrocytes were treated with RDX in complete growth medium. No cytotoxicity was observed up to the highest concentration evaluated of 495 uM. In a second experiment, the cells were treated with RDX in medium with or without serum. Apparent RDX cytotoxicity was observed for rat astrocytes in medium without serum, with >50% cell killing observed at the lowest concentration evaluated of 7.81 uM up to 62.5 uM and no cytotoxicity observed at the higher insoluble concentrations of 125 and 250 uM. As observed in the first experiment, no apparent cytotoxicity was observed for rat astrocytes in medium with serum. For human astrocytes, however, no cytotoxicity was observed in medium with or without serum. To evaluate the role of hepatic metabolism on RDX toxicity, a third experiment was performed to evaluate the role of an exogenous activation system, Aroclor-1254-induced rat liver S9, on RDX cytotoxicity. The exogenous S9 apparently increased the cytotoxicity of RDX in human astrocytes when treatment was performed in medium without serum. The results suggest that RDX toxicity may be attenuated by protein binding, and that hepatic metabolism may lead to the generation of toxic metabolites. These observations need to be further substantiated and defined to evaluate their physiological relevance (Abstract does not reflect U.S. Army Policy).

1053 EFFECTS OF PROPOFOL ON INTRACELLULAR CA²⁺ HOMEOSTASIS IN HUMAN ASTROCYTOMA CELLS.

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The effects of propofol, a short-acting general anesthetic, upon cell growth and Ca²⁺ signaling in a human astrocytic cell line (CCF-STTG1) were examined. Exposure of cells for 24 to 72 hr to graded concentrations of propofol resulted in a dose-dependent decrease in cell number with an inhibitory concentration of cell viability of 31.7 ± 1.2 uM. To evaluate the changes in intracellular Ca²⁺ homeostasis induced by propofol, cytoplasmic and mitochondrial Ca²⁺ were measured. Mitochondrial Ca²⁺ increased significantly at a propofol concentration lower than the IC₅₀ (10 uM for 24 hr, 1 uM for 72 hr) while cytoplasmic Ca²⁺ decreased significantly at the same concentrations. In addition, propofol diminished the Ca²⁺ response induced by addition of 10% fetal bovine serum to cells. To determine the source of the Ca²⁺ alterations induced by propofol, several pharmacologic agents that target intracellular Ca²⁺ homeostasis mechanisms were used. Nifedipine, an L-type Ca²⁺ channel blocker and estrogen, which is known to activate K⁺ channels, decreased the FBS-induced Ca²⁺ response of control cells to a level similar to propofol treated cells. However, diazoxide (a K⁺-ATP channel opener) administered 1 hr directly before FBS addition restored the FBS response in propofol treated cells to a level similar to control cells. In addition, diazoxide increased mitochondrial Ca²⁺ in control cells to a level comparable to propofol treated cells suggesting the activation of these channels by propofol treatment. Addition of 1 uM RU-360 (a selective blocker of the mitochondrial Ca²⁺ uniporter) for 30 min prior to propofol treatment had a minimal effect on control cells but significantly decreased the mitochondrial Ca²⁺ and increased the cytoplasmic Ca²⁺ in propofol-treated cells to control levels. These data suggest that voltage operated Ca²⁺ channels, mitochondrial Ca²⁺ and ATP-dependent K⁺ channels may be targets of propofol action in CCF cells and reversal of these actions result from treatment with K⁺-ATP channel activator and mitochondrial Ca²⁺ uniporter blocker.

1054 INITIATION OF APOPTOSIS AFTER 1, 3-DINITROBENZENE EXPOSURE IN IMMORTALIZED PRIMARY ASTROCYTES.

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Dinitrobenzene (1,3-DNB) is a neurotoxic compound shown to preferentially affect astrocytes within the auditory and vestibular nuclei. Although the exact mechanism of neuropathology is unknown, the lesion mirrors a group of compounds and metabolic disorders classified as Acute Energy Deprivation Syndromes (AEDS). In primary astrocytes, 1,3-DNB inhibits key metabolic enzymes and induces the mitochondrial permeability transition (MPT). We investigated the progression of 1,3-DNB-mediated cell death in order to better understand the pathological timeline and apoptotic mechanisms associated with this compound. Immortalized primary astrocytes (DITNC1) were exposed for various time points to 500uM 1,3-DNB with approximately 80% cell death occurring after 48h. Prior to death (24-48h), extracellular phosphatidylserine staining and DNA condensation were observed by fluorescence microscopy. Western blotting indicated caspase 9 and caspase 3 activation was apparent after 12h exposure. In order to determine if caspase activation was mitochondria dependent, cells were cotreated with 1,3-DNB

and MPT inhibitors (bongkreic acid (BA); cyclosporin A (CsA)) for 24h. Exposure to 0.25% dimethyl sulfoxide (DMSO), 10 μ M BA, and 1 μ M CsA alone showed no apparent activation of caspase 9 or 3. In contrast, cotreatment of 1,3-DNB with either MPT inhibitor reduced caspase 9 activation but significantly increased caspase 3 activation. Although caspase 9 appears to play a role in 1,3-DNB induced glial toxicity, this data suggests that it is not the primary initiating pathway. Further studies are necessary to determine if enhanced caspase 3 activation is due to effects independent of MPT inhibition such as metabolic impairment or whether induction of MPT may mediate protection in 1,3-dinitrobenzene-induced neurotoxicity. This work was supported by NIH T32 ES07062 and RO1 ES08846.

1055 ADDITIONAL INFORMATION FAVORING A NOVEL RECEPTOR ASSOCIATED WITH VOLTAGE-GATED SODIUM CHANNELS.

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Voltage-gated sodium channels (VGSC) are responsible for action potential initiation and propagation in excitable cells including neurons of the central nervous system. Typical VGSC are comprised of an α -subunit and 2 β -subunits. All known pharmacological agents that act on VGSC have receptor sites on the α -subunits. At least six distinct sites for neurotoxins and one for local anesthetics and related drugs have been identified (Cestele and Catterall 2000).

We presented preliminary results suggesting the presence of a novel receptor associated with VGSC characterized by the binding of an antitoxin, brevenal, produced by the dinoflagellate *Karenia brevis*, also known to make the site 5-specific brevetoxins (PbTx)(Gold *et al.*, 2005). Both unlabeled brevenal and the aldehyde-reduced brevenol displaced tritiated brevenol (³H-B) in rat brain synaptosomes but PbTx does not. Here, we further examine the basic characteristics of receptor identification using tritiated receptor binding experiments to support our findings. To show saturation of specific brevenal receptors, ³H-B was bound to rat brain synaptosomes in the presence and absence of brevenal/brevenol. Saturation and distribution of receptors were studied in synaptosomal preparations made of whole brain, cerebrum and cerebellum. Results indicate binding maxima (B_{max}) or the maximum number of binding sites and equilibrium dissociation constants (K_D), the concentration at which half the receptors are occupied, for the whole brain to be lower than those for the cerebrum and cerebellum, respectively. Finally, displacement studies involving ³H-B against several competitors including tetrodotoxin (site 1 neurotoxin), veratridine (site 2 neurotoxin), *Centruroides sculpturatus* venom (site 4 neurotoxin source), aconitine (site 2) and amiloride (inhibitor of sodium transport) demonstrate no inhibition. These findings indicate that ³H-brevenol does not bind to any of the tested receptor sites on VGSC and suggest a novel uncharacterized site on VGSC.

1056 IN VIVO ACRYLAMIDE EXPOSURE PRODUCES CUMULATIVE ADDUCTION OF SYNAPTIC PROTEIN THIOLS.

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We have previously demonstrated that in vivo acrylamide (ACR) intoxication results in the formation of adducts on cysteine residues of synaptic proteins. We have also demonstrated that synaptic protein thiol adduct burden correlates with motor dysfunction and decreased neurotransmitter uptake and release. Using Isotope Coded Affinity Tag (ICAT) labeling, we examined the synaptic proteome of rats treated orally with ACR (21mg/kg/day) for 7, 14 or 21 days. During this time rats progress to slight motor impairment. After 21 days of intoxication, the abundance of most cysteine containing peptides is similar, but more than 35 proteins exhibit at least a 25% reduction in available cysteines in treated rats. Many of these proteins are critical to normal synaptic function including N-ethylmaleimide sensitive factor (NSF), synaptophysin, synaptotagmin, syntaxin-7, complexin-2 and vATPase. Because reduced ICAT incorporation can occur as a result of decreased protein abundance as well as adduct formation, we used multidimensional LC/MS/MS to demonstrate ACR adducts on affected cysteines, strongly suggesting that reduced ICAT incorporation is due to ACR adduction. Differential effects of ACR treatment were observed on individual cysteines within a protein, indicating specificity of adduct formation. Because ACR is a relatively weak electrophile, adducts form primarily on protein thiol groups with high nucleophilic reactivity due to their local environment. In glutamine synthase, Cys346 labeling is reduced by more than 55% in ACR treated animals while Cys99 is only slightly reduced. Because ACR toxicity is a cumulative process, we examined changes in ICAT incorporation over time. Of the set of proteins common to all samples, some proteins exhibited cumulative changes, including syntaxin-7, VDAC and NSF. These proteins are likely critical targets of ACR toxicity in the nerve terminal. Supported by NIEHS grant ESO3830-20.

1057 LUMO ENERGIES PREDICT THE IN VITRO NEUROTOXIC POTENCIES OF TYPE-2 CONJUGATED ALKENES.

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Acrylamide (ACR) is a conjugated Type-2 alkene and a human neurotoxicant. Because pi electrons in a conjugated system are highly polarizable, ACR is a soft electrophile and will form adducts with soft biological nucleophiles, primarily sulfur. In a study of structure-toxicity relationships we showed that ACR and other Type-2 alkenes; e.g., acrolein (ACL), methylvinyl ketone (MVK), produced in vitro neurotoxicity (synaptosomal dysfunction) mediated by thiol adduct formation (LoPachin *et al.*, 2006). The rank order of neurotoxic potencies (ACL>MVK>ACR) suggested differences in softness among these conjugated carbonyl compounds. Reactions between soft electrophiles and soft nucleophiles is governed by frontier molecular orbital (FMO) interactions and thus, alkenes with low energy FMO's should more readily undergo adduct formation by accepting electrons from soft nucleophiles like thiols. Since the relevant FMO for electrophiles is the lowest unoccupied molecular orbital (LUMO), we calculated LUMO energies of the Type-2 alkenes using the SPARTAN Molecular Modeling program (Wavefunction, Inc). Results show that for each chemical in a series of Type-2 conjugated alkenes the respective LUMO energies were directly correlated to the corresponding IC50's for inhibition of synaptosomal transmitter uptake and sulfhydryl loss. In contrast, non-conjugated alkene or aldehyde analogs had higher LUMO energies and did not affect synaptosomal function or thiol content. Thus, the relative softness of conjugated alkenes is directly related to respective in vitro neurotoxic potencies. This suggests that LUMO energy calculations might be used to identify Type-2 alkenes with neurotoxic potential. Such screening is important because: 1) our findings indicate that, conjugated alkenes, as a chemical class, might produce neurotoxicity via a common mechanism involving thiol adduct formation and presynaptic toxicity and, 2) the neurotoxic risks associated with human Type-2 alkene exposure have been poorly delineated. (Sponsored by NIEHS ESO3830-20).

1058 CHARACTERIZATION OF THE N27-D_{2L} CELL LINE: ASSESSMENT AS A VIABLE CELL MODEL FOR INVESTIGATING DOPAMINERGIC D_{2L} RECEPTOR-COUPLED FUNCTIONS.

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Currently there is no dopaminergic cell model that genuinely reflects typical dopaminergic neurons as they would be found in the CNS. This is an impediment to many lines of research, including understanding of the functional mechanisms of action of intrinsic dopamine D₂ receptors. In this study we assess the characteristics of the N27 cell line in order to determine its utility as such a model system. First, we verified previous reports that this cell line synthesized dopamine and endogenously expressed the dopamine transporter. Using radioligand-based competition isotherms it was determined that the N27 cell line does not endogenously express any type of dopamine receptor. A FLAG-tagged hD_{2L} receptor was stably transfected into the cell line, and was found to couple to adenylate cyclase inhibition (a G $\alpha_{i/o}$ -mediated functional response). Real time PCR revealed the endogenous expression of a number of G protein isoforms as well as low levels of tyrosine hydroxylase and dopamine transporter. The cells, however, failed to show D_{2L}-mediated effects on dopamine uptake or release assessed using biochemical and electrochemical methods. In addition, agonist-induced D_{2L} receptor internalization also did not occur. The ability of this cell line to synthesize dopamine has made it a viable model for studying certain questions regarding the mechanisms that underlie the effects of neurotoxin activity, oxidative stress, ischemia and cell death in dopaminergic cells. It is our assessment, however, that this cell line is not a practical in vitro cell model for the study of dopaminergic function as it is regulated by D₂ receptors.

1059 LINKING REGULATORY TOXICOLOGICAL INFORMATION ON ENVIRONMENTAL CHEMICALS WITH HIGH-THROUGHPUT SCREENING (HTS) AND GENOMIC DATA.

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The application of high-throughput screening (HTS) and genomic technologies to environmental chemical classification and prioritization requires reference toxicological information for characterizing the associations between toxicological properties and HTS or genomic profiles. Regulatory toxicological data has been produced for thousands of environmental chemicals, and at EPA are used primarily in

hazard characterization and risk assessment of individual chemicals. In order to extend the use of this vast legacy toxicological information, the Toxicological Reference Database (ToxRefDB) captures toxicological endpoints, critical effects and relevant dose-response data from EPA Program Office evaluations in a relational database using a standardized vocabulary. Initially, ToxRefDB is being populated primarily with data for pesticide active chemicals. ToxRefDB provides the ability to cluster and group chemicals based on toxicological outcomes specific to study type, target organ, or effect categories (e.g., tumorigenicity). In addition, ToxRefDB will facilitate ranking of chemicals by relative potency based on specific endpoints, or grouping of chemicals based on mode or mechanism of action. These toxicity-based groupings can then be used to identify predictive HTS bioactivity profiles and genomic signatures. Thus ToxRefDB will be an essential computational tool providing an interpretive context for HTS and genomic data generated in EPA's ToxCast chemical prioritization program. This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy.

1060 THE USE OF NTP-HTS DATA FOR PREDICTIVE COMPUTATIONAL TOXICOLOGY.

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To explore efficient approaches to assessing the toxicity of environmental chemicals, the NIEHS National Toxicology Program (NTP) recently initiated a High Throughput Screening (HTS) Project. To date, HTS results for a set of 1,408 compounds tested in 6 cell viability assays have been released via PubChem. We explore herein the utility of the HTS data for predicting animal and human toxicity. Initially, we asked if these HTS results could enhance prediction models for rodent carcinogenicity. The Carcinogenic Potency Database (CPDB) includes 383 of the NTP-HTS compounds. Within this subset, HTS-actives have a relatively high correlation with rodent carcinogens (sensitivity 77%); however, HTS-inactives are less informative (specificity 30%). QSAR approaches were applied to 1,289 of the HTS compounds (230 actives and 1,059 inactives). External validation set consisted of 429 randomly excluded agents. The remaining 860 compounds were split into diverse training and test sets. A classification k Nearest Neighbor QSAR approach gave models with prediction accuracies for training, test and external validation sets as high as 88, 83, and 78%, respectively. Similar approaches to model carcinogenicity yielded a prediction accuracy of 53%. Importantly, the model accuracy for carcinogenicity increased to 65% after augmenting chemical descriptors with HTS data as biological descriptors. QSAR analysis of 93 known human carcinogens included in the HTS datasets afforded similarly significant models: HTS-actives had relatively high correlation with human carcinogens (sensitivity 80%) but HTS-inactives poorly correlated with human non-carcinogens (specificity 41%). These preliminary studies suggest that cheminformatics analysis of NTP HTS data, in conjunction with traditional QSAR approaches, may enhance human and animal toxicity prediction models. This abstract does not reflect EPA policy.

1061 TOXICO-CHEMINFORMATICS AND QSPR MODELING OF THE CARCINOGENIC POTENCY DATABASE.

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Combining chemical structure descriptors with systematically organized toxicity and biological activity databases is the next frontier for toxico-cheminformatics and quantitative structure-property relationship (QSPR) modeling in predictive toxicology. Formalized toxicity data models and public toxicity data schemas allow for flexible data mining and relational data searching across layers of chemical and biological information. Here, we analyze data in the Carcinogenic Potency Database (CPDB) that contains animal carcinogenicity bioassay data for over 1000 chemicals, with information on species, sex, target site, mutagenicity, tumor potency, etc. Such a database provides unique opportunities for development of QSPR models for subsets of compounds on the basis of biologically meaningful endpoints. To test the quality of the data and fitness of the methodology, the classification k Nearest Neighbor (kNN) QSPR modeling approach was applied to modeling mutagenicity within a CPDB subset of 693 compounds, where 70 randomly selected compounds were set aside as an external validation set. The compounds were split into diverse training set and test set with chemical descriptor models for predicting mutagenicity yielding prediction accuracies for training, test and external validation sets as high as 0.917, 0.847, and 0.893, respectively. Subsequent analyses use mutagenicity/carcinogenicity status as biological refinements for modeling carcinogenicity for

subsets of compounds within CPDB. We conclude that QSPR approaches combined with toxico-cheminformatics descriptors can enhance predictive toxicology models. This abstract does not reflect EPA policy.

1062 AN INTEGRATED PBPK-QSAR MODELING APPROACH FOR IN VITRO TO IN VIVO EXTRAPOLATION IN RATS.

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In vitro data on metabolism and partitioning may be integrated within PBPK models to provide simulations of the kinetics and bioaccumulation of chemicals in intact organisms. QSAR modeling of available in vitro data may be performed to predict metabolism rates and partition coefficients for developing in vivo PBPK models. The objective of the present study was to develop an integrated PBPK-QSAR modeling approach for the conduct of in vitro to in vivo extrapolation. For this purpose, data on rat blood:air partition coefficients (Pb) and fat:air partition coefficients (Pf) obtained using vial equilibration technique, as well as intrinsic metabolic clearance (Clint) obtained using rat liver slices for C5 to C10 volatile organics (VOCs) (n-alkanes, isoalkenes, n-alkenes, naphthenes and aromatic hydrocarbons) were compiled from the literature. Multilinear additive QSAR models for Pf, Pb and Clint were developed based on the number and nature of molecular fragments in these VOCs (CH₃, CH₂, CH, C, C=C, H, benzene ring and H in benzene ring structure) using a commercially available statistical software (SPSS 14.0). The mean experimental/estimated (exp/est) ratios ($\pm$ SD; range) were 1.01 ($\pm$ 0.28; 0.62–1.61) for Pf, 1.16 ($\pm$ 0.81; 0.43–4.44) for Pb, and 0.95 ($\pm$ 0.28; 0.55–1.63) for Clint. By accounting for the difference in the content of neutral lipids in fat and other tissues, the liver:air (exp/est: 1.25 $\pm$ 0.40 (0.78–2.09)) and muscle:air (est/exp: 0.97 $\pm$ 0.26 (0.53–1.48)) partition coefficients were predicted from Pf. Integrating the QSARs for Pf, Pb and Clint within a rat PBPK model, simulations of inhalation pharmacokinetics of several VOCs (ethylbenzene, t-butylbenzene, n-hexane and toluene) were generated on the basis of molecular structure, for a given exposure scenario. The integrated QSAR-PBPK modeling approach developed in this study should be useful for predicting in vivo kinetics and bioaccumulation of chemicals in rats under data-poor situations.

1063 DEVELOPMENTAL TOXICITY PREDICTIONS OF ETHYLENE GLYCOL ETHERS AND THEIR METABOLITES USING STRUCTURE-ACTIVITY RELATIONSHIP (SAR).

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Ethylene glycol mono-n-alkyl ethers (EGEs) are a family of organic solvents commonly found in industrial and household products which cause a wide range of toxic effects in laboratory animals including reproductive, hemato-, and developmental toxicity. EGE metabolites are considered to approximate the toxicity of their parent compounds. Developmental toxicity studies have been reviewed and evaluated for four EGEs: 2-methoxyethanol (EGME), 2-ethoxyethanol (EGEE), 2-propoxyethanol (EGPE), and 2-butoxyethanol (EGBE). Structure-activity relationships (SARs) have been successfully used to predict the biological activity of potential developmental toxicants. SAR was conducted to investigate the possible role of moieties in the developmental toxicity of Ethylene glycol mono-n-alkyl ethers and their metabolites (Alkoxy acetaldehyde, alkoxyacetic acid and alkoxyethyl acetate). SAR results indicate that the aliphatic oxygen group and the alkyl length may be associated with the developmental toxicity effects of the EGEs and their metabolites. The ranking of the developmental toxicity activity of these compounds was as follows: Alkoxyacetic acid > Alkoxyethanols > Alkoxyethyl acetates > Alkoxyacetaldehydes. This suggests that the presence and position of the ether linkage, and the molecular shape, are critical to the developmental toxicity of EGEs.

1064 PREDICTION OF BIOLOGICAL PARTITION COEFFICIENTS: CALCULATED MOLECULAR DESCRIPTORS VS EXPERIMENTALLY DETERMINED PROPERTIES.

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Biological partition coefficients are routinely used as input parameters in physiologically based pharmacokinetic (PBPK) models, which are developed for the assessment of chemical toxicity. In this study, predictive quantitative structure-activity relationship (QSAR) models for rat and human biological partition coefficients,

namely blood: air, fat: air, brain: air, liver: air, muscle: air, and kidney: air, were developed utilizing experimentally determined partition coefficients for 131 chemicals obtained from the literature and calculated molecular descriptors based solely on chemical structure. The descriptors were partitioned into four hierarchical classes, including topostructural, topochemical, 3-dimensional, and ab initio quantum chemical. Three types of regression methodologies—ridge regression, principal components regression, and partial least squares—were used comparatively in the development of these models. In addition to the structure-based models, ordinary least squares regression was used to develop comparative models based on experimentally determined properties including saline: air and olive oil: air partition coefficients. The results of the study indicate that many of the structure-based models are comparable or superior to their respective property-based models. This is an important result considering that structural descriptors can be calculated quickly and inexpensively for both existing chemicals and those not yet synthesized. With respect to the structure-based models, it was also found that ridge regression outperformed principal components regression and partial least squares regression, and that generally the topochemical descriptors alone produced models of good predictive ability. The descriptors found to be most influential in biological partitioning of chemicals include those which encode information regarding hydrogen bonding, polarity, and molecular size and shape.

1065 STRUCTURE-GUIDED VIRTUAL HIGH-THROUGHPUT SCREENING PRIORITIZATION MODELS OF ENVIRONMENTAL CHEMICALS ACTING AT NUCLEAR RECEPTORS.

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High-throughput screening (HTS) and applied chemical genomics methods are becoming essential quantitative in vitro components of environmental prioritization schemes. The use of molecular modeling and Virtual-HTS methods for (1) elucidating molecular-level spatial descriptions of potential toxicant/target interactions and (2) obtaining quantitatively relevant relative binding affinities will complement the other predictive toxicology approaches for prioritizing risk assessment.

We demonstrate the use of docking-pose populations-analysis (DPPA) on poses obtained from exhaustive search molecular docking (ESMD) screens and the use of DPPA in reference standard selection for calibrating and validating relative binding affinities (RBAs). In a test set of 104 estrogen receptor (ER) ligands our in silico approaches afford a correlation between theoretical and experimental relative binding affinities of $R^2 = 0.75$, substantially better than the norm for such an analysis (0.5 and lower). In addition we report a false negative assignment of less than 2%. Finally, using these methods we present affinity fingerprint matrices obtained from structure-guided virtual-HTS of the EPA's pesticide and high-production volume datasets against multiple nuclear receptors (PXR, RXR, CAR, LXR, FXR, ER, AR ...) and their respective isoforms.

The simple, efficient, cost-effective and complementary modeling methods we have investigated afford numerically relevant protein affinity data matrices for the human nuclear receptorome that may have a far reaching effect as an additional tool for prioritizing chemicals. [MRG is supported by NHEERL-DESE Training Agreement, EPA CT829471. [This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy].

1066 DEVELOPMENT OF QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS (QSARS) TO PREDICT TOXICITY FOR A VARIETY OF HUMAN AND ECOLOGICAL ENDPOINTS.

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In general, the accuracy of a predicted toxicity value increases with increase in similarity between the query chemical and the chemicals used to develop a QSAR model. A toxicity estimation methodology employing this finding has been developed. A hierarchical based clustering technique (based on Ward's method) will be used to generate a series of structurally similar groups of chemicals from a toxicity data set. The structural similarity will be defined in terms of more than 3000 physicochemical descriptors which include 2-dimensional descriptors (such as connectivity and E-state indices) and 3-dimensional descriptors (such as surface area and partial charges). A genetic algorithm based technique will be used to generate statistically valid QSAR models for each cluster (using the pool of descriptors described above). The toxicity for a given query compound will be estimated using the model generated from the cluster whose chemicals are the most structurally similar to the query compound.

Initially the methodology described above will be used to develop QSAR models for the acute oral rat toxicity end point. Upon successful validation, the tool is expected to predict toxicities of chemicals for other cancer and non-cancer endpoints. The descriptor generation, clustering, and model building algorithms have been validated against commercial software.

The toxicity models developed using the methodology in this study will be incorporated into a free web-based software tool (written in Java). The tool will enable a user to easily predict the toxicity of a query compound by simply entering its structure in a 2-D chemical sketcher, entering a SMILES string, or entering a CAS number.

1067 PREDICTING BIOCHEMICAL RESPONSES TO ENDOCRINE ACTIVE COMPOUNDS: MATHEMATICAL MODEL OF STEROIDOGENESIS IN SMALL FISH OVARIES.

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Sex steroids, which have an important role in a wide range of physiological processes, are synthesized primarily in the gonads and adrenal glands through a series of enzyme mediated reactions. The activity of steroidogenic enzymes can be altered by a variety of endocrine active compounds (EAC), some of which are therapeutics and others that are environmental contaminants. A mathematical model of the intraovarian metabolic network was developed to predict the synthesis and secretion of testosterone (T) and estradiol (E2), and the kinetics for steroidogenic enzyme inhibition by EAC. Model predictions were compared to data from an in vitro steroidogenesis assay with ovary preparations from a small fish model, the fathead minnow. Model parameters were estimated using an iterative optimization algorithm. Model predicted concentrations of T and E2 closely corresponded to the experimental time course data from control experiments. In experiments with the EAC, fadrozole (FAD), model predicted behavior was consistent with E2 data, but not fully supported by T data. To address this discrepancy between the data and model predictions, we revised our model by including the additional high dose inhibitory effects of FAD on the steroidogenic pathway as described in the literature. A relative sensitivity analysis of the model parameters identified specific transport and metabolic processes that most influence the steady state concentrations of T and E2 that included secretion of T from the ovary and conversion of T to E2. Our study demonstrates the feasibility of using the model to predict steady state T and E2 concentrations, in vitro. This capability could be useful for environmental health assessments and pharmaceutical development with EAC. This work was reviewed by the U.S. EPA and approved for publication but does not necessarily reflect Agency policy.

1068 A PREDICTIVE SYSTEM FOR COMPUTATIONAL MOLECULAR TOXICOLOGY BASED ON NEURAL NETWORKS.

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A Multi-Intelligent System for Toxicogenomic Applications (MISTA) program has been developed to assess human health impacts from pharmaceuticals and chemicals. The MISTA program includes a dynamic and thoroughly reviewed database, as well as LiverTox, a liver toxicity prediction tool that uses computational neural networks (CNNs) linked to modern computational chemistry and wavelet methods. Several techniques were employed to make high-accuracy predictions using (1) Quantitative Structure-Activity Relationships, (2) microarray data, (3) data from other gene expression assays, (4) total gene expression analysis, (5) subtraction hybridization, (6) serial gene analysis expression, (7) proteomics, and (8) metabolomics and metabolomics. This highly innovative approach ensures that LiverTox takes full advantage of other key in silico resources currently available to keep it up to date with new developments. The accuracy and flexibility of this approach has been demonstrated for predicting (1) Lipophilicity, log P, for a large number of diverse compounds, (2) acute inhalation toxicity, (3) Carcinogenic Potency, and (4) mutagenicity in Salmonella. LiverTox is expected to correctly predict liver toxicity at rates in the 90th percentile with some endpoints predicted at rates greater than the 95th percentile. In addition, LiverTox is designed to accommodate future development of toxicogenomic tools and techniques to improve and expand the prediction of toxicity endpoints in other body organs. As CNNs are designed for each new predictive organ toxicity module, the predictive platforms for each new module will be optimized by drawing on the strengths (or weaknesses) of the LiverTox predictive platform.

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1069 ESTIMATING SAFE WITHDRAWAL TIMES FOR DRUGS GIVEN TO FOOD-PRODUCING ANIMALS USING PBPK MODELING AND MONTE CARLO ANALYSIS.

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Contamination of meat and milk products with drugs/xenobiotics is not only a public health concern, but it can also influence consumer confidence in the safety of these food items. One important method in avoiding the occurrence of drug residues, is to improve estimates of withdrawal times (WDT); that is, the time it takes for the drug to be cleared from the animal's body to levels in meat and milk that are safe for human consumption. Our research group has utilized physiologically based pharmacokinetic (PBPK) models with Monte Carlo analysis to estimate safe WDT of oral sulfamethazine in swine. The PBPK model contained five tissue blocks and plasma compartment which were linked through the liver to a two-tissue compartment (liver and body) model for the N4-acetyl metabolite of sulfamethazine. Following sensitivity analysis, five kinetic parameters were subjected to Monte Carlo analysis. Each Monte Carlo run consisted of a series of 100 simulations for validation and 1000 simulations each to determine the meat WDT for each run. The resulting distributions from a single Monte Carlo analysis of 100 simulations were comparable to the distributions of an external data set and in vivo pilot study. WDT estimates were based on US FDA Center of Veterinary Medicine regulatory requirements. The calculated meat WDT covered the upper 95% confidence interval of the 99th percentile of the pig population. Our calculations derived a more conservative meat WDT (21 days) than the approved WDT (15 days) established by US FDA. This work demonstrated that the use of PBPK modeling with probabilistic analysis techniques such as Monte Carlo analysis can have applications in determining safe WDTs that are transparent and reproducible. Current work is focused on how other physiological parameters such as drug protein binding can influence WDT. This research was supported by a USDA CSREES grant.

1070 INTEGRATION OF MICROARRAY AND PROTEOMIC DATA WITH TRADITIONAL TOXICITY DATA REVEALS DISEASE PATHOGENESIS PATHWAYS IN MOUSE MODEL OF COPD.

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Chronic inflammation and long-term exposure to cigarette smoke (CS) are the leading causes of Chronic Obstructive Pulmonary Disease (COPD). Co-administration of lipopolysaccharide (LPS) with CS augments the degree of pulmonary inflammation and has been proposed as a mouse model for COPD progression. AKR/J mice were exposed to CS and/or LPS via inhalation for 3 weeks and pulmonary responses were evaluated. Microarray and mass spectrometry-based proteomic analyses of lung were performed to support the traditional toxicological endpoints of pathology and bronchoalveolar lavage (BAL) parameters such as cytology and cytokines. Data were integrated on gene/protein and pathway levels to characterize the relationship between the mouse model and reported human disease phenotypes. Concordance of gene expression to protein abundance was better for BAL cytokines ($r=0.54$) than for lung proteomic data ($r=0.37$). Transcription factor analysis demonstrated significant overlap for more than 80% of the significant gene and protein changes in the lung, with a regression coefficient of $r=0.84$. Statistical enrichment of biological processes from the combined data demonstrates that the combined CS/LPS treatment displays several hallmarks of human COPD, such as high levels of response to reactive oxygen species, high proteolytic activity, and dysregulation of calcium homeostasis. CS appears to suppress the innate immune response to LPS treatment through decreased cytokine production by macrophages, despite increased cell numbers in the BAL fluid. Also, increased numbers of apoptotic cells from CS and LPS measured by TUNEL staining appear to arise through distinct mechanisms, which synergize in the combined CS/LPS treatment group. Thus, it appears that inflammation without proper immune surveillance/regulation is leading to disruption of oxidant versus antioxidant and cell death versus proliferation balances in the lungs of CS/LPS mice, which is consistent with early stages of COPD development in humans.

1071 AGGLOMERATION STATE AFFECTS *IN VITRO* NANOPARTICLE DOSIMETRY AND TOXICITY ASSESSMENTS.

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In vitro cellular dose of nanoparticles is a function of the sedimentation and diffusional transport rates of the particles of interest. We have previously constructed a computational model of particle deposition kinetics and dosimetry in vitro for sin-

gle particles based on media and particle properties. However, the initial model analyses were conducted under the simplifying assumption that particles are non-interacting rather than agglomerating due to attractive forces or chemical or mechanical binding (as has been shown for metal oxides, C60 fullerenes, and carbon nanotubes). Agglomeration shifts the size class distribution of particles from its initial state to one with a larger mean and greater dispersity. Agglomerates have a higher mass but a larger surface area than the primary particles. The net impact of agglomeration is to lower both gravitational sedimentation (due to the increase in buoyancy from the increased surface area:mass ratio) and diffusion (due to the larger particle size). Agglomeration rate and extent were added to the dosimetry and kinetic model to improve basis for the calculation of deposited particle surface area. The difference between agglomerate and primary particle kinetics can yield cases where increased particle concentration can actually lower the delivered dose due to the higher rate of agglomerate formation. This model refinement improves predictions regarding the cellular dosimetry and the dynamics of cellular response. Funded through U.S. Department of Energy under Contract DE-AC05-76RL01830.

1072 PREDICTING AGE-DEPENDENT PHARMACOKINETICS OF SIX VOLATILE ORGANIC COMPOUNDS IN THE RAT UTILIZING PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING.

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The capability of physiologically-based pharmacokinetic (PBPK) models to incorporate age-appropriate physiological and chemical-specific parameters was utilized to predict changes in internal dosimetry for six volatile organic compounds (VOCs) across different ages of the rat. Typical 6 h animal inhalation exposures to 500 ppm benzene, chloroform, methyl ethyl ketone (MEK), methylene chloride, perchloroethylene, or trichloroethylene were simulated for postnatal day 10 (PND10), 2 month old (adult) and two year old (aged) rats. With the exception of MEK, predicted peak venous blood levels of VOCs in PND10 and aged rats were equal or up to 1.5-fold higher when compared to the adult rat. Predicted peak venous blood levels of MEK, the most water soluble VOC examined, were similar in the adult and aged rat, but were greater than 5-fold for PND10 rats, reflecting the lower metabolic capability and greater loading by more rapid ventilation. Steady state venous blood levels of VOCs, simulated by modeling constant exposure, were predicted to be achieved in the order PND10 > adult > aged, largely due to increasing fat volume. The dose metric, total amount of VOC metabolized per unit liver volume, was generally much lower in PND10 than adult rats. The blood:air partition coefficient, fat volume, and fat blood flow were identified to be the most important parameters accounting for the predicted differences in venous blood levels of VOCs between the aged and adult rat. The lower metabolic capability, largely due to smaller liver size, and higher ventilation rate per unit body weight of PND10 animals appear to contribute the most to the differences between PND10 and adult rats. This study highlights the pharmacokinetic parameters that may contribute to the differential susceptibility to the toxic effects of VOCs across lifestages. (This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy).

1073 INSIGHTS INTO THE DETOXIFICATION OF CYANIDE WITH SULFURATING AGENTS.

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The standard treatment of acute cyanide intoxication involves the use of sodium thiosulfate to convert cyanide into the significantly less toxic SCN which is excreted from the body. However, the exact details of this mechanism are currently unknown. This work seeks to probe the reaction between cyanide and various sulfurating agents in order to enhance our understanding of this important detoxification process. In this work we have addressed the following questions: 1. Does cyanide react with sulfurating agents directly to form SCN? Or, does isothiocyanide (SNC) form then rearrange to the observed thiocyanide? 2. Can a computational model be developed to evaluate the potential efficacy of sulfur donors? 3. Do Efficacy predictions change when examining thiosulfonates? To address these questions, we employed quantum mechanics. For the evaluation of the interconversion of SNC and SCN the method MPW1PW91/6-311++g(2d,p) was used. Calculation of the enthalpy of formation for the efficacy model was accomplished using the B3LYP/6-31g* level of theory. This level of theory was also used for the evaluation of thiosulfonates. Wave function files for each structure were evaluated using the AIM2000 program. Quantum mechanics calculations were carried out using the Gaussian03 program Revision C02. We have found the following: 1. In the gas phase, SCN is favored over SNC by nearly 36 kilocalories. Conversion of SNC to SCN occurs via an unusual two-step process with interconversion barriers approximately 2 Kcal/mol. Therefore, formation of SNC is highly unlikely; if such

were formed, it would spontaneously rearrange to SCN. 2. The enthalpy of formation for disulfides reacting with HCN is keenly dependent on their substituent organic groups. The bonding between sulfurs is almost exclusively between p-orbitals. That direct cleavage of disulfides by cyanide may be a good model for predicting the efficacy was indicated by the enthalpy values of several compounds, including those found in garlic.

1074 PREDICTION OF AIRFLOW AND PARTICLE DEPOSITION IN THE HUMAN LUNG.

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The study of particle transport and deposition in the human lung is critical in health risk assessment of air pollutants and pharmaceutical drug delivery. A number of computational fluid dynamics (CFD) studies have investigated particle deposition in the lung by identifying various parameters such as lung ventilation and particle characteristics that influence particle deposition behavior. In this study, CFD simulations of airflow and particle deposition in geometries representing the human tracheobronchial tree were performed. Two cases of interest were modeled: (1) Nine common airways of human lung including the trachea, main, lobar, and segmental bronchi connected as a branching network of cylindrical tubes with dimensions based on available morphometric measurement; (2) the same geometry with a lobe attached to the distal end of each segmental bronchus. A three dimensional computational model of the lung was created for each case. Airflow field in both geometries was solved numerically for a 4-second breathing cycle. To evaluate particle deposition patterns during inhalation, about 10,000 particles of sizes from 1nm to 10µm were injected at the inlet to the trachea. A Lagrangian particle tracking method was used that included the effect of random motion (Brownian motion). The resulting airflow and deposition patterns varied significantly between cases (1) and (2) which indicated the significance of realistic boundary characteristics for accurate assessment of flow field and particle deposition. Applications of these results could assist scientists in identifying vulnerable and preferred sites in the respiratory tract and would help to set standards for minimizing exposure to environmental pollutants and develop techniques to enhance particle deposition in drug delivery to improve effective inhalation therapy for lung illnesses.

1075 EFFECTIVE TORTUOSITY APPLIED TO INTER-SPECIES SCALING OF PARATHION DERMAL ABSORPTION IN BOVINE AND RAT SKIN.

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Skin contact is an important route of exposure to many industrial and agricultural chemicals, naturally occurring chemicals, therapeutic drugs, cosmetics and chemical warfare agents. Quantitative models that relate chemical exposure to internal dose are critical to our ability to estimate the risks and therapeutic potential associated with exposures to chemicals. The scarcity of dermal absorption data in humans forces a reliance on animal data in human risk assessment. Quantitative knowledge of inter species differences in dermal absorption is therefore essential to the reliable use of available data in risk assessment. Differences in parathion dermal absorption between bovine and rat skin were studied using flow-through diffusion cells over an 8-hour experimental period. The results were compared to differences in skin structural parameters between the two species using a physiologically based pharmacokinetic model of dermal absorption that includes a calculation of stratum corneum resistance based on effective tortuosity derived from quantifiable stratum corneum structural parameters. Bovine and rat skin were dosed with 20.5 µg/cm² and 21.6 µg/cm² 14-C parathion, respectively. The bovine skin studies indicated a peak parathion flux at 360 minutes and 8.76 % (SE 0.02 %) of the parathion dose was absorbed through the skin after 8 hours. In rat skin, peak flux was at 420 minutes and total absorption was only 2.03 % (SE 0.30 %). Assuming that diffusivity and partitioning parameters were similar between the species, scaling from the rat to the bovine data could be achieved by decreasing the number of corneocyte layers, decreasing the volume of the lipid-filled gap between corneocyte layers and increasing the diameter of corneocytes. The differences in structural parameter values required for scaling in the model were consistent with species differences in microscopically observable structural parameters, indicating potential value of the model for use in inter-species scaling.

1076 EVALUATION OF PHOTOTOXICITY ALERTS IN DEREK AND USE OF A FINGERPRINT-BASED DECISION TREE APPROACH TO IDENTIFY POTENTIAL NOVEL ALERTS.

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Phototoxicity refers to a wide range of adverse events that occur within the integumentary system (i.e. skin) in response to a combination of compound and absorbed light energy. Requests for information regarding the phototoxic potential of drugs

undergoing regulatory review have risen over the past few years. This has led to an increased desire among medicinal chemists to be able to predict and potentially use structural activity relationships (SAR) to select chemical structures not associated with this adverse effect. One of the screening options available early in the lifecycle of a program to identify phototoxicity potential has been the DEREK system – Lhasa Ltd). DEREK is an in silico solution which contains a chemical rule base linking chemical substructures with known associated adverse effects. The analysis presented here was performed in order to understand the predictive ability of DEREK's phototoxicity alerts. In this work, we have compiled and submitted to DEREK, compounds with defined phototoxicity profiles to assess its ability to identify phototoxicity potential. Results suggest that the system does a good job identifying compounds with well known phototoxicant substructures, while novel positive compounds were generally not flagged. Positive compounds that did not alert were evaluated further using a structural fingerprint-based decision tree approach to identify potentially undiscovered substructural alerts for phototoxicity. This analysis identified a single putative structural feature for follow-up and potential inclusion as a new rule within the rule base.

1077 NEW ORLEANS BASELINE SOIL MAPS OF LEAD, ARSENIC AND MERCURY: AN APPLIED TOXICOLOGY APPROACH FOR EXPOSURE PREVENTION.

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This urban soil metal research focuses on lead (Pb), mercury (Hg) and arsenic (As) in residential communities of New Orleans. The soil survey of metropolitan New Orleans (n = 5,467 samples stratified by 286 census tracts) was completed before Hurricane Katrina. Composite soil samples (n = 19) for each census tract were analyzed for Pb and As by ICP and for Hg by direct mercury analysis (Milestone DMA-80). The findings were integrated into a GIS system and the resulting maps show the spatial distribution of Pb, Hg and As in the city. The patterns for Pb and Hg are similar (correlation = 0.9) while the pattern for As is dissimilar to the other two metals (correlation = 0.6). The largest amounts of Pb and Hg (Pb ~1,000 mg/kg; Hg ~1,000 µg/kg) were in residential census tracts within the inner ring of communities surrounding the central business district. The sources of Pb were commercial products with large quantities of Pb such as paint, and vehicle fuels with Pb additives. Hg is associated with sulfur in fuels. Pb and As are associated with lead arsenate insecticides that were commonly used before the advent of synthetic organic insecticides. Arsenic is associated with CCA-treated wood (i.e., chromated copper arsenate). The soil metal maps of New Orleans show that the urban environment is a historic sink of metals and their pattern in soil delineates the chemical milieu of the city. These maps serve as a foundation for developing a science-based strategy for reducing the high childhood prevalence of chronic exposure (~ 14% in pre-Katrina New Orleans) and health effects associated with these toxic metals. They also supplied the critical information necessary to evaluate the environmental impact of flooding of New Orleans after Hurricane Katrina. Acknowledgement: This project was funded by an ATSDR/MHPF Cooperative Agreement to Xavier University of LA # 5 U50 TS 473408.

1078 ANALYSIS OF HEPATIC GENE EXPRESSION PATTERNS OF FISHER 344 RATS EXPOSED TO PB2+ USING AFFYMETRIX MICROARRAY AND TAQMAN RT-QPCR.

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Lead contamination of soil and drinking water represent a significant risk to human health, especially for developing infants. Methods employed in determining lead toxic effects on humans using animal models include; histopathological analysis, biochemical analysis such as glycogen levels, aminolevulinic acid (ALA) and DNA and RNA contents and more recently toxico-genomics analysis (study of gene and protein activity response to toxicants). In this study Affymetrix DNA Microarray analyses were used to assess transcriptional profiles due to dose levels and characterize changes in hepatic gene expression in response to Male Fisher 344 rats exposed to 0 ppm, 50 ppm and 500 ppm lead (as acetate) for up to 90 days. To validate gene expression data acquired by Affymetrix Microarray, Taqman RT-qPCR was used to quantify the relative expression levels of selected genes already known to play important roles in mediating Pb2+ toxicity and others identified in our study that might be equally important in this process. Using Taqman RT reagents and Assay-On-Demand offered by Applied Biosystems, the expression of *calm1*, *calm2*, *Alas1*, *Atp5o*, *Ppp3ca*, *Cyp3a13* and *Mapk1* were measured relative to β -Actin. Raw data was exported for normalized gene expression (NGE) analysis using data analysis for real-time PCR (DART-PCR) and relative expression software tool (REST©). Most of the transcripts in the 30d exposure group were up-regulated while most genes in the 90d treatment were down-regulated, relative to controls. *Calm1*, *Calm2*, *Atp5o*, *Ppp3ca* and *Mapk1* were all upregulated in the short treatment regime. For

the chronic exposure group, Calm1, Calm2, Atp5o, Ppp3ca, Mapk1 were all down-regulated except Calm2 in the 50 ppm treatment group, relative to controls. Similarly, Alas1 was negatively regulated in all treatment groups except the 90d 50 ppm group. Cyp3a13 was negatively regulated in all treatment groups during all time points. Confirming microarray results are the down-regulation of Calm2 -3.886, Alas1 -1.616, Atp5o -2.706 and Cyp3a13 -1.79 genes in the long term exposure group

1079 ASSOCIATIONS BETWEEN LONGITUDINAL MEASURES OF OCCUPATIONAL LEAD DOSE AND RENAL FUNCTION.

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Introduction: The exposure range associated with lead-related nephrotoxicity in the occupational setting has not been clearly established. With the exception of two small studies, prior research has been cross-sectional thus limiting inferences regarding causality.

Methods: We evaluated associations between lead dose and serum creatinine from three annual evaluations in a cohort of 803 Korean lead workers. Blood lead was measured via atomic absorption spectrophotometry; tibia lead was measured via ¹⁰⁹Cd X-ray fluorescence. Generalized estimating equations were used to evaluate annual change in serum creatinine in relation to tibia lead concentration at the beginning of each follow-up period and concurrent change in blood lead. Models were adjusted for age, sex, body mass index, hypertension, days between evaluations, current/former lead worker status, time since retirement, plant of employment, and baseline blood and tibia lead.

Results: 75.5% of the 575 participants who completed all three evaluations were male. At baseline, 7.7 % and 0.5 % reported diagnoses of hypertension and diabetes, respectively. Mean (SD) age and lead job duration were 41.4 (9.5) and 8.5 (6.3) years, respectively. Mean (SD) blood and tibia lead levels were 31.3 (14.1) µg/dL and 35.1 (32.6) µg/g bone mineral, respectively. Concurrent change in blood lead was associated with an annual increase in serum creatinine of 0.012 (95% confidence interval, 0.003, 0.020) mg/dL for each 10 µg/dL increase in blood lead per year. In contrast, tibia lead was not significantly associated with change in serum creatinine.

Conclusions: These results support the inference that occupational lead exposure contributes to renal function decline.

1080 ACUTE LUNG INFLAMMATORY RESPONSE FOLLOWING PHARYNGEAL ASPIRATION OF STAINLESS STEEL WELDING FUME OR SOLUBLE CHROMIUM IN A/J AND C57BL/6J MICE.

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Previous data from our laboratory showed a greater lung inflammatory response in tumor susceptible (A/J) versus resistant (C57BL/6J) mice to aspirated chromium-containing manual metal arc-stainless steel welding fume (WF) at 7 and 28 days post-exposure. To gain further insight into the mechanisms and time course underlying this inflammatory response to WF, an acute time point was completed. Mice were exposed by pharyngeal aspiration to four doses (one dose every three days), of 5mg/kg WF, 1.5mg/kg soluble chromium (S-Cr), or saline vehicle. Bronchoalveolar lavage (BAL) and histopathology were done 2 days after the fourth dose. Lung injury (lactate dehydrogenase [LDH] and albumin), cytokines (IFN-γ, IL-6, MCP-1, and TNF-α), inflammatory cell influx (% polymorphonuclear leukocytes [PMN] and lymphocytes), and nitric oxide (NO) were measured in the BAL. At 2 days, both strains had increased lung injury, cytokines, and % PMN following exposure to WF or S-Cr but only the WF-exposed A/J mice had significantly increased NO levels. The C57BL/6J had equal degrees of inflammatory cell influx, lung injury, and cytokines following exposure to either WF or S-Cr. In contrast, the A/J mice exhibited a more heightened response to WF than to S-Cr, which was significantly higher than the C57BL/6J. Lung lesions, observed by histopathology, ranging from multifocal, minimal to moderate in severity, were found to be similar between the two strains and the exposure groups. It was also observed that the exposed C57BL/6J mice had higher lymphocyte numbers compared to the susceptible A/J strain. In conclusion, the mechanisms responsible for the lung inflammatory response to WF versus S-Cr appear to differ in the A/J but not in the C57BL/6J mice. These data suggest components, other than chromium, in the stainless steel WF exert additional toxicological effects in the A/J strain.

1081 CHROMIUM(VI) INDUCES GENES THROUGH STAT1 TRANSACTIVATION OF INTERFERON-STIMULATED RESPONSE ELEMENTS.

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There is a traditional view that Cr(VI) suppresses inducible gene expression. However, recent evidence has shown that Cr(VI) stimulates cell signaling that activates nuclear translocation and DNA binding of the members of the STAT family of transcription factors. To investigate whether this binding leads to acute induction of STAT-driven genes, human airway epithelial (BEAS-2B) cells were transiently transfected with luciferase reporter constructs driven by STAT1 responsive ISRE or gamma interferon activated sites (GAS). Addition of 5 µM Cr(VI) stimulated only the ISRE reporter, suggesting that Cr(VI) mimics the action of interferon α/β. This was confirmed by demonstrating that Cr(VI) only induces the endogenous, ISRE driven gene, IRF7, but not IRF1, which is predominantly GAS site-driven. Induction of IRF7 mRNA levels occurred within 2 h of Cr(VI) exposure and Western analysis of nuclear proteins correlated this induction with a rapid increase in tyrosine phosphorylated STAT1. Cr(VI)-stimulated IRF7 mRNA expression was blocked in cells expressing STAT1 siRNA, but was not blocked in cells incubated with a neutralizing antibody to interferon α/β receptor (CD118). Cr(VI) inhibits certain gene induction by retaining histone deacetylase-1 (HDAC) activity in their proximal promoters (Wei, Y.D., et al. *J. Biol. Chem.* 279:4110-4119 2004). In contrast, Cr(VI)-stimulated transactivation of the ISRE reporter and induction of endogenous IRF-7 mRNA and was inhibited in cells previously incubated with the HDAC inhibitor sodium butyrate. These data demonstrate that Cr(VI) does induce acute phase genes and present a novel mechanism in which Cr(VI) acts at the level of cell signaling to activate STAT1 to induce interferon (IFN)-stimulated genes through a HDAC-dependent mechanism. *Supported by NIEHS grant ES10638.*

1082 MICROARRAY ANALYSIS OF PARTICULATE CHROMATE-INDUCED GENE CHANGES IN HUMAN LUNG CELLS.

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Hexavalent chromium (Cr(VI)) is a widespread environmental contaminant and a well-established human lung carcinogen with solubility playing an important role in its carcinogenicity. However, the carcinogenic mechanism remains unknown. Our study showed that particulate Cr(VI) is cytotoxic and genotoxic to human lung cells. Functional changes within the cell are believed to depend on the number of gene expression differences and the actual functional relationships between them. To identify genes that are induced by particulate Cr(VI) in human lung cells, microarray analysis was used allowing the measurement of the expression level of thousands of genes simultaneously. We treated the cells with lead chromate (0, 0.5 µg/cm²) for 24 hours and hybridized RNA onto oligo microarrays. After normalizing and filtering the data from two color oligo microarray scans, we found that 515 of a total of 887 genes have changed expression including 24 genes that are significantly up-regulated (fold change greater than or equal to 1.5), and 97 genes are significantly down-regulated (fold change less than or equal to 0.67). In addition, we identified a list of particulate Cr(VI) inducible genes which plays important role in cell cycling pathways. This work was supported by NIEHS grant ES10838 (J.P.W.).

1083 PARTICULATE HEXAVALENT CHROMIUM INDUCES CHROMOSOME INSTABILITY AND DECREASED EXPRESSION OF SPINDLE ASSEMBLY CHECKPOINT PROTEINS.

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The spindle assembly checkpoint (SAC) pathway ensures cells do not prematurely enter anaphase causing the formation of aneuploid cells. Aneuploidy is a type of chromosome instability (CIN) involving the loss or gain of chromosomes. CIN is a hallmark of lung cancer with 70-80% of lung tumors having severe aneuploidy including triploid and tetraploid complements and is proposed as an early event in carcinogenesis. Hexavalent chromium (Cr(VI)) is a known lung carcinogen. Cr(VI)-induced tumors exhibit chromosome instability (CIN), but the mechanisms underlying these effects are unknown. We have found chronic, low doses of lead chromate, a particulate Cr(VI) compound, causes increased aneuploidy with many aneuploid cells being able to continue to grow and persist. Specifically, after 120 h treatment of 5 µg/cm² lead chromate, 55% of the cells were aneuploid. Additionally, this treatment caused an increase in anaphase, often disorganized

anaphase, suggesting a SAC effect. We found further evidence that SAC was bypassed thru observations that lead chromate induced increases in premature centromere division and premature anaphase. To confirm the role of the SAC pathway in lead chromate-induced chromosome instability, we examined expression of key genes. After 120 h, 5 µg/cm² lead chromate caused decreased expression levels of SAC proteins, Cdc20 and Mad2. Lower levels of Cdc20 and Mad2 may allow for SAC bypass permitting cells with defective spindle assembly to enter into anaphase and divide forming aneuploid cells. These data suggest that one mechanism for lead chromate-induced carcinogenesis is through the bypass of the SAC pathway leading to the induction of CIN.

1084 RAD51D, XRCC3, AND RAD51C PROTECT CELLS FROM PARTICULATE CHROMATE-INDUCED CHROMOSOME INSTABILITY IN MAMMALIAN CELLS.

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Particulate hexavalent chromium (Cr(VI)) compounds are well-established human carcinogens. Chromosomal instability (CIN) is widely recognized as a characteristic of Cr(VI) induced tumors, however the mechanism of this effect is still unknown. We are investigating the hypothesis that homologous recombination (HR) repair of DNA double strand breaks protects cells from Cr(VI)-induced CIN. We investigated this repair pathway by utilizing cells that are defective in Rad51D, XRCC3, and Rad51C, known genes in the HR pathway and the ability of Cr(VI) to induce CIN in them. Lead chromate (LC), a particulate model for Cr, was more cytotoxic in repair-deficient cells than in the wild-type. There was a 1.2, 1.5 and 1.5-fold increase in the cytotoxic effects of LC in Rad51D, XRCC3 and Rad51C deficient cells, respectively. There was a concentration-dependent increase in chromosomal damage in all HR repair defective cells. The highest concentration, 1 µg/cm² LC, induced chromosome damage in 22, 20 and 46 percent of metaphases with the total damage in 100 metaphases reaching 36, 27 and 102 aberrations in Rad51D, XRCC3 and Rad51C, respectively. A notable shift in the spectrum of chromosomal damage occurred with a dramatic increase in chromatid exchanges in all mutant cells. For example, 1 µg/cm² induced 19, 20 and 32 exchanges in Rad51D, XRCC3 and Rad51C deficient cells respectively while in the wild-type cells there were no exchanges. These observations suggest that HR does indeed protect cells from Cr(VI)-induced CIN. This work was supported by NIEHS grant #ES10838 (J.P.W.).

1085 A GENETIC APPROACH TO DISCOVERING THE MOLECULAR MECHANISM OF MANGANESE TOXICITY.

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Manganese is an essential metal that can cause neurodegenerative disease upon chronic over-exposure. The molecular targets of manganese toxicity and pathways influencing sensitivity to manganese are not well understood. We studied this problem using baker's yeast, *Saccharomyces cerevisiae*, as a model system. We examined yeast with a genetic deletion of *pho80*, a cyclin that pairs with a non-essential cyclin dependent kinase to regulate phosphate metabolism. The genetic deletion of *pho80* (*pho80Δ*) causes the yeast to become very sensitive to manganese toxicity. Therefore we hypothesized that there is a connection between manganese toxicity and genes regulated by Pho80 and the phosphate metabolism pathway that are currently unknown. In order to discover the components downstream of Pho80 that influence the sensitivity of yeast to manganese and their function and thereby gain knowledge that may help us understand the molecular mechanism of manganese toxicity and its potential connection to phosphate metabolism, we conducted a genetic screen to find genes that reverse the *pho80Δ* deletion strains sensitivity to manganese. Five classes of genes were uncovered which can reverse the sensitivity of the *pho80Δ* strain to manganese. These include a new metal transport gene, and three genes involved in chromatin remodeling and DNA repair.

1086 MANGANESE (MN) IN SALIVA AS AN INDICATOR FOR OCCUPATIONAL EXPOSURE IN CHINESE SMELTING WORKERS.

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Exposure to Mn occurs in both environmental and occupational settings, leading to various degrees of neurotoxicity. This cross-sectional study was designed to test the hypothesis that occupational exposure to airborne Mn among active smelting

workers was associated with elevated metal concentrations, i.e., Mn and iron (Fe), in body fluids. Our goal is to investigate 100 subjects each in control, low- and high-Mn exposed workers. The cohort is located in Zunyi City, Southwest China. We have completed 23-42% of sample collections (n=42, 23, and 30 for control, low and high exposed workers, respectively). Subjects were randomly selected, based on airborne Mn levels and job classification, to control group (airborne Mn <0.01 mg/m³), low-exposed group (<0.1 mg/m³), or high-exposed group (>0.3 mg/m³). Blood, urine, saliva, and hair samples from all subjects were obtained to determine Mn and Fe levels by AAS. No age difference was identified among the 3 study groups. Mn concentrations in the whole blood (WB), plasma, urine, hair, and saliva in high-exposed workers were significantly higher than those in controls (p<0.05). Among low-exposed workers, significant differences were observed only in the WB, plasma, and hair. The increase in Mn levels in body fluids appeared to be associated with airborne Mn levels except for plasma Mn. Linear regression analyses revealed that saliva Mn concentration was significantly associated with years of employment (r=0.40, p<0.05). Mn saliva level was also significantly correlated with Mn WB concentration. (r=0.32, p<0.05). Additionally, we found that Fe levels in saliva and urine were significantly higher in high-exposed workers than in controls (p<0.05). These preliminary data indicate that among active smelting workers, Mn concentration in body fluids reflect environmental Mn exposure. Mn levels in saliva may represent a useful non-invasive indicator of Mn exposure. (NIEHS ES 08164 and DoD USAMRMC W81XWH-05-1-0239).

1087 CHELATION THERAPY OF MANGANESE (MN) INTOXICATION WITH P-AMINOSALICYLIC ACID (PAS) IN SPRAGUE-DAWLEY RATS.

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Our recent human studies indicate that PAS, a classic antituberculosis drug, can effectively treat severe Mn Parkinsonism with a promising prognosis (Jiang et al., JOEM 48:644, 2006). This study was designed to test the hypothesis that PAS may act as a chelating agent to remove Mn in targeted brain regions, giving rise to its clinical benefit. Groups of 8-10 male rats (220±10g) received saline or Mn (6 mg/kg daily, 5 days/wk, ip) for 4 wks followed by PAS injection (100 and 200 mg/kg as the low and high dose group, respectively, daily, 7 days/wk, sc) for another 3 wks. Mn concentrations in tissues and body fluids were determined by AAS. Exposure to Mn caused significantly increases of Mn concentrations in plasma, cerebrospinal fluid (CSF) and red blood cells (RBC) by 1.5, 2.2 and 25 fold, respectively, as compared to controls (p<0.05). Mn levels in striatum and frontal cortex were also markedly increased by 1.8 and 1.6 fold, respectively (p<0.05). Three-week treatment with PAS significantly reduced Mn concentrations in the RBC (down 21.1% compared to Mn-exposed animals, p<0.05 with high dose of PAS) and in the CSF (28.3%, p<0.05 with low dose of PAS), while it had no effect on plasma Mn levels. Among brain regional samples analyzed by far, the high-dose PAS treatment significantly reduced Mn levels in striatum (down 16.4%, p<0.05), but it did not affect Mn levels in frontal cortex. Further analysis of Mn as well as iron concentrations in the choroid plexus and other brain regions is currently in progress. These preliminary data suggest that PAS appears capable of chelating Mn and therefore mobilizing Mn from its brain storage area. Conceivably, PAS and possibly its analog may serve as a new category of chelating agents for treatment of Mn Parkinsonism. (Supported in part by NIEHS RO1 ES08146 and DoD USAMRMC W81XWH-05-1-0239)

1088 COBALT ACTIVATION OF THE LYSYL OXIDASE PROMOTER MEDIATED BY THE HYPOXIA-INDUCIBLE FACTOR 1.

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Cobalt (Co), an essential trace element critical for the synthesis of vitamin B12, stabilizes the hypoxia inducible transcription factor 1α (HIF 1α), which cooperates with HIF 1β, regulating the transcription of a gene in response to hypoxia. At high concentrations, Co is also toxic and carcinogenic for mammals. Lysyl oxidase (LO) is a key enzyme for stabilization of the extracellular matrix by crosslinking collagen and elastin. To elucidate LO transcriptional regulation, we cloned the 5'-flanking region (3,979 nt) of the rat LO gene and showed the maximal promoter activity obtained from the fragment containing 804 base pairs upstream of ATG. Computational analysis revealed at least 4 hypoxia-response element (HRE) consensus sequences (ACGTG) existing in this LO promoter region. CoCl₂ at 0-100 µM enhanced LO promoter activities in a dose-dependent manner in transfected RFL6 lung fibroblasts. The increased level of the HIF 1α mRNA was associated

with upregulation of LO transcripts in Co-treated cells. Cells co-transfected with the LO promoter-reporter construct and the HIF 1 α and HIF 1 β expression vectors in the presence of 100 μ M Co exhibited a 2.55-fold increase in the LO promoter activity in comparison to only a 1.32-fold increase in the LO promoter activity in the absence of Co. Dominant negative HIF 1 α inhibited LO promoter activities stimulated by Co. Electrophoretic mobility shift, oligonucleotide competition and in vitro translation of HIF 1 α assays indicated that only one HRE mapped at -194/-190 using the first nucleotide preceding the ATG codon as -1 was functionally active among 4 consensus. Site-directed mutation of this HRE significantly decreased the LO promoter-directed expression of the reporter gene. These results strongly support the conclusion that cobalt activation of the LO promoter is mediated by the HIF 1, a potential mechanism for Co-induced lung fibrosis in human (Supported by NIH grant R01-ES11340).

1089 DEPLETED URANIUM GENOTOXICITY IN HUMAN BRONCHIAL EPITHELIAL CELLS.

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Cellular oxidative stress has been implicated in the genotoxicity of many heavy metals. In addition, airway epithelial responses to metals may depend on the physical form of the metal. The aim of this study is to evaluate the genotoxicity of depleted uranium (DU) as soluble (uranyl acetate (UA)) and particulate (uranium oxide (U₃O₈)) uranium in human bronchial epithelial cells (16-HBE). 16-HBE cells were exposed, in vitro, to 300 ppb of soluble depleted uranium (UA) or insoluble depleted uranium (U₃O₈) for 2 – 4 hr. Oxidative stress was assessed in vitro by fluorescent microscopy using dihydroethidium (HE) (2 μ M). GSH and TNF- α levels were quantified to support the fluorescent microscopy assay. Results indicate that 16-HBE cells treated with UA and U₃O₈ induced minimal oxidative stress compared to untreated cells in 2 – 4 hr time points. However, the formation of apoptotic bodies in both UA and U₃O₈ treatments were observed after 4 hr. These results are consistent with our previous studies that did not observe oxidative stress in DU treated cells. Interestingly, there was an increase in GSH levels of DU treated cells compared to untreated cells. Measured TNF- α levels did not change with DU treatments. These results suggest that: (1) cellular oxidative stress may not be a major pathway of DU genotoxicity, and (2) DU induces apoptosis in vitro. This study is supported by NIH Grants CA096281, CA096320, ES006694, F31ES014971, and The Alfred P. Sloan Foundation.

1090 CUMULATIVE EXPOSURE ASSESSMENT IN DU EXPOSED GULF WAR SOLDIERS: RELATIONSHIP TO HEALTH EFFECTS.

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A cohort of Gulf War soldiers exposed to depleted uranium (DU) by inhalation, ingestion and embedded DU shrapnel has been followed since 1993 to determine whether DU exposure under combat conditions is associated with adverse health effects. Exposure assessment has been challenging due to the unique routes of exposure. The DU exposure metric used to date has been a 24 hr urine U concentration at the time of each surveillance visit. This measure represents a soldier's current level of exposure. Because U accumulates in tissues under chronic exposure conditions, and because soldiers with DU shrapnel have had high urine U levels for more than 15 years, a cumulative measure of exposure to DU has been determined using an area under the curve (AUC) calculation. Comparison of the two exposure metrics showed a close association ($R^2 = 0.827$; $p < 0.001$), however changes did occur in the assignment of soldiers to high vs low urine U groups used to statistically test differences in health measures. Thus, the relationship of exposure to health effects was examined using both exposure metrics based on the hypothesis that adverse effects in organs known to accumulate U would be more closely associated with cumulative U exposure. Results showed that high vs low DU group differences in renal proximal tubule function were not greater using the cumulative exposure metric, despite the expectation that renal U accumulation over time would increase effects on this organ. Analysis of chromosomal aberration (CA) data obtained by FISH, however, showed that differences in mean number of CAs between the high/low DU groups were larger and more significant when the cumulative exposure metric was used. Such a result was expected for this marker of stable aberrations. Results support the importance of considering both current and cumulative exposure to DU in this chronically exposed cohort for determining whether health changes over time are related to DU exposure. (Supported by Department of Veterans Affairs)

1091 COPPER REVERSES HYPERTROPHY IN PRIMARY CULTURES OF NEONATAL RAT CARDIOMYOCYTES.

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Previous studies have shown that dietary copper deficiency causes cardiac hypertrophy along with depression of vascular epithelial growth factor (VEGF) expression in mouse model. Copper replenishment in the diet reverses cardiac hypertrophy and restores VEGF expression. The present study was undertaken using primary cultures of neonatal rat cardiomyocytes to examine the effect of copper on phenylephrine (PE)-induced hypertrophy and the relation to VEGF signaling pathway. Cardiomyocytes were treated with 100 μ M PE for 48 h to develop cell hypertrophy which was characterized by increases in cell size and volume, and increases in cellular protein content. Flow cytometry was also applied to further characterize cell hypertrophy. Copper in the form of copper sulfate at a final concentration of 5 μ M in cultures was added at the end of PE treatment and presented in the cultures for 24 h. The PE-induced cell hypertrophy was not further increased during the additional 24 h exposure, but copper significantly reversed cell hypertrophy, as indicated by reduction in the size and volume, and decreases in protein content in the PE-induced hypertrophic cardiomyocytes. However, copper did not change the phenotype of non-PE-treated control cells. To determine the involvement of VEGF signaling pathway in the copper-induced regression of hypertrophy in cardiomyocytes, anti-VEGF antibody at 2 ng/ml was added in the cultures one hour before copper addition. In addition, VEGF at 200 ng/ml was used to replace copper. In the presence of anti-VEGF antibody, copper-induced regression of cell hypertrophy was blunted, and VEGF alone reversed cell hypertrophy as the same as copper did. These results thus demonstrate that copper regression of cardiomyocyte hypertrophy may require VEGF signaling pathway. This study provides novel insights into copper supplementation for alternative therapy for hypertrophic cardiomyopathy. Supported in part by NIH grants HL63760 and HL59225.

1092 THE EFFECTS OF COPPER DIMETHYLDITHIOCARBAMATE ON LONG-EVANS RATS.

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For several decades, chromated copper arsenate (CCA) has been the principal preservative for treated wood utilized in residential and nonresidential areas. However, the repercussions of heavy metals present in CCA on human health and the environment resulted in industry halting production of CCA-treated wood products for most residential uses at the end of 2003. Previous data from this lab showed that copper dimethyldithiocarbamate (CDDC), one of the copper-based alternatives to CCA, caused stress protein induction and lipid peroxidation in maternal hippocampi and newborn whole brains of Long-Evans rats as compared to control animals. Graphite furnace atomic absorbance spectrophotometry analysis demonstrated a dose-dependent increase in the bioaccumulation of copper in adult hippocampi and livers and newborn whole brains of Long-Evans rats. Bioaccumulation of copper in tissues has been linked to oxidative damage and lipid peroxidation. In this study, Long-Evans rats (220-270 g) were treated with 25, 50, or 75 mg/Kg CDDC by oral gavage for 3 weeks. Renal and liver tissues were collected and fixed for microscopy. Hematoxylin and eosin staining revealed that 50 and 75 mg/Kg CDDC exposure induced diffuse necrosis, loss of sinusoids, the presence of foam cells, and cytoplasmic vacuolization in the livers of Long-Evans rats. Swelling of hepatocytes was evident in the livers of rats treated with the 25 mg/Kg dose. In the kidneys, animals treated with 50 and 75 mg/Kg CDDC showed distortion and swelling of both the proximal and distal convoluted tubules, prominent retraction of the glomeruli with mesangial distortion and areas of parenchymal necrosis. Adult rats treated with the 25 mg/Kg dose of CDDC displayed glomerular retraction. It is concluded that a 3 week exposure to 25mg/Kg CDDC induces morphological changes in renal and liver tissues whereas exposure to 50 and 75 mg/Kg CDDC results in overt necrosis in the same tissues.

1093 FAILURE OF ANTIMONY TRIOXIDE TO INDUCE MICRONUCLEI OR CHROMOSOMAL ABERRATIONS IN RAT BONE MARROW AFTER SUB-CHRONIC ORAL DOSING.

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Antimony trioxide (ATO), Sb₂O₃, CAS 1309-64-4, is widely used as a flame retardant synergist in a number of household products, as a fining agent in glass manufacture, and as a catalyst in the manufacture of various types of polyester plastics. It does not induce point mutations in bacteria or mammalian cells, but is able to in-

duce chromosomal aberrations (CA) in cultured cells *in vitro*. Although no CA or micronuclei (MN) have been induced after acute oral dosing of mice, repeated oral dosing for either 14 or 21 days resulted in increased CA in one report but did not result in increased MN in another. In order to further investigate its *in vivo* genotoxicity, ATO was dosed orally to groups of rats for 21 days at 250, 500 and 1000 mg/kg/day. There were no clinical signs of toxicity in the ATO-exposed animals except for some reductions in body weight gain in the top dose group. Toxicokinetic measurements in a separate study confirmed bone marrow exposure, and at higher levels than would have been achieved by single oral dosing. Large numbers of cells were scored for CA (600 metaphases/sex/group) and MN (12000 PCE/sex/group) but frequencies of CA or MN in ATO-treated rats were very similar to controls at all doses, and not biologically or statistically different. These results provide further indication that ATO is not genotoxic to the bone marrow of rodents after 21 days of oral administration at high doses close to the maximum tolerated dose.

1094 ZINC DEFICIENCY ENHANCES OZONE-INDUCED INFLAMMATION.

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Previous studies have demonstrated that several factors, both intrinsic and extrinsic, can cause humans to be differentially susceptible to the adverse health effects of exposure to air pollutants, such as ozone. These factors can include age, ethnicity, gender, and nutritional status. Zinc is an essential trace element, and it is estimated that approximately 10% of the U.S. population consumes less than half of the recommended daily allowance of zinc. Few studies have addressed the effects of zinc deficiency on the health effects induced by ozone exposure. An *in vitro* model of human respiratory epithelial cells, grown under either zinc deficient or zinc adequate conditions, was used to determine whether intracellular levels of zinc affected the responsiveness to ozone. Zinc deficiency was confirmed by measuring the activity of the zinc-dependent enzyme, Cu/Zn superoxide dismutase. This study specifically examined the effects of zinc deficiency on ozone-induced inflammation (IL-8 production), NF-KB activation, and MAP kinase activation. Our data demonstrate that zinc deficiency enhanced ozone-induced production of IL-8 as well as the activation of NF-KB and p38 MAP kinase, while ozone-induced cytotoxicity was unaffected. Taken together these data suggest that deficiency in the nutritional trace element, zinc, can increase susceptibility to the adverse health effects induced by ozone exposure.

1095 EXPRESSION OF ZINC TRANSPORTER mRNA IN NORMAL HUMAN PROSTATE TISSUE, THE NORMAL PROSTATE CELL LINES, RWPE, AND HUMAN PROSTATE CARCINOMA CELL LINES.

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The prostate accumulates and stores the highest amount of zinc of all soft tissues within the body, yet during prostatic neoplasia, the zinc content of this organ falls to much lower levels. There are specialized membrane bound transporters that help to regulate the zinc homeostasis of the prostate. These transporters have been categorized into two families: ZnT (SLC30) and Zip (SLC39). Many of these genes have been newly discovered and their function and role is not known. The ZnT family pumps zinc out of the cell or into intracellular vesicles, decreasing the intracellular levels of zinc. In contrast, the Zip family increases the zinc concentration by transporting the metal into the cell or out of the vesicles. The gene sequence is known for each family member, which allows for the production of PCR primers and the ability to measure the amount of messenger RNA present within cultured cells and tissues. Real-time PCR was used to quantitatively measure the amount of mRNA present in normal prostate tissues, the normal prostate cell line, RWPE, and commonly utilized prostate carcinoma cell lines. Results show that the main efflux transporter, ZnT-1, is expressed in prostate tissues and cell lines, and within RWPE was highly inducible by zinc. ZnT-2 and ZnT-4, transporters responsible for vesicular sequestration were found to be variably expressed in tissues and cell lines. ZnT-5, -6, and -7, transporters thought to transport zinc into the cellular secretory pathway were expressed in all cell lines and tissues. ZnT-3 and -10 were not detected. The expression of the Zip class of transporters was equally as complex. Zip-1, a major plasma membrane uptake transporter was expressed in all prostate cell lines and most but not all prostate tissues. Many other uptake transporters were also expressed: Zip-3A, -6, -7, -8, -10, and -14. There was no expression of Zip-2, -4, or -5. These data indicate that the transport of zinc in the prostate is complex and involves a potentially redundant array of transporters.

1096 BRAIN AND BLOOD TIN LEVELS IN A DEVELOPMENTAL NEUROTOXICITY STUDY OF DIBUTYL TIN.

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Dibutyltin (DBT), a widely used plastic stabilizer, is detected in the environment and human tissues. While teratological and developmental effects are known, we could find no published report of DBT effects on the developing nervous system. As part of a developmental neurotoxicity study of DBT, we periodically collected samples to determine the distribution of tin (Sn). Pregnant Sprague-Dawley rats were exposed to 0, 10, or 25 ppm DBT in drinking water from gestational day 6 to weaning. Beginning on postnatal day (PND) 3, half of the litters were directly dosed (DD group) every 2 to 3 days via gavage with 0, 1, or 2.5 mg/kg DBT such that the dose level matched the water concentration (i.e., litters with 25 ppm DBT in the water received 2.5 mg/kg). For Sn analysis, brain and blood samples were collected from culled pups on PND2 (sex combined), from pups (both sexes) as well as the dams at weaning (PND21), and from adult offspring (both sexes) at PND93. Total Sn was quantified using ICP-MS. At all ages, brain Sn levels were higher than in blood. At culling and in the DD pups at weaning, Sn levels in both tissues were linearly related to dose. Weanling pups without direct dosing showed lower levels than either the culled pups or the dams, indicating that lactational exposure is minimal or negligible even while maternal exposure is ongoing. In the adults, there was still Sn present in the brains of DD rats, but only females had measurable blood Sn levels, perhaps indicating more rapid clearance from blood in the males. The remaining adults had very low, but still measurable, Sn in the brain but not at all in blood. Thus, during exposure to DBT in drinking water, Sn is placentally, but not lactationally, transferred to the offspring. Our data suggest that Sn is concentrated in the brain compared to the blood, and its elimination with cessation of exposure is protracted, on the order of months. This exposure to the developing brain suggests the potential for neurotoxic effects. This is an abstract of a proposed presentation and does not necessarily reflect US EPA policy.

1097 CHARACTERIZATION OF HEAVY METAL EFFECTS OF TUNGSTEN ALLOYS ON THE RAT AIRWAY.

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A number of direct fire munitions are made with tungsten alloys (WA) including armor piercing kinetic energy penetrators. When WA munitions strike hard targets, aerosol clouds containing respirable particles as small as 0.2 µm are formed. Since military personnel near impact sites may be acutely exposed to high concentrations of aerosolized metals, the effects of WA on the airway were investigated. Male Sprague-Dawley rats (n=36/group) were intratracheally instilled with 1, 5, 15 mg of WA1 (91.1% W, 6.0% Ni, 2.9% Co) or WA2 (97.2% W, 1.7% Ni, 1.1% Fe) suspended in 400 µl of sterile saline or saline alone (control). At select times (24 hours, 3 days, 7 days and 6 months), the lungs were lavaged with cold phosphate buffered saline and the bronchoalveolar lavage fluid (BALF) was analyzed for markers of inflammation and injury such as lactate dehydrogenase (LDH), total protein (TP), N-acetylglucosaminidase (NAG), cytokines, cell number, type, and viability. As was previously reported, significant positive trends were observed in the concentrations of TP, NAG and LDH in the BALF of the WA1 and WA2 (15mg & 5mg) from 24 hours to 7 days post-exposure. However, at 6 months the results were comparable to the controls. Cell differential counts were significantly different (P<0.001, n≥3) for both WA1 and WA2 (15mg) from 24 hours to 7 days but were comparable to the controls at 6 months. Macrophages were increased from 34.6% and 26.0% to 65.5% and 74.7% for WA1 and WA2 (15mg), respectively. Polymorphonuclear neutrophils (PMN) were decreased from 60.9% and 70.1% to 29.1% and 19.7% for WA1 and WA2 (15mg) respectively. The results suggest that inhalation of WA aerosols may have at minimum an acute effect on the airway. Investigation involving histology, genotoxicity and long-term systemic effects are on-going.

1098 BRAIN ALUMINUM INFLUX RATE DETERMINED WITH THE TRACER ²⁶AL IN THE RAT.

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Aluminum (Al) is a neurotoxicant. It can enter the brain through the blood-brain barrier (BBB). Al-transferrin and Al-citrate account for ~ 93 and 7% of plasma Al, respectively. It has been suggested that Al is transported across the BBB by transferrin-receptor mediated endocytosis and that Al citrate can cross the BBB. There is concern that Al-fluoride can enter the brain. The *in situ* brain perfusion method was used to determine brain Al entry rates. Fisher rats received perfusion, via a

carotid artery, at 10 ml/min for 1 min, of a solution containing, in mM: Na 153, K 4.2, (Ca 1.5, Mg 0.9 omitted for Al-citrate), glucose 9, and [^{14} C]-sucrose 0.2, at pH 7.4. To study Al-transferrin and Al-citrate, the perfusate had 1 μ M Al (containing 0.005 μ M ^{26} Al; 2.5 nCi/L) and 35 μ M transferrin (plus 15 μ M carbonate) or 99 μ M citrate. To study Al-fluoride, the perfusate had 3 μ M Al and 20 μ M fluoride, at pH 5.5. Speciation calculations suggested ~90% of the Al would be as Al-transferrin or Al-citrates and >80% as Al-fluorides. Control rat perfusate contained no Al. Results from rats with brain vascular space, determined from brain/perfusate ^{14} C, <25 μ l/gm brain were used. ^{26} Al was quantified by AMS. The influx rate constants (K_{in}) for Al-transferrin and Al-citrate were $\sim 2 \times 10^{-5}$ ml/sec/gm, whereas Al-fluoride was much higher. These rates are less than the K_{in} of 800 to 1340 and 200 to 400 reported for the Al ion and Al partially complexed with transferrin from a study also using the same method, but ^{27} Al, which is prone to interfering contamination (Jagaramudi and Melethil (1995), Pharm. Res. 12, S-350). These rates are greater than the 0.03 to 0.04 reported after a 50 hour Al perfusion, which provides time for significant brain Al efflux (Radunovic et al (1997) Biometals 10, 185-191). The Al-transferrin and Al-citrate results are intermediate to K_{in} values reported for iron (reviewed in Yokel (2006) J. Alz. Dis. 10 (2-3)). The results support conclusions that Al can enter the brain as Al-transferrin and as Al-citrate, and suggest Al-fluoride as well. Supported by NIH Grant R01 ES11305.

1099 REGULATION OF ZINC TRANSPORTERS IN ZEBRAFISH IN RESPONSE TO CHANGES IN ZINC STATUS.

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Zinc is an essential micronutrient and zinc deficiency is a global problem among human populations. However, dysregulation of zinc by cells can lead to zinc toxicity and this may be common particularly in neurodegenerative diseases. Fish can use the gills to take up zinc from the water and they regulate gill zinc uptake according to the water zinc concentration, which may vary five orders of magnitude. The objective of our research has been to study zinc transport in zebrafish as a model for vertebrate regulation of zinc in general. There are several transporters that control zinc in vertebrate tissues. In zebrafish, there are at least 8 genes encoding slc30 zinc transporters and 11 genes that encode zinc transporters of the slc39 gene family. With few exceptions, these appear to have direct orthologues in human. Whereas slc30 and slc39 proteins appear to be dedicated zinc transporters that move zinc out and in of the cytoplasm, respectively, TRPV6 is an epithelial calcium channel (ECaC) that is highly permeable to zinc. Expression of slc30 and slc39 transporters zebrafish is tissue specific with ovary showing the highest level of expression and muscle the lowest. In the gill of zinc deficient zebrafish, expression of slc30a1 was downregulated while slc30a5 and slc39a3 were upregulated. During treatment with zinc excess condition, the concentration of mRNA for slc30a1 was increased and those for slc30a5 and slc39a10 were decreased. Upregulation of slc30a1 during zinc exposure was likely mediated by MTF-1 binding to multiple MRE motifs in the 5'-flanking region. The decrease of slc39a10 mRNA in response to excess zinc was caused by repression of transcription as demonstrated by reporter-gene assays. This was in spite of the presence of four consensus MRE located within 1.2 kb upstream of the translation start site.

1100 MODULATION OF MRP-TRANSPORTERS BY HEAVY METALS IN HUMAN LUNG CELLS.

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Human lung has to cope with exposure to low doses of heavy metals from various sources and the chronic uptake might lead to adaptation. It is not known whether metals will modulate the expression of multidrug resistance associated proteins (MRP) and may thus interfere with the extrusion of metal complexes by MRPs. Primary cultures of normal bronchial epithelium (NHBE) and primary peripheral lung cell cultures (PLC) were grown from human lung tissue. We have conducted long term experiments with As(III), Cu(II) and Hg(II) salts in NHBEs cultured from three individuals (B182/1, B201/1, B222/1). The experimental conditions were selected in the MTT assay for well tolerated non toxic concentrations of these metals in PLCs, NHBEs and in two human tumor cell lines (H 322, H 358). Neither As (1 and 2.5 mM), Cu (1 and 2.5 mM) nor Hg (1, 2.5 and 5 mM) had substantial effects within 24 h on MRP 1, 3, 4, 5 RNA expression in H 322, H 358 and PLC. In NHBEs only MRP 5 RNA increased after Hg (24 h) and MRP 3 after Cu (24 h). Repeated exposure to As down-regulated MRP1, MRP3 and MRP4 in B201/1 (0.4 to 0.6fold). In cultures derived from B182/1 As led to an up-regulation of MRP3 (1.3 fold), MRP4 (1.8 to 2 fold) and MRP5 (2.3 fold). Cu

had no effect on MRPs, in B201/1 and B222/1 except MRP1 which was down regulated by Cu only in B201/1 (2 weeks). In B182/1 Cu increased expression of MRP3 and MRP4 (1.9 fold). Repeated exposure of NHBE (2 weeks) with Hg led to a down regulation of MRP1; MRP3, MRP4 and MRP5 in NHBE B201/1. In contrast treatment with Hg increased expression of MRP3 significantly in B182/1 and also that of MRP5 (1.7 fold) in B222/1.

Primary NHBEs are more sensitive towards metal stressors with respect to MRP regulation than tumor cell lines and PLCs. Clear inter individual differences became obvious for MRP RNA upregulation after arsenite, copper or mercury after repeated exposure which indicate that responders (B183/1) and non responders (B201/1; B222/1) might exist.

1101 RENAL ELIMINATION OF INORGANIC MERCURY AND THE ROLE OF THE MULTIDRUG RESISTANCE PROTEIN 2.

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One of the current therapies for inorganic mercury (Hg^{2+}) intoxication involves administration of a metal chelator, either 2, 3-dimercapto-1-propanesulfonic acid (DMPS) or meso-2, 3-dimercaptosuccinic acid (DMSA). Following exposure to these chelators, Hg^{2+} can be detected in the urine, presumably conjugated to DMPS or DMSA. The actual mechanisms involved in the renal elimination of these conjugates are unclear; however, indirect data implicate a role for the multidrug resistance protein 2 (MRP2), which is localized to the luminal membrane of proximal tubular cells. We hypothesize that MRP2 mediates the export of DMPS- or DMSA-S-conjugates of Hg^{2+} from these cells. To test this hypothesis, the disposition of Hg^{2+} was examined in control and TR- rats, which do not express MRP2. Rats were injected intravenously with 0.5 μ mol/kg/2ml $HgCl_2$, containing ^{203}Hg . After 24 hours, rats were injected with saline, DMPS, or DMSA. Blood, liver, and kidneys were harvested after 48 hours. The renal and hepatic burden of Hg^{2+} in the saline-injected TR- rats was significantly greater than that of the control rats. Accordingly, the content of Hg^{2+} in the urine and feces was less in the TR- rats than in controls. DMPS and DMSA significantly reduced the renal and hepatic content of Hg^{2+} in both groups of rats, with the greatest reduction in the controls. A significant increase in urinary and fecal excretion of Hg^{2+} , which was greater in the controls, was also observed. These data indicate that MRP2 plays a role in the DMPS- and DMSA-mediated elimination of Hg^{2+} from proximal tubular cells.

1102 THE MULTIDRUG RESISTANCE PROTEIN 2 AS A MECHANISM INVOLVED IN THE RENAL ELIMINATION OF ORGANIC MERCURY.

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Intoxication of organic mercury (CH_3Hg^+) is treated often by administering a metal chelator, either 2, 3-dimercapto-1-propanesulfonic acid (DMPS) or meso-2, 3-dimercaptosuccinic acid (DMSA). These chelators extract mercuric ions, likely as CH_3Hg^+ -chelator conjugates, from proximal tubular cells for excretion in urine. The actual mechanisms by which these conjugates are exported from cells have not been identified; however, it is likely that the multidrug resistance protein 2 (MRP2), localized in the luminal plasma membrane of proximal tubular cells, plays a role in this process. Therefore, the current study tested the hypothesis that MRP2 mediates the export of DMPS- or DMSA-S-conjugates of CH_3Hg^+ from proximal tubular cells. To test this hypothesis, the disposition of CH_3Hg^+ was examined in TR- rats, which have a mutation in the *mnp2* gene. Normal male Wistar and TR- rats were injected intravenously with 5 mg/kg/2ml CH_3HgCl , containing ^{203}Hg , followed 24 hours later with an injection of saline, DMPS, or DMSA. After an additional 24 hours, the renal and hepatic burden of CH_3Hg^+ was found to be significantly greater in saline-injected TR- rats than in corresponding controls. Accordingly, the CH_3Hg^+ content of the urine and feces was less in TR- rats than in controls. Treatment with DMPS or DMSA reduced significantly the body burden of CH_3Hg^+ in both groups of rats and increased the urinary and fecal excretion of this metal. Interestingly, there were still significant differences between the two groups of rats. These data implicate MRP2 in the DMPS- and DMSA-mediated elimination of CH_3Hg^+ from proximal tubular cells.

1103 MURINE PULMONARY PATHOLOGY AND ALTERATIONS IN SYSTEMIC IMMUNE FUNCTION FOLLOWING 7, 14, AND 30 DAY EXPOSURES TO INHALED MULTIWALL CARBON NANOTUBES (MWCNTS).

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Nanotoxicology has emerged as an important field in the evaluation of the health effects of various nanoparticles. There is current concern that humans may be exposed to nanoparticles, such as MWCNTs, by inhalation and contact exposures.

Previous studies have reported that MWCNTs produce pulmonary inflammation and pathology in rodent species. However, MWCNTs have not been studied for inhalation toxicity. Therefore, the purpose of the present studies was to conduct 7, 14, and 30 day exposures to an industrial MWCNT to assess potential lung and immune system toxicities. Adult Male C57Bl/6N mice (n= 5-7/group) were exposed to clean air or particle mass concentrations of 100, 300, or 1000µg/m³ MWCNTs 6 hours/day for 7, 14, or 30 consecutive days. Mice were sacrificed the morning after the last exposure. One group of mice was held 14 days after the 30 day exposure period. Pulmonary endpoints included white blood cell counts/differentials in bronchial alveolar lavage and tissue pathology. Immune function endpoints in splenocytes included T and B cell mitogenesis and the T cell-dependent antibody response to sheep erythrocytes analyzed ex-vivo. Following 14 days of exposure, animals subjected to 300 and 1000µg/m³ had suppressed antibody response as well as decreased T cell mitogen response. By 30 days exposure, T cell mitogen responses were back to normal; however in 1000µg/m³ exposed animals, B cell mitogen and antibody responses were increased. Gross pathology on high dose exposed lungs was altered compared to control lungs. Lavage fluid was taken from 30 day time point animals and showed that treatment resulted in influx of macrophages, and macrophage engulfment of black particulate. Current studies are aimed at elucidation of the mechanism of MWCNT-induced immune alterations. This work supported by NIEHS P30-0120720 and by EPA STAR Grant R832527.

1104 INDUCTION OF DNA DAMAGE AND MICRONUCLEI BY NANOSIZED TITANIUM DIOXIDE IN HUMAN BRONCHIAL EPITHELIAL CELLS.

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Nanoparticles are increasingly utilized in pharmaceuticals, cosmetics, biomedical, plastics, building materials, and other products. Because of their small size (diameter < 100 nm), nanoparticles readily pass through cell membranes and may possess unique physicochemical properties and show unpredictable toxic effects. Since the applications of nanoparticles are rapidly growing, information on their possible genotoxic properties is urgently needed. Nanoparticles might be genotoxic either directly or indirectly, possibly acting through oxidative stress and inflammatory responses. There are some indications that nanoparticles such as nanosized titanium dioxide can elicit inflammation and evoke the generation of reactive oxygen species and of lipid peroxidation products. The aim of the present study was to examine *in vitro* the potential genotoxicity of nanosized titanium dioxide used, e.g., in the production of cosmetics. As lungs cells are expected to be the primary target of nanoparticle effects, we treated human bronchial epithelial cells (BEAS-2B) with various concentrations (1-100 µg/cm²) of titanium(IV)oxide nanopowder (rutile, 99.5%). DNA strand breaks were assessed with the single cell gel electrophoresis (comet) assay. The induction of micronuclei was examined by the cytokinesis-block method 24, 48, and 72 h after the treatment. Titanium dioxide induced a dose-dependent increase in DNA damage, but the effect was not high in absolute terms. In the micronucleus assay, titanium dioxide produced no clear positive effects when tested up to 100 µg/cm². High concentrations were not readable due to titanium dioxide sedimented on the chamber slide. Thus, our results suggest that rutile, a phase of titanium dioxide known to be relatively poor in generating reactive oxygen species, is slightly genotoxic in the *in vitro* conditions examined.

1105 COMBINING A MAGNETIC NANOPARTICLE TECHNIQUE WITH THE DT40 CELL MODEL TO EVALUATE THE DNA DAMAGE RESPONSE TO TOBACCO SMOKE.

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Magnetic nanoparticles (MNPs) suspended in the air can serve as an excellent exposure technology for directly targeting atmospheric airborne matter onto a specific area of *in vitro* and *in vivo* biological systems. This innovative methodology could significantly advance the evaluation of the health impacts of atmospheric airborne matter and identify relevant biological responses. In this *in vitro* study, isogenic chicken DT40 B-lymphocyte cells deficient in a DNA damage response gene were exposed to MNPs coagulated with tobacco smoke residue (MNPs-TS) to evaluate the cellular response to tobacco smoke. Cells were first exposed to air-suspended MNPs-TS for thirty minutes in the presence of an external magnetic field, which directed particle deposition into the culture medium thereby increasing cellular exposure to TS constituents, including insoluble residues. Cells were then washed by PBS and were cultivated in fresh medium for ten cell replication cycles. Cell survival was then determined by an XTT-based cell proliferation assay at the end of cultivation. Wild-type DT40 cells showed a dose-dependent reduction in growth after MNPs-TS treatment but not after exposure to MNPs alone, without TS. It has been reported that nucleotide excision repair (NER) plays an important role in the

removal of PAH-modified base lesions. Therefore, we applied this exposure technique to DT40 cells deficient in xeroderma pigmentosum A (*xpa*), a gene involved in NER. MNPs-TS exposure slightly reduced cell survival in *xpa* mutant cells relative to wild-type DT40 cells. These results indicate that, when coupled with an appropriate biological system, this novel exposure technique is a valuable approach for evaluating toxicity caused by atmospheric combustion particles. (This research was supported in part by P42-ES05948, ES11746, P30-CA16086, and P30-ES10126 from the National Institutes of Health.)

1106 *IN VITRO* TOXICITY OF SILICA NANOPARTICLES IN HUMAN LUNG CANCER CELLS.

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The cytotoxicity of 15 nm and 46 nm silica nanoparticles was investigated by using crystalline silica (Min-U-Sil 5) as a positive control in cultured human bronchoalveolar carcinoma-derived cells. Exposure to 15 nm or 46 nm SiO₂ nanoparticles for 48 h at dosage levels between 10 and 100 µg/mL decreased cell viability in a dose-dependent manner. Both SiO₂ nanoparticles were more cytotoxic than Min-U-Sil 5; however, the cytotoxicities of 15 nm and 46 nm silica nanoparticles were not significantly different. The 15 nm SiO₂ nanoparticles were used to determine time-dependent cytotoxicity and oxidative stress responses. Cell viability decreased significantly as a function of both nanoparticle dosage (10-100 µg/mL) and exposure time (24 h, 48 h, and 72 h). Indicators of oxidative stress and cytotoxicity, including total reactive oxygen species (ROS), glutathione, malondialdehyde, and lactate dehydrogenase, were quantitatively assessed. Exposure to SiO₂ nanoparticles increased ROS levels and reduced glutathione levels. The increased production of malondialdehyde and lactate dehydrogenase release from the cells indicated lipid peroxidation and membrane damage. In summary, exposure to SiO₂ nanoparticles results in a dose-dependent cytotoxicity in cultural human bronchoalveolar carcinoma-derived cells that is closely correlated to increased oxidative stress.

1107 ASSESSING THE ADEQUACY OF *IN VITRO* CELL CULTURE SYSTEMS TO ACCURATELY PREDICT THE *IN VIVO* PULMONARY TOXICITY OF FINE AND NANO-SIZED PARTICULATES.

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Previous studies have reported little correlation between the relative lung toxicity of particle-types when comparing *in vivo* effects vs. *in vitro* cell culture exposures. This study was designed to assess the capacity of *in vitro* screening studies to predict *in vivo* pulmonary toxicity of several fine or nanoscale particle-types in rats. In the *in vivo* component, rats were exposed by intratracheal instillation to 1 or 5 mg/kg of the following particle-types: 1) carbonyl iron; 2) Min-U-Sil quartz; 3) amorphous silica; 4-5) nanoscale or fine zinc oxide. Following exposures, the lungs of exposed rats were lavaged and inflammation and cytotoxicity endpoints were measured at 24 hrs, 1 week, 1 and 3 months postexposure.

For the *in vitro* study, three different culture conditions were utilized. Cultures of 1) rat lung epithelial cells, 2) primary alveolar macrophages, as well as 3) alveolar macrophage – L2 lung epithelial cell co-cultures were incubated with the particle-types listed above and the culture fluids were evaluated for cytotoxicity endpoints (LDH, MTT) as well as inflammatory cytokines (MIP-2, TNF-α, and Interleukin-6). Initially, the particle-exposed, 3 different culture-types were assessed for cytotoxicity biomarkers at 4 different time periods – 1 hr, 4 hrs, 24 hrs or 48 hrs. Subsequently, MTT endpoints were measured following incubation for 4 or 24 hours and the cytokine secretions into the culture fluids were measured after treatment for 24 hours.

When considering the range of toxicity to 5 different particle-types, the comparisons of *in vivo* and *in vitro* measurements demonstrated little correlation, particularly when considering many of the variables assessed in this study – such as cell-types to be utilized, culture conditions and time course of exposure, as well as measured endpoints. It seems clear that *in vitro* cellular systems will need to be developed, standardized and validated (relative to *in vivo* effects) in order to provide useful screening data on the relative toxicity of inhaled particle-types.

1108 THE EFFECT OF NANO-SIZED TITANIUM DIOXIDE PARTICLES ON PULMONARY INFLAMMATION IN MICE.

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Strong evidence exist that ultrafine airborne particles (e.g. diesel exhaust particles) pose considerable health risks in the airways and lungs. However, there is extremely limited information of the potential health effects of engineered nanomaterials on

the airways. We used a mouse model to elucidate inflammatory effects of nano-sized titanium dioxide (TiO₂) particles in the lungs of BALB/c mice that were exposed to intranasally administered fine (particle size ca. 1µm) or nano-sized (diam. x length ca. 10nm x 40nm) TiO₂ at three different concentrations (1, 100, and 1000 µg/ml) twice a week for three weeks. Inflammatory cell infiltration was defined in the bronchoalveolar lavage (BAL) fluid. Expression of cytokines and chemokines relevant to inflammation in the lung tissue was assessed by using real-time PCR. Bronchial responsiveness to methacholine was determined by whole body plethysmography. An increase in the expression of chemokine MIP-2, a potent chemotactic factor for neutrophil recruitment, in the lung tissue of mice exposed to high concentrations (1000µg/ml) of nano-sized TiO₂ particles, when compared with mice exposed to the same concentration of coarser particles, was detected. In addition, increased infiltration of macrophages and neutrophils in the BAL was seen after the installation of high concentrations of TiO₂. The increase was lightly higher in mice exposed to nano-sized than to coarser particles. These data suggest that nano-sized particles of titanium dioxide may be more potent than larger particles in evoking inflammatory responses in the lungs of mice. Supported by The Finnish Work Environment Fund, project no 105097.

1109 A TWENTY-EIGHT DAYS INHALATION TOXICITY STUDY OF SILVER NANOPARTICLES IN SPRAGUE DAWLEY RATS.

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Silver nanoparticles (SNP), second most widely used nanomaterials, have been used in health, electronic, and home products. Although previous studies on silver dust, fume and silver compounds suggested some insights on their toxicity, the toxicity of SNPs, in which size and surface area are recognized as important determinants for toxicity, is little known about. Especially inhalation toxicity of SNPs is foremost information to protect workers and consumers' health, despite difficulties in inhalation study of NPs. Accordingly, we have designed a device to generate SNPs by evaporation/condensation, using a small ceramic heater. The generator was able to distribute desired concentrations of SNP to chambers containing experimental animals. The concentrations and distribution of the NP with respect to size can be measured directly by a differential mobility analyzer and an ultra condensation particle counter. SNPs were tested for 28 days for inhalation toxicity. Eight-week-old rats weighing about 283 g for male and 192 g for female were placed in to four groups (10 rats in each group); fresh air control, low (1.73×10⁴), middle (1.27×10⁵), and high-dose (1.32×10⁶ particles/cm³, 61 µg/m³). The animals were exposed to SNPs 6 hours each day, 5 days per week, for a total 4 weeks. The male and female rats did not show any significant changes in body weight depending on the concentration of SNPs during the 28-day of the experiment. There were no significant changes in hematology and blood biochemical values either both in male or female rats. Our initial results indicated that SNPs at a concentration near to current ACGIH silver dust (100 µg /m³) may not have significant health effect.

1110 PULMONARY AND CARDIOVASCULAR TOXICITY OF MULTIWALL CARBON NANOTUBES IN MICE.

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Manufactured nanoparticles possess unique properties and may have a potential to cause toxicity. In the present study, pulmonary and cardiovascular toxicity of multi-wall carbon nanotubes (CNT) was studied in apoE-deficient mice maintained on standard mouse diet. CNT (>95% purity, diameter 20-30nm and length ~50 micron) were suspended in sterile PBS and introduced into mice lungs @ 40µg/mouse, once/wk for 16 consecutive weeks by pharyngeal aspiration. Age-matched mice receiving sterile PBS served as controls. Twenty four hour urine samples were collected from animals after the 1st and 15th CNT treatment to assess oxidative stress. Animals were sacrificed on day-1 and day-7 after the last CNT treatment. Markers of pulmonary toxicity and inflammation were measured in bronchoalveolar lavage (BAL) by standard techniques. The formation of atherosclerotic lesions in the aorta was evaluated by *en face* lesion planimetry. The results showed significant increases in total BAL cells and polymorphonuclear neutrophils in CNT-treated mice on day-1 and day-7 post treatment. Analyses of cell-free BAL fluids showed significant increases in total proteins, LDH and mucin levels in CNT-treated mice given multiple treatments suggesting toxic and inflammatory re-

sponses. The lung tissue levels of CuZn-SOD and Mn-SOD proteins did not differ significantly in the control and CNT-treated groups. However, a significant increase in the urinary levels of 8-OHdG in mice within a day after the first CNT treatment indicated development of oxidative stress. Yet, multiple weekly exposures to CNT for 16 wks failed to influence the plasma cholesterol and the area of atherosclerotic lesions covering aortic intima of apoE-deficient mice. The results suggest that multiple exposures to CNT evoke a toxic and inflammatory response in the pulmonary system but are unable to promote atherosclerosis for up to 16 weeks in a susceptible mouse model of human atherosclerosis.

1111 INFLAMMATION AND FATE OF QUANTUM DOTS FOLLOWING PULMONARY TREATMENT OF RATS.

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Inhalation of nanoparticles may affect the respiratory, cardiovascular, and central nervous systems. The mechanism by which nanoparticles translocate from the air-spaces into the bloodstream is unknown. The goal was to investigate the fate and inflammatory effects of fluorescent quantum dots (30-50 nm; coated with carboxyl terminal groups) after pulmonary exposure. To monitor phagocytosis of quantum dots by non-primed and primed alveolar macrophages (AM) *in vitro*, AM were collected by bronchoalveolar lavage (BAL) from untreated rats or rats that had been treated by pulmonary inoculation with *L. monocytogenes*. AM were incubated for 2 hr with 0 (control), 2.5, or 25 µg/ml of quantum dots and mounted for laser scanning confocal microscopy (LSCM) or analyzed by flow cytometry to quantify phagocytosis. For *in vivo* studies, male Sprague-Dawley rats were intratracheally instilled with saline or quantum dots on day 0 at a dose of 12.5 µg/rat. At 2 hr post-instillation, and on days 1, 3, and 5, the left lungs were cryopreserved and sectioned for LSCM. BAL was performed on right lungs, and indicators of lung damage were measured. Collected lavage cells were differentiated to assess inflammation. Recovered AM also were mounted onto slides and analyzed by LSCM to assess uptake of the quantum dots *in vivo*. A concentration-dependent increase in the phagocytosis of the quantum dots by AM *in vitro* was observed. A greater number of quantum dots were phagocytized by the primed AM. At 2 hr post-instillation, quantum dots were located in the AM and airspaces, as well as on the epithelial surfaces, in the alveolar region. By 24 hr, most of the quantum dots had been phagocytized by AM, and localized areas of quantum particles were observed in interstitial areas of lung parenchyma. Lung injury and inflammation were found to be elevated in the BAL of animals treated with quantum dots on 1, 3 and 5 days post-exposure compared to saline controls. Due to their high intensity fluorescent signal, quantum dots appear to be appropriate for studying the pulmonary deposition and fate of nanoparticles.

1112 PULMONARY INFLAMMATORY AND SYSTEMIC IMMUNE RESPONSES TO INHALED OIL NANOCONDENSATES.

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This study was conducted to follow up on our previous finding of a statistical association between chemical components of oil and pulmonary toxicity of collected automobile and diesel exhaust that was extracted and instilled into rat lungs. Organic condensates derived from unburned or partially burned oil and fuel account for the majority of the particle emissions, especially nanoparticle emissions, from motor vehicles. Despite significant interest in the toxicity of nanoparticles in the environment, this class of materials has not been well studied. We report results from a recent study that characterized the pulmonary inflammation and systemic immune effects of inhaled organic condensates of used engine oil. Used 15w/40 (Shell, Rotella T) motor oil was obtained after 200 hours of operation in a 2000 Model Cummins 5.9L diesel engine operated on a slightly modified (no motoring) FTP engine cycle. Organic aerosol condensates of oil were generated by evaporation and condensation. C57Bl/6 mice (n=7) were exposed to oil droplets of approximately 20 nm in size in a nose-only inhalation exposure system. An equal number of mice were exposed to clean air as controls. Mice were exposed for 7 consecutive days, 6 hr/day at a particle mass concentration of 300 µg/m³ and number count of ~1 x 10⁶ particles/cm³. On day 7, the mice were sacrificed. Lungs were lavaged for determination of total and differential inflammatory cell populations and indicators of toxicity. Lung tissue pathology was assessed by light microscopy of hematoxylin/eosin stained sections. Biochemical indicators of inflammation and oxidant stress were analyzed in lung tissue. Immunotoxicity was analyzed by splenic B and T cell proliferation in response to mitogens. We observed modest to no response in most of the pulmonary indicators of toxicity and oxidant stress. However, we did observe significant changes in B and T cell proliferation. Supported by DOE FreedomCar and Vehicle Technology Program.

1113 COMPARATIVE *IN VITRO* PULMONARY TOXICITY OF ENGINEERED, MANUFACTURED, AND ENVIRONMENTAL NANOPARTICLES.

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Engineered nanomaterials display many unique physicochemical properties for a variety of applications and due to their novel properties/applications may have unique routes of exposure and toxicity. This study examines the: 1) ability of the MTT assay to generate false positives or negatives when examining the cellular toxicity of single wall carbon nanotubes (SWCNTs); 2) comparative cellular toxicity using BEAS 2B human airway cell line of three different commercially manufactured SWCNTs (#1-3), nanographite fiber (nGF), ultrafine carbon black particles (ufCB), diesel exhaust particles (DEPs), and residual oil fly ash particles (ROFA); and 3) relationship between nanoparticle surface reactivity and *in vitro* pulmonary toxicity. The MTT assay did not produce either false positive or negative results since SWCNTs alone were negative in this assay and did not bind the reduced formazan dye. BEAS 2B cells were exposed to various doses of each nanoparticle and their viability assessed using the MTT assay at 24 hr post-exposure. These studies identified a hierarchy of BEAS 2B cellular toxicity with SWCNT#1 (ED50= 12.5 ug/ml) = SWCNT#2 > SWCNT#3 (ED50=50 ug/ml) >> ROFA (ED50=75 ug/ml) > DEP (ED50=100 ug/ml) > nGF (ED50>200 ug/ml) = ufCB. Nanoparticles were tested for their ability to generate thiobarbituric acid reactive substances in an acellular assay and demonstrated the following hierarchy of reactivity: ROFA >> DEP > ufCB = SWCNTs #1- #3. These results demonstrate that engineered nanoparticles display a significantly greater *in vitro* pulmonary toxicity when compared to manufactured and environmental particles. In addition, SWCNTs *in vitro* pulmonary toxicity did not correlate with their "reactivity". (Funding: USEPA-NCSU Cooperative Training Agreement CT 829470. This abstract does not necessarily reflect EPA Policy)

1114 LUNG TOXICITY STUDIES WITH NANOSCALE AND FINE-SIZED QUARTZ PARTICLES IN RATS: TOXICITY IS NOT DEPENDENT UPON PARTICLE SIZE BUT ON SURFACE CHARACTERISTICS.

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Pulmonary toxicology studies in rats demonstrate that nanoparticles are more toxic than fine-sized particles of similar chemistry. This study, however, provides evidence to contradict this theory. The aims of the study were 1) to compare the toxicity of synthetic 50 nm nanoquartz-I particles vs. (mined) Min-U-Sil quartz (~500 nm); the toxicity of synthetic 12 nm nanoquartz-II particles vs. (mined) Min-U-Sil (~500 nm) vs. (synthetic) fine quartz particles (300 nm); and 2) to evaluate the surface activities among the samples as they relate to toxicity. Well characterized samples were tested for surface activity and hemolytic potential. In addition, groups of rats were instilled either with doses of 1 or 5 mg/kg of carbonyl iron or various alpha-quartz particle-types in phosphate buffered saline solution and subsequently assessed using bronchoalveolar lavage fluid biomarkers, cell proliferation, and histopathological evaluation of lung tissue at 24 hrs, 1 week, 1 month and 3 months postexposure.

Exposures to the various alpha-quartz particles produced differential degrees of pulmonary inflammation and cytotoxicity, which were not always consistent with particle size but correlated with surface activity, particularly hemolytic potential. Lung tissue evaluations of three of the quartz samples demonstrated "typical" quartz-related effects – dose-dependent lung inflammatory macrophage accumulation responses concomitant with early development of pulmonary fibrosis. The various alpha-quartz related effects were similar qualitatively but with different potencies. The range of particle-related toxicities and histopathologic effects in descending order were nanoscale quartz II = Min-U-Sil quartz > fine quartz > nanoscale quartz I > carbonyl iron particles. The results demonstrate that the pulmonary toxicities of α -quartz particles appear to correlate better with surface activity than particle size and surface area.

1115 DISPERSION SIGNIFICANTLY ENHANCES THE PULMONARY TOXICITY OF SINGLE WALLED CARBON NANOTUBES.

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All nanomaterials have a fundamental dimension less than 100 nanometers. However, agglomerates of nanomaterials, micrometers in diameter, are the more common form of the material. This is particularly evident in prior studies of the

pulmonary toxicity of single walled carbon nanotubes (SWCNT) where large granulomatous lesions encased by epithelioid macrophages were produced by large agglomerates of SWCNT (~15 μ m diameter). In this study, a highly dispersed preparation of SWCNT with a mean geometric diameter of 1.1 μ m was given by pharyngeal aspiration to C57BL/6 mice in order to determine the pulmonary toxicity. Lung responses were studied by lavage and morphometry of lung sections at 1 hour, 1 day, 7 days and 1 month after a single SWCNT exposure of 10 μ g/mouse. A PBS aspiration group served as the negative control. Examination of lung sections and lavage cell differential demonstrated an early, transient neutrophilic, inflammatory phase which rapidly resolved and was similar to that observed with large agglomerates. However, no granulomatous lesions or epithelioid macrophages were detected. Electron microscopy demonstrated a highly dispersed, interstitial distribution of SWCNT deposits by 1 day. The deposits were generally less than 1 μ m. Alveolar macrophage phagocytosis of SWCNT was minimal at all times. Morphometric measurement of the Sirius red staining in sections was used to assess the connective tissue response. The average thickness of Sirius red stained connective tissue in alveolar regions, excluding alveoli adjacent to CNT deposits, was 0.10 $\pm$ 0.02, 0.10 $\pm$ 0.03, 0.09 $\pm$ 0.02, 0.51 $\pm$ 0.05 and 1.10 $\pm$ 0.35 μ m (mean $\pm$ SE N=6) for PBS, 1 hour, 1 day, 7 days and 1 month groups, respectively. The results demonstrate that dispersed SWCNT are rapidly incorporated into the alveolar interstitium and produce a generalized fibrotic response. Alveolar macrophage clearance is not a significant component of the lung response.

1116 INTERACTION BETWEEN CARBON NANOTUBES AND OZONE IN THE INDUCTION OF PULMONARY TOXICITY IN MICE.

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Manufactured nanoparticles have received considerable attention recently due to their potential to cause pulmonary injury. Ozone (O₃), on the other hand is a well defined air pollutant known to produce acute and chronic toxicity to the respiratory system. Whether pre-exposure to nanoparticles influences the O₃ toxicity is not well studied. To examine the extent of toxicological interactions between such particulate and gas exposures, we determined acute pulmonary effects of a 3-hr ozone exposure (0.5ppm) in female C57Bl mice administered 12 hrs earlier a single dose of 20 μ g multiwall carbon nanotubes (CNT) by pharyngeal aspiration. A total of four groups were compared: 1) PBS/air-control, 2) PBS/O₃, 3) CNT/air, and 4) CNT/O₃. Analyses of the bronchoalveolar lavage fluid (BALF) and lung tissue samples collected at 5 and 24 hrs post-O₃ exposure were performed for various markers of cytotoxicity and inflammation using standard ELISA and immunoblot procedures. The results showed a pronounced cellular response and increase in various cytotoxicity/inflammatory markers in the lungs of CNT-exposed mice. Ozone, by itself, produced minimal effects, but in CNT-exposed animals there was a significant increase in total BAL cells and polymorphonuclear neutrophils. Additionally, protein, LDH, TNF α , IL-1 β and mucin levels in BALF at 5 and 24 hrs were higher in CNT-exposed animals than corresponding air-exposed controls or animals exposed to O₃ alone. A comparable increase over the controls was also observed in the CNT/O₃ group but neither an additive nor a synergistic interaction was observed in mice that received sequential exposures to CNT and ozone. In fact some CNT-induced cytotoxic/inflammatory responses were attenuated in mice following exposure to both CNT and a low level of ozone. These results are opposite to enhanced responses that we were anticipating and may represent the development of "cross tolerance" reported by others for some sequentially administered pollutants.

1117 *IN VIVO* PULMONARY TOXICITY STUDY IN RAT'S WITH FULLERENE WATER SUSPENSIONS (NANO-C60 & C60(OH)24).

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Due to the increased attention on nanoparticle toxicity, specifically that of water-suspendable fullerene systems, we studied the *in vivo* pulmonary effects of rats intracheally instilled with an aggregated form of C60, termed nano-C60, and a highly water soluble derivative, C60(OH)24. Previously, it was reported that "the cytotoxicity of water-soluble fullerene species are a sensitive function of their surface derivatization". In this regard, *in vitro* toxicity studies with human dermal fibroblasts and human lung epithelial cells indicated that the pristine nano-C60 particles were significantly more toxic than the fully derivatized C60 particulates. We set out to validate this *in vitro* finding using an *in vivo* pulmonary bioassay system, wherein rats were exposed to 1.5 or 3 mg/kg of the two C60 forms or a quartz positive reference particulate control. In addition to BAL fluid analyses, oxidative damage was also probed. Results demonstrated that increased lipid peroxidation was measured

in the lung recovered cells of pristine nano-C60 exposed rats at the 1 day time point, but decreased over time. However, no increases in lung cell inflammatory responses, i.e. % neutrophils, were measured between the two forms of C60 at any of the postexposure time points evaluated. We show that under ambient conditions in water, fullerenes can generate carbon centered radicals detected by electron spin resonance spectroscopy. These oxygen radicals may be responsible for increased lipid peroxidation at the 1 day time point. This work demonstrates both the difficulty in interpreting/extrapolating in vitro toxicity measurements and highlights the complexities associated with probing the toxicological response of nanoparticle systems.

1118 NANO-PARTICLES - A SHORT-TERM INHALATION TEST WITH TiO₂ IN RATS LINKED TO *IN VITRO* SYSTEMS.

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Inhalation exposure is the route of most concern for nano-particles. There is that nano-particles in aerosols agglomerate to larger clusters affecting their deposition, distribution and effects in the lung.

Various testing methods are currently used to estimate the effects of nano-scaled materials in the lung, i.e. inhalation, intra-tracheal instillation and various cell culture systems. Compared to inhalation studies, these systems are relatively simple but the exposure is, considering the complex processes in nano-particle aerosols. Alternative exposure routes will need validation by inhalation studies covering the appropriate endpoints.

Therefore an inhalation study with rats was performed with exposure to 50, 10 and 2 mg/m³ nano-TiO₂ for 6 hours on 5 days. At the end of the exposure, and two and twelve days thereafter, animals were examined for an extensive set of parameters: Organ burdens were estimated in 7 tissues and electron microscopy was used to characterize the particles deposited in the tissues.

The particles in the atmosphere had a median diameter of 2 µm and less than 1% were actually in the nano-range (<100 nm), indicating agglomeration. Agglomerates in airways and alveoli (extra-cellularly and within activated macrophages) had a similar size distribution. 10 organs were examined by histopathology. S-phase response and apoptosis was examined in the lung.

Cytokine profiles, indicators of oxidative stress (malondialdehyde, carbonylmethyllysine, 8OHdG) and indicators of complement activation, as well as standard clinical pathology parameters were analyzed in lung lavage fluid and blood.

Signs of inflammatory response were observed in the lung and the results correlated well with results from a previous subchronic inhalation study. However, the wide range of examined parameters in this study, revealed a much more differentiated picture of the effects of nano-TiO₂ in the lung. The results will be discussed in detail and compared to intra-tracheal instillation studies as well as tests with cell cultures.

1119 ROLE OF PARTICLE AGGLOMERATION IN NANOPARTICLE TOXICITY.

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Particle agglomeration can affect the physical characteristics of airborne particles and influence deposition and toxicity. We hypothesized that there will be a difference in the toxicity of fresh (predominantly singlet) vs. aged (predominantly agglomerated) carbon nanoparticles. We measured the agglomeration rate of freshly generated carbon nanoparticles and determined whether agglomeration is affected by residence time, particle charge, or humidity. To examine the role of these parameters on carbon nanoparticle toxicity, we exposed BALB/C mice for 5 hrs to filtered air or carbon nanoparticles generated by spark discharge and examined lung injury and inflammation 24 hrs after exposure. Freshly generated carbon nanoparticles (60 nm, count median diameter, CMD, with a minor peak around 10 nm) produced significantly greater lung inflammation compared to nanoparticles aged approximately 3 minutes (260 nm CMD). This fresh nanoparticle-induced inflammation was concentration dependent with lavage fluid neutrophils at 2%, 4%, 14%, and 35% for 0, 1, 2.5, and 5 mg/m³ nanoparticle concentrations, respectively. There was little difference, however, in total protein concentrations in the lavage fluid of mice exposed to fresh vs. aged nanoparticles. Moreover, despite the role of particle charge in the physics of agglomeration processes, there was no significant difference in the response of mice exposed to charged nanoparticles vs. nanoparticles that were brought to charge equilibrium with a Kr source. Thus, the aging of freshly generated carbon nanoparticles has a profound effect on toxicity but it is unclear if this decrease in toxicity is due solely to particle agglomeration or changes in surface chemistry of the carbon nanoparticles. Ongoing studies are examining the effect of: 1) particle agglomeration on the toxicity of nanoparticles generated from metals and 2) low and high humidity on the pulmonary toxicity of freshly generated carbon nanoparticles.

1120 AN IMPROVED METHOD TO PREPARE SUSPENSIONS OF NANOPARTICLES FOR TREATMENT OF LUNG CELLS IN CULTURE OR *IN VIVO* EXPOSURE BY PHARYNGEAL ASPIRATION OR INTRATRACHEAL INSTILLATION.

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Occupational health risks associated with the production and use of nanomaterials are at this time undefined. Therefore, a need to conduct studies examining the health effects of nanoparticle exposure is of great importance. However, nanoparticles agglomerate and clump in solution, making it difficult to accurately deliver nano-sized particles in an in vivo or in vitro experimental procedure. Therefore, a dispersal method which does not alter the biological activity of the particles surface and is not toxic to the lungs was developed. Experiments were conducted to determine the best method to suspend nanosized particles. Ultrafine and fine carbon black and titanium dioxide were suspended in a variety of suspension medias including phosphate buffered saline (PBS), rat and mouse bronchoalveolar lavage fluid (BALF), and dipalmitoyl phosphatidylcholine (DPPC). To assess and compare how the various suspension medias dispersed the particles, images were taken using light microscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The results of this study show that PBS is not a satisfactory media to prepare particle suspensions of nanosized particles. However, BALF is an excellent vehicle in which to suspend ultrafine particles. The use of protein alone or DPPC alone, in concentrations found in BALF, did not result in satisfactory dispersions. However, the combinations of protein plus DPPC are satisfactory, although slightly less effective, substitute for BALF.

The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy.

1121 ARE POLY(ETHYLENE GLYCOL)-FUNCTIONALIZED NANOPARTICLES BIOCOMPATIBLE?

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Nanoparticles engineered for biomedical application need to be biocompatible to avoid adverse health effects. Surface-coating with poly(ethylene glycol) (PEG) is being used for this purpose. Since pulmonary delivery of nanoparticles is of great interest for therapeutic treatments targeting both pulmonary and extrapulmonary tissues, we sought to evaluate in the respiratory tract the biocompatibility of different nanoparticle coatings. We looked at the effect of two commonly used coatings, PEG (Mw = 5 kg/mol) and serum albumin (SA, Mw = 66 kg/mol), on pulmonary inflammation, cytotoxicity, and particle translocation. Single doses of saline and citrate-stabilized-, PEG-coated- or SA-coated- 50nm Au particles were intratracheally microsprayed into male F-344 rats. 24-hours after delivery, Au from all particle-exposed groups was detected in all regions of the lung analyzed, with the smallest amount being found in regional lymph nodes and the highest amount being found in lung tissue and cells of the bronchoalveolar lavage fluid (BALF). Rats exposed to SA- and PEG-coated Au, relative to citrate-stabilized Au, showed higher Au concentration in the BALF supernatant (p<0.05). This indicates that surface coatings prevent particle uptake by mononuclear phagocytic cells. Surprisingly, PEG-, but not SA-coated Au particles induced large increases in biochemical markers of cell toxicity (2-fold, p<0.05), polymorphonuclear neutrophils (60-fold, p<0.0001), and lymphocytes (10-fold, p<0.01), as measured in the BALF one day after exposure. These findings of severe pulmonary inflammation due to PEGylation differ with the generally held view of PEG biocompatibility. Future studies with different PEG-nanoparticle formulations will determine the mechanism of this unexpectedly high inflammatory response.

1122 COMPARATIVE SOLUBILITY OF NANOPARTICLES AND BULK OXIDES OF MAGNESIUM IN LUNG SIMULANT FLUIDS.

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Nanoparticles (NP), with > 1 dimension < 100 nanometers (nm) can clear airborne smoke particles. NP deposit in the deep lung, evade phagocytosis (< 1,000 nm diameter) and enter the lung interstitial space; if dissolved rapidly, we predict minimal health effects. We compared the solubility of Nanoactive [NA]TM MgO Plus

and Na^{TM} MgO; NanoScale, Manhattan, KS) with bulk macrocrystalline (MC) MgO at 50 mg/L and 250 mg/L MgO. Dissolved magnesium was measured using ion coupled plasma (ICP) – atomic emission spectroscopy (AES); Accuris-141, Fisons Instruments, Beverly, MA at 3, 6, 12, and 24 minutes, as well as 1, 3, 24, 48, 72, 96 and 120 hours (hr) in Hanks Basic Salt Solution (HBSS) and Dulbecco's Modified Eagles Medium (DMEM) to simulate lung epithelial lining fluid (ELF). Incubation was at 37°C and 37°C in the presence of 5% CO_2 . To maintain better contact of CO_2 , samples for HBSS and DMEM with CO_2 were taken at 15 and 30 min and at similar times through 120. We hypothesized that both NA MgO particles would be dissolved more quickly in fluids simulating lung ELF. This was true only for the smallest nanocrystalline size (4–6 nm Na^{TM} MgO Plus) relative to MC- and Na^{TM} MgO (nanocrystalline size 8 nm) at < 1 hour of dissolution. Secondly, we hypothesized that the composition of lung ELF would affect MgO solubility; dissolved DMEM > HBSS at 24–120 hours and proportional to the HCO_3^- concentration to simulate lung ELF ($p < 0.001$). Dissolution of HBSS in the presence of 5% CO_2 was greater than HBSS alone. For HBSS and DMEM alone or in the presence of 5% CO_2 , > 50% of the MgO dissolved was in the first 3 hr. These data suggested that significant rapid dissolution would occur for MgO deposited in deep lung that evaded phagocytosis, this minimized risk of health effects. This research was partially funded through the award of a contract from the Marine Corps Systems Command to M2 Technologies Inc.

1123 THE MECHANISM OF SOLUBILITY OF MAGNESIUM OXIDE NANOPARTICLES AND NANORODS IN FLUIDS THAT SIMULATE LUNG EPITHELIAL LINING FLUIDS.

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Nanoparticles (NP), with > 1 dimension < 200 nanometers (nm) clear airborne smoke particles. Nanorods (NR) tumble in air and obscure visibility. Particles deposit in deep lung, evade phagocytosis at < 1,000 nm diameter (D) and enter the lung interstitium; if they dissolve rapidly, we predict minimal health effects. We compared the solubility of nanoactive $[\text{Na}]^{\text{TM}}$ magnesium oxide (MgO) Plus nanocrystalline (NC) size 4–6 nm and Na^{TM} MgO, NC size 8 nm NP with bulk (MC) MgO and NanoScale (Manhattan, KS) nanorods with D ~ 200 nm and an aspect ratio (L/D) ~ 35 at 50 mg/L and 250 mg/L MgO. Solubility was measured in Hanks Basic Salt Solution (HBSS) to simulate lung epithelial lining fluid (ELF) at 37°C and 37°C in the presence of 5% CO_2 . Dissolved magnesium was measured using ion coupled plasma (ICP) – atomic emission spectroscopy (AES); Accuris-141, Fisons Instruments, Beverly, MA at 3, 6, 12, and 24 minutes, as well as 1, 3, 24, 48, 72, 96 and 120 hours (hr) for HBSS. A pH was measured at the same times as Mg. We hypothesized lung ELF HCO_3^- would affect MgO solubility; dissolved DMEM > HBSS at 24–120 hours and proportional to the HCO_3^- concentration ($p < 0.001$). We hypothesized the chemical form and dissolution of MgO would affect pH; pH increased from 9.5 to 10.6 for HBSS. Mg carbonate was formed as indicated by > 1/2 the MgO solubility at < 24 hr, and a pH increase. $\text{MgCO}_3 \cdot 3\text{H}_2\text{O}$ is stable at 37°C and 40 torr PCO_2 . We hypothesized based on NR shape that solubility/unit mass would be less (~1/length) than for NP, yet no differences in MgO solubility were noted, suggesting that NR shortened during solubility. That almost no MgO remained predicted no health effects from inhaled MgO. This research was partially funded through the award of a contract from the Marine Corps Systems Command to M2 Technologies Inc.

1124 ACCUMULATION RATES OF 26 NM DIAMETER PARTICLES BY MACROPHAGES AND EPITHELIAL CELLS.

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The rate that nanoparticles are accumulated by cells should reflect differences in molecular and cellular function. For fluorescent 26 nm diameter polystyrene latex (PSL) beads this expectation was tested with an epithelial cell line (A549 — Human lung adenocarcinoma alveolar type-2-like epithelial cells) and macrophages (alveolar macrophages from 12-week old F-344 female rats). The cells were mounted on a heated stage for observation with a confocal Laser Scanning Microscope (LSM 510 META microscope; Zeiss, Thornwood, NY). A549 cells were grown overnight in the wells of a cover-glass chamber at approximately 40,000 cells per well (The surface area of each well was approximately 0.8 cm²). The macrophages were obtained from 12-week-old F-344 female rats by bronchial-alveolar lavage. (Recovered cells — 97–99% macrophages — were resuspended in medium at a final concentration of 2 or 6.6 x 10⁵ macrophages/ml, allowed to attach to the well surface, and immediately exposed to PSL beads.) Cells were exposed to approximately 2x10⁷ beads per cell. During a 5 or 25 minute time course

the focal plane of the LSM detector was adjusted to step — optical-slice by optical-slice — through the entire volume of a macrophage or epithelial cell. The LSM was calibrated before and after each time course (Moss and Wong, *Inhal Toxicol* 18:711–716, 2006). For both cell types the uptake rate during the first minute was 10 to 20 beads per 10 ms. For the macrophages the uptake rate during the next 25 minutes was constant for a specific macrophage; although, between macrophages the uptake rates ranged from 2 to 10 beads per 10 ms. For the epithelial cells there was a plateau: During minutes 1 to 5 the uptake rates ranged from 1 to 2 beads per 10 ms. Subsequently, for exposure times longer than 6 minutes, the uptake rate ranged from 2 to 8 beads per 10 ms.

1125 ENHANCED TOXICITY OF CHARGED CARBON NANOTUBES AND ULTRAFINE CARBON BLACK PARTICLES.

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Man-made carbonaceous nano-particles such as single and multi-walled carbon nano-tubes (CNT) and ultra-fine carbon black (UFCB) particles are finding increasing applications in industry, but their potential toxic effects is of concern. In aqueous media, these particles cluster in large aggregates and their interactions with lung cells is rather inefficient. Nonetheless, these particulates have a time and dose dependent toxic effect on lung epithelial cells in culture. Recently, a method to solubilize carbon nano-tubes by heating the nano-tubes in a microwave oven with a mixture of sulfuric and nitric acid under high pressure, was described in order to improve the dispersal property and utility of SWCNTs (Wang et al *J Am Chem Soc* 128, 95, 2006). By using this procedure we generated particles that dispersed much more efficiently in aqueous media because of the introduction of negatively charged sulfonate and carboxy groups on carbon atoms. Compared to control untreated preparations of nano-particles, acid functionalized carbon nano-tubes as well as ultrafine carbon black were significantly more toxic to LA4 lung epithelial cells in culture as measured by the reduction in cell viability. Microscopic examination showed increased uptake of acid functionalized nano particles (AFNP) by the epithelial cells and flow cytometric studies indicated a significant decrease in the proportion of LA4 cells in S phase of the cell cycle. Pretreatment with poly L-lysine, a polymer that is positively charged and hence could neutralize the negative charge on AFNPs, resulted in abrogation of the toxic effects of AFNPs. These results indicate that dispersability and charge are important criteria in determining the toxicity of nano-particles to lung epithelial cells. This abstract does not reflect EPA policy.

1126 TARGETED DELIVERY OF PHOSPHATIDYL SERINE-COATED SINGLE WALLED CARBON NANOTUBES TO MACROPHAGES.

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Non-functionalized single walled carbon nanotubes (SWCNT) are not effectively taken-up by phagocytes. In contrast, apoptotic cells with externalized phosphatidylserine (PS) and non-apoptotic cells enriched with PS on their surface are well recognized by macrophages resulting in the suppression of their ROS and RNS production. We hypothesized that coating of SWCNT with PS could interface them with macrophages and stimulate their recognition and engulfment. SEM imaging revealed engulfment of PS-SWCNT by RAW264.7 macrophages and protrusions of SWCNT fibers through the surface into the interior of macrophages. Non-coated and PC-SWCNT were less attractive targets for the macrophages. TEM evaluations of the uptake of SWCNT showed that RAW264.7 macrophages expeditiously phagocytosed PS-SWCNT while less pronounced engulfment of PC-SWCNT and non-coated SWCNT was detected. In addition, macrophages co-incubated with PS-SWCNT had more endocytotic vesicles with entrapped SWCNT compared to macrophages exposed to PC-SWCNT or non-coated SWCNT. PS-SWCNT pretreated with Annexin V were phagocytosed at a much lower frequency. PS-SWCNT (but not PC-SWCNT) effectively suppressed LPS-induced production of $\text{TNF-}\alpha$ by macrophages. Interestingly, in vivo, PS-SWCNTs caused a more robust inflammatory response as compared with PC-SWCNT or non-coated SWCNT. This was documented by a greater total number of cells and PMNs as well as by increased production of $\text{TNF-}\alpha$. PS-SWCNT were preferably phagocytosed by macrophages obtained from BAL as compared to PC-SWCNT or non-coated SWCNT. Supported by NIOSH OH008282, NIH HL70755, HL70807, Human Frontier Science Program.

1127 TISSUE DISTRIBUTION OF FLUORESCENT NANOPARTICLES AFTER INTRAVENOUS INJECTION OR PHARYNGEAL INSTILLATION INTO THE AIRWAYS OF RATS.

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Rats were exposed to nanoparticles via IV tail vein injection or pharyngeal instillation into the airways to compare the effects of route of exposure and particle size on particle distribution over time. F344 female rats were exposed to saline, 40nm or 100nm polystyrene nanoparticles loaded with an infrared or far red fluorescing dye. One hour or 1, 7 and 28 days after exposure, whole blood was collected, the organs perfused with saline and tissues excised and homogenized for measure of fluorescent signal (normalized to organ weight). The total fluorescent signal of the tissue was calculated as the difference from control. 97% of the total signal was found in the liver and spleen from rats injected with 100nm particles; 3% of signal was found in gut, lung, heart, kidney, uterus + ovaries, blood and brain. A statistical significant drop in signal was measured in the liver, spleen, gut and lungs over time; signal from brain tissue was absent by day 7. Similarly, >95% of signal was found in the liver and spleen from rats injected with 40nm particles; remaining signal was detected in brain, gut, lung, heart, kidney and uterus + ovaries. A statistical significant drop in signal was measured in the liver, spleen, gut and lungs; all signals were absent in the brain, heart, kidney and uterus + ovaries by day 28. In contrast, the lung retained >99% of the fluorescent signal over 28 days from rats instilled with 100nm or 40nm particles. Statistical significant drop in signal was detected in the gut 1 hour and 1 day after exposure with no signal at days 7 and 28. Very low fluorescent signal was detected in the spleen and perfusate of 1 to 2 rats over time in both the 100nm and 40nm groups. The significant retention of signal in lung tissue suggests little movement from the airways to other organs over the 28 day observation period. These data show a difference in the tissue distribution of 40nm and 100nm particles in rats exposed via IV injection or by instillation into the airways.

1128 NOSE-TO-BRAIN TRANSLOCATION OF INHALED NANOPARTICLES.

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There is clear epidemiological evidence associating the particulate component of air pollution with adverse health effects such as respiratory distress, elevated blood pressure, heart attack, and stroke. The mechanisms underlying this association are not well understood. However, there is evidence to suggest that particle size may be an important feature of toxicity, and that the smallest particles (100 nm or less in diameter) may have the greatest toxic potential. Dosimetry studies have shown that the majority of such particles are trapped in the nose.

To test the hypothesis that the nasal cavity contributes to the observed particulate-associated health effects by serving as a port of entry to the central nervous system, we exposed adult mice to aerosolized fluorescent semi-conductor nanocrystals (quantum dots) for 3 hours in a nose-only inhalation chamber delivering a mean particle concentration of 1.5 ppb. The CdSe-ZnS quantum dots were encapsulated in phospholipid micelles to minimize aggregation and improve aqueous solubility. Mice were sacrificed 2, 2.5, 4.5, and 5 hours post-exposure. Brains, lungs, and nasal cavities were fixed for histological analysis. Sectioned paraffin-embedded tissues were imaged using standard fluorescence and confocal microscopy methods tuned for the detection of dots emitting maximally at 580 nm. Quantum dots were detected in a punctate pattern throughout the olfactory epithelium of the nasal cavity and in the olfactory bulb region of the brain. No dots were detected in the sham control mice.

To our knowledge, this is the first reported use of quantum dots as a model particle in an inhalation exposure and the first evidence of them translocating to the central nervous system by this route.

1129 CROSS REACTIVITY IN ALLERGIC ASTHMA-LIKE RESPONSES BETWEEN MOLD AND HOUSE DUST MITE IN MICE.

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Molds are ubiquitous in the environment and exposures to molds contribute to various human diseases including allergic asthma. Some mold allergens have been implicated as the causal agent for allergic asthma. Western blot analysis demonstrated IgE-binding cross-reactivity among mold extracts. However, the physiological relevance of this cross-reactivity to exacerbate asthma-like responses in house dust mite (HDM) and other mold sensitized mice is not well characterized. We hypothesized

that mold extracts can exacerbate allergic asthma-like responses in a BALB/c mouse model. Mice were sensitized to HDM or *Penicillium chrysogenum* extract (PCE) by intratracheal aspiration two times in 14 days. Seven days later, both HDM- and PCE-sensitized mice were challenged to mold extracts (PCE, *Stachybotrys chartarum* extract [SCE], or *Metarhizium anisopliae* crude antigen [MACA]) and HDM. Two days after challenge, serum and bronchoalveolar lavage fluid (BALF) were collected and analyzed for allergic and immunologic responses. Immediate airway responses were also assessed by whole-body plethysmography. Both HDM-sensitized, MACA-challenged mice and PCE-sensitized, MACA or HDM-challenged mice had pulmonary edema. PCE-sensitized, MACA-challenged mice had cellular damage in the lung. BALF total cell counts, especially macrophage counts, and BALF IL-5 levels were significantly increased in PCE-sensitized, MACA-challenged mice compared to PCE-sensitized mice. Serum total IgE levels were elevated compared to controls but were not different among sensitized mice regardless of challenge. Immediate airway responses were significantly elevated in HDM-, PCE-sensitized and mold-challenged mice compared to those of control mice. Taken together, mold extracts can exacerbate allergic responses in HDM-, PCE-sensitized mice including inflammatory, Th-2 cell, and airway responses through cross reactivity. (This abstract does not reflect EPA policy.)

1130 TESTING THE RESPONSE OF PROTEINS IN THE LOCAL LYMPH NODE ASSAY.

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No animal model has been validated for assessing the ability of protein-based preparations to behave as skin sensitizers. Still some authorities request skin sensitization data on materials such as industrial enzymes. As the local lymph node assay (LLNA) is regarded as the preferred model, the aim of the present work was to gain experience with the model and see whether it could provide useful information, even though the model was validated only for chemicals. The standard protocol was used, in which groups of CBA/Ca mice (n=5) received 3 consecutive daily topical applications of test material at 3 dose levels. The following protein allergens were tested: A protease and an amylase from *B. licheniformis*, a protease from *B. subtilis*, and Ovalbumin. The contact allergen hexyl cinnamic aldehyde and the vehicles were positive and negative controls. As the test materials were water soluble, the work included the investigation of several vehicles. The result was that some of the protein-based preparations were classified as skin sensitizers. The vehicle Propylene glycol was the most suitable for some of the test materials, while ethanol/water was best for others. The classification of enzymes as contact sensitizers is consistent with existing data from the guinea pig skin sensitization models, but conflicts with human data as it is well documented that enzymes are not skin sensitizers in man. Enzymes are known to be human respiratory allergens however and it is of note that the LLNA does not discriminate between chemical contact and respiratory sensitizers. Thus the results demonstrate a need to add other parameters to the traditional endpoint of tritiated thymidine incorporation if the model is to discriminate skin allergens from respiratory allergens such as enzymes. Cytokine profiling already achieves this for chemicals. Our efforts continue to investigate cytokine or lymph node cell markers that could discriminate the predominant T-helper 2 cell activation of protein respiratory allergens in contrast to the T-helper 1 cell activation after exposure to chemical contact allergens.

1131 DIGESTIBILITY AND ORAL TOLERANCE IN A MOUSE MODEL FOR FOOD ALLERGY.

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An animal model for food allergy is needed to test novel proteins produced through biotechnology for potential allergenicity. We demonstrate that mice can distinguish allergens from non-allergens when exposed to foods orally, both in terms of oral tolerance and allergic antibody responses generated by concomitant exposure to cholera toxin. Female 8-10 week-old C3H/HeJ mice were orally treated with 1, 2, or 5 mg of roasted peanut, egg white, or spinach extract, with or without cholera toxin, two or four times at weekly intervals. Peanut was the most potent antigen in eliciting allergic antibody responses, followed by egg white and spinach. Differences among antibody responses to allergens and non-allergens were greatest at low doses over a shorter dosing regimen. Alternatively, animals were orally exposed once to various food extracts or purified soluble or encapsulated ovalbumin prior to parental immunization to gauge induction of oral tolerance. Tolerance was observed in animals exposed to soluble ovalbumin, but not encapsulated ovalbumin or peanut extract. These differences appear to be related to digestibility or solubility, as indicated by the lack of oral tolerance to proteins known to be indigestible (peanut) or rendered indigestible by encapsulation (ovalbumin). Allergic antibody responses in mice orally exposed to food extracts with cholera toxin also reflect differences in

digestibility observed for these antigens, and parallel allergic responses observed in humans. Oral administration of proteins to mice with adjuvant may be a good model for predicting allergenicity of novel proteins. (This abstract does not reflect EPA policy.)

1132 UNDERSTANDING ALLERGENIC PROPERTIES OF PROTEINS: PROTEASE ALLERGEN AND INDUCTION OF M-CSF FROM HUMAN AIRWAY EPITHELIAL CELLS.

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Type I respiratory allergy is a Th2 dominated immune response to a protein allergen with development of allergen-specific IgE. The initial interaction of allergen with airway epithelial cells may be crucial in determining whether a Th2-dominated (vs Th1-dominated) immune response will be generated. In previous studies we showed that protease allergens (Alcalase®, Savinase®, BPN'Y217L) induced, in a dose dependent manner, the production of M-CSF from human airway type II-like bronchial epithelial cell line A549 grown submerged or in an air/liquid interface. In this study we assessed whether other human airway epithelial cells also produced M-CSF in response to exposure to protease allergens. The SV40-transformed human bronchial epithelial cell BEAS-2B was incubated with 0.1 - 10 ug/ml Alcalase, Savinase and Der p 1 for 24 hours. M-CSF was detected in culture supernatant of BEAS-2B cells treated with Alcalase as low as 0.1 ug/ml and was maximal between 1 - 10 ug/ml. Normal, human derived tracheal/bronchial epithelial cells from a non-asthmatic healthy individual and from an asthmatic individual (EpiAirway Tissue Model System) were exposed to 1 - 100 ug/ml Alcalase for 24 hours. M-CSF was produced by both cell types with no difference between asthmatic and non-asthmatic epithelial cell responses. Neither the primary human airway epithelial cells nor the BEAS-2B cells generated M-CSF upon exposure to 100 or 10 ug/ml BSA, respectively. These data show that M-CSF production may be a universal response of human airway epithelial cells when exposed to protease proteins and that the A549 air/liquid interface model may be a good surrogate for future studies on understanding the allergenic properties of protease allergens.

1133 ENVIRONMENTAL TOBACCO SMOKE SYNERGIZES WITH PROGESTERONE TO ENHANCE ALLERGIC ASTHMA IN A MOUSE MODEL.

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Background: Exposure to Environmental Tobacco Smoke (ETS) is known to both trigger and exacerbate the asthmatic phenotype. Previous studies performed by our laboratory have shown that exposure to ETS enhances the immune response to allergens by increasing T-helper 2 (TH2) type cytokines. Females have a higher incidence of asthma than males after the onset of puberty. This suggests a hormonal component to the development of the disease. Progesterone has previously been shown to illicit TH2 type immune response similar to that seen in allergic asthma. **Objective:** To determine if the combination of exposure to ETS and progesterone would further exacerbate the immune response in a mouse model of allergic asthma. **Methods:** Female mice were ovariectomized and then implanted with time-release progesterone pellets. Mice were housed in either ETS or Filtered Air (FA) chambers and half were exposed to aerosolized house dust mite allergen (HDMA). Bronchoalveolar lavage was performed for cell differentials; lung and spleen cells were harvested to compare IL-4 and IFN- γ production by ELISPOT. **Results:** Progesterone pellet implantation resulted in significantly increased serum progesterone levels vs. placebo treated mice, $p < 0.0001$. Serum total IgE levels were significantly greater in progesterone vs. non-progesterone treated animals that were also exposed to HDMA; these levels were further exacerbated by ETS exposure. HDMA/ETS treated animals had higher eosinophilia in lavage than all other groups. Lung homogenate cells from HDMA/Progesterone treated animals stimulated with Concanavalin A produced significantly more IL-4 compared to HDMA/Placebo treated animals (200 ± 17.6 vs. 146 ± 17.5 spots/well, $p < 0.01$ in ETS exposed animals and 221 ± 28.9 vs. 167 ± 23.4 spots/well, $p < 0.01$ in animals housed in FA). **Conclusion:** Increased serum progesterone levels exacerbate the allergic asthmatic phenotype in a mouse model. These effects are further exacerbated by the addition of environmental tobacco smoke.

1134 ENHANCEMENT OF ALLERGIC REACTION BY TRICHLOROETHYLENE EXPOSURE WITH DRINKING WATER IN MICE.

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The number of allergic patients has been increasing rapidly in industrialized countries in last 2 or 3 decades. The causes of rapid increase of the patients are considered to be due to environmental chemicals. As the contamination of ground water

by chloride organic chemicals has been social problem, we paid attention to the chemicals that have toxicity and persistency. We reported that these exposures enhanced passive sensitized reaction such as passive cutaneous anaphylaxis (PCA) reaction, and trichloroethylene (TCE) had the strongest effect among the chemicals. In this study we investigated a relationship between TCE exposure and active sensitized reaction. BALB/c mice were given TCE (0.03 mg/L and 3 mg/L) as drinking water for 2 or 4 weeks, and mice were immunized with ovalbumin (OVA) twice. On the final day in TCE exposing period, active cutaneous anaphylaxis (ACA) reaction was elicited in the ear of mice by injecting of OVA. Blood and spleen were collected from each mouse under diethylether anesthesia. Antigen-specific IgE and histamine levels in serum were measured and T lymphocyte proliferation in spleen cells was assayed. As a result ACA reaction was enhanced by TCE exposure in a dose-dependent manner. OVA specific IgE level in mice exposed to 0.03 mg/L TCE for 4 weeks was enhanced compared with control. Spleen T cell proliferation was enhanced in mice exposed to 3 mg/L TCE. From these results, TCE exposure is considered to relate with T lymphocyte proliferation, not with antigen-specific IgE production. TCE exposure by drinking water may increase allergic patients and exacerbate allergic disease.

1135 VALIDATION OF LLNA-DA ASSAY FOR ASSESSING SKIN SENSITIZATION POTENTIAL.

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Objectives: The local lymph node assay (LLNA) is a stand alone test method using mice to assess skin sensitization potential of chemicals. The LLNA-DA is a non-radioisotopic modification of the LLNA, in which ATP instead of 3H-thymidine is measured for lymphocyte proliferation. The objectives of this study are to experimentally evaluate the inter-laboratory reproducibility and predictability of LLNA-DA.

Materials and Methods: 10 laboratories participated in the study to conduct experiment. Twelve chemicals were uniformly selected from strong-, mild-, weak- or non-sensitizers. 3 chemicals were examined by all laboratories, while 9 chemicals were examined by 3 laboratories under a balanced allocation design. The cut-off value for the stimulation index (SI) was set as 3.0.

Results and Discussion: The results for common 3 chemicals and 5 allocated chemicals were consistent with small variances in SI. Achieved sensitivity, specificity and concordance were 87.5% (7/8), 100% (3/3) and 90.9% (10/11), respectively, which showed good reproducibility and predictability.

References: Yamashita K. et al. Development of a modified local lymph node assay using ATP measurement as an endpoint. Alternatives to Animal Testing and Experimentation, 11 (2005), 136-144.

1136 USE OF AN ENHANCED LOCAL LYMPH NODE ASSAY TO CORRECTLY CLASSIFY IRRITANTS AND FALSE POSITIVE SUBSTANCES.

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A major weakness of the local lymph node assay is that irritants can produce increases in the stimulation index (SI<3) in a manner analogous to true sensitizers or allergens. Due to the short-duration of the LLNA, many known human irritants are not detected as such during the course of the test (ear swelling and edema). The prototypical false positive irritant in the LLNA is SLS at concentrations above 20-25%. Herein we use an enhanced LLNA with immunophenotypic endpoints and measurements of ear thickness to characterize, identify, and correctly classify additional irritants, including benzalkonium chloride and ethylenediamine. Treatment with these irritants can result in maximum SI values from 5.4 to 13.3. Using flow cytometric analysis of surface markers on lymphocytes, including B220, CD3, I-Ak (MHC), and CD69, we are able to distinguish true sensitizers from these from

other anomalous test substances that increase Stimulation Index by unknown mechanisms. None of the false positive irritants exhibited the hallmarks of true sensitizers, specifically increases in percent B220+, I-Ak + and cd69/iak++ cells. In contrast, irritants tended to decrease the percent of B-lymphocytes while increasing the percent of T-lymphocytes in the draining lymph node. Thus, in the event that irritancy is present or suspected as a known property of the test substance, we herein define a tier-testing approach which integrates additional endpoints including ear thickness measurements and immunophenotypic analysis. This Enhanced LLNA (E-LLNA) allows a more thorough assessment and accurate classification of problematic substances.

1137 CHARACTERIZATION OF PHOTO-ALLERGIC PROPERTIES OF PSORALENS USING A FLOW CYTOMETRY-BASED PHOTO-LLNA WITH IMMUNOPHENOTYPE MARKERS.

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The phototoxicity of psoralens, specifically 8-methoxypsoralen (8-MOP) and 5-methoxypsoralen (5-MOP), commonly found in teas, celery, parsley and at least 12 other plant species, evoke a variety of phototoxic effects, including photo-genotoxicity, photo-irritation (dermatitis) and therapeutic uses as in PUVA therapy for psoriasis. Historically, 8-MOP has been classified as a very strong photo-irritant, in both in vivo and in vitro phototoxicity assays. There are sporadic reports of the photo-sensitization potential of the psoralens, however, due to their potent dermal irritation properties, it is difficult to establish their allergenic properties based on skin erythema or edema endpoints. To determine if psoralens possess photo-allergic (DTH) properties, in addition to their irritative effect, we employed a modified LLNA using solar simulated UVA light source following topical application 0.01% to 1.0% 8-MOP or 5-MOP). All concentrations above 0.03% were photo-irritating, causing maximum ear swelling up to 97%, accompanied by dose dependent increases in the Stimulation Index (SI) up to 13.7 for 8-MOP and 10.9 for 5-MOP. The positive photo-sensitization responses were all accompanied by dermal irritation. Using flow cytometric measurement of %B and T lymphocytes, as well as % CD69 and MHC+ subsets, we confirmed that both 8-MOP and 5-MOP increased the % of B cells and CD69/MHC positive lymphocytes, consistent with the profile of dermal sensitizers. In addition, the typical decrease in %T cells induced by irritants, was not observed, but rather the opposite, further supporting the strong allergenic potential of these psoralens.

1138 EFFECT OF UVB LIGHT ON THE DETECTION OF PHOTOSENSITIZING POTENTIAL OF CHEMICALS WITH THE MURINE UV-LLNA.

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We investigated the influence of UVB light on the detection of photosensitizing potential of chemicals and drugs in the murine local lymph node assay (LLNA). Two light sources were used: a mercury vapor lamp ($\lambda_{max}=365nm$; UVB filter < 320nm) and a xenon lamp simulating natural sun light including UVB. Female BALB/c mice were treated either topically on the dorsum of the ears or orally for 3 days followed by exposure to light. In a first experiment mice received 3 daily UVB doses of 0.02, 0.1, 0.5 J/cm² (mercury vapor lamp w/o UVB-filter) including controls without light or a UVA exposure w/o UVB. A light dose-dependent skin irritation (ear weight) and hyperplasia of the ear draining LN (weights and cell counts) was observed on day 4 with a NOEL of 0.02 J/cm² UVB. UVA light (5 and 10 J/cm²) from the xenon lamp induced skin photoirritation due to the lamp's UVB (0.15 and 0.3 J/cm²), however no effects were seen in the LN on necropsy day 4. Sacrifice of similarly treated mice on day 9 revealed that LN hyperplasia had developed over this time period. With topical tetrachlorosalicylanilide (TCSA, a contact photoallergen), we could demonstrate the confounding activity of UVB (Xenon) in the detection of chemically induced photoirritation of the skin after 4 days, whereas the LN showed the same photoreactivity to TCSA regardless whether UVB was included or not. Oral application of Sparfloxacin (20, 60, 180mg/kg b.w.), a photo-sensitizing antibiotic, led to dose-dependent increase in ear weight and LN hyperplasia on day 4, when animals were exposed to UVA light (10 J/cm²). Using the sunlight simulator in the same experimental setting, similar confounding activity of UVB was observed in the skin, whereas the LN activation was not affected by the UVB. After 9 days, confounding activity of UVB was clearly visible in the ear skin and the ear-draining LN at the high dose of Sparfloxacin. Our results demonstrate that the use of sun light simulators in in vivo photosensitization testing is possible, however with careful adjustment of experimental conditions.

1139 IDENTIFICATION OF SKIN SENSITIZING CHEMICALS IN A REDUCED LLNA.

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For the identification of potential skin sensitizing substances, the local lymph node assay (LLNA) has, for many years, been accepted as a full replacement for guinea pig methods. In part this was due to the scientific advantages provided by the LLNA, but also due to the significant animal welfare benefits this method offers. However, the desire to reduce further animal usage is in conflict with the need to complete toxicological evaluations of tens of thousands of chemicals under new European legislation (REACH: Registration, Evaluation and Authorisation of Chemicals). Consequently, we have conducted a retrospective evaluation of a recently published LLNA dataset comprising 211 chemicals. The work addressed whether it is possible to obtain a satisfactory identification of skin sensitization hazard using only a comparison of the top dose group with the concurrent vehicle treated control. In these analyses, negative results were not accepted as secure negatives if the top dose group was treated with <10% of the test chemical. The results indicated that a reduced LLNA employing on the top dose group (as per OECD Guideline 429) would provide about 99% accurate identification of sensitizers and non-sensitizers: 166 of 168 sensitizers were correctly identified, as were all non-sensitizers (42/42). Only one chemical was tested below 10% and, being negative, had a result which was regarded for these purposes as invalid. Thus, overall, the reduced LLNA gave 208/210 predictions that were identical to the full assay, including a 100% accurate identification of non-sensitizers; in only a single instance was the interpretation inconclusive due to the use of a low test concentration. In our view, it would be possible, for certain specific purposes, and in some circumstances, to consider the utility of a reduced LLNA as a contribution to a reduced requirement for animal numbers.

1140 THE IMPACT OF REDUCING ANIMAL NUMBERS IN THE LOCAL LYMPH NODE ASSAY.

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New European legislation requires complete toxicological profiles for a large number of chemicals, including evaluations for skin sensitization potential. The Local Lymph Node Assay (LLNA) is an accepted alternative to guinea pig methods for skin sensitization testing which provides important animal welfare benefits. OECD guideline 429 for the LLNA requires 3 test concentrations and a vehicle control group with a minimum of 4 animals per group. Conduct of the assay in this manner to satisfy REACH will result in significant animal usage. Therefore the possibility of conducting the assay as a screening test with a single high dose test group and a concurrent vehicle control has been evaluated and was found to provide an accurate identification of sensitizers and non-sensitizers. To reduce further animal usage, the impact of conducting this LLNA screen using 2 mice per group has been explored. To determine the effect on the stimulation index (SI), 34 datasets with individual animal data comprising 23 chemicals were used. SIs were calculated based on every possible combination of 2 control group dpm values and 2 high-dose dpm values. For most studies, there were 5 control values and 5 high dose values, so there were 10 possible pairs of control values and 10 possible pairs of high dose values and therefore 100 possible sets of 4 values (2 control + 2 high-dose). Of the sensitizer datasets, 20 of 26 had 96% or more of the possible SI combinations greater than 3. The sensitizer with the lowest number of positive combinations was the very weak allergen, isopropyl myristate, at 61%. Excluding the 3 sodium lauryl sulfate datasets, a reported false positive in the LLNA, all non-allergen datasets had $\leq 13\%$ positive combinations. Based on this analysis, conduct of a cut-down LLNA with 2 mice per group provides a means for further reduction of animal usage.

1141 EVALUATION OF HIGH THROUGHPUT METHODS TO SCREEN FOR GENE EXPRESSION CHANGES FOR PREDICTING THE SKIN SENSITIZATION POTENTIAL OF CHEMICALS.

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Twenty nine genes that are regulated following the interaction of chemical allergens with peripheral blood-derived dendritic cells (PBMC-DC) have been identified as potential endpoint measures for use in an in vitro assay for assessing the skin sensi-

tization potential of chemicals. These genes, derived using real-time PCR (RT-PCR), now require further evaluation with a larger test chemical set. For this purpose two multiplexed assays using fluorescent microspheres based on Luminex® xMAP® technology are being evaluated as potential high throughput put screening (HTS) methods, the Radix bead assay and Panomics QuantiGene® Plex. The Radix assay uses a single 25-mer oligonucleotide probe selected from the Affymetrix GeneChip® probe set linked to xMAP® beads and biotinylated cRNA targets as input samples. The QuantiGene® Plex platform uses probe sets based on automated probe selection criteria, xMAP® beads, branched DNA signal amplification technology and total RNA as input samples. The performance of these platforms was evaluated in studies conducted using RNA obtained from PBMC-DC treated with doses of the contact allergen dinitrobenzenesulfonic acid. In both assays, for all genes there was agreement between the bead data and the RT-PCR data in the direction of the gene expression change, either up- or down-regulated, relative to control for at least one of two allergen doses. However, for most genes, the RT-PCR treatment-versus-control fold-change values tended to be larger than the Radix assay fold-changes. Run-to-run variability of the Radix assay was assessed and overall, the fold-changes were quite similar. Studies with the QuantiGene® Plex platform showed a better correlation of fold change values with RT-PCR, especially for genes with large changes across treatment. Based on this initial examination both platforms appear to be potential HTS methods for evaluating gene expression changes, with the QuantiGene® Plex data having a better correlation with RT-PCR data.

1142 ECPA INTERLABORATORY STUDY INVESTIGATING THE SUITABILITY OF AN AQUEOUS VEHICLE IN THE MOUSE LOCAL LYMPH NODE ASSAY.

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The mouse LLNA has become broadly accepted and is requested as the primary animal test for evaluating the dermal sensitization potential of chemicals. As pesticides formulations are typically a specific complex blend of chemicals, traditional vehicles prescribed for the LLNA can be incompatible with these formulations leading to inaccurate test results and hazard identification. The objective of this study was to evaluate the effectiveness of an aqueous dilution of Pluronic® L92 block copolymer surfactant (L92) as a vehicle in the mouse LLNA amongst 5 labs using 3 chemicals with known sensitization potential, hexylcinnamaldehyde (HCA), 36.5% formaldehyde in water (FOR) and potassium dichromate (PDC). Identical LLNA protocols and test material lots were used. Female CBA mice were treated on 3 consecutive days with HCA (3 – 30%), FOR (1 – 20%) or PDC (0.02 – 0.5%) diluted in 1% L92. After 2 days rest, lymph nodes were harvested for evaluation of cell proliferation (³H-thymidine incorporation). All labs observed positive LLNA responses (SI > 3) for the 3 chemicals. For HCA, the range of concentrations (6.7 – 17.6%) calculated to elicit 3-fold increases in proliferation (EC3 values) were similar with that reported (8%) using acetone and olive oil. PDC elicited EC3 values (0.06 – 0.33%) which encompass that reported using L92 (0.17%) or DMSO (0.06 – 0.18%). EC3 values for 36.5% FOR ranged from 3.8 – 12.3% v/v. These data reproduced previous results using L92 and are only slightly above the EC3 measured with FOR in acetone. LLNA results using 1% L92 as a vehicle were reproducible amongst 5 labs and support the use of 1% L92 as a vehicle in the mouse LLNA. This work was sponsored and funded by the European Crop Protection Association.

1143 USE OF AN EX VIVO LOCAL LYMPH NODE ASSAY FOR CONTACT HYPERSENSITIVITY ASSESSMENT.

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The murine local lymph node assay (LLNA) is used to assess the contact hypersensitivity potential of compounds. In the standard assay, mice are treated topically with test compound to the dorsum of both ears on Days 1-3. The induction of a hypersensitivity response is assessed on Day 6 by injecting ³H-thymidine into a tail vein, and measuring thymidine incorporation into DNA of lymph node cells draining the ears. The *ex vivo* LLNA is conducted similarly except lymphocyte proliferation is assessed after *in vitro* incubation of lymph node cells with ³H-thymidine, which significantly reduces the amount of radioactive waste. We recently developed this alternative method with the contact sensitizers hexylcinnamic aldehyde and oxazolone, and the nonsensitizer methyl salicylate. In the current study, additional compounds were tested to further validate the assay for hazard assessment of con-

tact hypersensitivity, and to evaluate the ability of the assay to estimate allergenic potency. Female BALB/c mice were treated on Days 1-3 with 2 nonsensitizers (4'-methoxyacetophenone, diethyl phthalate), 3 weak sensitizers (hydroxycitronellal, eugenol, citral), 2 moderate sensitizers (isoeugenol, phenyl benzoate), and 1 strong sensitizer (dinitrochlorobenzene). Hexylcinnamic aldehyde also was tested, and served as a positive control. On Day 6, lymph nodes were removed, and single-cell suspensions were prepared. Cells were incubated overnight with ³H-thymidine. The *ex vivo* LLNA accurately estimated the relative potency of the compounds tested. The EC3 values were as follows: 4'-methoxyacetophenone (>50%), diethyl phthalate (>50%), hydroxycitronellal (20.4%), eugenol (13.6%), citral (8.9%), isoeugenol (3.8%), hexylcinnamic aldehyde (2.7%), phenyl benzoate (2%) and dinitrochlorobenzene (0.02%). These results were consistent with published reports using the standard LLNA, and provided further evidence that supports the use of this approach for hazard assessment of contact dermatitis. Further, these data demonstrated that the *ex vivo* LLNA could be used to estimate allergenic potency.

1144 COMPARATIVE EVALUATION OF THE *IN VITRO* SKIN SENSITIZATION TEST; HUMAN CELL LINE ACTIVATION TEST (H-CLAT) WITH LLNA AND HUMAN DATA.

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We have reported that a 24-hr exposure of THP-1 cells (human monocytic leukemia cell line) to sensitizers was sufficient to induce augmented CD86 and/or CD54 expression, and we have developed *in vitro* skin sensitization test named h-CLAT on the basis of that finding. Our previous inter-laboratory study in Japan revealed that the protocol of h-CLAT is easy to transfer, and inter-laboratory reproducibility is basically good. The aim of this study is to confirm the predictive capacity of h-CLAT for skin sensitization activity by employing a larger number of chemicals. In this study, more than 60 known chemicals were evaluated with h-CLAT. We selected these chemicals according to the results of LLNA in the following categories: extreme, strong, moderate, and weak sensitizers, and non-sensitizers. Correspondence with LLNA was about 90%. h-CLAT could predict not only extreme and strong sensitizers, but also moderate and weak sensitizers as positive. There were a few false-negatives (e.g., benzoyl peroxide, hexyl cinnamic aldehyde) and false-positives (e.g., 1-bromobutane, diethylphthalate), but most chemicals were evaluated correctly. Among sensitizers, some enhanced both CD86 and CD54, and some enhanced only one of them. The use of the combination of CD86 and CD54 as a positive indicator improved the accuracy of the test. We calculated the estimated concentration to induce CD86 or CD54 expression with RFI ≥ 150% for CD86 (EC150) or RFI ≥ 200% for CD54 (EC200) in h-CLAT and compared these values with the EC3 values of LLNA. Especially, there was a good correlation between EC150 of CD86 and EC3. These data suggest that h-CLAT could be useful not only for hazard identification, but also for estimating the allergic potency of chemicals. Furthermore, comparing h-CLAT with human test data, the correspondence was more than 80% (for about 50 chemicals). In conclusion, h-CLAT is a useful cell-based *in vitro* skin sensitization method.

1145 USE OF A B CELL MARKER TO DISCRIMINATE BETWEEN THE IRRITANT AND ALLERGENIC POTENTIAL OF D-LIMONENE.

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The local lymph node assay (LLNA) is a validated method for the identification of potential contact allergens. History has shown the LLNA to be a reliable model, though a small number of irritants result in false positives. The use of a B cell marker, specifically the % increase in B220⁺ cells in the draining lymph nodes, has been demonstrated to discriminate between allergens and irritants in the LLNA. *d*-Limonene is a cyclic terpene that rapidly autoxidizes. The oxidation products are known to be contact allergens, while un-oxidized/high purity *d*-limonene (HDL) is not considered sensitizing. However, HDL has been reported to result in positive responses in the LLNA and is a strong irritant in human dermal primary irritation tests. These facts have lead to the question 'Are HDL responses in the LLNA irritant or allergenic?'. To answer this we conducted a series of LLNAs utilizing the B220 endpoint. Groups of mice (n=5, CBA/Ca) were treated on the ears for 3 consecutive days with neat HDL or 50% HDL in one of four vehicles - ethanol (EtOH), 1:3 EtOH:diethyl phthalate (DEP), 3:1 EtOH:DEP, DEP. Controls received dinitrochlorobenzene (DNCB, allergen); Benzalkonium chloride (BZC, irritant), vehicle or left naïve. Draining lymph nodes were excised on day 6; a single cell suspension was prepared and stained with fluorescent-labeled anti-B220 antibody. The suspensions were analyzed by flow cytometry. A % test:vehicle B220⁺ ratio of > 1.25 is typically indicative of an allergenic response. In the present study the 1.25 cutoff could not be relied upon, due to a slight decrease in the background B220⁺ population. Therefore, each group was compared to the appropriate controls on its own

merits. There was good discrimination between the responses to BZC (mean B220⁺ ratio = 1.48) compared to DNCB (mean B220⁺ ratio = 3.09). Both neat (mean B220⁺ ratio = 1.57) and each of the 50% solutions (mean B220⁺ ratio = 1.44) of HDL resulted in B220⁺ ratios similar to that of BZC, indicating that HDL was acting as an irritant. The vehicle employed appeared to play no role in the response.

1146 PLASMACYTOID DENDRITIC CELLS AS A TOOL FOR TESTING ALLERGENICITY OF CHEMICALS.

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A predictive test system for assessing the allergenicity potential of chemicals will have enormous significance in industries involved in cosmetic, personal hygiene and topical medication products and in the fields of dermatology and immunology. Since dendritic cells/Langerhans cells (DC/LC) are the first cells responsible for sampling skin and mucosal surfaces for changes in the antigen microenvironment, we investigated whether phenotypic and functional changes to subset of DC, plasmacytoid dendritic cells (pDC), could be used to identify contact allergens. To achieve this goal, normal human DC were generated from CD34⁺ progenitor cells and were cryopreserved. Frozen DC were thawed and the pDC fraction (CD123⁺/CD11c⁻) was collected using FACS sorting. The pDC were cultured, expanded, and pulsed with chemical allergens (n=12) or irritants (n=7). Sub-toxic concentrations of each chemical was determined using FACS analysis on propidium iodide stained cells. Concentrations that resulted in >75% cell viability were used in the study. Results showed that exposure of pDC (n=6 donors) to allergens induced an increase (≥ 1.5 fold) in surface expression of CD86 for 11 of the 12 allergens; however, 6 of 7 irritants did not result in increased CD86 expression. Based on these findings, a prediction model was developed with a sensitivity of 92%, specificity of 86%, and an accuracy of 89%. Increased levels of released IL-6 were also detected in culture supernatants of allergen-pulsed pDC (6 of 6) but not from cultures of non-allergen-treated pDC. In conclusion, the use of CD86 expression on pDC appears to be a sensitive and specific predictor of allergenicity of chemicals. When compared with existing animal models, the assay is advantageous because high throughput screening of chemicals using cells of human origin is possible at low cost.

1147 ROLE OF CELL-SURFACE THIOLS IN ACTIVATION OF HAPTEN-TREATED THP-1 CELLS AND EVALUATION AS A BIOMARKER FOR IN VITRO SENSITIZATION TEST.

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It is known that in the induction phase of skin sensitization, antigen-presenting cells (APC), including dendritic cells (DCs), migrate into lymph nodes and present antigens to T cells after having been activated by haptens. Furthermore, the activation is accompanied with the augmentation of CD86 expression. Human monocytic cell line THP-1 is used as an indicator for predicting skin sensitization potential because its response to haptens is similar to that of DCs. Although p38 mitogen-activated protein kinases (MAPKs) and intracellular redox imbalance play crucial roles in the activation of THP-1 by skin sensitizers, the trigger of cell activation has not been identified. Therefore, we examined whether haptens induce THP-1 activation directly or indirectly. 2,4-Dinitrochlorobenzene (DNCB), but not DNP-BSA conjugate, induced CD86 expression. DNCB and nickel sulfate (NiSO₄) induced related changes of cell-surface thiols and phosphorylation of p38 MAPK. However, DNCB is membrane-permeable, and so its direct effect may not be confined to cell membrane proteins. Furthermore, we found that CD86 expression and macrophage inflammatory protein-1 β (MIP-1 β) production were augmented by the membrane-impermeable thiol blocker 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), and these changes were suppressed by an inhibitor of the p38 MAPK pathway, SB203580. These results suggest that cell-surface thiols are one of the triggers of cell activation. Finally, we evaluated about 60 chemicals, including allergens and non-allergens. When tentative criteria for a significant change of surface thiols (RFI $\leq$ 85% and RFI $\geq$ 115 %) were applied, the accuracy of in vitro assay vs. in vivo assay was 80 %. Taken together, our data indicate that cell-surface thiols might be useful as a biomarker for an in vitro sensitization assay.

1148 THE PREDICTION AND CONFIRMATION OF POTENTIAL RESPIRATORY SENSITIZERS GENERATED FROM INDOOR AIR SAMPLES.

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Of the 89 million people in the US working in indoor office environments, between 35 and 60 million experience symptoms such as eye, nose and throat irritation, headache, and fatigue. Several investigations into these complaints have as-

cribed the effects to volatile organic compounds (VOC) emitted from building materials, cleaning formulations or other consumer products. Additional compounds can result from these VOC's, being transformed by hydroxyl or nitrate radicals and ozone, present in indoor environments. Several dicarbonyls, such as glyoxal (GX), methylglyoxal (MG), glycolaldehyde (GA) and diacetyl (DA) have been identified as VOC reaction products of indoor environment chemistry. The sensitization potential of these compounds was assessed using three quantitative structure-activity relationship (QSAR) programs. DEREK for Windows and Logistic Regression predicted all compounds as sensitizers, while TOPKAT 6.1 predicted all compounds except for MG. Next the compounds were tested in a combined irritancy and local lymph node assay (LLNA). Mice were exposed dermally to GX (0.35-1.5%) MG (0.05-1%), GA (0.8-3.2%) or DA (1.25-12%). All compounds except for GX were found to be irritants. All compounds were found to be sensitizers resulting in EC3 values of 0.74, 0.18, 1.8, and 1.9%, respectively. No changes in organ, body weight, or serum IgE levels were observed following exposure to the compounds. MG, GX, and GA did not significantly increase IgE+B220⁺ cell populations in the draining lymph nodes, but 5% DA resulted in significant increases in both B220⁺ and IgE+B220⁺ cells. The four compounds generated by indoor air chemistry were predicted by QSAR analysis to be sensitizers and confirmed using an animal model. The identification of these compounds as sensitizers may help to explain some of the health effects associated with indoor air complaints.

"The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the National Institute for Occupational Safety and Health."

1149 EVALUATION OF THE SENSITIZATION POTENTIAL OF CHEMICALS USING IL-8 RELEASE IN NAÏVE THP-1 CELLS.

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At present, the assessment of the allergenic potential of chemicals is carried out using experimental models. Over the last decade, several in vitro methods have been proposed to identify the potential of chemicals to induce skin sensitization to meet current animal welfare and public opinions. Many attempts have been made to develop in vitro sensitization tests using primary dendritic cells derived from peripheral blood mononuclear cells or from CD34⁺ hematopoietic progenitor cells. The major limitations of such tests are donor-to-donor variability, low levels in the source, and a possible shortage of human sources.

The aim of this study was to establish an in vitro test to identify chemical allergens using the human monocytic leukemia cell line, THP-1, in order to avoid some of these difficulties. Many chemokines, including eotaxin, macrophage inflammatory protein-1 α , RANTES and interleukin (IL)-8 have all been consistently reported to be up-regulated in allergic inflammation. Among them, we investigated whether IL-8 production can provide a methodology for the detection of allergens. THP-1 cells were exposed to contact allergens (nickel sulfate, p-phenylenediamine, dinitrochlorobenzene, cinnamaldehyde, tetramethylthiuram disulfide), to respiratory allergens (diphenylmethane diisocyanate, trimellitic anhydride, ammonium hexachloroplatinate) and to irritants (sodium lauryl sulphate, salicylic acid, phenol). Following 48 h of incubation, IL-8 production was significantly increased after stimulation with all allergens tested, with the exception of trimellitic anhydride, whereas irritant exposure failed to induced IL-8 release. The lack of IL-8 production by trimellitic anhydride can be explained by the readily hydrolysis of this chemical in water to trimellitic acid, which is not an allergen.

In conclusion, IL-8 production by THP-1 cells provides a promising in vitro tool for identification and discrimination between allergens and irritants.

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1150 CAN LOW MOLECULAR WEIGHT (LMW) CONTACT ALLERGENS INDUCE ALLERGY IN THE RESPIRATORY TRACT?

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Most LMW allergens cause contact allergy in the skin, whereas only a small number are known to cause respiratory allergy. These respiratory allergens are all positive in the LLNA and are thus able to induce skin sensitization as well. The question therefore is: can skin allergens induce allergy in the respiratory tract when inhaled, and, if so, what are the consequences? BN rats were sensitized by two dermal applications of dinitrochlorobenzene (DNCB; 1% at day 0; 0.5% at day 7). Controls received the vehicle. The challenge consisted of nose-only inhalation of 20 mg/m³ of

DNCB for 15 min, at day 21. Four groups were used: unsensitized/unchallenged or -/-; +/-; -/+ and +/+. The following parameters were examined: breathing frequency and pattern, total IgE, specific IgG, histopathology of the respiratory tract and bronchoalveolar lavage (BAL) cells. Lungs were examined immunohistochemically or were analyzed for clusters of activated genes (Affymetrix microarrays). Sensitization via the skin induced upregulation of a cluster of immune-related genes in lung. Inhalation challenge with DNCB in sensitized animals did not provoke respiratory allergy based on breathing parameters, airway inflammation, IgE, and gene activation. However, the total cell number in BAL in the +/- group was about 3 times higher than in the other 3 groups, largely due to an increase in eosinophils. Thus, the immune system of the BN rat recognizes the contact allergen DNCB but is only minimally affected by the compound. This implies that the hazard of inhaling contact allergens may be of a completely different magnitude than that of typical respiratory allergens. It is important to realize that this is based on a single inhalation exposure. To substantiate the conclusion, the response upon repeated exposure should be investigated, because, if 'contact' allergy can be evoked in the respiratory tract by allergens like DNCB, it is possibly an insidious process. The research was funded by CEFIC-LRI

1151 THE RESPIRATORY LOCAL LYMPH NODE ASSAY (RESPIRATORY LLNA) AS A TOOL TO STUDY RESPIRATORY SENSITIZERS.

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The LLNA is used to test the potential of low molecular weight (LMW) compounds to induce sensitization via the skin. All LMW respiratory allergens tested, have also tested positive in this dermal assay. However, it is unknown if potential and potency via this route are comparable to those via the inhalation route. In the present study, BALB/c mice were exposed nose-only to trimellitic anhydride (TMA; 30 mg/m³), phthalic anhydride (PA; 15 mg/m³), hexamethylene diisocyanate (HDI; 15 mg/m³), or toluene diisocyanate (TDI; 7.5 mg/m³), or to 30 mg/m³ of dinitrochlorobenzene (DNCB) or oxazolone (OXA). The animals were exposed during 3 days for 45, 90, 180 or 360 min/day. The animals were necropsied 3 days after the last exposure, the draining lymph nodes (LNs) were excised and harvested cells were processed with 3H-thymidine to determine proliferation. For comparison, the dermal LLNA was performed as well. Negative (vehicle) controls were exposed by ear application to acetone-olive oil (4:1) and by inhalation to acetone. In addition, cytokine production (IFN- γ and IL-4) was measured in the LNs using ELISA. All allergens induced grossly observed enlargement of and proliferation in the mandibular LNs draining the upper respiratory tract. Increased INF- γ was found with all allergens tested: DNCB, OXA, TMA and HDI, whereas increased IL-4 was found with the typical respiratory allergens TMA and HDI only. With regard to potency ranking: the typical contact allergens DNCB and OXA were at least of comparable potency as TMA in the respiratory LLNA. In analogy to the dermal LLNA, the present results in the respiratory LLNA suggest that strong contact allergens such as DNCB and OXA can also act as potent sensitizers by inhalation. The risk of humans of course depends on exposure. With regard to cytokine production IL-4, but not IFN- γ , seems to be able to discriminate between typical respiratory and contact allergens. This research is funded by the Dutch Ministries of VWS and SZW.

1152 LACK OF EFFECT OF PHTHALATES ON TYPE 2 CYTOKINE EXPRESSION PROFILES.

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The prevalence of IgE-mediated allergic diseases (including respiratory allergy and asthma) in Western Europe and the US has been increasing over the last decades. It has been suggested that one possible cause is the presence in the environment of chemicals that may act as adjuvants, including certain commonly used phthalate plasticizers, such as di-(2-ethylhexyl) phthalate (DEHP) or diisononyl phthalate (DINP). The induction of IgE antibody is orchestrated by the development of T helper (Th) cells with a type 2 cytokine secretion profile (interleukins [IL]-4, -5, -10 and -13). In the current experiments we have examined the ability of the phthalates DEHP and DINP to influence cytokine production by activated murine lymph node cells (LNC). LNC preparations were isolated after *in vivo* priming by subcutaneous injection of the antigen keyhole limpet hemocyanin (KLH) and alum (an adjuvant for IgE responses). Culture of KLH-primed LNC with KLH for 96 hr resulted in marked lymphocyte activation characterised by vigorous proliferation and expression of a preferential Th2 type cytokine profile with relatively low levels

of Th1 cytokines interferon γ and IL-12. Co-culture with DEHP or DINP (0.1 to 1000 μ M) in the presence of various concentrations of KLH (incorporating suboptimal doses for both read outs) failed to impact on either proliferation or cytokine secretion profiles. These data indicate that the reported adjuvant effects of phthalates are not as a result of the ability of these chemicals to modulate cytokine secretion profiles of activated LNC.

1153 DIACETYL, AN ARTIFICIAL FLAVORING COMPOUND IN POPCORN, INDUCES CONTACT SENSITIZATION IN FEMALE BALB/C MICE.

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Diacetyl (2,3-butanedione: BTD) is a natural by-product of secondary or malolactic fermentation. BTD is used for artificial flavoring, which adds the characteristic flavor most commonly associated with butter and cream in food products (e.g., popcorn). It has been reported that exposure to diacetyl can result in damages to the skin, eyes, mucous membrane and respiratory system. The objective of the present study was to evaluate the hypersensitivity inducing potential of BTD using Local Lymph Node Assay (LLNA), Irritancy Test (IRR), Mouse Ear Swelling Test (MEST), flow cytometric analysis, and cytokine production. Female BALB/c mice were exposed to BTD at levels of 1%, 2.5%, 5%, 10% and 25% (w/v) in acetone: olive oil (4:1 AOO) vehicle by dermal application in LLNA and IRR. In the MEST assay, animals were sensitized with either 10% or 25% (w/v in AOO) and challenged with 25% BTD. There were no significant changes in the body weight for BTD treatment groups in any of the studies when compared to the vehicle controls. An increase in the proliferation of auricular lymph node cells was observed at all BTD, reaching the level of 3-fold increases at the 10% and 25% concentration as evaluated by LLNA. However, BTD did not cause irritant effects at any concentrations. In the MEST assay, the animals which were sensitized with either 10% or 25% BTD and challenged with 25% BTD exhibited significant increases in the percent ear swelling when compared to the challenge only group at both 24 and 48-h post challenge. In addition, a significant increase in the percentage of B lymphocytes (B220+) was also observed. Furthermore, dermal exposure to BTD (25%) for 3 days also significantly increased the production of IL-13 and IFN- γ , evaluated by ELISA, in the auricular draining lymph node cells after concanavalin A stimulation for 24 h in culture. Taken together, BTD can be classified as a sensitizer at the concentrations tested in female BALB/c mice (Supported by the NIEHS NO1-ES-05454).

1154 EVALUATION OF THE SYSTEMIC SENSITIZATION POTENTIAL OF 2, 3 BUTANEDIONE (DIACETYL) IN BALB/C MICE.

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Inhalation of and contact with high concentrations of diacetyl (2,3 butanedione)-containing butter flavor seasoning vapors have been associated with an increased prevalence of airway obstruction (asthma, bronchitis, bronchiolitis obliterans), skin irritation and severe fatigue among employees at microwave popcorn plants. Several reports suggest that diacetyl targets the respiratory epithelium, leading to inflammation, fibrosis, necrosis and irreversible tissue damage. C57/BL6 mice exposed to diacetyl by inhalation developed rhinitis, laryngitis and bronchitis, while mice exposed by oropharyngeal aspiration developed foci of pulmonary fibrosis without inflammation. We are using a BALB/c mouse hypersensitivity model to investigate the mechanisms of diacetyl-induced hypersensitivity. Diacetyl stimulated lymph node cell proliferation following subcutaneous exposure in a dose dependant manner at concentrations as low as 2.5 mg/kg/day. At 25 mg/kg/day diacetyl, an increase in total cell number of all lymphocyte sub-populations tested (CD3+ T-cell, B220+ B-cell, CD4+ T-helper cell, CD8+ cytotoxic T-cell, Mac3+ macrophage) was observed in both lymph nodes and spleen, indicating possible generalized systemic inflammation. At 10 mg/kg/day diacetyl, only B-cells were altered in the spleen. However, in the draining lymph nodes, both T-cell (CD3+, CD4+ and CD8+) and B-cell numbers were elevated. Diacetyl also regulated synthesis of several inflammatory cytokines, including IL-1 α and IL-4 in lymph nodes and spleen, and IL-2, IL-10, MIG, and TNF- α in lymph nodes. The lymph node cytokine profile suggests alteration of both Th1 and Th2 cytokines. Collectively these data suggest that exposure to diacetyl induces inflammation, systemic hypersensitivity, and functional changes in immune responses. Further investigation will more fully elucidate the mediators, mechanisms and cell populations involved, and may offer potential strategies for therapeutic intervention in exposed humans.

1155 AGE AND SEX CONTRIBUTE TO ASTHMA HETEROGENEITY IN ANIMAL MODELS.

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Asthma is a lung disorder characterized by eosinophil infiltration and airway hyper-responsiveness (AHR). However, the predominance of airway obstruction vs inflammation differs in asthmatics, with numerous asthma phenotypes being described (eosinophilic, neutrophilic, childhood onset, steroid-resistant, etc). The purpose of the present study was to assess contributions of age and sex to the profile of asthma symptoms in guinea pig and mouse models of asthma. Female and male Hartley guinea pigs and Balb/c mice were sensitized and challenged with ovalbumin (OVA) before sexual maturity (Y/Y) or after sexual maturity (A/A) and eosinophil infiltration and AHR determined. For mice, Y/Y and A/A animals were sensitized 19 or 82 days postnatal, respectively. With guinea pigs, Y/Y and A/A animals were sensitized at 9 or 57-84 days postnatal, respectively. Eosinophil peroxidase in homogenized lung was used as an indicator of eosinophilia. In guinea pigs, AHR was assessed by measurement of pulmonary resistance to i.v. methacholine in anesthetized animals. In mice, AHR to aerosolized methacholine was determined using unrestrained barometric plethysmography to measure PenH. In the guinea pig, 24 hours after OVA challenge, eosinophils increased significantly in the lung in Y/Y females with minimal airway hyperresponsiveness detected. In contrast, A/A female guinea pigs accumulated very few eosinophils into the lung after OVA challenge, but the airway hyperresponsiveness was marked. In contrast to guinea pigs, OVA challenge of mice resulted in greater increases in eosinophils in A/A animals than Y/Y animals whether male or female. Marked AHR was evident in both Y/Y and A/A mice. These studies clearly indicate that age and sex contribute to asthma heterogeneity and need to be carefully considered when using animal models of asthma. (Support: Department of Defense CDMRP; DAMD 17-02-1-0191).

1156 ZN/CU SUPEROXIDE DISMUTASE AUTOANTIBODY IN CEMENTED TUNGSTEN CARBIDE WORKERS.

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Asthma and hard metal pneumoconiosis have been associated with exposure to aerosols containing nickel, cobalt, and tungsten in the cemented tungsten carbide industry. Only a few studies reported metal-specific IgE in sera of hard metal aerosol exposed workers, but antibody prevalence was very low. In the present study, sera from 140 cemented tungsten carbide workers were screened for specific IgG against chromium (Cr³⁺), copper (Cu²⁺), cobalt (Co²⁺), zinc (Zn²⁺) and nickel (Ni²⁺), haptenized to albumin (HSA) and superoxide dismutase (SOD). SOD was used as a protein carrier for haptenization, because its physiologically active form is Zn/Cu-SOD and it is an important endogenous antioxidant enzyme. Antigens were characterized using MALDI-TOF mass spectrometry (MS). Mass spectra of haptenized SOD showed 0-3 Co, 0-3 Zn, and 0-1 Ni ions bound per SOD molecule. Quantification of specific binding of Cu was not possible due to the large numbers of copper ions bound to SOD, consistent with non-specific binding. Haptenization to HSA was evident by color change resulting from binding of the metals to HSA. Specific chromium and cobalt IgGs were not found using either of the haptenized proteins. Casein was not sufficient to block non-specific IgG binding from 19 sera. These sera were re-screened using bovine serum albumin blocked ELISA plates. Within this subgroup, specific-IgG was found for Ni-SOD in one worker, and IgG specific to both Cu- and Zn-SOD was found in the sera of 10 workers. Anti-apo-SOD IgG was not found. This is the first report of autoantibody against Zn/Cu-SOD. It is not known if the 7.1% prevalence rate of anti-Zn/Cu-SOD IgG in this group of cemented tungsten carbide workers is excessive. The relationship of Zn/Cu-SOD autoantibody to reported respiratory symptoms and respiratory disease has not yet been established in this workforce. The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent agency determination or policy.

1157 THE ROLE OF THIOLS AND DISULFIDE FORMATION IN MERCAPTOTENZOTHIAZOLE ALLERGENICITY.

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The rubber accelerator, 2-mercaptobenzothiazole (MBT), can cause allergic contact dermatitis (ACD) from gloves and other rubber products, but the chemical mechanism leading to MBT's allergenicity is unknown. It was hypothesized that the thiol

group is critical to MBT's covalent binding/haptenation to nucleophilic protein residues. Oxidative transformation of MBT to the disulfide 2, 2'- dithiobis(benzothiazole) (MBTS) was observed within the glove matrix when hypochlorous acid, iodine or hydrogen peroxide were used as oxidants. Cysteine reduced MBTS to MBT with subsequent formation of the mixed disulfide 2-amino-3-(benzothiazol-2-yl disulfanyl)-propionic acid. Simultaneous reduction of MBTS and disulfide formation with Cys34 on bovine serum albumin was observed, suggesting a potential route of protein haptenation through covalent bonding between cysteinyl residues on proteins and the MBT/MBTS thiol moiety. MBT metabolites were not found following incubation with isoniazid and dexamethasone-induced rat liver microsomes and a NADPH regeneration system. Guinea pigs were sensitized to MBT using a modified guinea pig maximization assay (GPMT) and cross-reactivity towards MBTS and the free thiol lacking or blocked compounds benzothiazole (BT), 2-hydroxybenzothiazole (HBT) and 2-(methylthio)benzothiazole (MTBT) assessed. MBT and MBTS, but not BT, HBT or MTBT elicited ACD in MBT-sensitized animals. Both chemical and experimental animal studies demonstrate that the thiol group is required for MBT allergenicity and also suggest that MBT first undergoes non-enzyme mediated oxidation to MBTS with subsequent haptenation via the formation of mixed disulfides with sulfhydryl groups on proteins. This work was supported by an interagency agreement with the NIEHS (Y1-E50001-06). The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent agency determination or policy.

1158 ANTI-INFLAMMATORY ACTIVITY OF Fisetin IN HUMAN MAST CELLS (HMC-1).

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Mast cells play an important role in the pathogenesis of allergic diseases through the release of inflammatory mediators such as, histamine, cysteinyl leukotrienes, cytokines, and chemokines. Flavonoids, like fisetin are naturally occurring molecules with antioxidant, cytoprotective, and anti-inflammatory actions. The aim of our study was to examine whether fisetin modulates the inflammatory reaction in a stimulated human mast cell (HMC-1). HMC-1 cells were stimulated with 40 nM of phorbol-12-myristate 13-acetate plus 1 µM of calcium ionophore A23187 (PMACI). The expression of pro-inflammatory cytokines was measured by reverse transcriptase-polymerase chain reaction. The nuclear transcription factor (NF)-κB promoter-mediated luciferase activity and the DNA-binding activity were detected by luciferase assay and electrophoretic mobility shift assay. Nuclear translocation of p65 NF-κB and phosphorylation of mitogen-activated protein kinases such as, p38, extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) were detected by the Western blotting. Fisetin decreased the gene expression and production of PMACI-stimulated tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-4, IL-6 and IL-8 in HMC-1. Fisetin suppressed NF-κB activation induced by PMACI, leading to expression of IκB-α phosphorylation and degradation. Fisetin also suppressed NF-κB promoter-mediated luciferase activity and DNA-binding activity. In addition, fisetin inhibited PMACI-induced phosphorylation of p38 MAPK, ERK, and JNK. We observed that the selective inhibitors of p38 MAPK, ERK, JNK and NF-κB reduced PMACI-induced cytokine expression in activated HMC-1 cells. These data display that the inhibitory effect of fisetin on the pro-inflammatory cytokines was p38 MAPK, JNK, ERK and NF-κB dependent. Pharmacological actions of fisetin produce new suggestion that fisetin is a potential medicine for treatment of inflammatory diseases through the down-regulation of mast cell activation.

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1159 DOSE METRICS IN THE ACQUISITION OF SKIN SENSITIZATION : THRESHOLDS AND DOSE PER UNIT AREA.

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Allergic contact dermatitis is a common occupational and environmental health problem. Skin sensitization is acquired following topical exposure of an inherently susceptible individual to an appropriate amount of a contact allergen; that required to induce a cutaneous immune response of the magnitude necessary for a degree of immunological priming (sensitization) that will result in a dermal inflammatory reaction being provoked following subsequent exposure. The amount of chemical necessary to result in the acquisition of skin sensitization will be determined primarily by two factors: (a) the inherent potency of the contact allergen, and (b) the con-

ditions of exposure (the site, extent, frequency and duration of exposure). The available human and experimental animal data derived from contact allergens of varying potencies demonstrate that under most normal conditions of exposure it is the dose per unit area of chemical, rather than the total delivered dose, that has the over-riding impact on the effectiveness of sensitization. The interpretation is that there is a certain critical level of chemical allergen and LC availability for transport required in the area of skin from which there is lymphatic drainage to a single lymph node, or to a single series of lymph nodes. The exception to this rule is when the area of the application site drops below a certain critical level. Under such circumstances, there will be insufficient numbers of primed LC transporting antigen to the draining lymph node to reach the threshold of activation necessary for an effective immune response.

1160 CHARACTERIZATION OF MALE REPRODUCTIVE TOXICANTS IN AN IN VITRO 3-D SERTOLI CELL/GONOCYTE CO-CULTURES.

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The development of *in vitro* models for testicular toxicity can provide important mechanistic tools for the assessment of reproductive toxicity induced by environmental agents. In our previous study, we reported a significantly improved 3D-Sertoli cell/gonocyte co-culture (SGC) system that utilizes an extracellular matrix Matrigel (ECM) applied as an overlay instead of a substratum. Our findings have demonstrated that application of an overlay of ECM to SGC permitted the formation of testicular-like multilayered architectural structure that mimics *in vivo* characteristics of seminiferous tubules. The purpose of this study is to refine and characterize our *in vitro* 3-D SGC culture using testicular specific toxicants and comparing molecular and cellular response endpoints. A range of known *in vivo* male reproductive toxic phthalate esters such as dibutyl phthalate (DBP), diethylhexyl phthalate (DEHP), dipentyl phthalate (DPP) and three non-developmental testicular toxic phthalates such as diethyl phthalate (DEP), dimethyl phthalate (DMP), dioctyl tere-phthalate (DOTP) were evaluated. We compared their differential effects on morphology and cell viability 24 h after treatment. We also compared their differential impacts on cell stress signaling pathways such as phosphorylated p38, SAPK/JNK by Western blot analysis using phospho-specific antibodies. We observed that treatments with phthalates that had been identified as developmental testicular toxicants induced significantly more dose-dependent morphological changes and decrease in cell viability than those treated with non-developmentally toxic phthalates. Our results support the expansion of 3-D SGC as an *in vitro* model useful for assessment of male reproductive developmental toxicity. (This work was supported in part by EPA R826886, NIEHS 1P01ES09601, R01-ES1063, P30 ES07033 and Colgate-Palmolive Grants for Alternative Research, Society of Toxicology)

1161 BOVINE SPERMATOZOA: AN IN VITRO MODEL FOR REPRODUCTIVE TOXICITY?

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As part of the EU ReProTect project, an *in vitro* method is developed to test chemicals for their toxic effect on thawed mature bovine spermatozoa using Computer Assisted Sperm Analysis (CASA). Motility parameters and membrane integrity were used to determine toxicity.

Bovine mature spermatozoa were thawed and exposed to chemicals for one hour at 37°C. After incubation, motility parameters were determined in a dose range finding study (DRF) at concentrations of 10, 100 and 1000 µg/ml. Based on the outcome of the DRF a main test was performed using seven concentrations in a narrowed range to determine the motility IC50 for each compound. Positive and negative controls were included. A viability test was performed to distinguish between deactivation of sperm motility and cell death due to unspecific toxicity. Approximately 30 chemicals were tested at two laboratories. The IC50 for motility ranged from 0.06mM to 6 mM. In 20% of the chemicals the IC50 was not reached within the interval tested.

This test appeared to be a simple, fast and consistent method to predict potential impact of chemicals on male fertility using endpoints motility and membrane integrity. Since, for some chemicals, percentages viable cells differed from percentages motile cells, inclusion of a viability test seems useful in order to set up a prediction model. Besides, specific motility parameters (e.g. velocity) easily measured with CASA could give useful information on working mechanisms of specific chemicals. Whether this *in vitro* test could be used as part of high throughput *in vitro* tests in reproductive toxicological evaluation should be evaluated within the EU ReProTect project.

1162

COMBINATION OF GROWTH FACTORS AND EXTRACELLULAR MATRIX COMPONENTS INDUCE EPIDERMAL/EPITHELIAL DIFFERENTIATION IN MOUSE EMBRYONIC STEM CELLS. A.R.CALABRO AND F.A.BARILE ST JOHN'S UNIVERSITY COLLEGE OF PHARMACY AND ALLIED HEALTH PROFESSIONS DEPARTMENT OF PHARMACEUTICAL SCIENCES, TOXICOLOGY DIVISION, JAMAICA, NEW YORK 11439.

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Mouse embryonic stem (ES) cells (ES-D3, ATCC) are undifferentiated pluripotent cells derived from the inner cell mass of mouse blastocysts capable of forming embryoid bodies (EBs) as an intermediate stage of differentiation. Since several growth factors (GFs) direct differentiation of EBs, we examined the effect of four GFs (EGF, KGF, TGFβ1, and amphiregulin) on the expression of primers for gene markers (OCT3/4, for undifferentiated cells; AFP, for EBs; ARNT and EGFR, for differentiated cells; TGFβR2, for transformed cells) in cells cultured on filter inserts and coated with extracellular matrix (ECM) proteins (collagen types I&IV, fibronectin, laminin). Gene expression was detected using RT-PCR from RNA purified from confluent ES cells after 14-16 days on the inserts. The results suggest that, in general, EBs preferentially differentiate in response to specific combinations of GFs and ECM components, and express the gene markers accordingly. The data supports our previous paracellular permeability and acute cytotoxicity studies suggesting that specific GFs and ECM proteins induce formation of epidermal-like membranes with high transepithelial electrical resistance. Thus, the cell culture insert model represents a novel approach for inducing membrane formation in drug screening and cytotoxicity testing protocols.

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EXPRESSION AND FUNCTIONALITY OF P-GLYCOPROTEIN IN HUMAN AND RAT TROPHOBLASTS USING *IN VITRO* PRIMOCULTURES BEGHIN D.1, 2, DELONGEAS J.-L.2, CLAUDE N.2, FARINOTTI R.1 AND GIL S.1 1 DEPARTMENT OF CLINICAL PHARMACY (UPRES EA2706), PARIS XI UNIVERSITY, FRANCE. 2 DRUG SAFETY ASSESSMENT, SERVIER GROUP, GIDY AND PARIS, FRANCE.

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The placenta assumes several functions during pregnancy which are essential for the fetal development and plays an important role in drugs metabolism. However a better understanding is needed in order to evaluate some pharmacological and toxicological aspects of xenobiotics used during pregnancy. It is now well established that P-glycoprotein (P-gp) participates in the efflux of maternal drugs thereby reducing chemical fetal exposure.

The goal of our study was to evaluate expression and functionality of P-gp in human and rat trophoblasts using *in vitro* primoculture models.

P-gp expression was evaluated by western blot analysis and activity by rhodamine uptake in presence or absence of the inhibitor cyclosporine A. Human and rat trophoblasts were cultured 5 and 7 days, respectively.

i) Differences in P-gp expression were observed in primocultures: in human trophoblasts, P-gp expression was stable during the first 3 days then decreased. In rat trophoblasts, a time dependent increase in P-gp expression was notified from day 2 to day 4 followed by a plateau.

ii) Activity of P-gp was confirmed in both placental primocultures, by a 60% inhibition with Cyclosporine A.

iii) Moreover, expression and activity of P-gp was also observed with cryopreserved rat trophoblasts.

This preliminary study shows that these models are able to quantify the expression and the activity of P-gp both in human and rat trophoblast primocultures. These primocultures seem promising for a better understanding of the role of efflux transporters in the toxicity of drugs administered during pregnancy but need to be confirmed in larger series.

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FORMATION OF INTACT MEMBRANES WITH HIGH TRANSEPITHELIAL ELECTRICAL RESISTANCE IN MOUSE EMBRYONIC STEM CELLS.

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Mouse embryonic pluripotent stem cells (mES, ES-D3) were induced to form membrane structures in cell culture inserts, using combinations of extracellular matrix (EM) components and growth factors (GFs). The ability of the stem cells to

form intact membranes and construct tight junctions (TJs) was assessed by measuring transepithelial electrical resistance (TEER) and passage of TJ markers. Confirmation of differentiation and formation of epidermal/epithelial-like membranes is supported by the expression of gene markers for embryoid bodies (EBs), differentiated and undifferentiated cells. Further evidence for formation of TJs in differentiated cultures is demonstrated by immunohistochemical localization of TJ markers occludin and F-actin. Mouse ES cells were inoculated on mitomycin-inactivated 3T3 mouse embryonic fibroblasts plus LIF factor, to maintain the undifferentiated state, after which mES cells were stimulated to form EBs. After 4 days EBs were transferred to culture inserts coated separately with EM components (collagen I or IV, fibronectin and laminin) in the presence of a GF (amphiregulin, EGF, KGF or TGF β 1). After 10 to 14 days, TEER was measured at 700 Ω -cm². After exposure to 19 chemicals for 24-h, cell viability and paracellular permeability (PP) were measured. The data indicate that cell viability decreases before the integrity of the membrane is compromised. Also, TEER measurements inversely correlated with increased passage of TJ markers for most chemicals. We conclude that mES cells can be induced to form TJs in coated inserts after treatment with specific GFs as evidenced by progression of high TEER values comparable to those seen previously with Caco-2 cells. The extent and direction of differentiation is time- and GF-dependent. This represents the first study describing the manipulation of mES cells toward specific tissue organization, with potential for use as an *in vitro* model for biological membrane formation and cytotoxicity testing.

1165 RESULTS OF A PROTOCOL STANDARDIZATION STUDY FOR THE LUMI-CELL® ESTROGEN RECEPTOR (ER) TRANSCRIPTIONAL ACTIVATION (TA) BIOASSAY.

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In 2003, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) concluded that none of the ER TA assays considered for inclusion in the EPA Endocrine Disruptor Screening Programs Tier 1 battery had been adequately validated. NICEATM subsequently conducted a study to standardize protocols to be used in formal validation studies for the XDS LUMI-CELL® ER TA test method, a proposed Tier 1 *in vitro* screening assay for the detection of ER agonists and antagonists. The objective of the study was to develop standardized protocols that can be easily transferred to other laboratories and be used to obtain reproducible results for the detection of ER agonists and antagonists. The study included optimization of the use of reference standards and controls, and compared quantitative and qualitative methods for evaluating cytotoxicity. The intralaboratory reproducibility and accuracy of the standardized protocols was demonstrated using eight coded substances covering a range of ER agonist activities, including negatives (atrazine [ATZ], bisphenol A [BPA], bisphenol B [BBP], corticosterone [COR], o,p'-DDT [DDT], diethylstilbestrol [DES], 17 α -ethinyl estradiol [EE], and flavone [FLA]) and eight coded substances covering a range of ER antagonist activities, including negatives (butylbenzyl phthalate [BBP], dibenzo[a,h]anthracene [DBA], flavone [FLA], genistein [GEN], nonylphenol [NON], progesterone [PRO], o,p'-DDT [DDT], and tamoxifen [TAM]). EE, DES, BPA, BBP, DDT, and FLA were classified as estrogenic agonists, while ATZ and COR did not induce a significant ER TA response. TAM, DBA, FLA, and GEN, were classified as antagonists, while BBP, PRO, NON, and DDT did not affect ER TA response. This study demonstrated the intralaboratory reproducibility and accuracy of this test method and indicated its readiness for a multi-laboratory validation study. Supported by NIEHS Contract N01-ES-85424.

1166 NICEATM/ECVAM/JACVAM MULTI-PHASED INTERNATIONAL VALIDATION STUDY OF A STABLY-TRANSFECTED ESTROGEN RECEPTOR (ER) TRANSCRIPTIONAL ACTIVATION (TA) TEST METHOD.

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The U.S. EPA proposed Tier 1 endocrine disruptor screening program (EDSP) includes validated *in vitro* test methods to determine if chemicals bind to the ER. A stably transfected ER TA method (LUMI-CELL®, Xenobiotic Detection Systems, Inc.) to detect ER agonists and antagonists was subsequently nominated to the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and recommended for validation studies. NICEATM, ECVAM, and JACVAM designed and initiated a four phase international validation study to evaluate the reproducibility and accuracy of the LUMI-CELL® ER bioassay. The study is using three laboratories, one each in Japan, the U.S., and Europe. A standardized test method protocol incorporating ICCVAM recommended ER TA essential test

method components is being used to evaluate 78 coded reference substances recommended by ICCVAM for validation of *in vitro* ER test methods. The first phase will evaluate positive and vehicle controls and establish test acceptance standards for each laboratory. The second phase will evaluate 12 coded reference substances in each lab in two stages. Intra- and inter laboratory reproducibility and accuracy will be assessed during and after each of the first two phases. Excessive variation and discordance will be investigated and protocols modified accordingly. The third phase will evaluate the performance of the optimized test method protocol using the remaining coded 41 minimum validation substances. The final phase will test the final 25 substances in one laboratory. Performance standards will be developed to serve as the basis for determining if similar ER TA methods have comparable or better performance. This multi-phased approach is expected to identify and resolve sources of variation early in the validation process and to generate a highly reproducible test method protocol for international regulatory use. Supported by NIEHS Contract N01-ES-85424.

1167 ICCVAM REVISED RECOMMENDED SUBSTANCE LIST FOR THE EVALUATION OF *IN VITRO* TEST METHODS FOR IDENTIFYING POTENTIAL ENDOCRINE DISRUPTORS.

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NICEATM recently reviewed the commercial availability and cost for the 78 substances recommended by ICCVAM for use in *in vitro* ER and AR binding and TA validation studies. A minimum of 44 substances are recommended for AR binding and TA assays, while a minimum of 53 substances are recommended for ER binding and TA assays. This review indicated that three substances [anastrozole, CGS 18320B, fadrozole] are not commercially available, one substance has restricted commercial availability [ICI 182,780], and the cost to conduct a validation study with six others [actinomycin D, hydroxyflutamide, 4-hydroxytamoxifen, methyltrienolone, 12-O-tetradecanoylphorbol-13-acetate, zearalenone] was considered to be relatively expensive (>\$2000/lab). ICCVAM subsequently replaced the four original substances that are not commercially available or have restricted availability with ones having similar ER and AR activity profiles [4-hydroxyandrostenedione, chrysin, dicofol, raloxifene HCl]. Suitable replacements (19-nortestosterone and resveratrol) were identified to replace two of the six expensive substances, methyltrienolone and zearalenone, respectively. NICEATM also preferred to replace the other four expensive substances but was unable to identify suitable replacements because of their unique activity profiles and/or chemical/physical properties. The revised list of reference substances has now been published*, and is being used for validation of *in vitro* ER TA methods by NICEATM, ECVAM, and JACVAM. Supported by NIEHS Contract N01-ES-85424.

*Addendum to the ICCVAM Evaluation of *In Vitro* Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays." available at <http://iccvam.niehs.nih.gov>.

1168 CYTOTOXICITY IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS EXPOSED *IN VITRO* TO GLYPHOSATE.

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Glyphosate is a broad-spectrum, non-selective herbicide and commonly used in the world to eliminate weeds in agricultural and forest settings. Studies evaluating glyphosate toxicity in animals and environment have been controversial. Additionally, it has been reported that commercial formulations based on glyphosate, are more toxic than pure glyphosate. Accordingly, the purpose of this study was to evaluate cytotoxicity of glyphosate (technical grade) and the commercial formulation Roundup on human peripheral blood mononuclear cells (PBMCs). PBMCs were isolated from healthy volunteers using ficoll-hypaque and exposed to different concentrations of glyphosate (Sigma-Aldrich) and commercial Roundup for 24, 48, 72 and 96h. After treatment, cell viability was determined by trypan blue exclusion method and reduction of XTT reagent (Sigma). We found that both glyphosate (Sigma) and Roundup were cytotoxic to human PBMCs and that cell viability decreased in a concentration-dependent manner. Cytotoxicity of Roundup was significantly higher than cytotoxicity of glyphosate (Sigma), since we found that LC50 (50 lethal concentration) analyzed with trypan blue exclusion method at 24h was 56,4 μ g/ml for Roundup and 1,64 mg/ml for glyphosate (Sigma). Nevertheless, evaluation of cytotoxicity using XTT reagent revealed that PBMCs treated with concentrations of glyphosate (Sigma) equal or higher than

400 µg/ml were metabolically inactive. In summary, these results confirm the cytotoxic effects to human cells reported for glyphosate and the commercial preparations based on it. Also, our results corroborate that commercial formulations are more cytotoxic than the active component, which supports the idea that additives present in commercial formulations, such as Roundup, play a crucial role in the toxicity attributed to glyphosate. Project code: 1419-04-16299. Supported by Colciencias Grant # 282-2004 awarded to Niradiz Reyes, PhD.

1169 FRONTLOADING THE EVALUATION OF BONE MARROW TOXICITY USING A THREE-TIERED APPROACH.

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Bone marrow toxicity is a cause of late stage compound attrition in the pharmaceutical industry, but is typically not assessed prior to the pre-Candidate Drug (CD) phase. The objective of this project was to develop assays such that chemistry-associated liabilities can be evaluated earlier in drug-discovery. To this end, a three-tiered approach has been developed that enables a large number of compounds to be evaluated around early 'Lead Optimisation' phase with more thorough analyses during latter stages of drug discovery. Multiple assays using cell lines representative of broad bone marrow lineages (myeloid, erythroid, lymphoid and stromal) and a non-hematopoietic cell line were developed to assess chemical series (Tier 1). There was a good correlation between the mouse myeloid cell line and the mouse 35mm dish Colony Forming Unit-GM (CFU-GM) assays ($r^2=0.8046$). Additionally, compounds with lineage-specific in vivo bone marrow toxicity showed similar lineage specificity with these tier 1 assays. For example, chloramphenicol, an erythrototoxicant, had IC50 values of 8mM in the erythroid cell line assay and >100mM for the other cell line assays. CFU-assays (Tier 2) are then run to qualify and confirm the findings of tier 1. Tier 2 consists of mouse, rat, dog and human 35mm dish CFU-GM assays to predict species sensitivity. However, these assays are tedious and time-consuming, enabling only a limited number of compounds to be evaluated each month. A mouse 96-well CFU-GM assay that correlated well with the mouse 35mm dish CFU-GM assay ($r^2=0.7666$) was developed to increase throughput capabilities. Results from the tier 1 & 2 assays may also trigger frontloading in vivo studies that are evaluated using flow cytometry (Tier 3). In summary, a three-tiered approach allows compounds with chemistry-related bone marrow toxicity to be eliminated early from the research project pipeline while still evaluating in vivo bone marrow toxicity later in drug discovery.

1170 MODULATION OF THE 3'IgH REGULATORY REGION, A PROSPECTIVE *IN VITRO* SCREENING TOOL FOR IDENTIFYING POTENTIAL IMMUNOTOXICANTS.

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The immune system is critical to human survival. However, assessing alterations of immune function by potential immunotoxicants is complicated by the diffuse nature of the immune system which is composed of various effector cells each with differing effector functions. Current immunotoxicity testing is limited to animal studies. We have developed a model which may provide an in vitro alternative to animal studies in identifying immunotoxicants that specifically target B cell function (i.e., alteration of immunoglobulin (Ig) expression and antibody secretion). This model consists of a well-characterized B cell line, CH12.LX, which appears to appropriately model primary B cell function. We have stably transfected the CH12.LX cell line with a transgene regulated by an enhancer, the 3'Ig heavy chain regulatory region (3'IgH RR), purported to control Ig heavy chain gene expression. Our previous work has identified the 3'IgH RR as a sensitive target of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) possibly mediated by activation of the aryl hydrocarbon receptor (AhR) signaling pathway. We have tested our model with several structurally diverse chemicals shown to activate the AhR signaling pathway and found an association between AhR activation and inhibition of both 3'IgH RR activation and Ig protein expression. We have also tested two chemicals previously shown to activate humoral immunity through non-AhR receptors. Results indicate that our model appropriately identifies immunomodulators of two receptor signaling pathways each leading to altered immunoglobulin expression. To further validate our in vitro model and assess its applicability in identifying potential B cell immunotoxicants, future studies include evaluating chemicals that have been previously screened for immunotoxicity but likely have little information regarding their mechanism of action. (Supported by the Colgate-Palmolive Grants for Alternative Research and the Boonshoft School of Medicine, WSU)

1171 USE OF THE COMET ASSAY TO DETECT GENOTOXIC EFFECTS IN RECONSTITUTED HUMAN SKIN.

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The 7th amendment to the EU Cosmetics Directive obliges the cosmetics industry to phase out animal-based genotoxicity experiments by March 2009. However, the current in vitro alternatives have serious drawbacks in terms of specificity and metabolic competence. The rate of false positives (positive results for non-carcinogens) lies above 70 % if at least two standard tests are included in the battery. Therefore, the development of reliable alternatives is a must for the cosmetic industry. The skin is the main route of exposure for cosmetics and the similarity to human skin predestinates 3D skin based models as a potential replacement for in vivo testing. These models, unlike standard cell culture assays, possess in vivo skin features like the barrier function of the stratum corneum and relevant metabolizing capacity.

As the Comet assay is able to detect a broad spectrum of primary DNA damage, we applied this methodology to human reconstituted skin models. In this procedure, the critical step is the generation of single cells without causing primary DNA damage. Although several standard enzymes could not be used due to their DNA damaging properties, a protocol to gently disintegrate the epidermis was successfully developed. The results show that single cells with a low background of DNA damage can reproducibly be generated with our method. Moreover, the application of the standard positive control methyl methane sulfonate to the stratum corneum of the 3D model caused a significant increase in percentage of tail DNA as compared to the negative control.

Based on the results generated so far we propose the Comet assay applied to 3D human skin models as a useful tool to evaluate genotoxicity of dermally applied compounds. For the micronucleus assay this has been shown earlier by Curren et al, *Mutat Res* (2006). For both methods the robustness of the protocol, transferability of the method, reproducibility between different labs as well as the sensitivity and specificity for a set of chemicals will be studied in a project sponsored by the European Cosmetic Toiletry and Perfumery Association (COLIPA).

1172 BHAS CELL TRANSFORMATION ASSAY AS A PREDICTOR OF CARCINOGENICITY.

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Carcinogenesis is a multistep process which involves sequential genetic alterations in a single target cell ultimately leading to malignant transformation and tumour development. Carcinogen-induced alterations in DNA as well as non-genotoxic mechanisms can play a causal role. In vitro cell transformation tests using BALB/c3T3 or C3H10T1/2 cells simulate the process of animal two-stage carcinogenesis. A limitation of these methods however is the time (4-8 weeks) required for focus formation as a morphological indicator of transformation. Thus Sasaki et al developed the cell line Bhas 42 by transfecting BALB/c 3T3 cells with the v-Ha-ras oncogene. Using these cells, transformed foci can be efficiently induced within 2-3 weeks by treatment with initiating and promoting agents.

The Bhas-system was established and seven known carcinogens and non-carcinogens with results from other cell transformation assays (SHE- and BALB/c 3T3 assay) were compared to that of the Bhas 42 assay. By adding a metabolic activation system (S9 mix) all seven compounds showed corresponding results compared to the bioassay data.

By selective trypsinization separate cultures could be established from foci cells and from normal cells. Parallel staining of microfilaments and nuclei clearly displayed a dense and multilayered piling-up of cells in foci. Thus alterations in adhesion protein profiles and enzyme activities modulating cellular adhesion and migration seem to be involved in the process of focus formation. In various tumour an increased cholinesterase activity is found. Thus the cholinesterase activity was investigated by a selective stain. A higher activity and intensity was found in the transformed foci compared to normal cells. This may be a promising candidate for the identification of molecular markers leading to a more objective scoring of foci and a greater acceptance of cell transformation tests in general.

1173 MECHANISMS OF DMN-INDUCED HEPATIC PRENEOPLASIA IN THE F344 RAT VERSUS THE MEDAKA FISH MODEL.

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To determine the roles of transforming growth factor-β1 (TGF-β1) and hepatic stellate cells (HSCs) in hepatic fibrosis and liver carcinogenesis, we performed parallel dimethylnitrosamine (DMN) exposures in F344 rats (drinking water) and

Japanese medaka (ambient water). DMN, an alkylating carcinogen, is used to model fibrosis/cirrhosis and neoplasia in the rat liver. TGF- β 1 has been associated with hepatic neoplasia and fibrosis in humans and rodents. Liver injury induces HSCs to transdifferentiate to myofibroblast-like cells. Activated TGF- β 1 stimulates transdifferentiated HSCs to produce collagen type-1. TGF- β 1 has tumor suppressor activity through growth-inhibition and apoptosis of hepatocytes. Mutations in the TGF- β 1 pathway have been associated with hepatic neoplasia. TGF- β 1's role as a tumor suppressor protein has been documented in humans and rodents, and DMN-induced lesions in rodents are TGF- β 1 dependent. DMN is also a potent hepatotoxin and carcinogen in fish and is associated with hepatocellular and biliary carcinomas. Mutagenesis studies in DMN-exposed medaka have demonstrated DNA lesions similar to those seen in rodents, but the mechanisms by which liver morphologic changes occur are unknown. In our study, fish were exposed to 0, 10, 25, 50, or 100 ppm DMN and rats to 0, 0.1, 1, 5, 10, and 25 ppm DMN for 14 days. Post-exposure, specimens were intermittently sacrificed for histopathology and immunohistochemistry. In the medaka, results included: hepatocellular necrosis, apoptosis, regeneration, and dysplasia; HSC and spindle cell proliferation; and hepatocyte TGF- β 1 expression. An association between TGF- β 1 and hepatic lesions in DMN-exposed fish suggests a similar dependence to that in rodents. Direct comparisons between animal models at different phyletic levels will help facilitate the interspecies extrapolations that are so vital in toxicological risk assessment.

1174 PERMEATION OF BENZENEARSONATES PROVIDES SUFFICIENT CONCENTRATIONS TO CAUSE ALTERATION IN CACO-2 CELL PROLIFERATION.

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Roxarsone (4-hydroxy-3-nitrobenzenearsonate), used to control microbial infections and to promote growth in poultry, has been approved by FDA since the 1960s as a feed additive. More than 90% of the additive is excreted. This has resulted in over 2 million pounds of Roxarsone and its metabolites being introduced into waterways. USGS and EPA have declared Roxarsone an organic water contaminant. Roxarsone's reduction product 3-amino-4-hydroxy-benzenearsonate (AHBA) has been identified in the literature as a metabolite, and is a potential substrate for multiple enzymes. The presumed detoxification N-acetylation reaction by NAT1 is poor for AHBA. Bioactivation can be catalyzed by oxidative enzymes such as the cytochrome P450s. Our *in vitro* experiments have confirmed the NADPH-dependent oxidation of AHBA, catalyzed by cloned human CYP 3A4, 1A2, and 2E1 isoforms. Previously we found a proliferative effect of these compounds on Caco-2 cells at 1.0-10 μ M levels as monitored by flow cytometry and [³H]-thymidine uptake into DNA. To assess potential human impact of these compounds, it was important to study their absorption characteristics in the gastrointestinal tract. For this study of intestinal absorption, human Caco-2 adenocarcinoma cells were grown to confluency on collagen-coated Transwells, then dosed on the apical side. After dosing at 10-50 μ M for 24-48 hours, media from apical and basolateral sides were collected and analyzed by HPLC. Roxarsone permeation of 1.4 μ M (14%) to basolateral side can be detected from a 10 μ M dose (48 hrs). Acetarsone permeation of 2.55 μ M (10.2%) to basolateral side can be detected from a 25 μ M dose (24 hrs). Therefore, it appears that the benzenearsonates permeate to the basolateral side at concentrations that are capable of stimulating cell proliferation. (Support: NIGMS/NIH GM008241-220S1-220S2; NIH/RIMI MD00215-01; RCMI RR 03032; MBRS/GM-028248; MIE/NASA NCC8-227).

1175 TARGET IDENTIFICATION OF DRUG-INDUCED MITOCHONDRIAL TOXICITY BY ANTIDIABETIC AND ANTILIPEDOMIC DRUGS.

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Almost all pharmaceuticals are prescribed with a risk-benefit analysis implicit in the clinician's decision where side effects are weighed against therapeutic potential. Recent data are increasingly implicating deleterious effects on mitochondrial function in the etiology of iatrogenic toxicities. For example, experiments on isolated mitochondria reveal that many prescription drugs with a variety of untoward side effects uncouple or inhibit the mitochondrial electron transport system from oxidative phosphorylation.

Although such assessments can identify compounds with a potential mitochondrial liability, the existing techniques do not identify specific targets or mechanism(s) responsible for dysfunction and this is critical to understand in both mechanistic work and for the future monitoring of possible liabilities *in vivo*. Here, we report

the use of our five previously described MitoProfile™ assays in identifying possible targets of antidiabetic and antilipemic drugs. We confirmed the complex I and V inhibition at high drug concentrations previously reported for certain fibrates. In addition we found, that certain glitazones not only inhibited complex I, but also targeted complex IV and V with higher potency. Several statins also targeted complex IV and V. Thus the use of the MitoProfile™ assays provided mechanistic information, which when applied to the anti diabetic and antilipemic drugs together, focuses attention on the potential enhanced toxicities when these classes of drugs are used in concert.

1176 ISOLATED MITOCHONDRIAL RESPIRATION AND VIABILITY OF GALACTOSE-GROWN HEPG2 CELLS PROVIDE ROBUST ASSESSMENT OF DRUG-INDUCED MITOCHONDRIAL TOXICITY.

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Despite regulatory vigilance, untoward toxicity and other side effects of ethical pharmaceuticals remain a major health concern. Mitochondrial integrity is essential for viability of aerobically poised cells, and many of these drugs have deleterious mitochondrial effects leading to toxicity of the liver, cardiovascular system, skeletal muscle, nervous system, and kidneys. Recent technological developments are fostering large-scale assessments of potential mitochondrial liabilities of drugs with an eye towards improving safety profiles.

We have shown preliminary data suggesting that mitochondrial toxicity can be readily detected in isolated mitochondria, and in galactose-grown (OXPHOS competent) intact HepG2 cells, using novel fluorescent oxygen sensors. Here we provide an extensive validation of both models using >100 commercially available drugs, both with and without reported *in vivo* toxicities and mitochondrial liabilities. Drugs included were: hepato-, cardio-, and myo-toxicants, compounds with no known toxicity, as well as compounds that require bioactivation. Data from isolated mitochondria provided mostly mechanistic insights, such as uncoupling and/or OXPHOS inhibition, and also correlated well with the cell-based assay. On the other hand, the cell-based assays provided the necessary toxicity readout and distinguished between primary and secondary mitochondrial effects. Many compounds with previously unreported clinical liabilities also induced mitochondrial dysfunction. Post-facto examination of adverse event reports revealed that such drugs frequently induce lactic acidosis, exercise intolerance, fever or sensory impairment, among others, which are all clinical manifestations of mitochondrial pathologies having known genetic etiologies. Together, these assays provide a powerful predictive tool for understanding the mechanism(s) underlying the toxicities encountered with certain drugs.

1177 ASSESSMENT OF COMPOUND INDUCED HEART, GASTROINTESTINAL TRACT, LIVER, AND KIDNEY TOXICITY IN ZEBRAFISH.

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Zebrafish have several advantages for assessing drug induced organ toxicity, including transparency, rapid development, easy drug delivery, and use of small quantity of drug per experiment. Here, we describe *in vivo* assays for assessing drug induced organ toxicity, including heart, gastrointestinal tract, liver, and kidney. Developmental or non-developmental toxicity can be assessed by choosing the appropriate zebrafish stage and drug dosing regimen.

Toxicity evaluation is conducted by assessing a series of physiologic and morphologic endpoints. Compound effects on heart rate and A/V ratio are assessed using a stop watch or a video camera. Contractility and circulation can be visualized in the transparent animal. To determine compound-induced effects on the intestine, the morphology of the epithelial layer, bleeding, and structural abnormalities are visually examined. To assess liver damage, size can be quantified using image analysis. Overt hepatic necrosis can also be visually assessed and apoptosis can be analyzed using acridine orange staining. Level of carboxylase enzymes containing biotin covalently bound as co-enzyme can be assessed in the liver and gut. In addition, after drug treatment, abnormal CYP gene expression, indicating cytotoxicity and mutagenicity, can be assessed by *in situ* hybridization. After staining with alcian blue, pancreas size can be measured by image analysis. In addition, level of carboxypeptidase, a digestive enzyme, can be determined using a whole animal ELISA. Nephrotoxicity can be visually assessed by examining kidney cysts and trunk edema; renal tubules and nephrons can be examined after antibody staining. Glomerular filtration rate can also be measured.

After compound treatment, the phenotypes of common organ toxicity in zebrafish present a striking correlation with results in mammals. Studies in this model organism are designed to complement in vitro and rodent methods for assessing toxicity.

1178 MODELING THE NEUROTOXICITY OF AN EXCITATORY AMINO ACID RECEPTOR POTENTIATOR IN ZEBRAFISH.

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The zebrafish has several features that make it an attractive model system for developmental neurotoxicity, including conserved genetics, anatomical structures, biological pathways, and neuronal cell types with other vertebrates. To explore the applicability of zebrafish as a model for neurotoxicity screening in pre-clinical drug safety assessment, we investigated nervous system effects caused by exposure to an excitatory amino acid potentiator in zebrafish larva. The similarities and differences between responses of zebrafish larva and those reported in earlier rat studies were evaluated from three aspects: behavior, histology, and the expression patterns of target receptors. After exposure, zebrafish larvae exhibited characteristic changes in locomotor behavior, such as increased motility and tremor, which were consistent with behavior abnormalities observed previously in rat. Histologic changes within the central nervous system after exposure ranged from decreased size and abnormal shape of the cerebellum or of the entire brain, with dilated ventricles and overall decreased cellularity in the most severely affected larvae. Histologic changes previously reported in rat were limited to necrosis of cerebellar Purkinje cell neurons. Interestingly, no major morphological changes were detected during the first three days in zebrafish development by BODIPY-ceramide staining, suggesting temporal specificity in lesion progression. In addition, primary sequence, syntenic and phylogenetic relationships between zebrafish and human receptors were characterized. All zebrafish receptors were increasingly expressed in a linear manner as assessed using quantitative real time PCR assay, with subtype specific expression in telencephalon, cerebellum and myelencephalon. Altogether, this work provides evidence that zebrafish is a valuable model not only for neurotoxicity screening, but also for dissecting the molecular mechanisms underlying the neurotoxicity of excitatory amino acid potentiators.

1179 FINAL RESULTS OF THE NICEATM/ECVAM VALIDATION STUDY OF *IN VITRO* CYTOTOXICITY TEST METHODS FOR ESTIMATING RAT ACUTE ORAL TOXICITY.

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A multi-laboratory validation study evaluated the reduction in animal use and deaths produced by using in vitro neutral red uptake (NRU) basal cytotoxicity test methods to predict starting doses for acute oral toxicity test methods [Up-and-Down Procedure (UDP) and Acute Toxic Class (ATC) method]. Regression models developed from in vitro NRU IC50 values (from testing up to 68 coded chemicals in BALB/c 3T3 murine fibroblasts or normal human keratinocytes) and the corresponding rat oral LD50 values from the Registry of Cytotoxicity were used to estimate LD50 values to determine starting doses for computer simulated UDP and ATC testing. For each chemical, the number of animals used and that died using the default starting dose or the IC50-determined starting dose for each NRU method was computed. Average animal savings when using the NRU methods were 5-8% for the UDP and 5-10% for the ATC method, and were highest for chemicals with low in vivo toxicity (11-21% for the UDP [for LD50 > 2000 mg/kg] and 17-28% for the ATC [for LD50 ≥ 5000 mg/kg]). Compared with the default starting dose, the IC50-based starting doses produced fewer deaths (0.5-0.6 animal) for the ATC but not for the UDP. The recommended IC50-LD50 molar unit regression cannot be used with mixtures. A weight unit regression produced comparable animal savings but has not been tested with mixtures. This demonstrates that using data from in vitro cytotoxicity methods can reduce the number of animals required for acute oral toxicity determinations. Supported by: N01-ES-35504, N01-ES-75408; EPA IAG DW-75-93893601-0; European Commission 19416-2002-04 F2ED ISP GB.

1180 CYTOTOXICITY ASSAYS USING HL-60 CELLS AND COMPARISON WITH RODENT 4-DAY TOXICITY DATA.

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To estimate the rodent 4-day repeated dose toxicity, cytotoxicity of 93 pharmaceutical compounds in Mitsubishi Pharma Corporation (MPC) was examined. At first, to determine a sensitive parameter and a cell line, cytotoxicity of 9 compounds (acetaminophen, acetylsalicylic acid, sodium phenobarbital, isoniazid, verapamil hydrochloride, orphenadrine hydrochloride, quinidine sulphate, chloramphenicol, and caffeine) was evaluated in the HL-60 and Hep G2 cells at 3 endpoints (ATP, Alamar Blue, and LDH) after a 24h-treatment. In these experiments, the HL-60 ATP assay was the most sensitive. Furthermore, 46 MPC compounds were evaluated on ATP in HL-60 and Hep G2 cells. Cytotoxicity of 40 compounds was more potent in the HL-60 cells than the Hep G2 cells. As for the rest 6 compounds, the cytotoxicity was equivalent in both cells. Therefore, cytotoxicity data in the HL-60 ATP assay was compared with the 4-day toxicity data on 93 MPC compounds. Among the 37 compounds detected lethality in the 4-day toxicity studies, 33 compounds could be provided IC50s (the median was 9.9 uM) and the rest 4 compounds revealed to be less than 50% on ATP depletion at 100 uM. A compound group classified into IC50s < 1 uM showed 10 mg/kg of the minimum approximately lethal dose (ALD), and mainly induced hepatotoxicity, gastrointestinal (GI) toxicity, and myelosuppression. A compound group with IC50s ranging from 1 to 30 uM showed 30 mg/kg of the minimum ALD, and induced the hepatic and GI toxicity. An IC50 ranking of the compounds tested in HL-60 cells was comparable to the potency of the rodent toxicity except some compounds that were insufficiently absorbed in the rodents. These data show that the HL-60 ATP assay may be a useful method in estimating toxicity before the rodent 4-day toxicity study in the early phase of drug discovery.

1181 *IN VITRO* PREDICTION OF TOXIC POTENTIAL BASED ON MULTIPLE PARAMETER CYTOTOXICITY ANALYSIS.

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One frequently cited reason for the discontinuation of a compound is unacceptable toxicity, therefore an early and appropriate prediction of toxicity would greatly contribute to the drug discovery process. To develop and validate a cell-based model to predict toxicity of novel compounds, we employed a multiplexed approach using multiple parameters to detect cell membrane integrity, mitochondrial membrane potential, and glutathione status, which were intended to be representative of different mechanisms of toxicity. The model was applied to drug metabolizing-competent (primary cultured rat hepatocytes) and non-competent (CHO) cells. Using cytotoxicity measurements from cells treated with 18 reference compounds, threshold values were derived to predict the development of toxicity in vivo. These included TC50 ≤ 100 uM for cell membrane permeability, ≥ 250% loss in mitochondrial membrane potential, and TC50 ≤ 100 uM in glutathione concentration. The second step involved evaluating the performance of the model across 27 additional compounds with varying degrees of toxicity. For positive assay results, a similar percentage of test compounds affected membrane integrity and mitochondrial membrane potential, whereas decrease in glutathione was observed for a lesser proportion of compounds. The parameters derived from the training set accurately identified the ability of test compounds to induce toxicity with approximately 80% sensitivity. When examined across classes of molecules, the model was more predictive for certain classes of toxicants, i.e. hepatotoxicants vs. inflammatory-mediated toxicities. This study demonstrates that in vitro data can be used as a sensitive method for the early prediction of compound-induced toxicity.

1182 ESTIMATING SYSTEMIC TOXICITY IN VITRO USING AN ADENOSINE TRIPHOSPHATE CYTOTOXICITY (ATP) ASSAY IN NORMAL HUMAN EPIDERMAL KERATINOCYTES.

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Ekwall *et al* (ATLA 17:83-100, 1989) have proposed that ≈80% of chemical-induced systemic toxicity is the result of disruption of basic cellular processes common to most cell types in the body, and that systemic toxicity for many chemicals could be estimated in *in vitro* cultures. Accordingly, Strickland *et al* (The Toxicologist, Abs#761, 2003) reported on a joint European/USA validation study initiated to evaluate two cytotoxicity assays using normal human epidermal keratinocytes (NHEK) and BALB/c 3T3 with a neutral red uptake (NRU) viability

endpoint to predict acute systemic toxicity. We have previously reported on a proof of concept study to evaluate the ATP endpoint to assess viability of NHEK using the same cell culture and test chemical exposure protocol optimized during the aforementioned validation wherein 20 coded chemicals were tested in 3 valid assays (The Toxicologist, Abs#1958, 2006). Cytotoxicity was measured as a dose-dependent reduction in ATP from which an IC_{50} was determined. The relationship between the ATP IC_{50} values *in vitro* and published human 50% lethal serum concentrations (LC_{50}) was presented [$r^2 = 0.887$]. Recently, 50 of the chemicals tested in the NHEK cytotoxicity validation study with the NRU endpoint were tested using the ATP endpoint. The relationship between the ATP IC_{50} values *in vitro* and published rodent oral LD_{50} values was determined [$\log LD_{50} \text{ mmol/kg} = 0.461(\log IC_{50} \text{ mM}) + 0.449$; $r^2 = 0.367$] and is similar to the prediction model published in the Registry of Cytotoxicity (Halle, 1998). A comparison of the ATP IC_{50} values showed a high correlation to the NRU $_{50}$ values obtained in the NHEK cytotoxicity validation study with the NRU endpoint ($r^2 = 0.930$). The results demonstrate that the NHEK assay with the ATP endpoint may be used to predict systemic toxicity, or rodent oral LD_{50} doses, and that the ATP endpoint is an acceptable alternative to the NRU endpoint.

1183 EFFECT OF SUBACUTE DAILY REPEATED EXPOSURE OF 18 CHEMICALS ON CELL VIABILITY, DNA SYNTHESIS, AND RECOVERY OF HUMAN INTESTINAL EPITHELIAL CELLS.

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The effect of repeated administration of 18 cytotoxic compounds on human immortal intestinal epithelial cell cultures was investigated using Caco-2 cells. Confluent monolayers were exposed for 48-h and 96-h repeated daily concentrations of chemicals. Effects were monitored using MTT cell viability and paracellular permeability (PP) assays. IC_{50} s were calculated from concentration-effect curves and the data was subjected to Students' t-test for paired sample means. In addition, confluent monolayers were radiolabeled with [^3H]-thymidine for the last 24-h of a 48-h exposure period and during a post-48-h recovery phase. Cell viability data suggests that there was a significant difference between the IC_{50} s of the 48-h & 96-h repeated dosing schedules. PP data indicates a significant difference between 72-h single dosing and 96-h repeated dosing. In fact, IC_{50} s from 96-h repeated exposure were consistently lower than 48-h repeated exposures. Other comparisons, including 48-h repeated dosing vs. 24-h single dosing (from previous data), and 96-h repeated dosing vs. 72-h single dosing (from previous data), did not demonstrate statistical significance. Also, uptake of [^3H]-thymidine was significantly greater in recovery experiments suggesting that the DNA repair mechanisms are at least partially responsible for cell recovery. The study demonstrates that repeated daily dosing of cultured cells for at least 96-h is necessary to simulate *in vivo* chronic exposure to chemicals.

1184 A HIGH-THROUGHPUT METHOD FOR ASSESSING CHEMICAL TOXICITY USING A *C. ELEGANS* FEEDING RESPONSE ASSAY.

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Feeding in *Caenorhabditis elegans* is a complex behavior controlled by an integrated and well-regulated neuromuscular system. *C. elegans* feed via constant pharyngeal pumping while moving through the environment. Exposures to neurotoxic chemicals have been shown to affect feeding in *C. elegans* making this response a sensitive endpoint in toxicological testing. Previous feeding assays required microscopic examinations of individual nematodes, limiting the number of samples that could be observed. We present a method that uses flow cytometry technology that can measure the size and fluorescence characteristics of hundreds of nematodes per minute. After chemical exposures, *C. elegans* are allowed to feed on red fluorescent microspheres such that accumulated red fluorescence is used as a measure of feeding response when compared to non-exposed control nematodes.

To validate our system, we first compared the feeding responses in the *C. elegans* wild type N2 strain with 18 different Eat mutant strains that are known to express abnormal pharyngeal pumping. The Eat mutations known to affect feeding most severely exhibited the lowest feeding responses with our assay. Next, we assayed two chemicals known to either stimulate (serotonin) or uncouple pharyngeal pumping (arecoline) in the presence or absence of a food source. Concentration-dependent increases in feeding responses after serotonin exposures were dependent on food availability while feeding responses decreased in arecoline-exposed nematodes regardless of the presence of food.

Using our feeding assay, the effects of more than 50 chemicals on the feeding response of *C. elegans* have been assessed. Cadmium and chlorpyrifos, potent neurotoxins, will be used to illustrate the effects of toxicants on *C. elegans* feeding. Cadmium chloride exposures led to a sharp drop in the feeding response above 200 μM while feeding of chlorpyrifos-exposed nematodes decreased in a concentration-dependent fashion with an EC_{50} of 2 μM .

1185 CHEMICAL COMPOUND PROFILING OF CELL-BASED TOXICITY ASSAYS USING QUANTITATIVE HIGH-THROUGHPUT SCREENING.

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Traditionally, toxicological profiles of drugs or chemicals rely on *in vivo* studies in laboratory animals. While these profiles provide useful information about the safety of chemical compounds, this approach is expensive, low-throughput, and inconsistently predictive of human biology and pathophysiology. To overcome these limitations, we have begun to develop a battery of cell-based assays to profile the toxicity of chemical compounds in a variety of cell types using quantitative high throughput screening (qHTS). The ultimate goal is to identify *in vitro* chemical signatures that could act as predictive surrogates for *in vivo* toxicity. Importantly, and distinct from conventional single concentration HTS screening, qHTS is capable of generating high-confidence positive and negative activity data, making it suitable for the comprehensive profiling of potentially toxic compounds. Thus far, the activity of 1,408 compounds from the National Toxicology Program (NTP) has been determined in assays for cytotoxicity and caspase 3/7 activity. Corresponding human and rodent cell lines derived from 6 tissues that are common targets of xenobiotic toxicity (liver, blood, kidney, brain, lung, and skin) were used in these assays. Data analysis has identified compounds that are toxic to all cell types at similar concentrations, as well as compounds that exhibit selective toxicity to particular cell types. In addition, some compounds appear to utilize the caspase 3/7 pathway as a mechanism for cytotoxicity, while others exhibit cytotoxicity independent of this pathway. These proof-of-principle data are being used to scale-up the profiling effort to incorporate more assay conditions, assay and cell types, and compounds. The resulting large data sets will provide a rich source of information for the development of *in vitro* toxicological signatures and the prediction of toxic effects of new chemical entities.

1186 THE NATIONAL TOXICOLOGY PROGRAM (NTP) HIGH THROUGHPUT SCREENING (HTS) INITIATIVE: CURRENT STATUS AND FUTURE DIRECTIONS.

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Since mid-2005, the NTP has participated in the NIH Molecular Libraries Initiative (MLI) by collaborating directly with the NIH Chemical Genomics Center (NCGC) to use quantitative HTS (qHTS) assays to test compounds for activity against defined targets. This collaboration benefits to both programs by adding toxicity testing capabilities to the MLI, and by allowing rapid implementation of NTP's HTS program designed to screen large numbers of compounds for activity against targets and pathways believed to have toxicological relevance (e.g., oxidative stress, inflammation, apoptosis). The NTP plans to link HTS-produced toxicity data to data from standard *in vivo* toxicological assays, with the goal of identifying mechanisms of action requiring additional investigation, developing predictive models for biological response, and prioritizing substances for further evaluation. In addition to the collaboration with NCGC, a medium throughput screen using *Caenorhabditis elegans* has been established to provide *in vivo* data on the same chemicals screened at the NCGC. Another aspect to this collaborative effort is a joint NTP/EPA "faculty" that shares ideas and strategies and provides a broader venue for scientific debate on HTS. The NTP had provided 1353 unique compounds (55 in duplicate to assess assay reproducibility) with known toxicological effects to the NCGC (see C. Smith et al. poster). The NTP has also provided six cell-based HTS assays to the NCGC; these assays evaluate cytotoxicity (2), caspase activation (3), and a cell membrane efflux pump (1). The first set of NTP compounds has been tested in several of these assays in up to 7 human and 6 rodent cell types (see M. Xia et al. Poster). The resulting data are posted on PubChem and are being evaluated internally by the NTP for patterns of response potentially indicative of toxicological effects. Efforts are underway to identify other potentially useful assays and the second set of ~1408 compounds.

1187 CHEMICAL SELECTION FOR NTP'S HIGH THROUGHPUT SCREENING INITIATIVE - ROUND 1.

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In support of a new HTS Initiative, the NTP is collaborating with the NIH Chemical Genomics Center (NCGC) to use quantitative HTS (qHTS) assays to test compounds for activity against defined biological targets (see Tice et al. Poster). This collaboration benefits both programs by adding toxicity testing capabilities to the NIH Molecular Libraries Initiative (MLI), and by allowing rapid implementation of NTP's HTS program designed to screen large numbers of compounds for activity against targets and pathways with toxicological relevance (e.g., oxidative stress, inflammation, apoptosis). The NTP will link HTS-produced toxicity data to data from traditional toxicity assays, with the goal of identifying mechanisms of action requiring additional investigation, developing predictive models for biological response, and prioritizing substances for further evaluation. For the first round of testing, the NTP provided 1408 compounds (1353 unique, 55 duplicates to assess assay reproducibility) selected because they were soluble in dimethylsulfoxide at 10 mM and because they were associated with publicly available bioassay results. Most of these compounds originated as nominations to the NTP for toxicity testing of various types, and of these, virtually all have been tested in the Salmonella mutagenicity battery, while many have been studied in reproductive and chronic rodent bioassays. Also, reference sets of compounds proposed for developing *in vitro* assays for endocrine disruption or ocular toxicity were included. The set sent to the NCGC include nearly every chemical class of small molecules imaginable. Molecular weights ranged from approximately 100 to 400. Functionally, the set includes solvents, fire retardants, preservatives, flavoring agents, plasticizers, therapeutic agents, inorganic and organic pollutants, drinking water disinfection byproducts, pesticides and natural products. A second set of compounds is currently being chosen based on the knowledge gained from this first set.

1188 MODE OF ACTION-BASED EVALUATION OF RAPID CELL-BASED TOXICITY SENSORS.

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Water supplies are potential targets for contamination with a broad spectrum of industrial and agricultural chemicals. Rather than relying on biosensor technologies that identify specific analytes or a very limited number of analytes in water samples, current research is focusing on the use of rapid responses of cell-based systems to identify toxicity associated with a broad range of chemicals. The goal of the current work was to develop a prioritized list of candidate toxicity sensor endpoints for further evaluation or development by: (1) identifying new technologies and novel modes of action that can detect broad classes of chemicals relevant to human health risk prediction; (2) critically evaluating cell or macromolecule-based processes that may serve as suitable endpoints for rapid toxicity assessment of water; (3) ranking candidate endpoints based on a set of criteria; and (4) evaluating the strengths and limitations of various toxicity assays. About 150 cell-based assay technologies were identified and categorized by relevant modes of action or toxicological endpoints. Based on this analysis, current assays are likely to provide adequate detection in the human acute toxicity range of chemicals that act through well-conserved cell biology pathways. For example, agents that cause chemical or physical effects on cell membranes affect cellular energetics or cause cell death through formation of reactive electrophiles that bind nonspecifically. However, other specific cellular or tissue-specific targets may not be well represented by such general assays. Our analysis indicates that assays that target key neuroreceptors, ion transport mechanisms, and selected signal transduction pathways as well as whole tissue or systemic responses would benefit most from further research as potential toxicity biosensor assays.

1189 CYTOTOXICITY OF ORGANOSELENIUM COMPOUNDS IN NORMAL, IMMORTALIZED AND TRANSFORMED HUMAN BREAST CELLS CO-CULTURED IN A NOVEL CELL CULTURE SYSTEM, IDMOC.

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We report here an application of a novel cell culture system, the Integrated Discrete Multiple Organ Culture (IdMOC) in the evaluation of the cytotoxicity of anticancer agents towards the target cancer cells, and the nontarget normal cells. The IdMOC utilizes a wells-in-a-well concept, allowing multiple cell types to be cultured as physically discrete cultures but interconnected by an overlying medium. In this study, we evaluated the anticancer agent methylseleninic acid (MSA) and its inactive structural analogs Se-methylselenocysteine (MSC) and selenomethionine (SM). Human normal mammary epithelial cells (HMEC) were cocultured with an

immortalized mammary epithelial cell line (MCF10A) and a mammary adenocarcinoma cell line (MCF10AT). The IdMOC therefore models a breast tissue containing both a normal epithelium and cancer cell populations. The cells were treated with the selenium compounds for 24 hours up to the highest concentration of 100 μ M. MTT metabolism was used as a viability endpoint. MSA was found to be cytotoxic to all three cell types, while MSC and SM had no apparent cytotoxicity. The results show that MSA had slightly higher cytotoxicity towards the transformed cells than towards the normal cells. The calculated EC₅₀, EC₉₀ and EC₉₉ values were 14, 51 and 104 μ M for HMEC; 4, 24 and 53 μ M for MCF10A, and 8, 30, and 61 μ M for MCF10AT. The results show IdMOC can distinguish between cytotoxic (MSA) and noncytotoxic agents (MSC, SM) and, more importantly, can provide information towards differential cytotoxic effects towards normal and transformed cells.

1190 APPLICATION OF PREDICTION MODELS TO THE DEVELOPMENT OF A PEPTIDE REACTIVITY ASSAY FOR SCREENING CONTACT ALLERGENS.

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Currently, the skin sensitization potential of chemicals is assessed using the Local Lymph Node Assay (LLNA). In the interest of reducing animal use, *in vitro* alternatives to skin sensitization testing are under development. One characteristic of a chemical allergen is its ability to react with proteins prior to the induction of skin sensitization. The majority of chemical allergens is electrophilic and as such react with nucleophilic amino acids like cysteine or lysine. In order to determine if reactivity correlates with sensitization potential, 82 chemicals representing allergens of different potencies (weak to extreme) and non-sensitizers were evaluated for their ability to react with glutathione (GSH) or two synthetic peptides containing either cysteine or lysine. Following a 15 minute reaction time for GSH or a 24 hour reaction period for the 2 synthetic peptides, the samples were analyzed by HPLC. UV detection was used to monitor the depletion of GSH or the peptide following reaction. To determine whether reactivity can be used as a potential skin sensitization screening tool, the peptide reactivity data were analyzed against existing LLNA data using recursive partitioning methodology in order to build a classification tree that allowed a ranking of reactivity. An optimal split variable and a corresponding threshold value were selected to rank peptide reactivity as minimal, low, moderate and high using the available LLNA data on each of the tested chemicals. Non-allergens and weak allergens demonstrate minimal to low reactivity whereas moderate to extreme allergens demonstrate moderate to high reactivity. Finally, classifying minimal reactivity as non-sensitizers and low, moderate and high reactivity as sensitizers it was determined that models based on cysteine alone or with GSH and/or lysine have accuracies of greater than 87%. These models are currently ongoing further evaluation using additional test chemicals.

1191 INTER-LABORATORY EVALUATION OF A CYSTEINE AND GLUTATHIONE (GSH) PEPTIDE REACTIVITY ASSAY FOR SCREENING SKIN ALLERGENS.

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A direct peptide reactivity assay has been developed as a potential screening assay for assessing skin sensitization potential. This assay involves the examination of peptide depletion by HPLC after incubation of the peptide with chemicals. Previous results using 52 allergens and 30 non-allergens have demonstrated a good correlation between reactivity and allergenic potency. In order for the assay to gain acceptance, it is necessary to demonstrate that the assay is robust and transferable. To investigate this an inter-laboratory study was designed to evaluate the peptide reactivity of 15 chemicals (4 non-sensitizers and 11 sensitizers). In order to determine whether reactivity can be used as a potential skin sensitization screening tool, measurements of peptide reactivity were taken for the cysteine peptide at a 1:10 or 1:50 ratio and GSH at a 1:10 ratio. The majority of the 15 chemicals showed similar peptide depletion values for both the GSH and cysteine peptides. The results indicate that the more potent allergens give the greatest amount of peptide depletion. The data were analyzed using recursive partitioning methodology that incorporates a classification tree. The algorithm involves starting with the complete data set and seeking to partition it into separate subgroups. An optimal split variable and a corresponding threshold value were selected to rank reactivity as minimal, low, moderate and high using available Local Lymph Node Assay data on each of the tested chemicals. Classifying minimal reactivity as non-sensitizers and low, moderate and high reactivity as sensitizers it was determined that Kao and P&G classified 15/15 and 14/15, respectively. This ranking of peptide reactivity allows the potential to use the data in combination with other *in vitro* data (e.g., cell-based work, skin penetration data) in a battery approach for replacement of *in vivo* skin sensitization testing.

1192 DEVELOPMENT AND PRELIMINARY EVALUATION OF A MONOCLONAL ANTIBODY BASED ASSAY FOR DETECTING PROTEIN REACTIVE CHEMICALS.

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In order to elicit a sensitization reaction, a chemical has to penetrate into the skin, react with proteins, be presented by antigen presenting cells and be recognized as an antigenic haptenized peptide by immune cells. Since a correlation between protein reactivity and skin sensitization has been established, the measurement of the protein binding properties of a chemical may represent a promising endpoint for the development of an *in vitro* approach to detect the sensitizing potential of chemicals. With the support of The European Cosmetic Toiletry and Perfumery Association (Colipa) we have developed monoclonal antibodies (MoAbs) against CLIP (class II-associated invariant-chain) peptides with Lysine or Cysteine substituted side chains. These synthetic CLIP peptides represent a functional target for reactive chemicals. Modification of the side chains by a test chemical or its metabolites (haptenization) can be detected as a decrease of the binding affinity of the MoAbs specific for the unmodified CLIP peptides. In this assay, the synthetic CLIP peptides coated on Dextran beads are first incubated with a range of test chemical concentrations for 1 to 30 hours. The beads are then transferred and coated to 96 wells plates, and incubated with the specific MoAb-peroxidase conjugates in an ELISA-like assay format. First results demonstrate that modification (haptenization) by test chemicals of the sulfhydryl or amino functional group in the peptide-side-chain led to a concentration dependent loss of reactivity when the appropriate matching pairs of monoclonal antibody - peptide dextran-conjugate are used. Further work is ongoing in order to test more chemicals and to establish a correlation between the protein binding capacity measured in our assay and the sensitization potential of a chemical.

1193 MEASUREMENT OF CD86 EXPRESSION AND IL-8 SECRETION FOR THE DETECTION OF CONTACT ALLERGENS IN THE U937 CELL LINE.

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The human myeloid cell line U937 is being evaluated as a test system for the detection of contact allergens based on the CD86 expression and IL-8 secretion. In a previous *in vitro* test protocol called "U937 activation test", we demonstrated the capacity of this cell line to discriminate sensitizers from non-sensitizers according to CD86 expression and gene expression of interleukin-1 β (IL-1 β) and IL-8 at three different time points (work recently submitted for publication). In the present study, we evaluated a simplified version of the test procedure by analyzing the expression of CD86 by flow cytometry and the secretion of IL-8 by Enzyme-Linked Immunosorbent Assay (ELISA) after a 72 hrs exposure period. About 20 chemicals have been evaluated in this new assay format. Most of sensitizers induced a dose-dependent increase in CD86 expression and IL-8 production. Most of non-sensitizers induced no relevant increase in the expression of these two markers. Based on the relative CD86 and IL-8 expression levels, a simple and empirically defined approach is proposed to evaluate the sensitizing potential of each chemical. These *in vitro* data were finally compared with the *in vivo* data obtained by the local lymph node assay (LLNA). We conclude that the combination of CD86 protein expression and IL-8 secretion allowed to discriminate sensitizers from non-sensitizers, indicating that the U937 cell line is a promising test system that may contribute to reduce animal testing.

1194 METHOD OPTIMIZATION OF A PEROXIDASE PEPTIDE REACTIVITY ASSAY FOR SCREENING THE SKIN SENSITIZATION POTENTIAL OF PROHAPTENS.

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Reactivity of electrophilic sensitizers (haptens) to protein nucleophiles is considered a necessary event in the induction skin sensitization. With the desire to develop non-animal screening methods to reduce our reliance on animal testing, an *in vitro* model that measures the reactivity of chemical allergens to nucleophilic peptides (e.g., cysteine) was developed. Analysis of 82 chemicals, including sensitizers and non-sensitizers, confirmed the correlation between reactivity and sensitization potential. A challenge of this assay is that some chemical sensitizers are non-reactive (prohaptens) and require bioactivation to a reactive metabolite (electrophilic hapten) prior to initiating a skin sensitization response *in vivo*. By incorporating a

mechanism for metabolic activation of prohaptens, the use of peptide reactivity data would improve prospective evaluations of novel chemicals and potentially reduce misclassifications due to false negatives. The purpose of this work was to optimize assay parameters for determining the reactivity of prohaptens using peroxidase and peroxide. An anchoring approach was employed to evaluate the effect of altered experimental conditions as well as incorporating appropriate controls to compensate for loss of peptide monomer due to the effects of peroxidase and peroxide. Incubation time, enzyme concentration and pre- and post-incubation treatment of chemical reactions with dithiothreitol were examined using prototypical prohaptens. Results indicate that peptide reactivity profiles in the presence and absence of peroxidase and peroxide is a useful approach for identifying prohaptens from directly reactive haptens. Enhancement of reactivity has been demonstrated with classic prohaptens such as cinnamyl alcohol, eugenol, and 3-aminophenol. Additional testing will be required to determine the usefulness of this approach for predicting the skin sensitization potential of novel compounds in a battery approach.

1195 IN VITRO GENOTOXICITY-TESTING USING THE PHENION® FULL THICKNESS SKIN MODEL.

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Currently existing *in vitro* genotoxicity assays demonstrate high sensitivity but low specificity resulting in a high number of false positive results. Additionally, with regard to cosmetic ingredient and product testing, the most relevant tissue for genotoxicity testing is considered to be human skin, the primary target. Therefore we established a genotoxicity assay using the Phenion® Full Thickness Skin Model featuring tissue- and species-specific metabolism. The model consists of a dermal and an epidermal equivalent and represents a physiologically relevant model for testing the influence of xenobiotics. Our work focuses on the Micronucleus Assay for detecting the genotoxic impact of substances after topical application. This assay accounts for persisting damages of chromosomal structure or deficient segregation. A protocol was established for isolating both keratinocytes and fibroblasts from the *in vitro* skin model. First results with isolated keratinocytes are presented showing the suitability of this test system.

1196 PERFORMANCE OF EPISKIN™ AND EPIDERM™, TWO IN VITRO SKIN IRRITATION TEST METHODS, USING THE EPA AND GHS HAZARD CLASSIFICATION SYSTEMS.

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The European Centre for the Validation of Alternative Methods (ECVAM) recently completed a skin irritation validation study (SIVS) to evaluate EPISKIN™ and EpiDerm™, two test methods using *in vitro* human skin constructs optimized to detect skin irritation, as potential replacements for the *in vivo* rabbit dermal irritation test when using the EU hazard classification system. Because U.S. and United Nations regulatory hazard classification systems differ from the EU system in having three categories vs two, NICEATM/ICCVAM conducted additional analyses of the SIVS database using decision criteria based on those developed by ECVAM. The accuracy of the two test methods to identify skin irritants using the EPA and GHS systems ranged from 43-58%, depending on the test method used and classification system employed. The major limitation is their inability to identify mild and moderate irritants, which are over- and under-predicted up to 87% and 14% of the time, respectively, depending on the test method [and with or without the inclusion of IL-1 α analysis] and the classification system used. However, when predictivity for the irritant category according to the EPA and GHS systems is compared to predictivity for the mild irritant and nonirritant combined categories, the false negative rate for EPISKIN™ is 0% for both classification systems vs 33%-35% for EpiDerm™, and the false positive rate for EPISKIN™ is 37%-47% vs 22%-28% for EpiDerm™. Because the false negative rate is 0%, EPISKIN™ may be useful as a screening test to identify substances that are not irritants (e.g., GHS Category 2/EPA Category II). However, the performance characteristics for these *in vitro* test methods, when using three-category hazard classification systems, indicate that further optimization would be needed before they can be used to fully support U.S. regulatory decisions. Supported by NIEHS contract N01-ES-35504.

1197 EVALUATION OF EYE IRRITATION POTENTIAL USING IN VITRO SHORT TIME EXPOSURE (STE) METHOD.

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There is a greater urgency to develop non-animal safety tests in view of the forthcoming EU ban on *in vivo* testing (7th amendment to the Cosmetic Directive). For the evaluation of eye irritation, some *in vitro* assays are used, but none are accepted

as an OECD test. Previously we reported a good predictive capacity for eye irritation of personal care and household products using Short Time Exposure (STE) method. The method involved assessing cytotoxicity with SIRC (rabbit corneal cell line) cells following a one 5 minute dose treatment. In the current study, the aim was to confirm the predictive capacity of the STE method for assessing eye irritation of cosmetic ingredients and chemicals in the ECETOC database. First, we selected test concentrations with good correlation between the Draize MAS and cell viability in the STE using 31 cosmetic ingredients. Physiological saline or EMEM/FBS medium was the vehicle and test concentration was 0.05%. We repeated the process for 20 more chemicals. For some organic solvents (e.g. acetone, toluene), mineral oil was used as the vehicle and test dose was 5%. The results of the 51 chemicals were analyzed. Chemicals with a MAS ≥ 15 for Draize test or cell viability $\leq 70\%$ for STE method were classified as irritants. Those chemicals outside of these parameters were classified as non-irritants. The accuracy of this classification for the 51 chemicals was about 78%. For our second goal, an inter-laboratory study was conducted with 31 cosmetic ingredients to confirm inter-laboratory reproducibility using same protocol, treatment dose, and cell lot. The two laboratories obtained identical data classifications for our STE method. These data suggest that the STE method has a good predictive capacity, easy transferability, and good reproducibility. The advantage of using this method as a useful *in vitro* eye irritation test is that it is a easy (5 min., one-dose) and fast method to use plus and it has the ability to evaluate eye irritation potential of both products and ingredients.

1198 ASSESSMENT OF OCULAR RESPONSE TO COMPOUNDS USING AN *IN VITRO* HUMAN CORNEAL EPITHELIAL CULTURE MODEL.

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These studies were designed to evaluate the performance of the Clonetics® Human Corneal Epithelial Model in assessing ocular barrier function and irritancy response. Trans epithelial electrical resistance (TEER) was used to assess barrier function of the models. TEER measurement results for multiple lots of models averaged >1700 ohms $\times$ cm², which approaches the values observed in *ex vivo* corneal tissue. Test chemicals for evaluating ocular irritancy response were selected from the ECETOC Eye Irritation Reference Chemicals Data Bank (Second Edition). The cytotoxicity response of the model system to 34 test compounds at concentrations correlating to a full range of ocular irritancy categories was evaluated. Time-to-toxicity was determined using the MTT cytotoxicity assay. The compounds were applied topically at sequential time points to calculate the time at which 50% of cells are rendered non-viable (ET50). In order to distinguish irritants from non-irritants, a prediction model cutoff was identified at ET50 = 15 minutes. The ET50 results were compared with Draize Modified Maximum Average Scores (MMAS), and with the irritancy categories of the Globally Harmonized System of Classification and Labeling of Chemicals (GHS). Irritancy response showed sensitivity (percent of irritating chemicals identified correctly) of 100% (11/11), and specificity (percent of non-irritating chemicals identified correctly) of 70% (16/23), and an overall concordance of 79% (27/34). Lot-to-lot consistency in tissue response was demonstrated with statistical analysis of irritancy data of the positive control (0.3% Triton X-100). These results indicate that the Clonetics Human Corneal Epithelial Model is a robust platform for the evaluation of corneal epithelial function and response.

1199 USE OF THE EPIOCLAR ASSAY FOR PRECLINICAL QUALIFICATION OF FORMULAS FOR HUMAN CLINICAL STUDIES.

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Cosmetic products applied around the eyes must be extremely mild because of the sensitivity of the skin in this region and the chronic use of such products. The topical application, assay using the EpiOcular tissue construct (Ghassemi et al 1997), is being extensively evaluated for the prediction of product mildness. In this study, we report the correlation between ET50 (exposure time required to reduce cell viability to 50%) responses in the EpiOcular assay and eye-area irritation in human clinical testing. Formulations were applied neat to the EpiOcular construct (MatTek) for up to 4 hours or 20 hours (depending on the expected degree of irritancy) and the ET50 of the water-treated controls were measured using tetrazolium dye (MTT) reduction. The EpiOcular assay was used as part of the preclinical qualification of products for subsequent *in vivo* studies. The *in vivo* studies were safety-in-use studies performed to evaluate eye irritation potential in individuals with self-perceived sensitive eyes or who were contact lens wearers. These studies are intended to evaluate eye-area products under "real-world use." These studies were conducted under the supervision of an ophthalmologist. The ophthalmologic evaluation included a gross exam, slit-lamp exam, and visual acuity exam. After baseline exams, subjects were instructed to use the eye area formulation over the next several weeks. Final examinations were conducted to determine eye irritation after the use period. The eye irritation scale ranged from no irritation (0) to severe irritation (4).

Ten eye area products were evaluated in the Epiocular assay with ET50 values ranging from >240 min to >1440 min. Products with ET50 values >240 min were evaluated clinically and showed no to minimal irritation in the clinical studies. These observations establish that the data from an epiocular assay lend support to the results of an *in vivo* assessment of the product. In addition, the lack of ocular irritation potential established, by these studies also indicate that the product is not likely to induce dermal irritation.

1200 EVALUATION OF VIABILITY MEASUREMENT (MTT) AND HISTOLOGY AS PARAMETERS FOR THE *IN VITRO* ASSESSMENT OF THE EYE IRRITATION POTENTIAL OF HAIR DYES WITH A RECONSTITUTED HUMAN CORNEAL EPITHELIUM MODEL.

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The evaluation of the human corneal epithelium (HCE) SkinEthic model to assess acute ocular irritation of chemicals has been the subject of a formal prevalidation study. This model was shown to be able to discriminate between irritants and non-irritants by means of a prediction model based on viability measurement in the MTT assay. In a preliminary study using this *in vitro* approach with six hair dyes, we had evaluated the *in vivo/in vitro* concordance by comparing MTT viability and histology with existing *in vivo* Draize data and had concluded that the use of the HCE model with evaluation of histology as additional endpoint was a promising tool for the detection of the ocular irritation potential of hair dyes. In this study, the applicability of this *in vitro* approach (using both MTT and histology) for hair dyes has been further evaluated *in vitro* only, with additional dyes with different coloring and/or physico-chemical properties. The materials were applied topically, either neat or diluted, for different time periods onto the surface of HCE constructs. First results demonstrate that MTT can be used to differentiate between cytotoxic and non-cytotoxic hair dyes. Possible interference of the dyes with the test system was evaluated to exclude artefacts. Preliminary histology results show that the assessment of the time-dependent morphological changes can also be useful to discriminate the dyes based on their capacity to damage the corneal epithelium structures, either as a confirmatory endpoint, or if needed, as a possible replacement of MTT.

1201 AN ALTERNATIVE MODEL OF CORNEAL IRRITATION AND REVERSIBILITY IN EXCISED PORCINE CORNEAS.

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We have developed an alternative to the Draize Eye Irritation Test in rabbits. The Porcine Corneal Opacity Reversibility Assay (PorCORA) determines eye irritancy as well as reversibility. Although a number of alternative assays exist for the determination of irritancy (eg. BCOP, HET-CAM, CAMVA, EpiOcular), none address the important endpoint of reversibility. The failure of present assays to determine reversibility may account for the delay in regulatory acceptance of other alternative methods because recovery time is used to categorize potential irritancy.

The assay can be used to evaluate chemicals for ocular irritancy and reversibility using excised porcine corneas (usually discarded by the meat industry). Excised corneas were maintained for over three weeks with minimal morphological changes to the corneal epithelium. Corneas were treated one day after excision with 100% EtOH, 3% SDS, 1N NaOH or 1% Benzalkonium Chloride. The area of damage on the corneal surface was visualized with brief fluorescein staining under UV illumination. This visualization method is not destructive to the sample enabling repeated measurements of fluorescein retention on the same tissue. With the exception of NaOH, the area of damage decreased over time in culture. Histological analysis showed re-epithelialization of the tissues where they had been previously damaged by chemical irritants. The decrease in fluorescein retention area correlated favorably with the time to recovery in published Draize rabbit eye test results. The PorCORA may fill the gap that is left by current alternative eye irritation assays since it has the potential to determine recovery after initial eye irritation. The PorCORA, is a good candidate for a tier testing strategy to replace the Draize eye test.

1202 THE COMPARATIVE TOXICOGENOMICS DATABASE: CONNECTING CHEMICALS, GENES AND DISEASES.

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The etiology of most chronic diseases involves interactions between environmental factors and genes that modulate important physiological processes. One critical environmental factor is chemical exposure. Understanding interactions between

chemicals and genes/proteins at the molecular level will help resolve questions concerning disease predisposition, therapeutic drug interactions, and health risks associated with differential susceptibility to exposures. Towards this end, we are developing the Comparative Toxicogenomics Database (CTD; <http://ctd.mdibl.org>) as a public resource to integrate and curate diverse data to promote the understanding of chemical interactions with genes and proteins.

CTD currently incorporates 59,000 chemicals, 1.2 million sequences (with Gene Ontology annotations), 83,000 taxonomic terms, 6,000 human diseases, and 78,000 references to produce a unique database for the cross-species analysis of chemical, gene/protein, and disease interactions. We also now manually curate the literature to capture specific details of every chemical-gene/protein interaction using searchable controlled vocabularies. To date, more than 22,000 interactions have been curated for 2,200 chemicals and 3,500 genes. Currently, over 650 genes have both a disease association and a curated chemical interaction, opening up the way to explore putative chemical-disease connections. User-friendly query pages and vocabulary browsers allow scientists to ask sophisticated questions and explore the data in detail from a chemical, gene, and disease perspective; all results can be downloaded in spreadsheets for further analysis.

CTD is a public information resource that supports hypothesis-driven research about chemical actions, enhances understanding about complex chemical-gene/protein networks and their impact on human health, and helps turn dispersed toxicology information into knowledge.

1203 CEBS 2.0.7 - INTEGRATING TOXICOLOGY AND TOXICOGENOMICS.

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The Chemical Effects in Biological Systems (CEBS) Knowledgebase has been under development at the National Institute of Environmental Health Science (NIEHS) for four years. Earlier versions of CEBS have been reported, and now we report the completion of CEBS version 2, which fully integrates study design and toxicity data with data obtained from microarray and proteomics studies. Using CEBS v.2, the user can filter the database on the basis of biological responses of subjects and other characteristics of the subject or study, and then navigate to the microarray domain in CEBS for analysis of the associated gene expression data. Additionally, CEBS users can annotate lists of genes using more than 50 public annotation resources, managed by the caBIO engine developed by the NCICB. Gene lists may result from analysis within CEBS or may be entered by the user. CEBS contains both private and public data; public data, including toxicity findings, microarray data and proteomics images and spectra can be downloaded from CEBS. CEBS now resides at the NIEHS, on three state-of-the-art servers, and has been subjected to multiple cycles of stress testing. CEBS can be accessed at <http://cebs.niehs.nih.gov>.

1204 TOXICOGENOMICS APPLICATIONS OF THE BIOMEDICAL INVESTIGATION DATABASE (BID).

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Several collaborative efforts to create large, public toxicogenomics datasets are underway, including efforts by members of the HESI Genomics Committee, and the NTP High Throughput Screening Faculty. Data exchange among laboratories reveals that while inter-laboratory agreement is generally high, a variety of metadata are needed to properly interpret the collaborative data set. Such metadata are described in the Chemical Effects in Biological Systems (CEBS) Data Dictionary (Tox.Sci. 2006). The BID, Biomedical Investigation Database, is used to manage and curate data prior to loading into a public repository such as CEBS (<http://cebs.niehs.nih.gov>). BID has been shared with major data contributors such as Health Canada, for use behind their firewall prior to publishing data in CEBS. BID is a flexible data repository for *any* deposited data, and at the moment houses toxicogenomics data, PCR data from genetic toxicology studies performed by ILSI HESI members, and cheminformatics data from high throughput screening carried out by the NTP and the National Chemical Genomics Center (NCGC). Looking

towards the future, BID development parallels the development of OBI, Ontology for Biomedical Investigations, an ontology under development by the OBI Working Group (<http://fugo.sourceforge.net/>). Once completed and fully integrated with OBI, BID will be accessible via the semantic web. Additional information about BID is at <http://www.niehs.nih.gov/cebs-df/>.

1205 ADVERSE EFFECTS OF PHARMACEUTICALS: I. CONSTRUCTION OF A RELATIONAL DATABASE OF ADVERSE HUMAN EFFECTS USING FDA ARCHIVES, PHARMAPENDIUM, AND PUBLIC SOURCES.

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The Informatics and Computational Safety Analysis Staff at the US FDA's Center for Drug Evaluation and Research has created a database of pharmaceutical adverse effect (AE) and toxicology findings linked to pharmaceutical chemical structure, drug class, clinical indication, receptor target, and estimated patient exposure. The database is being used to develop quantitative structure activity relationship (QSAR) models and to identify possible mechanisms for AEs (see related poster). The pre-clinical, clinical, and post-marketing observations contained in the database were obtained from FDA's Spontaneous Reporting System, PharmaPendium software, and public sources using Micromedex Integrated Index. PharmaPendium includes information from FDA drug approval packages, Meyler's Side Effects of Drugs, drug monographs from Mosby's Drug Consult, FDA's Adverse Event Reporting System, and the published literature. The data describing drug class, clinical indication, and pharmacological target were derived from PharmaPendium and the literature, with the addition of predicted target sites from the Prous Institute for Biomedical Research's BioEpisteme mechanism of action module. In order to integrate the data from multiple sources, PharmaPendium's standard vocabulary was employed for all AE endpoints and pharmaceutical generic names. The database now contains 4,849 unique pharmaceutical compounds and 11,211 AE endpoints. To account for variations in the AE reports due to different patient populations for each drug, we estimated patient exposure based on the number of reports for each drug expressed as a percentage of the total number of reports in the AE database. This denominator was applied to data from all endpoints to identify chemicals that could be scored positive in the training data sets of QSAR models predicting AEs.

1206 ADVERSE EFFECTS OF PHARMACEUTICALS: II. PREDICTION OF HUMAN LIVER AND KIDNEY EFFECTS USING AN ADVERSE EFFECTS DATABASE, MC4PC, AND BIOEPISTEME.

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The Informatics and Computational Safety Analysis Staff at the US FDA's Center for Drug Evaluation and Research conducted an investigation employing in silico methods to predict possible human liver and kidney adverse effects (AEs) of drugs. The investigation used an AE database (see related poster) combined with a weight of evidence (WoE) paradigm to identify drugs with significant findings. Quantitative structure activity relationship (QSAR) models were constructed using the MC4PC (MultiCASE, Inc.) and BioEpisteme (Prous Institute for Biomedical Research) programs to predict the activities of drug molecules. The number of AE reports for each drug was expressed as a percentage of the total number of reports in the AE database to provide a background (expected) level of reporting. Using a WoE approach, a biologically significant finding was defined as one having positive findings for more than one toxicologically related endpoint, a method which was validated using data on drugs withdrawn from the market with significant post-marketing AEs. Fragment-based MC4PC models were constructed using liver and kidney AE data sets and model predictive performance was optimized using 10% Leave-Many-Out internal validation experiments to yield high specificity (>85%) and receiver operating coefficients (>2.0). Molecular descriptor-based BioEpisteme models were used to predict the most probable mechanisms of action of a drug molecule based upon 3D molecular conformations and a training set of known drug receptor site targets reported in >200,000 drug patents. BioEpisteme predictions were validated by comparing known and predicted drug receptor site targets. Results are presented for adverse human liver and kidney effects in terms of MC4PC model predictive performance and BioEpisteme predicted mechanisms for drug toxicity experiments.

1207 THE MOUSE GENOME INFORMATICS DATABASE: INTEGRATING SEQUENCE, GENE FUNCTION, AND MOUSE MUTANT PHENOTYPE DATA.

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The Mouse Genome Informatics Database (MGI, <http://www.informatics.jax.org>) is a free international resource for the genetics, genomics, and biology of the laboratory mouse. MGI includes annotations of descriptions of mouse mutant phenotypes and relationships of genes and gene products to molecular function, biological process, and cellular component using controlled vocabularies. These annotations are integrated with other biological data in MGI allowing users to search, compare, and analyze data that are not effectively retrieved through text searches. To access these integrated resources, database query forms are structured to allow complex queries that can simultaneously include parameters for phenotype, expression, biochemical function, subcellular location, sequence, and genome location. Complex queries can be posed to identify candidate genes using select phenotype characteristics, gene functions and/or gene expression profile. For example one can ask "What genes are expressed in the liver, have mutations resulting in increased susceptibility to induced tumors, and are known to be involved in the cell cycle?" MGI may also be used to identify strains with sequence differences (SNPs) in select genes or chromosome regions that can then be evaluated for differences in susceptibility to one or more compounds. In addition MGI provides access to prototype tools that use Gene Ontology (GO) annotations to aid in microarray analysis and candidate gene identification. Finally, MGI links to the International Mouse Strain Resource to aid in locating and obtaining mice. Integration and the use of structured vocabularies for annotations assists in robust and accurate data mining when posing such complex questions in both computational and individual formats at MGI.

The Mouse Genome Database (MGD) at MGI is supported by NIH/NHGRI grant HG000330.

1208 EXAMINATION OF GENETIC NETWORKS THAT REGULATE GENE EXPRESSION IN LIVER USING COMPLEX TRAIT ANALYSIS.

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Liver is a primary site for metabolism of nutrients, drugs and chemical agents. While metabolic pathways are complex and tightly regulated, genetic variation among individuals introduces yet another level of complexity into research on liver disease. This study aimed to dissect genetic networks that control liver-specific gene expression by combining large-scale mRNA expression analysis and gene mapping in a reference population of BXD recombinant inbred strains for which extensive SNP, haplotype and phenotypic data is publicly available. We profiled gene expression in livers of naive mice of both sexes from C57BL/6J, DBA/2J, B6D2F1, and 37 BXD strains, using Agilent oligonucleotide arrays. This data was used to map quantitative trait loci (QTLs) associated with variation in expression of about 19,000 transcripts. We identified polymorphic cis- and trans-acting loci, including several loci that control expression of large numbers of genes in liver, by comparing the physical transcript position with the location of the controlling QTL. The data is available through public web-based resource (www.genenetwork.org) that allows custom genetic linkage analysis, identification of co-regulated transcripts and correlated phenotypes, cross-tissue and -species comparison, as well as testing of a broad array of hypotheses.

1209 QUANTIFICATION OF GENOMIC DATA: VALUE-ADDED ASSESSMENT AND APPLICABILITY TO TOXICOLOGICALLY SIGNIFICANT ENDPOINTS.

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The use of genomic methodologies has emerged as a valuable tool to assess toxicological responses through its ability to measure the expression patterns of thousands of genes within a single experiment. Translating genomic information into metrics that are easily used within toxicological and risk assessments, however, has been an

ongoing issue and difficult problem. In addition, the complexity of array data itself creates challenges for interpretation and subsequent application in areas such as risk assessment. Several research groups have proposed methods to address these issues, including the use of Gene Ontology (GO) mapping and pathway analysis, approaches that allows for the interpretation of array data within the context of biological or pathway response. The recently developed GO-Quant method (Yu et al 2006), takes the application of these methods further by creating an avenue for quantitative assessment of toxicogenomic data within specific biological pathways. This type of analysis gives quantitative functional information in terms of dose- and time providing a powerful tool for genomic interpretation and global pathway response. To further characterize and assess the general applicability of this approach, we applied the GO-Quant method to genomic data sets for endocrine disruptors found within the publicly available National Center for Biotechnology Information (NCBI) database. Our results delineate examples of the specific quantitative metrics that can be gained through the use of GO-Quant such as the characterization of effect levels (i.e. benchmark dose). This ability allows for improved toxicological interpretations, such as mode of action and biological pathway response, information critical to the development of risk assessment profiles.

1210 AUTOMATED QUANTITATIVE TOXICOGENOMIC DOSE-RESPONSE MODELING.

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Toxicogenomics applied to dose-response studies yields hundreds of putative biomarkers of exposure and toxicity. Through phenotypic anchoring, functional annotation, and pathway analysis differential gene expression associated with toxicity may be identified, allowing investigators to develop novel quantitative, mechanistically-based biomarkers of toxicity to more accurately monitor and assess risk. Typically linear or other low-dose extrapolation models are fit manually to identify points of departure, however, this is difficult and time-consuming when many toxicogenomic responses (e.g., differential gene expression) do not exhibit sigmoidal, linear, or exponential dose-response characteristics. To facilitate high throughput model-fitting, we developed the ToxResponseModeler, a Java application capable of fitting: 1) sigmoidal, 2) linear, 3) quadratic, 4) exponential, and 5) modified gaussian function models. The ToxResponseModeler identifies the most suitable model for each gene using the particle swarm optimization algorithm (PSO) to identify the optimal set of parameters to model the response of interest. The best fitting models are then compared, and the optimal model is chosen for each gene based on the Euclidean distance between the model predicted and observed data. The nth percent effective dose (EDn), including points of departure, may be calculated using the optimal model. Vehicle-based points of departure are also calculated based on the intersections of the 95% or 99% confidence intervals for the vehicle and dose-response data. Collectively, this yields a point of departure with a known confidence interval, based on measurement variance. The utility of ToxResponseModeler is demonstrated using published toxicogenomic dose-response data from the livers of TCDD treated C57BL/6 mice. This work has been supported by NIEHS Suprefund grant P42 ES04911.

1211 ELUCIDATION OF DIFFERENTIAL RESPONSE NETWORKS FROM TOXICOGENOMICS DATA.

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We describe a novel approach for the analysis of toxicogenomics data and elucidation of biological networks impacted by drug treatment. Approximately 15,000 interconnected modules were generated from manually assembled human pathways maps in MetaCore (GeneGo, inc.). Next, microarray expression data from rats livers chronically exposed to phenobarbital, mestranol and tamoxifen were mapped onto these modules. Using different statistical and clustering procedures, we have identified the sets of pathways featuring "synchronous", highly correlated expression among multiple repeats of the same treatment while showing strong anti-correlation across different treatments. We identified a large number of pathways specifically differentiating phenobarbital from mestranol and tamoxifen treatments, and fewer pathways distinguishing tamoxifen from mestranol treatments. Reconstruction of network modules that distinguish chemical treatments also provides insights into mechanisms of such differences. Unlike traditional statistical and clustering procedures of gene expression profiling, our method is applicable to raw expression data, regardless of amplitude and statistical significance of expression change at individual data points. Statistical validity of expression data is determined

by signal correlation when mapping to functional pathways, not at the level of individual signals. This advance allows to substantially increase the pool of differentially expressed gene in analysis and avoid statistical bias in microarray gene expression studies.

1212 NONPARAMETRIC AND GRAPHICAL METHODS REVEAL NEW GENE PATTERNS IN LOW DOSE ARSENIC MICROARRAY EXPERIMENTS.

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Gene microarrays can reveal informative patterns of gene regulation, providing a means to test hypotheses, generate new mechanistic hypotheses, and identify potential biomarkers of exposure, susceptibility and effect. However, applying these approaches to experiments using low doses or where there are modest treatment effects can yield non-informative, unstable results. Small changes in statistical parameters can lead to large changes in candidate gene list composition, and replicate experiments using the same approach may lead to lists that exhibit poor overlap. Using simpler, more direct alternatives to commonly used microarray statistics reduced these problems. We found that graphical techniques such as scatterplots of raw expression values, coupled with methods that allow for data that fails to follow a normal distribution, achieved better concordance between replicate experiments than standard parametric methods such as SAM. There is a strong biological and biochemical foundation for why gene regulation might behave in a non-parametric rather than a parametric manner, particularly in whole animal systems. Our methods found individual genes and groups of biologically related genes that the more common approaches missed, for example, identifying genes that are both up down and up-regulated, whereas SAM found only up-regulation. Comparison of these methods also shows how certain assumptions about data distribution can result in lists that are too short (false negatives) or long lists biased in favor of genes that do not show treatment specific effects (false positives) in low dose arsenic gene expression changes in mouse lung and Daphnia. (NIH-NIEHS ES07373, NSF DEB-0221837)

1213 IDENTIFICATION OF GENE EXPRESSION SIGNATURE IN BLOOD ASSOCIATED WITH ACETAMINOPHEN-INDUCED LIVER TOXICITY.

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Acetaminophen (APAP) is a widely used analgesic-antipyretic drug and its overdose (OD) is a leading cause of drug induced liver injury (DILI) and related fatalities. Current methods for diagnosing DILI are highly inadequate. This necessitates the discovery of novel biomarkers which may also shed light on mechanistic basis of liver toxicity.

We explored whether peripheral blood gene expression (PBGE) profiles could (1) distinguish individuals exposed to therapeutic or toxic doses of APAP from placebo; and (2) mimic the gene expression changes occurring in rat liver due to APAP treatment.

We analyzed PBGE resulting from 3 studies: (1) 6 subjects (4g APAP) + 3 placebo. PBGE was determined 24/48hr post-dosing when blood APAP levels were undetectable. (2) 5 subjects with OD (>10g APAP) with undetectable APAP levels at time of PBGE. (3) Gene expression from rat livers 12/24hrs post-dosing (1500/2500mg/kg APAP); using Agilent oligonucleotide arrays. PBGE was analyzed using Significance Analysis of Microarray (SAM) and enrichment of biological processes was examined using Significance Analysis of Function & Expression (SAFE). Prediction Analysis of Microarray (PAM) was used to identify genes that can predict APAP exposure.

We identified a minimal (16) and a maximal (6640) set of genes which can identify subjects exposed to APAP (either 4g or OD) with a predictive accuracy of 100% & 86%, respectively. Of the 6440, 1248 genes have orthologs in rat; and 474 of them differentially expressed in the same direction in rat liver & human blood. Within 474 genes, we find induction of apoptosis (p=0.007), ATP binding & synthesis (p=0.012), & cellular defense response (p=0.056) among the most significantly enriched biological processes.

We report a set of genes for which PBGE can (1) distinguish individuals exposed to APAP from controls with high accuracy even in absence of detectable APAP levels; and (2) mimic the changes occurring in liver. This approach can be helpful in diagnosing DILI and investigating mechanistic basis of toxicity by APAP and possibly other drugs.

1214 EVALUATION OF METRICS FOR ASSESSMENT OF MICROARRAY ASSAY PERFORMANCE USING A MIXED TISSUE RNA REFERENCE SAMPLE.

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Data quality and comparability remain an issue with microarray technology, due in part to incomplete application of proper methods for performance monitoring. We have previously introduced a rat mixed tissue RNA reference sample that allows defined target ratios to be measured on multiple microarray platforms (Nucleic Acids Res. 33: e187, 2005). Using this material, we evaluated several metrics for monitoring and standardizing the performance of laboratories that run microarray assays. A quantitative assessment of performance should include measurements of assay selectivity and sensitivity as these parameters underlie most types of data analysis. The area under the curve (AUC) from receiver-operator characteristic (ROC) plots was found to be a good overall indicator of performance. More detailed information on precision and accuracy was derived from metrics that evaluated the reproducibility of the observed ratios and their deviation from target values. The applicability of these metrics to different testing paradigms, such as proficiency testing, process drift monitoring, and protocol optimization was also explored. The establishment of suitable metrics to assess the performance of microarray assays is essential for effective use of this technology in clinical and regulatory applications.

1215 SOURCES OF VARIABILITY IN BASELINE GENE EXPRESSION IN RAT LIVER AND KIDNEY.

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Toxicogenomic studies are typically variable in design, but the impact of variations in study design and conduct on control animal gene expression has not been well characterized. A working group of the Health and Environmental Sciences Institute (HESI) Technical Committee on the Application of Genomics in Mechanism Based Risk Assessment was formed to assess baseline gene expression variability from microarray data derived from control or vehicle-treated animals across multiple studies and sites. Eighteen sites voluntarily contributed a total of 536 Affymetrix data sets of rat liver and kidney samples from control animals. Thirty-five biological and technical factors were obtained for each animal, describing a wide range of study characteristics. Thirteen of these factors with complete data were evaluated in detail for their contribution to total variability using multivariate statistical and graphical techniques as well as pattern analysis of the expression data. Certain factors emerged as key sources of variability, including gender, organ section, strain, and fasting, although a significant degree of confounding occurred due to the observational nature of the data and missing value patterns. The most variable and least variable probe sets within and between factors were identified and classified according to their gene ontology annotation. Better characterization of gene expression variability in control animals will aid in the design of toxicogenomic studies and in the interpretation of their results.

1216 IDENTIFICATION OF BIOLOGICALLY RELEVANT GENES USING A DATABASE OF RAT LIVER AND KIDNEY BASELINE GENE EXPRESSION.

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Microarray data from independent labs and studies can be compared to potentially identify toxicologically and biologically relevant genes. The Baseline Animal Database working group of HESI was formed to assess baseline gene expression from microarray data derived from control or vehicle-treated rats across multiple studies and sites. We determined whether this database could be used to identify genes clearly associated with a number of biological factors that were part of the database. We used Extracting Patterns and Identifying Genes (EPiG) to identify gender-specific and fasting-specific genes from each institute in which the factor could be examined in isolation. Gender-specific genes were identified in liver and kidney from a total of 5 different institutes using a total of 134 liver and 58 kidney

transcript profiles. A total of 266 liver genes and 313 kidney genes were identified that exhibited similar behavior (both direction of change and degree of gender difference) in at least 2/4 and 2/3 institutes, respectively. Surprisingly, approximately 1/3 of the kidney genes were regulated in an opposite manner in liver. The gender genes overlapped with well-characterized genes from earlier studies. Using in silico approaches, we identified a number of known response elements in the promoters of co-regulated genes including those involved in determining gender-specific gene expression. Fasting-specific genes were also identified in a similar manner and significantly overlapped with those that were known to be regulated by fasting in rodents. Our results demonstrate that biologically relevant genes that are associated with a number of biological variables common to toxicology studies can be identified using a well annotated database of microarray data from multiple sources. This abstract does not necessarily reflect EPA policy.

1217 TARGET KINASE PROFILING DATABASE: A TOOL FOR THE MECHANISTIC ASSESSMENT OF POTENTIAL TOXICITY FINDINGS AND THEIR HUMAN RELEVANCE.

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Pharmaceutical research is showing increasing interest in protein-kinases as pharmacological targets, and in developing molecules that are selective or pan-target protein-kinases inhibitors in many therapeutic areas. Since the majority of kinase inhibitors in development act via an ATP competitive mechanism, there is significant potential for cross-reactivity. Each compound is generally tested experimentally against members of diverse protein-kinases families in order to assess molecular specificity and to identify early off-target interactions. Typically, when a compound is active not only on the targeted kinase but cross-reacts with some other kinases, two types of pharmacological effects can be observed: the "on-target" effect, due to the interaction with the targeted enzyme, and the "off-target" effect, due to the interaction of the compound with a highly homologous or different kinase. During the early phase of the drug development process, a number of in vitro assays are performed to test cross-reactivity. At the same time, gene expression profiling of the kinases in healthy tissues derived from both human and pre-clinical animal species can be performed in order to predict the possible toxic effects due to their inhibition in a particular organ.

In the present work, we measured by Real-Time PCR the gene expression profile for 162 kinases in human, 146 in mouse, 110 in rat, 36 in dog and 25 in nonhuman primate, across approximately 50 healthy tissues. Our aim was to build an expression profiling database that prompted hypotheses on potential toxic liabilities when inhibiting a particular kinase or a set of kinases in a specific tissue. The resulting database is a valuable tool that proves useful in assessing the presence or absence of a kinase in a particular tissue, in comparing different expression profiles among species, in identifying new target protein kinases, and in building mechanistic hypotheses to understand and predict species sensitivity and human relevance of a candidate drug's side effects.

1218 COMPARATIVE NETWORK ANALYSIS OF THE TOXICANT DOSE RESPONSE: ACETAMINOPHEN TOXICITY.

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Chemical exposure profiling with microarray and high content screening technologies is increasingly used to investigate the mode of action of toxicants. Databases of these profiles linked to phenotypic endpoints of toxicity are being established to support comparative and predictive modeling of new compounds. This modeling requires new computational approaches to extract the biological significance from the large amount of noisy data in these microarray profiles. Previously we have demonstrated that toxicant exposure data can be mapped to a system-wide gene interaction network in which differentially expressed genes identify toxicant-specific gene network signatures. We now extend this approach to functionally characterize the dose response of a reference hepatotoxicant, acetaminophen (APAP) using two manually curated databases (Ingenuity Pathways Analysis, IPA, and GeneGo MetaCore, MC). As a proof of concept for our comparative network approach, we performed an integrated analysis of several published APAP microarray profiling datasets that cover the dose response range from sub-toxic (50 mg/kg, 24 hr.) to acutely toxic (4250 mg/kg, 24 hr.) exposure in the rat liver model. We demonstrate that dose-specific gene network signatures can be identified that reveal the dynamic, modular nature of the response to APAP exposure. At a sub-toxic APAP dose, a protective module of Phase II detoxification pathways is linked to an oxidative stress response module by a signaling hub of highly connected stress response and nuclear receptor genes. In contrast, as the APAP dose increases to an acutely

toxic exposure, linked modules for the DNA damage and inflammatory responses become activated. Significantly, our network-based approach allows the dynamics of the toxicant dose response to be visualized as statistically validated, functional modules supporting subsequent biomarker and drug target discovery, key considerations for future development of the IPA and MC platforms.

1219 DEVELOPMENT OF A NMR-BASED METABOLOMIC ANALYSIS METHODOLOGY FOR TOXICOLOGY.

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Metabolomics is the simultaneous measurement of metabolites from endogenous and exogenous chemicals, which may be used to identify putative biomarkers of exposure and toxicity. Currently, most metabolomics studies focus on using pattern recognition techniques to cluster spectrometric peaks, but most fail to statistically identify peaks associated with exposure. We have developed a data analysis and processing methodology for Nuclear Magnetic Resonance (NMR) spectrometry to 1) identify and eliminate spectral regions with no signal, 2) statistically characterize the significance of differentially expressed metabolite signals, and 3) quantify the change in these signals. The method identifies spectral regions with no signal by scanning spectra with a low-level threshold. Detection Theory is used to produce probabilistic estimates of the presence of a treatment effect, based on either a minimum Bayesian risk cost or a constant false alarm rate. The treatment effect is then quantified by either absolute or relative (fold) changes of the significant bins. As an example, hepatic lipid extracts from mice dosed with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were analyzed using ¹³C NMR. Noise screening eliminated channels for which there was no signal in both control and treatment replicates, a reduction from 1024 to 192 active bins. The Bayesian-cost significance metric was then used to reduce the data further to 77 channels that had a high probability of treatment effect. We ranked these bins both by absolute and by fold change to identify those channels showing the largest effect. These results are valuable as they stand, or can serve as a screened basis for further classification and identification analysis. Supported by NIH R21 GM75838.

1220 DEVELOPMENT OF A COMPUTATIONAL TOXICOLOGY COURSE AS A COMPONENT OF THE MOLECULAR TOXICOLOGY CURRICULUM AT UC BERKELEY.

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A Computational Toxicology course taught in the instructional laboratory of the College of Natural Resources Geospatial Imaging and Informatics Facility, is required as part of the curriculum of the Molecular Toxicology major at the University of California, Berkeley. This course is the first of its type in toxicology curricula in the United States. The course focuses on both environmental and physiological risk assessments of unknown chemicals using a variety of computational methods. Using both SAR and QSAR approaches, toxicophore identification and elucidation of mechanism of toxic action are emphasized. The software programs used are freely available on the internet or commercially-available via subscriptions. The programs can be categorized into: (1) predictive software where no new data are required and results were thus based on existing databases within the software for specific toxicological endpoints, (2) data mining software where correlations between chemical structures and toxicity are obtained by entering data from published datasets, (3) model building software that allows for the generation of QSAR predictive models, and (4) systems biology software that can infer pharmacodynamic pathways of chemicals in human biological systems based on published *in vitro* and animal studies. A detailed laboratory manual was developed for this course, which can serve as both an instructional tool and a guide for the design of a similar course. The manual provides instructions on how to use the different computational approaches to systematically identify toxicophore components and the molecular actions of unknown compounds.

1221 TOXIPEDIA – CONNECTING SCIENCE AND PEOPLE.

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One of the great challenges facing the toxicological sciences is communicating scientific information in a manner that creates an informed public. Toxicological research and experience provides a seemingly overwhelming, and expanding, amount

of information. To keep abreast of the growing amount of information on any one agent or issue, scientists and public health professionals must specialize. Currently there is no easy way for specialist to easily share their knowledge with the public or even other scientists. Toxipedia (www.toxipedia.org) is a new wiki-site concept that is designed to encourage toxicology specialists and public health professionals to share their knowledge of agents that affect human and environmental health. The goal of toxipedia is to be a definitive, yet accessible, information resource on the hazards and history of chemical and physical agents, regulatory requirements, and risk management. As an inter-related web, toxipedia is a new tool for scientific information exchange and communication to and with the public, the media, students, NGOs, legislators, and practicing professionals. This unique platform provides an interrelated web of information that grows as the toxicological sciences advance and reshapes how we communicate scientific information. The accuracy and credibility of Toxipedia will be supported by subject-specific associate editors using a four-tiered review system. The highest level of review will require peer review, not unlike peer-reviewed professional publications, that can be periodically updated through a rigorous review process. There will be extensive reference list that will encourage users to explore the primary literature. Lower levels of review will have progressively less oversight coupled with review-level disclaimers. In this session you will learn more about the goals and structure of Toxipedia, how to contribute to this wiki site, and how to be part of a growing movement to share our knowledge for the common good. Providing information on toxipedia is a way to satisfy our ethical responsibility to share knowledge and a primary goal of SOT to "advance the science of toxicology to enhance human, animal and environmental health".

1222 HUMAN HEALTH RISK ASSESSMENT TOOLS: SUPPORTING THE RISK ASSESSMENT PROCESS AT THE NATIONAL HOMELAND SECURITY RESEARCH CENTER.

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EPA's National Homeland Security Research Center (NHSRC), Threat and Consequence Assessment Division (TCAD), evaluates the human health risks associated with the release of contaminants into the environment. To support this evaluation, TCAD develops risk assessment methods and tools and risk communication techniques that will be of practical assistance to government agencies and first responders dealing with threats from chemical, biological, and radiological contamination. Two tools being developed to assist EPA first responders, risk assessors, and decision-makers are the Data Dictionary (DD) and the Emergency Consequence Assessment Tool (ECAT).

The DD includes a compilation of primary and secondary data on toxicity, infectivity, dose-response, and health effects for NHSRC's priority list of chemical, biological, and radiological agents.

The ECAT is an interactive web-based software tool designed to provide a wide range of emergency response officials with accurate information in a rapid manner during a major environmental crisis caused by a terrorist attack or natural disaster. Both these tools will undergo rigorous internal and external peer review to ensure scientific credibility and technical quality. In combination, these tools offer a powerful means to quickly and efficiently gather pertinent information on the health risks posed by agents that are of primary concern to the Nation's security. These tools will play an important role in understanding human health risks and will provide the means for risk assessors and managers within and outside the Agency to make informed decisions that can be utilized to conduct innovative human health risk assessments.

1223 THE INTERAGENCY COORDINATING COMMITTEE ON THE VALIDATION OF ALTERNATIVE METHODS (ICCVAM) RECOMMENDATIONS FOR THE USE OF *IN VITRO* CYTOTOXICITY TEST METHODS TO REDUCE ANIMAL USE FOR ACUTE ORAL TOXICITY TESTING.

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Cytotoxicity methods are proposed as a way to reduce animal use by using the *in vitro* data to estimate starting doses for *in vivo* acute oral toxicity studies (i.e., the Up-and-Down Procedure [UDP] and the Acute Toxic Class [ATC] method). ICCVAM evaluated two *in vitro* methods proposed for this purpose, and published recommendations on their usefulness and limitations. ICCVAM reviewed validation study results for two *in vitro* neutral red uptake (NRU) basal cytotoxicity test methods (BALB/c murine fibroblasts [3T3] and primary normal human epidermal ker-

atinocytes [NHK]) used for estimating the LD50 of 72 substances. Animal savings were calculated using the *in vitro* estimates for the starting doses for the UDP and ATC method. Based on computer simulations and compared to using default starting doses, use of these *in vitro* methods reduced animal use from 5 to 28%, (0.5 to 3.3 animals) per test with savings greatest for chemicals that do not require acute oral toxicity hazard classification. These NRU test methods correctly predicted (29-31%) the UN Globally Harmonised System (GHS) acute oral toxicity category for 67-68 substances. ICCVAM concluded that the two NRU test methods are not sufficiently accurate to predict acute oral toxicity for the purpose of hazard classification. However, ICCVAM recommends that the *in vitro* test methods be considered in a weight-of-evidence approach to determine the starting dose for the UDP and the ATC. Consistent with U.S. Animal Welfare Policies and Guidelines, ICCVAM further recommends that such *in vitro* test methods be considered and used before testing is conducted using animals. The ICCVAM evaluation report also provides recommended test method protocols, performance standards, and future studies that might advance the use of *in vitro* methods for estimating acute systemic toxicity (<http://iccvam.niehs.nih.gov/>). Supported by NIEHS contract N01-ES 35504.

1224 EFFECT OF TOPICAL ANESTHETIC PRE-TREATMENT ON *IN VIVO* OCULAR IRRITATION HAZARD CLASSIFICATION.

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The ocular irritation or corrosion potential of substances to which humans may be exposed has been evaluated since 1944 using the Draize rabbit eye test. Due to the potential for pain and distress that may occur in rabbits after application of a severely irritating or corrosive substance, alternative approaches have been proposed and developed to reduce the number of such substances that require animal testing (e.g., a weight-of-evidence approach, use of topical ocular anesthetics prior to test substance administration). This evaluation focuses on the effect of topical application of 0.5% (w/v) tetracaine hydrochloride on the irritancy potential of 97 proprietary formulations, tested in 339 rabbits, evaluated using a sequential testing scheme. In this testing scheme, the first rabbit did not receive topical anesthetic pre-treatment. If a rabbit appeared to exhibit pain or suffering after formulation administration, subsequent rabbits were pre-treated with the topical anesthetic. For all formulations, the final rabbit tested was pre-treated with the topical anesthetic. Irritancy classifications were assigned to each rabbit according to three regulatory hazard classification systems (i.e., EU, EPA, GHS). Although none of the observed differences were statistically significant, rabbits pre-treated with anesthesia appeared to produce slightly more severe responses for all three hazard classification systems than rabbits that were not pre-treated. Further, studies indicated that anesthetic pre-treatment had no impact on the variability of rabbit irritancy classifications for the same formulation. Finally, analyses indicated that anesthetic pre-treatment did not significantly increase the number of days needed for opacity, iris, or conjunctival lesions to fully reverse. Combined, these findings support the routine use of 0.5% (w/v) tetracaine hydrochloride as a pre-treatment in the *in vivo* Draize rabbit eye test. ILS, Inc. staff supported by NIEHS contract N01-ES-35504.

1225 ICCVAM/NICEATM/ECVAM SCIENTIFIC WORKSHOP ON ALTERNATIVE METHODS TO REFINE, REDUCE AND REPLACE THE MOUSE LD50 ASSAY FOR BOTULINUM TOXIN TESTING.

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A mouse LD50 assay is used by U.S. and European regulatory agencies to detect botulinum toxin in food and environmental samples, and to assess the potency of therapeutic toxin. Recent advances provide new prospects for the development and validation of alternative methods that may be faster, more accurate, and may refine (cause less pain and distress), reduce, and replace animal use. ICCVAM, NICEATM, and ECVAM co-sponsored a public workshop in November 2006 to review the state-of-the-science and current knowledge of alternatives to the mouse LD50 assay. The workshop reviewed public health needs, current regulatory requirements, structural aspects and mechanisms of action of botulinum toxin, and specific alternative methods and approaches for their potential to reduce, refine, and replace the mouse LD50 assay. Specific alternative methods considered included *in vitro* (e.g., endopeptidase assays, cell-based methods), *ex vivo* (e.g., mouse phrenic nerve-hemidiaphragm assay), and *in vivo* test methods (e.g., mouse hind limb assay, mouse abdominal ptosis assay). Alternative approaches considered included evaluation of earlier more humane endpoints. Panel discussion outcomes

identified hindrances to further development of alternative methods, including knowledge gaps, and suggested future research initiatives to address them. Panel discussion addressed the current development and validation status of specific alternative methods and approaches, their strengths and limitations, and their potential to reduce, refine, and replace the mouse LD50 assay. Implementation of some of these alternative methods could decrease the number of animals tested and reduce animal pain and suffering. ILS, Inc. staff supported by NIEHS contract N01-ES 35504.

1226 ICCVAM RECOMMENDATIONS ON THE USE OF FOUR *IN VITRO* TEST METHODS FOR THE CLASSIFICATION OF OCULAR CORROSIVES AND SEVERE IRRITANTS.

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Animal welfare concerns about the Draize rabbit eye test have led to the development of numerous proposed alternative *in vitro* test methods. ICCVAM recently completed an evaluation on the validation status of and published recommendations for four of these methods for identifying ocular corrosives and severe irritants: IRE, ICE, BCOP, and HET-CAM. *In vivo* results were classified based on regulatory eye hazard classification systems and *in vitro* results were classified based on decision criteria obtained from literature reviews or personal communications with test method developers. Accuracy statistics for each *in vitro* test method were similar across all regulatory classification systems (i.e., EPA, EU, GHS). Where data permitted, reliability analyses indicated that the methods were generally reproducible within and between laboratories. Based on the available data, ICCVAM recommends that BCOP and ICE can be used in appropriate circumstances and with certain limitations as screening tests for the detection of ocular corrosives and severe irritants in a tiered-testing scheme, using a weight-of-evidence approach. Since test method performance varied for different chemical and physical classes, ICCVAM recommends that the current database and performance be reviewed to determine the suitability of a method for specific testing situations. Consistent with U.S. Animal Welfare Regulations, ICCVAM further recommends that *in vitro* ocular test methods be considered for specific testing situations prior to testing in animals and that an alternative test method be used when appropriate. When used in this manner, these methods should reduce the pain and distress associated with testing ocular corrosives and severe irritants. The ICCVAM report also provides recommended test method protocols and substances for validation studies, and recommendations for future studies that might improve the usefulness of these test methods. Supported by NIEHS contract N01-ES-35504.

1227 EVALUATION OF CURRENT EPA CANCER POTENCY ESTIMATES BASED ON THE 2005 CANCER GUIDELINES.

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Cancer potency estimates (cancer slope factors and unit risk factors) developed by the EPA are widely used in human health risk assessment and regulatory decision making. The EPA's Final Guidelines for Carcinogen Risk Assessment, released in March 2005, heralded several significant changes to the development of cancer potency estimates. This guidance describes what types of toxicity data are suitable for making a determination about whether a chemical should be evaluated as a human carcinogen. It also describes the methods used to develop human potency estimates based on available toxicity data. Although there is a formal peer review process for re-evaluating existing potency estimates under the new Guidelines, progress is slow. This results in a situation in which potency estimates developed based on older guidance and methodologies are still being used to make regulatory decisions. All cancer potency estimates published in the EPA's Integrated Risk Information System (IRIS) database were evaluated. Of the chemicals with cancer potency estimates listed in IRIS that are based on animal data, only a few have potency estimates developed using the new Guidelines. Most current animal toxicity data were scaled to a human equivalent dose based on the outdated method of body weight raised to the 2/3 power. Revised cancer potency estimates are provided using the current recommended scaling factor of body weight raised to the 3/4 power. This new scaling factor alone lowers slope factors by 1.9-fold for values based on mouse studies. For several chemicals, the data previously used to derive potency estimates are likely insufficient to warrant quantitative cancer potency estimates under the new Guidelines. These chemicals are identified with respect to their potentially unreliable cancer potency estimates.

1228 THE PRESENCE OF DRUG IN CONTROL SAMPLES: MINIMIZING RISK OF CONTAMINATION IN TOXICOLOGY STUDIES.

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In 1999, during the assessment of a centralized application, serious concerns were raised on mouse and rat carcinogenicity studies where variable amounts of test substance were detected in samples from control animals. One of these studies was considered invalid and had to be repeated. Since then, several applications have been identified where samples from control animals showed some levels of test substance in pivotal repeated dose, reproductive and carcinogenicity studies. The source of contamination could not be identified and an appropriate risk assessment related to carcinogenicity and reproductive toxicology could not be performed because safety margins could not be established. As a consequence, this issue has led to delays in Marketing Authorization, and in some cases to the issuing of "Warning letters" from the FDA, especially in cases of bioequivalence studies. An EFPIA survey conducted in 2003 showed that contamination occurs in toxicology studies regardless of the route of administration used, the dose levels or the duration of the treatments. As nowadays, analytical methods are becoming more and more sensitive, the possibility of detecting test substance in controls is increased. Industry has recommended best practices to reduce this event, but it is not a realistic expectation that it will be possible to completely eliminate it. The causes may be multiple and it is often not possible to identify the source and/or the precise cause of the presence of drug in control samples. Therefore, a weight of evidence approach is needed to evaluate the validity of the study data in assessing risk to humans. With this aim, an EMEA guideline came into operation on September 2005. Potential sources of contamination, internal policies, and training of personnel are presented in this poster, and data from bioanalysis conducted during the 2005-2006 toxicology studies performed in our department are also shown. Conclusions and best practices to address this issue based on our acquired experience are drawn.

1229 ACCEPTABLE LEVELS OF RISK - A SURVEY OF STATE REGULATIONS.

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Risk-based corrective action (RBCA) is a process for remedial decision-making predicated on the concept that there is an acceptable level of risk from residual contamination in the environment. For carcinogens, the National Oil and Hazardous Substances Pollution Contingency Plan authorized the EPA to use a risk range between 1 in 1,000,000 (1E-06) to 1 in 10,000 (1E-04) for determining when remediation is required at contaminated sites based on the belief that this range represents de minimus excess risks to the public. With the maturation of risk assessment methodology, many states have adopted their own RBCA programs. Most of these programs rely heavily on guidance from the EPA and/or the American Society for Testing and Materials (ASTM) for their basic framework. However, there is significant disparity in the way that acceptable risk is handled among various programs. A comprehensive review of U.S. state environmental agency regulatory program approaches for establishing acceptable risk was conducted. Sources included agency guidance documents, state legislation, and phone interviews with state regulators. Detailed information was identified for most states. Of the states with their own RBCA programs or guidance, more than three quarters have established chemical-specific default remediation goals using a target cancer risk of 1E-06 while the remainder set these default levels using a target risk of 1E-05. Most states have guidance on managing cumulative site risks and more than half of these have either explicitly or implicitly adopted EPA's target risk range. However, a significant number have opted to manage cumulative risks at a bright-line 1E-05 level and at least one state does so at a 1E-06 level.

1230 A TECHNICAL ANALYSIS OF THE PROPOSED REACH REGULATION.

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REACH (Registration, Evaluation, and Authorization of Chemicals) is the European Union's proposed strategy for the management of risk to human health and the environment posed by chemical substances. At its core, REACH will require producers and users of chemical substances to register these uses in a volume-triggered system. It demands the submission of chemical assessment reports containing information on the hazards, exposures and risks associated with the uses of chemical substances for review by the competent authorities and government-appointed expert committees. Chemicals deemed to be of very high concern, e.g., carcinogens, mutagens or substances toxic to reproduction, will trigger a complex authorization procedure in which the ultimate political goal is to substitute those

chemicals with safer alternatives. REACH is now in its final stages of the political review process. It is expected that the political and regulatory hurdles will be cleared by the end of 2006 and REACH will enter into force in mid' 2007. In parallel with the regulatory review, stakeholder expert working groups have been charged to develop the necessary guidance and support tools that are essential to help industry and regulators understand their tasks and fulfill their obligations under REACH. These guidance documents produced through various REACH Implementation Projects are essential for the workability, smooth transition and successful implementation of REACH. This paper will present the results of a study to characterize the key requirements, obligations and review processes specified under REACH. Specific focus will be on the identification of the toxicological data requirements as specified in Annexes VII to X of the current REACH proposal, the role of alternative methodologies such as in vitro testing, (Q)SARs (structural activity relationships) and read-across. Moreover, it analyses the possibilities of chemical grouping and the application of 'weight of evidence' approach to justify data waiving, and the methodological approaches applied to characterize risks associated with the use of chemical substances.

1231 CONSIDERATION OF THERAPEUTIC DOSE FOR DEVELOPMENT OF PROVISIONAL REFERENCE DOSE (P-RFD) FOR NITROGLYCERIN.

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Nitroglycerin, has been used for over 100 years in the treatment of angina pectoris (myocardial ischemia). The mechanism of action involves vasodilatation, thereby, lowering blood pressure and reducing cardiac load. In addition to clinical usage, contact with nitroglycerin can occur through occupational exposure in the explosive industries and through contamination of drinking water. There is no information to determine the minimum effective dose of nitroglycerin to assess the safe level of exposure in sensitive individuals. The lowest available single oral dose of sustained-release nitroglycerin is 2.5 mg (equivalent to 0.036 mg/kg-day) with a recommended maximum total daily oral dose of 38 mg/day. Adverse effects, such as headache, nausea and hypotension associated with therapeutic and occupational exposure to nitroglycerin are secondary to actions on the cardiovascular system, with vasodilatation as the initiating event. Therefore vasodilatation can be considered as the critical effect upon which to base the p-RfD. Furthermore, due to rapid elimination of nitroglycerin, bioaccumulation is not expected in long-term exposure, thus suggesting the possibility that the adverse effect level for long-term exposures may not be different than the adverse effect level for acute exposure. No evidence is available to suggest that chronic exposure to therapeutic doses of nitroglycerin by any route of exposure results in toxicity to the liver or other organs, as reported in animal studies. This presentation will discuss approaches for using a therapeutic dose to support risk assessment of exposures at contaminated site(s).

1232 EXPANDING THE ROLE OF EXPERT PEER INVOLVEMENT IN RISK ASSESSMENT: PART 1, A COMPREHENSIVE PROCESS.

J. Patterson¹, J. E. Strawson¹, M. E. Meek² and R. Liteplo². ¹TERA, Cincinnati, OH and ²Health Canada, Ottawa, ON, Canada. Sponsor: M. Dourson.

Many organizations (OMB, 2004; IPCS, 2005; IARC, 2006; Health Canada, 2006) use peer review to obtain external opinions on the scientific and technical merit, as well as the strength of assumptions and conclusions of risk assessment documentation. Typically, peer review is employed at the end of the document development process; however, by incorporating peer involvement earlier in the process, organizations can improve the overall quality of their risk assessment documents. This poster examines peer involvement techniques and presents a comprehensive process for expanding the use peer involvement in risk assessment to insure completeness and quality at various stages in a risk assessment document's development. Peer involvement includes peer input, peer consultation, and peer review. Peer input involves solicitation of information, data, or issues at an early stage. Peer consultation may be a formal or informal process to gather expert peer advice on a specific issue or work product during its development. Peer review is a formal, external, and independent review of an intended final work document. Techniques for peer involvement include data call ins, expert elicitation, requests for written comments, open workshops, conference calls, public meetings, peer consultation meetings, and formal peer review meetings. Four broad principles are important for consideration and organization of peer involvement activities – independence, inclusion of appropriate experts, transparency, and a robust scientific process. Attention to these principles insures that expert peer involvement is credible and valuable.

1233 EXPANDING THE ROLE OF EXPERT PEER INVOLVEMENT IN RISK ASSESSMENT: PART 2, USE OF PEER EXPERTS IN CEPA EXISTING SUBSTANCES PROGRAM.

M. E. Meek¹, R. Liteplo¹, J. Patterson² and J. E. Strawson². ¹Health Canada, Ottawa, ON, Canada and ²TERA, Cincinnati, OH. Sponsor: M. Dourson.

Part 1 of this study described a process for expanding the role of peer involvement in risk assessment that would improve the overall quality of risk assessment products. This poster describes examples of how this process has been used for risk assessments under the Canadian Environmental Protection Act (CEPA). Canada is the first country to introduce a legislative requirement for systematic priority setting of all chemicals in commerce, necessitating the categorization and prioritization health risk assessments for approximately 23,000 substances. This has provided opportunity to expand the incorporation of the input of peers within the program both for the development of methodology and assessments of individual chemicals and/or groups. Peer input has been used to guide in the development of priority setting tools such as ComET (Complex Exposure Tool), resulting in participation of multiple stakeholders and acquisition of data not previously available. Peer consultations have assisted in the design of methodologies for development of the weight of evidence component of the Complex Hazard Tool (ComHaz), as well as advice on chemical specific risk issues, such as design of studies for aluminum. These peer consultations focused development and stimulated additional thought thereafter, which has contributed to development of predictive tools not only to address the Canadian but other jurisdiction's mandates. Peer review has been used for chemical assessments to evaluate the adequacy of coverage and identify if key information or data are missing, as well as review of supporting documentation, hazard characterization, and dose-response analysis. These peer reviews have resulted in maximal utilization of available data in drawing conclusions, verified the need for additional analyses of key studies, and have led to the conduct of more appropriate investigations to inform conclusions.

1234 BIOLOGICAL EVALUATION OF THE OREGON WATER QUALITY CRITERIA: NEW APPROACHES IN ANALYZING PROTECTION OF ENDANGERED SPECIES.

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The Clean Water Act requires the U.S. Environmental Protection Agency (EPA) to develop water quality criteria and approve water quality standards for the protection of aquatic life. Under the Endangered Species Act (ESA), U.S. federal agencies are required to ensure that their actions are not likely to jeopardize the continued existence of threatened or endangered species. EPA's approval of state water quality standards is considered an action that could potentially jeopardize threatened or endangered species, thus requiring consultation with natural resource agencies under the ESA. The state of Oregon recently submitted new or revised water quality criteria for 19 freshwater chemicals and 13 saltwater chemicals to EPA's Region 10 office for approval. Four major types of evaluations were performed by EPA Region 10 to evaluate potential adverse effects of water quality criteria on the 16 threatened or endangered aquatic species (14 fish, 2 aquatic invertebrates) and 11 threatened or endangered wildlife species in Oregon (5 aquatic-dependent birds, 6 marine mammals). These evaluations analyzed effects on listed aquatic species from direct exposure to chemicals in water; effects on prey of listed aquatic species; effects on listed aquatic species from multiple routes of exposure to criteria chemicals (i.e. from direct water column toxicity and dietary toxicity due to ingestion of contaminated prey); and toxicity to listed wildlife species whose diet consists of an appreciable proportion of aquatic prey potentially contaminated from exposure to criteria concentrations. New approaches were used to analyze effect concentrations in listed aquatic species including Interspecies Correlation Estimation (ICE) models and species sensitivity distributions. Descriptions of the methodology used for each type of evaluation and how this relates to determining potential adverse effects of criteria approval on ESA listed species will be presented for representative chemicals.

1235 BEYOND RISK ASSESSMENT – INCORPORATING OUR VALUES AND ETHICS IN PRECAUTIONARY ASSESSMENT.

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Risk assessment is an expression of our values and ethical decision-making. Classical risk assessment was defined in a 1983 report by the National Research Council as including four basic elements: hazard identification, dose-response assessment, exposure assessment, and finally risk characterization. Initial efforts were focused on defining a numeric probability for developing cancer, which evolved

into quantitative risk assessment. This approach become highly technical and dominated by experts in risk assessment. While the initial goals of risk assessment were laudable, it has degenerated into defining how much hazard is tolerable or is necessary in any given situation. Quantitative risk assessment has a number of weaknesses. It was said "We should remember that risk assessment data can be like the captured spy: If you torture it long enough, it will tell you anything you want to know." (William Ruckelshaus (1st administrator of U.S. EPA) 1984.) The goal of precautionary assessment (PA) is to move beyond risk assessment and allow communities and individual to incorporate their knowledge, values and ethics into a more comprehensive evaluation of a hazardous condition. The PA combines the philosophy and ethics of the precautionary principle with the standard scientific evaluation of the hazards. Precautionary assessment contains three basic elements: a) community and social issues, b) exposure, and c) hazard and toxicity. Each element is broken down into a series of questions that are scored numerically to produce a summary score for each element. The PA is designed to help place the knowledge available within the context of the community. In contrast to the traditional risk assessment, the PA is a more comprehensive approach to evaluating the human and environmental health risks. Overall, the PA is a more reasonable, rational, and responsible approach to evaluating risk of hazardous chemicals.

1236 CRITICAL ROLES OF COACTIVATORS AND COREPRESSORS DURING TRANSCRIPTIONAL ACTIVATION BY XENOBIOTIC RECEPTORS.

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The aryl hydrocarbon receptor (a member of the basic-loop-helix-PAS family of transcription factors), peroxisome proliferator-activated receptor α (PPAR α), estrogen receptor, and pregnane X receptor (PXR) are xenobiotic activated nuclear receptors. Each receptor can bind with a distinct set of xenobiotics to mediate biological/toxicological effects in species from fish to human. For each activated receptor, toxicity is initiated to a large degree through transcriptional activation/repression of a particular set of target genes by the liganded receptor. In most cases, the ability of the DNA bound receptor to modulate gene regulation is dependent on its interaction with coactivator and corepressor proteins. Some of these coactivators and corepressors covalently modify histones, thereby relieving or enhancing their repressive effects on transcription. Over the past several years, there has been intense investigation to identify the specific coactivator and repressor proteins that interact with nuclear receptors and to determine how they impact gene regulation. Interestingly, it appears that many divergent receptor proteins associate with similar coactivator/corepressors proteins to modulate gene regulation. In addition, recent findings suggest that inter-individual response to xenobiotic ligands may be related to variations in coactivator or corepressor expression or activity. These variations may underlie tissue-specific differences in sensitivity to xenobiotics during development, and in adulthood. Studies have also demonstrated that there is functional cross-talk between different xenobiotic receptors that can be mediated by coactivators or corepressors. Thus, this session is aimed at exploring the direct interaction of several toxicologically relevant receptors with coactivator/corepressors proteins and how these interactions impact response of humans and other mammals to xenobiotics.

1237 ROLE OF CO-REPRESSOR SMRT IN REGULATION OF PREGNANE X RECEPTOR (PXR).

J. Chen, *Pharmacology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ*. Sponsor: R. Pollenz.

The orphan nuclear receptor PXR (pregnane X receptor, also known as SXR) is expressed primarily in liver and intestine, and coordinates hepatic responses to prevent liver injury induced by toxins, such as rifampicin and clotrimazole. It is well documented that PXR activates cytochrome P450 (CYP) gene expression by recruiting transcriptional co-activators such as p160's. However, little is known about the function of unliganded PXR and the role of co-repressors in regulation of PXR activity. We have recently found that the co-repressor SMRT (silencing mediator of retinoid and thyroid hormone receptors) may play a role in regulating the activity of unliganded PXR by direct interaction. The PXR-SMRT interaction is generally sensitive to species-specific ligands, causing an exchange of the co-repressor with co-activator. Homology models of intact LBD with two SMRT nuclear receptor (NR)-interacting domains have also been constructed. These modeling studies reveal key interactions that account for the binding preference. Furthermore, preferential association of PXR with distinct SMRT isoforms was observed. Our data suggest that the co-repressor SMRT may regulate the transcriptional activity of unliganded PXR. Thus, co-repressors may play a role in regulating PXR function in response to environmental toxins.

1238 LIGAND-DEPENDENT COACTIVATOR RECRUITMENT BY PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPARs).

J. Vanden Heuvel, *Veterinary and Biomedical Sciences, Penn State University, University Park, PA.*

The regulation of gene expression by nuclear receptors (NR) is dependent on the recruitment of coactivators to the transcriptional complex. With many NRs, the ability to interact with these proteins is ligand-specific. In these studies we examined the ligand-dependent recruitment of coactivator proteins to peroxisome proliferator-activated receptor α (PPAR α , NR1C1). Of the several coactivators that were tested, PPAR binding protein (PBP), PPAR γ coactivator 1 α (PGC-1 α), steroid receptor coactivator-1 (SRC-1) and CBP/p300-interacting transactivator with ED-rich tail 2 (CITED2) were the most efficacious at affecting PPAR α activity in the presence of the peroxisome proliferator (PP) Wy-14,643. Interestingly, overexpression of PBP or PGC-1 α resulted in a more pronounced increase in PPAR α activity when activated by fatty acids relative to xenobiotic PPs. In contrast, CITED2 and SRC-1 had equal effects on PPAR α activity whether activated by Wy-14,643 or fatty acids. When expression of PGC-1 α , SRC-1 or PBP is diminished through the use of RNAi plasmids, Wy-14,643-induced PPAR α activity is only affected by PGC-1 α RNAi whereas conjugated linoleic acid (CLA) and ciprofibrate are affected by decreasing expression of all three coactivators. A LXXLL containing peptide library was screened for its ability to interact with PPAR α under various ligand treatments using a mammalian two-hybrid assay. The affinity of these peptides to interact with PPAR α was dependent on the ligand examined. To further define coactivator recruitment to PPAR α , a portion of the ligand-dependent transactivation domain (AF2, 456LHPLL) was mutated (456LHPAA). This mutation resulted in a dominant negative construct that inhibited PPRE-driven transcription. Together, this research shows that coactivator recruitment to PPAR α is dependent on the ligand used to activate the receptor.

1239 MODULATION OF ARYL HYDROCARBON RECEPTOR-DEPENDENT TRANSCRIPTION BY ESTROGEN RECEPTOR ALPHA.

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New mechanisms of AhR/ER α crosstalk have recently been described that have profound implications on AhR and ER α signal transduction. We have shown that dioxin induces recruitment of ER α to the AhR regulated promoter, cytochrome P4501A1. ER α recruitment was enhanced by co-treatment with 17 β -estradiol (E2), but E2 alone had no effect. Analysis of several AhR agonists revealed ligand-specific oscillatory recruitment of AhR and ER α , a differential ability to recruit ER α to AhR target genes, and that the influence of E2 co-treatment on ER α promoter occupancy exhibited ligand and promoter specificity. For example, 3,3'-diindolylmethane (DIM) strongly induced promoter occupancy of ER α to CYP1A1 and CYP1B1, which was not further increased by co-treatment with E2. Although co-treatment with E2 had no effect on CYP1A1 mRNA levels, siRNA mediated suppression of ER α significantly inhibited dioxin-induced CYP1A1 mRNA expression. Chromatin immunoprecipitation assays revealed this to be due to reduced promoter occupancy of AhR at CYP1A1. ER α directly interacts with AhR via a sequence motif in its N-terminal ligand-independent activation domain (AF1). Deletion of this domain prevented TCDD-induced recruitment of ER α to CYP1A1, highlighting the importance of the AF1 domain in mediating interactions between AhR and ER α . Currently, we view the recruitment of ER α at AhR as two distinct processes; (1) unliganded ER α (no E2) acts as an AhR ligand-dependent coregulator of AhR transcription; and (2) the increased recruitment of ER α by cotreatment of AhR agonist and E2 represents a mechanism for AhR-dependent inhibition of ER α activity by sequestering E2-bound ER α away from ER α target genes. The signals that induce ER α recruitment to AhR target genes and the biological consequences of these findings remain elusive, but provide further evidence for strong functional crosstalk between these two receptor systems. This work was supported by the David and Astrid Hagelén Foundation and the Swedish Cancer Fund.

1240 COACTIVATOR RECRUITMENT AND HISTONE MODIFICATION DURING DIOXIN INDUCTION OF HUMAN CYP1A1, CYP1B1 AND CYP2S1.

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The inducibilities of CYP1A1, CYP1B1 and CYP2S1 by 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) differ from one another in different tissues and organs, and the maximally induced levels of these P450s can also differ substantially

from one another. Differences in these parameters also occur in established mammalian cell lines. We have found that these differences between cell lines can occur even when the dioxin-dependent recruitment of the aryl hydrocarbon receptor (AHR) and aryl hydrocarbon receptor nuclear translocator (ARNT) proteins to the enhancer regions of the individual P450 genes are equivalent. However, we have found that these differences, in some instances, correlate with differences in transcriptional coactivator recruitment to and/or covalent histone modifications at, the P450 genes in question. We are manipulating the expression levels of particular coactivator proteins in order to address the notion that differential coactivator recruitment is causally related to the variation in inducibility and maximally induced levels of the P450s in the cell lines. These investigations are providing further insight into the molecular mechanism underlying tissue-specific variations in P450 induction, which are, in turn, likely to be associated with tissue-specific variations in sensitivities to xenobiotics.



1241 EMERGING TECHNOLOGIES IN IMAGING FOR TOXICOLOGY.

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This session will present overviews of exciting new and emerging imaging modalities and technologies that will be of interest to toxicologists. These are new technologies and rapid advances in existing technologies that will provide powerful new tools for toxicologists to address important new questions in toxicology and the biomedical sciences. Speakers will present overviews of the new technologies, potential applications to the field of toxicology, and sample data. The first presentation will describe new applications of in vivo EPR imaging for non-invasive spatial mapping of tissue oxygenation and redox status in small animals. The second presentation will present new applications of in situ molecular imaging and profiling of proteins and drugs in tissues by mass spectrometry for biological and clinical research. The third presentation will present advances and applications in imaging by microCT and microPET scanning for small animal in vivo imaging. The fourth presentation will focus on non-invasive in vivo optical imaging techniques for disease diagnosis and therapeutic monitoring. The fifth presentation will discuss the utilization of virtual microscopy for high throughput image analysis for automatic assessment of morphological changes.



1242 IN VIVO ELECTRON PARAMAGNETIC RESONANCE (EPR) IMAGING.

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Electron paramagnetic resonance (EPR) imaging is an emerging technique for non-invasive spatial mapping of tissue oxygenation and redox status in living small animals, such as mice and rats. With the recent improvement of low frequency EPR instrumentation and the synthesis of better imaging agents, many exciting results have been obtained. This presentation will highlight the latest developments in EPR imaging techniques and some of recent results, with particular emphasis on their application in toxicology research. EPR and MRI imaging are both based on the same principle of paramagnetic resonance, and each can provide unique information that is complementary to each other. Utilized effectively together, they are more powerful than being used alone. Advances for in vivo EPR imaging now permit synthesis with MRI imaging and these two imaging modalities can now be utilized together in vivo (and also in combination with histological studies in post-mortem studies). Integration of these two complementary imaging modalities to create novel image reconstructions will be discussed along with applications for toxicology research.



1243 IN SITU MOLECULAR PROFILING/IMAGING OF PROTEINS BY MALDI MS.

R. Caprioli. *Vanderbilt University, Nashville, TN.* Sponsor: C. Marcus.

Imaging Mass Spectrometry (IMS) is a new molecular discovery technology that takes advantage of the methodology and instrumentation of MALDI mass spectrometry. It can be used to locate specific molecules such as drugs, lipids, peptides and proteins directly from the surface of fresh frozen tissue sections. Frozen tissue specimens are cut in very thin (~10 µm) sections and thaw-mounted on flat metallic target plates. Matrix can be manually or automatically deposited on the sections. Molecular profiles recovered upon analysis typically contain from 300 to 500 or more distinct signals in the m/z range beyond 200,000. When imaging from a tissue section, matrix is deposited in a homogeneous manner minimizing the lateral

dispersion of molecules of interest. This can be achieved by automatically printing arrays of small droplets. Each microspot is then automatically analyzed generating a mass spectrum. When monitoring the intensity of a signal within the data array, a two-dimensional ion density map (or image) can be reconstructed giving information on the location and relative abundance of a given analyte.

IMS is an effective discovery tool for the qualitative and quantitative analysis of molecular signatures that may differentiate unhealthy and normal tissues and for identification of potential protein markers in disease progression. In this regard, histology directed profiling permits higher sample throughput and reproducibility. The visual specificity of histology is combined with the positioning accuracy of the robotic microdispenser to direct placement of matrix drops onto specific cells with high placement accuracy. The ability to construct 3-D molecular images will also be shown. In addition, IMS technology has been successfully applied to drug targeting and metabolic studies and the measurement of concomitant protein changes in specific tissues after systemic drug administration. Identification of statistically significant protein markers can be identified in high throughput mode by mass spectrometry based proteomic approaches.



1244 MICROCT SCANNING WITH MOLECULARLY TARGETED CONTRAST AGENTS.

J. Weichert, B. Durkee, J. Grudzinski, G. Ton and M. Longino. *Departments of Radiology and Medical Physics, University of Wisconsin-Madison, Madison, WI.* Sponsor: C. Marcus.

The recent explosion in the generation of spontaneous models of disease including transgenic mice and rats has fueled the rapid development of high-resolution, 3-dimensional noninvasive, small animal imaging technologies, including microCT and microPET. MicroCT is now capable of providing exquisite 3D anatomic detail at resolutions exceeding 20 microns in live mice and rats, whereas microPET scanning affords 3D localization of radiolabeled molecular imaging agents. Acquisition times of several minutes preclude the use of conventional CT contrast agents in preclinical studies conducted on high resolution microCT scanners. Our lab has developed two new long-acting hepatocyte and vascular microCT-contrast agents which enhance the soft tissue capabilities of microCT significantly, thus permitting novel applications of this technology for rendering 3D tissue images in living animals. The design of these agents is based on a biochemical approach, whereby naturally occurring compounds known to be stored and/or metabolized in the organ or tissue of interest serve as carriers for the radiologic moiety. These types of novel targeted molecular imaging agents are extending the utility of CT, MRI, and PET imaging including potential applications to the field of toxicology. Moreover, recent introduction of multimodal hybrid scanners, including the world's first commercial microCT/microPET hybrid scanner (Siemens Inveon) now allows automatic dual modality image coregistration so that the functional PET image can be precisely located within the anatomic CT framework. The anatomic and functional strengths of microCT and microPET will be stressed and exemplified by 3-D liver images from intact live mice obtained with our novel liver contrast agent, and 3D "fly through" imaging of biological structures including blood vessels and colon will be presented, accompanied by discussions of how this new imaging approaches might be utilized in the field of toxicology.



1245 ADVANCED OPTICAL IMAGING FOR TOXICOLOGY, PATHOLOGY, MEDICINE AND SURGERY.

D. L. Farkas. *Surgery, Cedars-Sinai Medical Center, Los Angeles, CA.* Sponsor: C. Marcus.

Toxicology and pathology are becoming more quantitative and molecular, medicine is benefiting from developments in biology, and surgery is moving towards minimally invasive intervention. These advances necessitate new approaches, and biophotonics represents a major new area of hope and growth (1,2). The use of photonic tools and methods will be reviewed, with emphasis on our advances.

The central challenge is the identification and characterization of abnormalities. Photonic imaging has been lagging in clinical acceptance, in spite technological advances in laboratory research and preclinical trials (3), and a clear need for the kind of resolution (spatial, temporal, spectral) and specificity that optical imaging alone can offer. Our approach integrates the use of light intensity, coherence, polarization, temporal gating, and spectral features into a multimode implementation, to improve imaging-based quantitation and sensitivity in vivo, and build a new clinical armamentarium (4). One goal is mesoscopic imaging, enabling microscopic-level spatial resolution within the body without forsaking specificity and temporal resolution, while emphasizing imaging methods that do not require contrast agents (5).

Results presented will also include the imaging of very fast calcium transients (6); early cancer detection by spectral imaging (5); hyperspectral Mie scattering imaging for endoscopy (7); improved OCT imaging (8); monitoring of stem cell fate in vivo (9) and the design of an advanced multimode imaging endoscope.

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1246 THE USE OF VIRTUAL MICROSCOPY FOR HIGH THROUGHPUT IMAGE ANALYSIS.

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Digitizing a glass slide at a high magnification (20x to 40x) so the representative pixels can be manipulated into stitched virtual images at smaller magnifications is starting to revolutionize anatomic pathology. Virtual microscopy is evolving from a teaching tool to a diagnostic method as accurate as the standard light microscope. An added benefit of these digital images is that they allow for high throughput image analysis. This presentation will describe virtual microscopy and the adaptation of virtual images to object based morphometry. High throughput image analysis on virtual images requires an investment for a high resolution image scanner, servers to store large data files, a program for image analysis adapted to virtual images and computers to analyze the data. Equally important is establishing a team of pathologists, computer technicians, laboratory scientists and statisticians to optimize the computer environment, standardize the slides from which the images will be obtained and define data analysis parameters. However, once established, this technology allows for an almost "set it and forget it" quantitative evaluation of microscopic slides. This is largely because virtual images allow for the capture of an area of interest at one magnification and its analysis in greater detail at a higher magnification. To demonstrate the potential of this emerging technology, lessons learned from attempts at its implementation will be presented. These will include methods to standardize the image digitized, benefits of evaluating immunostained versus hematoxylin and eosin stained sections and establishing a balance between magnification, image compression, speed of image acquisition/analysis and storage costs. Finally, we will demonstrate examples of the successful use of image analysis on virtual images of the skin, immune system and pancreas.

1247 AN OVERVIEW OF THE USE OF WILDLIFE SPECIES IN IMMUNOTOXICOLOGY.

J. DeWitt¹ and J. T. Zelikoff². ¹*Curriculum in Toxicology, University of North Carolina, Research Triangle Park, NC* and ²*Nelson Institute of Environmental Medicine, New York University School of Medicine, Tuxedo, NY*.

Although rodents have been the traditional models of choice in immunotoxicology, phyla outside of the murine family can serve as warnings of pollutant immunotoxicity or of emerging infectious diseases that could affect humans. For example, marine organisms warned of the immunotoxic effects of tributyltin oxide, an agent used in marine antifouling paints and the mute swan proved a sensitive sentinel for avian influenza. Alternative, most commonly wildlife species, are undeniably valuable for evaluating immunotoxicity of a particular phyla, however, the question remains as to whether information gained from such species is applicable across phyla for the purposes of evaluating risks to ecosystems, to humans, or to both. To determine the applicability of wildlife species to the risk assessment process, the breadth of the knowledge base about the immune systems of non-rodent species, the relative costs and benefits of using non-rodent species to assess immunotoxicity, and the phylogenetic extent of immune system pathology and dysfunction must first be understood.

1248 THE INVERTEBRATE IMMUNE SYSTEM AS A TARGET ORGAN OF TOXICITY.

T. S. Galloway. *school of biological sciences, university of plymouth, Plymouth, United Kingdom*. Sponsor: J. DeWitt.

In contrast to the complexity of the vertebrate immune system, the relative simplicity of invertebrate immune systems makes them a potentially sensitive and accessible means of monitoring the effects of immunotoxic chemicals and the com-

plex interactions that ultimately affect host resistance. In this presentation, the effects of chemicals on immune function in different invertebrate species will be considered and biomarkers of immunotoxicity identified. Recent research results will be presented in which links between endocrine and immune toxicities are explored, using the periwinkle *Littorina littorea* as a case study. The presentation will finish with a discussion of the relevance of the results for environmental monitoring programmes.

1249 CHEMICAL-INDUCED IMMUNOTOXICITY IN FISH: TRANSLATION ACROSS PHyla FOR PREDICTING RISK.

J. T. Zelikoff¹, E. Carlson² and J. E. Duffy¹. ¹*environmental medicine, new york university school of medicine, Tuxedo, NY* and ²*Toxicology, General Electric Global Research, Niskayuna, NY*.

Fish share a number of immunological properties with their mammalian counterparts including phenotypically similar immune cells, lymphoid tissue structure and function, biologic mediators, and the ability to mount innate and adaptive immune responses. Because of these similarities, fish are emerging as an alternate species for assessing immunotoxicity in higher vertebrates. In this presentation, the effects of aromatic hydrocarbons such as benzo(a)pyrene (BaP) and coplanar and noncoplanar polychlorinated biphenyl (PCB) congeners on the immune system of laboratory fish models, will be examined. Mechanistic underpinnings of the observed effects will be discussed for each agent and used to link chemical-induced immune dysfunction across species. Just how these mechanistic similarities between fish and mammals can be used to establish risk will also be conferred.

1250 ASSESSING IMMUNOLOGICAL HEALTH IN WILD BIRDS: GENERAL, INTEGRATIVE, AND MECHANISTIC METHODS AND ECOLOGICAL RELEVANCE.

K. A. Grasman. *Biology, Calvin College, Grand Rapids, MI*. Sponsor: J. DeWitt.

Over the past 4 decades numerous studies have shown that environmental contaminants can alter immune function in wild birds. Methods employed in these studies have ranged from general indicators of immunological status (e.g., blood cell counts, immune organ masses) to functional assays (e.g., sheep red blood cell hemagglutination test, phytohemagglutinin skin test). The latter, which integrate the functions of multiple cell types, are similar to assays used to screen for immunotoxicity in laboratory rodents. While often difficult to implement in field studies, cellular and molecular methods (e.g., lymphocyte culture, apoptosis assays, genomic methods) hold much potential for monitoring immunological effects and providing mechanistic information to link these effects to particular contaminants. Increasing evidence in the field of immunotoxicology demonstrates the ecological relevance of reduced immune function in wild birds, including increased rates of infection and decreased rates of survival and colonization of new habitats.

1251 DEVELOPMENT AND UTILIZATION OF A STANDARDIZED SUITE OF IMMUNE ASSAYS IN HEALTHY AND NON-HEALTHY BOTTLENOSE DOLPHIN.

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Increases in the incidence of mass mortality events (MMEs) involving bottlenose dolphins (BND) has lead to concern over their health. Many of these mortality events are thought to be caused by infectious microbes such as morbillivirus. It is unclear if these agents were the primary cause of the MMEs or if compromised immunity, related to contaminant exposures, caused increased susceptibility to infectious agents. Studies with terrestrial and marine mammals have shown that various contaminant classes suppress immune function. A suite of assays for assessment of immune function in marine mammals, that can be performed from non-lethal blood collection was developed based on the NTP Immunotoxicity testing scheme. Samples from approximately 200 resident bottlenose dolphin in Indian River Lagoon, FL and Charleston, SC were collected during the summers of 2003-2005 and assessed for immune function using the developed suite. Each location has different primary contaminant classes of concern based on tissue residue analysis. Associations between contaminants and immunity were determined. Additionally, dolphins in Indian River Lagoon were noted to have higher rates of gastritis in 2003, which corresponded with associated immune data, and they were also found

in general to have higher rates of skin disease than animals in Charleston. Immune function is currently being assessed in relation to various disease types such as Lobomycosis. Information from this study allows us to begin to assess the utility of dolphins as sentinel species for identifying estuarine areas of concern for human health and allows us to begin to determine associations between contaminants, immune, function, and diseases in populations and ecosystems.



1252 INCORPORATING IMMUNOLOGY INTO ECOLOGICAL RISK ASSESSMENT.

D. Nacci. *U.S. EPA, Narragansett, RI*. Sponsor: J. DeWitt.

Immunological effects of chemical exposures are seldom considered qualitatively and almost never considered quantitatively in ecological risk assessments (ERAs). However, the impacts of chemicals on immunological competence must be accounted for because disease is one of the most important factors regulating wildlife populations. Here, we describe a research strategy to enhance the incorporation of immunology into ERAs. First, basic knowledge about immunological variation across species will benefit ERAs and human health RAs, as well. From this knowledge, functional tests of immunological responses may be used to infer mechanisms for categories of chemicals of concern, and ultimately develop inter-species extrapolations. Of particular importance are cellular and molecular methods for which samples may be obtained nondestructively, and whose results may be relevant across a relatively broad range of species. Secondly, methods are needed to extrapolate from measurement endpoints at lower levels of biological organization to ecologically relevant (population-level) effects. Whole animal challenges using acute exposures to pathological agents in combination with mechanistic indicators may provide quantitative links between immunological changes and functional responses. But these exposure methods may not provide insight into responses under real world (low level exposure) conditions. Population ecologists and ecotoxicologists need to consider epidemiological methods that take account occasional, sometimes catastrophic effects of infectious disease, to begin prediction and testing of population-level outcomes associated with chemical effects on immunological performance. In summary, research is needed to extrapolate immunological effects taxonomically and across levels of biological organization. Interdisciplinary collaborations will be necessary to integrate theory and practice from the fields of immunology, biology, and ecology to inform ERAs about potential impacts of chemicals on disease susceptibility and responses in wildlife and aquatic populations.



1253 PHYSIOLOGICAL PARAMETERS AND PBPK MODELING FOR CHILDREN'S RISK ASSESSMENT.

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Evaluation of potential risks for children has become a regular aspect of many regulatory activities in state and Federal agencies including the U.S. Environmental Protection Agency and Food and Drug Administration. Pharmacokinetics plays a key role as the interface between external exposure and internal dosimetry for environmental compounds, dietary constituents, and pharmaceuticals. A range of pharmacokinetic modeling approaches are used, in part, dependent upon the availability of in vitro or in vivo data for the compound of interest. Physiologically based pharmacokinetic (PBPK) modeling is a valuable tool for estimating internal dosimetry across ages based upon information describing physiological, metabolic, and other chemical specific parameters. While standard values for adult physiological parameters have been compiled and published, physiological parameter values for pregnancy (mother and fetus), lactation (mother and offspring), and growing offspring are less readily available. Compilations of information in the literature and collection of new laboratory data are ongoing for physiological parameters including tissue volumes, blood flows, liver metabolism, and kidney function. Models are being developed to describe the pharmacokinetics of volatile organics, pesticides, and pharmaceuticals in young rats and humans. Modeling children's variability is also important. These efforts are designed to improve estimates of internal dosimetry for toxicological studies of pregnant and developing animals and for use in risk assessments for infants and children. (This abstract does not present US EPA policy.)



1254 CHILDREN'S RISK ASSESSMENT AND PBPK MODELING.

C. Thompson and S. Barone. *National Center for Environmental Assessment, U.S. Environmental Protection Agency, Washington, DC*.

Federal agencies are increasingly employing PBPK models as a preferred approach for performing extrapolations in risk assessment. In this context, susceptible populations, particularly children, are receiving greater focus in risk assessment than in

the past. While much of this focus represents a shift within the scientific and risk assessment community, federal laws (e.g. Food Quality Protection Act, FQPA) have also helped foster a focus on children's health protection. In addition to testing programs, the U.S. EPA is developing more formalized approaches for evaluating risks to children's health. For instance, the U.S. EPA "Supplemental Guidance for Assessing Cancer Susceptibility from Early-Life Stage Exposure to Carcinogens" and "A Framework for Assessing Health Risks of Environmental Exposures to Children" address how risks to early life stages can be addressed within the existing risk assessment framework by encouraging the consideration of data in children and young test animals. By incorporating pharmacokinetic and pharmacodynamic differences (and variability) across ages and species, PBPK models represent a powerful tool to help evaluate the life stages most sensitive to an environmental toxicant of interest. Not only are these models useful in assessing risks to environmental toxicants, but also have the potential for improving pharmaceutical safety – by relating effective doses in clinical trials to likely safe and effective doses in groups (e.g. children) that rarely participate in such studies. Despite these new and innovative efforts, challenges remain for protecting children's health: collection of accurate physiological parameter values, development of accurate algorithms for predicting values based on indexes (e.g. height, age, etc.), ontogeny of xenobiotic metabolizing enzyme systems and transporters, body and tissue composition, and more). The views expressed herein are solely those of the authors and do not represent the policies and/or endorsement of the U.S. Environmental Protection Agency.



1255 THE PHYSIOLOGICAL PARAMETERS FOR EARLY LIFE STAGES DATABASE.

R. Gentry¹ and S. Olin². ¹*ENVIRON, Ruston, LA* and ²*ILSI, Washington, DC*.

Recent initiatives with regards to children's health have resulted in the development of physiologically-based pharmacokinetic (PBPK) models to address the question of whether children of a specific age receive a higher dose than adults for a given chemical exposure. As the use of these models in regulatory risk assessment increases to attempt to understand age-specific risk, it is critical that these models reflect age-specific animal and human physiological processes. Currently available PBPK models demonstrate a lack of consistency in the age-specific parameters incorporated. This identified the need for the development of a central database made available to both modelers and risk assessors for the use of PBPK or biologically-based dose-response (BDDR) modeling. Use of consistent physiological parameters across modelers will decrease the uncertainty that may be introduced by using parameters from different sources. A multi-year multi-sector effort, coordinated in part through ILSI, has been ongoing to develop a database of physiological and pharmacokinetic parameters in the neonatal and developing rat and mouse, as well as for early lifestages in humans. The goal throughout the development of the database has been to identify the most appropriate parameters, insuring accuracy and scientific quality as well as the identification priority data needs. In the case of rats, in addition to parameter identification, comparisons have been made of critical parameters across selected strains, the results of which indicated that combinations of information across strains may be appropriate and may help to form a comprehensive dataset needed for PBPK modeling in the neonatal rat. In the development of the human database, case studies have also been conducted for kidney function and liver volume/blood flow in the perinatal period. The intent is that this database will provide not only a source in which researchers and modelers can readily access the information on age-specific physiological parameters, but that this database will be a "living" database, to which researchers or modelers will contribute data to in the future.



1256 AGE-DEPENDENT PBPK MODEL FOR THE PYRETHROID, DELTAMETHRIN (DLT), IN RATS.

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Pyrethroid insecticides have largely replaced organophosphates in the U.S., but very little is known about their ADME in adults or children. Our objectives were to: 1) characterize the age-dependency of the metabolism and systemic disposition of DLT, a potent neurotoxicant, in preweanling, adolescent and adult S-D rats; and 2) utilize these data and other age-related input parameters to develop an age-specific PBPK model for DLT. Metabolic inactivation of DLT by hepatic P450s and hepatic and plasma carboxylesterases progressively increased with age. Blood and brain DLT concentrations varied inversely with age, as did the incidence and severity of acute neurotoxicity. Organ and body weights (BW) were recorded for rats from 1 – 280 days of age. A generalized Michaelis-Menten (GMM) BW growth model was used to forecast organ weights/volumes of specific age groups. The GMM equation yielded much more accurate predictions of organ weights than the standard allometric approach. An adult PBPK model was developed and refined, such that it reliably simulated DLT time-courses in blood, plasma, brain and fat for different iv and oral doses. Input of age-specific parameters resulted in reliable

blood and tissue time-course predictions in immature rats. These data suggest that age-dependent differences in DLT dosimetry account for some but not all of the susceptibility of preweanling rats to DLT acute neurotoxicity. This first-generation model can be improved and utilized for other pyrethroids, as more is learned about basic processes that govern the internal dosimetry of this class of chemicals. Such models can be used to predict total pyrethroid concentrations in different immature brain regions for aggregate and cumulative exposures to pyrethroid mixtures. (Supported by U.S. EPA STAR Grant R830800). Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.



1257 MODELING VOLATILE ORGANIC COMPOUND PHARMACOKINETICS IN RAT PUPS.

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Toxicity studies in developing animals exposed to volatile organics are important for risk assessments of cancer, neurotoxicity, and other endpoints. PBPK model predictions of internal dosimetry in young rats were compared to adult animals for benzene, chloroform (CHL), methylene chloride, methyl ethyl ketone (MEK), perchloroethylene, and trichloroethylene. Modeling used partition coefficients for six volatile organic compounds measured in tissues from pups (postnatal day 10) and young adult (60 days), which were similar across ages (e.g., MEK) or varied up to 50% (CHL). Literature data on developing enzyme systems and physiological parameters for growing rats provided other parameter values. Steady state blood levels for all the chemicals except MEK are predicted to be <50% higher in pups than adults. MEK was >6-fold higher in pups than adults due to loading by more rapid ventilation per unit body weight, but limited exhalation of this water soluble compound. Although the blood:air partition coefficient is the major determinant of steady state blood concentration, it is as much as 50% lower in pups than adults. The higher pup blood levels reflect more rapid ventilation and decreased metabolism due to lower fractional liver volume. Pups come to steady state more rapidly than adults largely due to smaller fat volume. Brain concentrations are generally predicted to be slightly higher in pups than adults, except for MEK reflecting its much higher blood levels. The dose metric, total amount metabolized normalized for liver volume (AM/VL), was generally much lower in pups than adults. This modeling indicates that differences across ages reflect the integrated effect of all the relevant physiological, metabolic, and biochemical determinants of pharmacokinetics rather than the changes in the most sensitive parameter. (This abstract does not represent US EPA policy.)



1258 MODELING OF HUMAN PHYSIOLOGICAL AND METABOLIC VARIABILITY DURING EARLY LIFE STAGES.

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Pharmacokinetic modeling in children needs to account for the substantial variability in tissue volumes, blood flow rates and metabolizing enzyme levels observed during childhood. Availability of data on physiological parameters and liver metabolic capacity of individual children allows modeling to simulate individual variability in internal dose within the population. This presentation demonstrates the usefulness of physiologically-based pharmacokinetic (PBPK) models for predicting the inter-individual variability of internal dose of volatile organic chemicals in children, using toluene as the model substance. PBPK models were constructed for children (newborn to 17 years old; n=116) on the basis of subject-specific CYP2E1 protein levels obtained from autopsy samples. The physiological flows and volumes of organs (except liver) were computed as a function of body weight of children, using higher order polynomial equations. Liver volume for each subject was known. On the basis of subject-specific and age-specific data on physiology and metabolic capacity in children, the PBPK model provided simulations of kinetics and internal dose of toluene for various exposure scenarios. The modeling results indicated that the variability in the rate of metabolism and the area under the concentration vs time curve for toluene would be greater in the neonatal group than in older children and adults. This marked variability in the neonate group would appear to be a direct consequence of the variability of CYP2E1 levels and the age-dependency of the limiting factor of metabolism (i.e., enzyme levels in neonates vs liver blood flow in older children). Overall, the PBPK modeling approach allows the integration of subject-specific and age-specific data on the variability of metabolic and physiological factors to assess their combined impact on the variability of tissue dose of chemicals in children.



1259 THE VASCULAR SYSTEM AS A TARGET OF METAL TOXICITY.

W. Prozialek and J. Edwards. *Pharmacology, Midwestern University, Downers Grove, IL.*

The functioning of the vascular system involves complex interactions among the vascular endothelium, smooth muscle, the immune system and the nervous system. The toxic metals Cd, As and Pb can target the vascular system at multiple levels and these vascular effects appear to play pivotal roles in mediating the pathophysiologic effects of the metals in specific target organs. Acute exposure to Cd can cause selective increases in microvascular permeability and hemorrhagic injury in some organs but not others. One possible factor in determining if a vascular bed is sensitive to these Cd effects may be the expression of the *Slc39a8* gene, which encodes the ZIP8 ion transporter. This transporter may play a key role in mediating Cd toxicity in Cd-sensitive organs such as the testis and kidney. Additional studies indicate that Cd, at non-cytotoxic concentrations of 10-100 nM, can inhibit chemotaxis and vascular endothelial cell tube formation. These Cd-induced angiostatic effects may be mediated through disruption of VE-cadherin, a Ca-dependent cell adhesion molecule. Other studies have shown that exposure to arsenic in drinking water increases the incidence of cardiovascular diseases and is associated with blood vessel growth and remodeling in vivo. Ingestion of water containing toxicologically relevant concentrations of As promotes capillarization of liver sinusoidal endothelium. Since capillarization is a hallmark precursor for liver fibrosis and contributes to an imbalance of lipid metabolism, this effect of As on the liver endothelial cells may be a pathogenic mechanism underlying arsenic-related vascular diseases. Other studies indicate that early exposure to Pb, may be a factor in the development of hypertension in genetically-prone individuals. It has been suggested that Pb-induced alterations in vascular smooth muscle intracellular Ca signaling may contribute to hypertension by enhancing vascular contractility through actions on L-type Ca channels. Taken together, these findings indicate that the vascular system may be a critical target of metal toxicity in vivo.



1260 IDENTIFICATION OF A GENE RESPONSIBLE FOR CADMIUM-INDUCED VASCULAR DAMAGE.

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Cadmium (Cd²⁺, Cd) is a nonessential metal, an environmental toxicant, and a lung carcinogen in humans. Cd induces testicular necrosis in all species having testes. Of 45 inbred mouse strains, 31 are sensitive and 14 are resistant (autosomal recessive mutant trait) to Cd-induced testicular necrosis. Screening BXD/Ty recombinant inbred lines and using B6 x D2 genetic crosses, we narrowed down the *Cdm* locus on a ~24-cM region on Chr 3 ultimately to an 880-kb region, which includes the *Slc39a8* gene, encoding the ZIP8 transporter. By Northern or Q-PCR analysis, ZIP8 mRNA levels are the same in testis, kidney, liver, intestine, lung and brain of both sensitive and resistant inbred strains; however, by in situ hybridization, we showed a robust ZIP8 mRNA signal in vascular endothelial cells of the testis in two sensitive, but not in two resistant, inbred mouse strains [PNAS 2005; 102: 3401]. From a 129/Sv BAC (bacterial artificial chromosome) library, we isolated a 168.7-kb clone containing only the *Slc39a8* gene (63.8 kb) plus 87 kb of 5'-flanking and 18 kb of 3'-flanking sequences. We inserted the (Cd-sensitive) transgene onto the B6 (Cd-resistant) background: the transgene converts the phenotype from resistance to sensitivity (testicular necrosis) in BTZIP8-3 mice. These data unequivocally confirm that *Slc39a8* is the *Cdm* locus, and that regulatory sequences conferring this organ- and cell-type-specific expression of ZIP8 are contained within the 168,720 bp of this BAC. The blood-testis barrier differs from the blood-brain barrier, and both are different from any other endothelial vascular cells. We have now found that ZIP8 probably functions normally as a Zn²⁺ or Mn²⁺/HCO₃⁻ symporter and is present at high levels on the apical side of kidney proximal tubule epithelial cells; its high affinity for the hitchhiker Cd leads to Cd-induced renal proximal tubular damage and osteomalacia. The mechanism of organ damage by Cd therefore differs between testis and kidney—Supported in part by NIH grants R01 ES10416 and P30 ES06096.



1261 A NOVEL ANGIOSTATIC FUNCTION FOR CADMIUM ACTING THROUGH VE-CADHERIN.

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This study originated with the observation that human endothelial cells (ECs) grown in a monolayer culture physically separate from each other in the presence of cadmium (Cd). The vascular endothelium appears to be a primary target for Cd,

yet very little is known about the effects of Cd on angiogenesis, and even less is understood in regards to a potential mechanism. We hypothesized that Cd disrupts angiogenesis through decreasing cell surface VE-cadherin. ECs were exposed to Cd at various doses and tested in viability assays, chemotaxis assays, and tube formation assays, in addition to localizing VE-cadherin via immunofluorescence. Toxicity studies in the presence of 0.1% FBS suggest that a dose of 100 μ M Cd has no deleterious consequences after a 24 hr exposure, whereas a dose of 1 mM Cd appears to kill approximately 50% of macrovascular ECs. Despite the absence of a toxic effect, doses as low as 10 μ M Cd significantly decreased migration of ECs to a powerful chemoattractant. Similarly, the presence of as little as 1 μ M Cd significantly disrupts EC tube formation ($p < 0.05$). To eliminate the possibility that Cd was acting on the Matrigel, we pre-incubated ECs with Cd and then performed the assay in the absence of the heavy metal. Our results demonstrate that doses as low as 100 nM Cd can significantly disrupt PMA-induced tube formation when the assay is performed in the absence of Cd. Finally, immunolocalization studies reveal consistent VE-cadherin expression around the perimeter of confluent ECs that are not Cd-treated. In contrast, VE-cadherin expression is significantly decreased following an overnight incubation with doses of Cd ranging from 100 nM to 100 μ M. We conclude that Cd acts in an angiostatic manner on ECs, having low toxicity, yet significantly disrupting EC migration and tube formation. Moreover, Cd disrupts VE-cadherin expression on the cell surface of ECs, an adhesion molecule that is critical for angiogenesis.



1262 ARSENIC-INDUCED ENDOTHELIAL CELL ACTIVATION AND VASCULAR REMODELING.

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The vascular effects of drinking water arsenic (AsIII) are a global public health concern that contribute to disease in millions of people. Arsenic (AsIII) causes ischemic vascular diseases, atherosclerosis, hypertension, and diabetes. In addition, the angiogenic effects of AsIII contribute to tumorigenesis. The mechanisms for the pathogenic effects and health risks of chronic low to moderate AsIII exposures remain unresolved. However, research identifying AsIII health risks and filling the knowledge gaps for mechanisms of AsIII action has been complicated by a lack of sensitive animal models. Previous *in vivo* mouse studies from this laboratory demonstrate that 5-500 ppb AsIII in drinking water stimulated vessel growth in surrogate models of angiogenesis. To examine whether AsIII affected pathogenic endothelial cell activation and vascular remodeling in an endogenous bed, mice were fed drinking water containing 250 ppb AsIII for 1, 2, and 5 wk and then evaluated for changes in liver sinusoidal and peribiliary blood vessels. These exposures did not affect the overall health of the animals and did not cause cytotoxicity in the liver. However, AsIII caused significant vascular remodeling with sinusoidal endothelial cell (SEC) defenestration, increased SEC capillarization, vascularization of the peribiliary vascular plexus, and constriction of hepatic arterioles. In addition to ultrastructural changes SEC morphological, quantitative immunofluorescence revealed increased sinusoidal PECAM-1 and laminin-1 protein expression suggesting gain of adherens junctions and a basement membrane. After 5 wk of exposure, these vessel changes were accompanied by increased staining of CD45/CD68 positive inflammatory cells. *Ex vivo* exposure of primary isolates of mouse SEC demonstrated dose-dependent defenestration and capillarization in response to AsIII. In conclusion, these data demonstrate that SEC capillarization is a sensitive, early human-relevant marker of AsIII-stimulated vascular dysfunction in intact mice.



1263 MECHANISM OF PB-INDUCED HYPERTENSION: ROLE OF Ca^{2+} HOMEOSTASIS.

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Evidence suggests that chronic low-level Pb exposure can cause small, though persistent and significant elevations in mean arterial blood pressure. The mechanisms by which this occurs are not known. We hypothesize that alterations in intracellular Ca signaling induced by Pb contribute to hypertension by enhancing vascular contractility through actions on L-type Ca channels. Pregnant female spontaneously hypertensive rats (SHR) were given 100 ppm Pb in their drinking water, or water alone. After weaning at 21 d, one group of pups received only tap water to drink, the other received tap water containing 100 ppm Pb acetate. This procedure generated 4 groups: WW received only water perinatally and post-weaning; PbW received Pb perinatally but water only after weaning; WPb received water perinatally but Pb post-weaning; and PbPb received Pb both perinatally and post-weaning. Blood Pb levels were undetectable in WW and PbW rats; they ranged from 0-12.5 μ g/dL in PbPb and WPb rats. SHR became hypertensive as expected, but no significant differences occurred among treatment groups in either blood pressure or body weight over the entire 6 mo study period. Radiotelemetric recordings of blood pressure were collected. Prior to treatment with L-type Ca channel blocker nifedipine,

basal blood pressure was not affected in any Pb exposure group. However, treatment with nifedipine for one wk caused a differential pattern of blood pressure response based on the timing of Pb exposure. Rats exposed to Pb perinatally (PbPb and PbW) had a markedly larger decline in daily average blood pressure during nifedipine treatment than did control rats (WW) or rats exposed to Pb only after weaning (WPb). Hydralazine, a nonspecific vasodilator caused equivalent reductions in blood pressure in control and Pb treated rats. Thus the enhanced response to nifedipine in Pb-treated animals is not due to nonspecific actions. These preliminary data suggest that perinatal exposure to Pb can produce alterations in L-type Ca channel function sufficient to modify cardiovascular responses to Ca channel blockers into adulthood. Supported by NIH grant R21ES012349-01.



1264 WOMEN'S HEALTH INITIATIVE: LESSONS LEARNED AND FUTURE DIRECTIONS.

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The Women's Health Initiative (WHI) was one of the largest research studies devoted entirely to issues of postmenopausal women. Over 160,000 women aged 50-79 were enrolled at 40 clinical sites across the United States. WHI was comprised of two separate trials of menopausal hormone therapy (MHT), a diet intervention trial, and a trial of calcium and vitamin supplementation, plus an observational cohort. Issues examined included cardiovascular disease, cancer, bone fractures, and memory/cognition. WHI results have been instrumental in changing clinical practice and provided valuable insights into women's healthcare. They have also been the source of considerable controversy. This symposium will provide insight into results obtained and directions for future research that have emerged for this exceptional undertaking.



1265 BREAST AND COLORECTAL CANCER: EFFECTS OF MENOPAUSAL HORMONES, A LOW-FAT EATING PATTERN AND DIETARY SUPPLEMENTS (CA + VITAMIN D).

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The WHI trials of combined estrogen plus progestin in women with a uterus (E+P trial), estrogen only in women post-hysterectomy (E-Alone trial), and a low-fat eating pattern intervention (diet modification, DM, trial), as well as the trial of Calcium and Vitamin D supplements (CaD trial), investigated breast and colorectal cancer outcomes. Invasive breast cancer was significantly increased by conjugated equine estrogens (CEE) combined with daily medroxyprogesterone acetate (MPA) over the average 5.6 year follow-up period, and played a major role in the early stopping of the trial, whereas there was no increase in breast cancer with CEE only during 7.1 years of follow-up in the E-Alone trial. Colorectal cancer, on the other hand, was significantly reduced by CEE+MPA, but not affected by CEE in the respective MHT trials. Although the 9% reduction in breast cancer in women assigned to the low-fat diet failed to reach significance and there was no difference in colorectal cancer, there was a significant reduction in breast cancer in women who had the highest initial dietary fat intake, and there was a significant reduction in polyps/adenomas in the overall DM intervention group versus control. In contrast, there was no evidence that calcium and vitamin D supplements benefited colorectal cancer or polyps. This presentation will provide information on cancer outcomes in subgroups which may be of particular interest to women and their clinicians regarding menopausal hormones and diet.



1266 FRACTURE PREVENTION: MENOPAUSAL HORMONE THERAPY AND DIETARY SUPPLEMENTS (CA + VITAMIN D).

J. McGowan. *Musculoskeletal Diseases, NIAMS/NIH, Bethesda, MD.* Sponsor: M. Bhattacharyya.

Two of the randomized clinical trials in the Women's Health Initiative investigated fracture outcomes. In both parts of the trials of menopausal hormone therapy (MHT) – that is, both with estrogen alone as well as combination estrogen/progestin – hip, spine and total fractures were reduced. In the trial of calcium and vitamin D there was a modest effect of the dietary supplements on bone density at the hip but not at other sites. Hip but not other fractures were reduced in women over 60 and in adherent women but not in the overall group. Assessing and communicating the results of these trials to the public has been very challenging. MHT with very positive effects on fracture has some risks that must also be considered. Calcium and vitamin D supplements had modest effects in the overall population

of healthy postmenopausal women. Nevertheless, adding more calcium and vitamin D to the diet of particular groups can reduce the rate of hip fractures. This presentation will discuss the options for osteoporosis and fracture prevention in the light of the Women's Health Initiative.



1267 CARDIOVASCULAR DISEASE AND MHT: READING THE FINE PRINT.

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In the last 10 years, postmenopausal estrogen and progesterone have gone from therapies essential for women to stay "feminine forever" to drugs with significant side effects to be used with caution if at all. Contributing to this paradigm shift were the results of the Women Health Initiative, the largest trial of postmenopausal hormone therapy. Although stopped early for increased risk, the results are conflicting for some cardiovascular disease outcomes when treatment with estrogen alone is compared to estrogen plus progesterone. This presentation will compare the cardiovascular results of the two arms and present possible hypotheses for the differences and similarities seen.



1268 AGE-ASSOCIATED COGNITIVE DECLINE: EFFECTS OF HORMONE THERAPY.

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A major concern of most aging adults is that they will suffer from memory loss, severe cognitive impairment or even some form of dementia. As people live longer, there has been a significant increase in the number of people who suffer from various forms of cognitive impairment and dementia, with the concomitant growth in economic, health and family burdens associated with these debilitating conditions. In response to the increased numbers of older adults with cognitive decline, there has been a growing demand for effective methods to treat and prevent this health problem. Based on observational studies in women, and data from animal and basic science studies, post-menopausal hormone therapy (HT) appeared to address this growing need and led to an increased use of HT "off label" for the prevention and treatment of cognitive decline. The WHI Memory Study (WHIMS) provided the first, long-term, randomized placebo controlled clinical trial designed to investigate the effects of hormone therapy on cognitive functioning, mild cognitive impairment, and dementia in post-menopausal women. In a study of 7,500 women 65 years and older, it was learned that estrogen plus progestin or estrogen alone do not protect against cognitive decline or dementia; rather, they increase women's risk for cognitive decline and all-cause dementia. These results led to questions that are currently under investigation, including: What happens to women's cognitive functioning once HT is stopped? What are the underlying mechanisms that might explain the increased risk of dementia associated with HT and what will the WHIM-MRI study be able to tell us? Are there subgroups of women who may be more susceptible to the negative effects of HT on cognition? And, is there a "window of opportunity" among younger post-menopausal women where HT is beneficial rather than harmful?



1269 COMMUNICATING SCIENCE: WRITING FOR TECHNICAL AND LAY AUDIENCES.

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Research in toxicology often produces results that are significant for regulators and the general public as well as scientists. In addition to technical reports, written material is needed for information resources such as web sites, press releases, prospectus blurbs, and package inserts, making written communication skills essential for toxicologists. One key to the success of a scientific document is the scientist's style which should structure the content in a logical and persuasive fashion, include informative and persuasive illustrations, and express ideas in clear, connected sentences. Getting research published in a good journal is a fundamental requirement for all scientists. A good scientific paper should be clearly focused from title to discussion, provide adequate information regarding how experiments were carried out and make it easy for any scientist to understand the significance of the work. Yet bridging the gap between technical publication and other public media can be difficult. The lay media often have a tough time digesting complex scientific jargon.

Scientists, fearing their message will be lost in the translation, may be reluctant to share their research. These barriers prevent interesting and important science developments from making the news. Exploring how the media evaluate science news helps break down these barriers and encourages scientists to accurately translate their message to a lay audience. Discussion among the participants in this session will address practical questions regarding how one translates scientific jargon into a form that non-professionals can appreciate, why non-professionals should care about the latest advances in toxicology, and how toxicologists can clarify messages for the larger public.

1270 CHEMICALLY INDUCED CELL DEATH AND PROLIFERATION DURING MULTIPLE GENERATIONS OF HUMAN PROXIMAL TUBULAR (HPT) CELLS.

L. H. Lash and D. A. Putt. *Pharmacology, Wayne State University Sch. Med., Detroit, MI.*

Primary cultures of hPT cells undergo a range of responses when exposed to the nephrotoxic and nephrocarcinogenic metabolite of trichloroethylene, S-(1,2-dichlorovinyl)-L-cysteine (DCVC), which vary according to dose and time and include cell death by necrosis or apoptosis, growth arrest, and enhanced proliferation. To investigate the ability of DCVC to induce proliferation, longer exposure times than are possible with primary cell cultures are needed. Therefore, we studied the responses to hPT cells cultured and passaged for up to six generations. At several passages up to passage 6, morphology, cyokeratin and vimentin staining, protein and DNA synthesis, cytotoxicity and expression of heat shock protein 27 (Hsp27) and p53 in response to DCVC or two oxidants, tert-butyl hydroperoxide (tBH) or methyl vinyl ketone (MVK), were studied. hPT cells in either primary culture (hPT-p) or at passages 1, 2, 4, or 6 (hPT-1, -2, -4, or -6) exhibited similar morphology and staining for cyokeratins. Growth rate of cells slowed moderately through the second passage (time to confluence increased 25% to 100%) but slowed markedly (time to confluence increased > 250%) thereafter. Confluent hPT cells, from primary cultures through passage 4, exhibited similar sensitivity to DCVC-induced necrosis or apoptosis. In contrast, sensitivity to both tBH and MVK was markedly increased in hPT-2 and hPT-4 cells as compared with hPT-p and hPT-1 cells. Decreased activities of glutathione peroxidase and reductase in later passages may contribute to the greater sensitivity to injury from oxidants. Modest staining for vimentin, increased fraction of S-phase cells, and increased fluorescence of MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide) was observed in hPT-p through hPT-4 when these cells were exposed to 10 μ M DCVC, indicating growth and proliferation. These results suggest that hPT cell cultures can be used over several passages to investigate mechanisms of DCVC-induced cell proliferation, although changes occur in sensitivity of cells to oxidants.

1271 GENE EXPRESSION CHANGES IN HUMAN RENAL PROXIMAL TUBULE CELLS (HRPTC) EXPOSED TO LOW CONCENTRATIONS OF S-(1, 2-DICHLOROVINYL)-L-CYSTEINE (DCVC), A METABOLITE OF TRICHLOROETHYLENE.

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Trichloroethylene (TCE) is an important industrial solvent that causes renal injury in rats and mice following chronic exposure. Primary HRPTC were exposed daily to DCVC, a minor metabolite of TCE, for 10 days. Confluent cells 8-10 days after seeding were exposed to DCVC at 0.1 to 10 μ M. Cytotoxicity was determined using MTT following 1, 3, 7 and 10 days exposure. RNA was isolated from cells exposed to medium alone or DCVC 0.1 μ M or 1 μ M for 10 days and the integrity and concentration determined. Gene expression levels were measured using Affymetrix human genome U133A Gene chips. Comparisons were conducted for each DCVC concentration with respect to control treatment derived from the same sample. Daily doses of 10 μ M DCVC/day for 7 days resulted in 75% loss of MTT activity, which was confirmed microscopically as extensive cell necrosis. No significant loss in MTT activity was seen at 3 μ M/day over 10 days, with no loss at 1 and 0.1 μ M/day over 10 days. The IC50 for DCVC was 7.5 μ M after 7 and 10 days. Two doses 0.1 and 1 μ M DCVC were used to examine changes in gene expression. Using the criterion of a mean 2-fold change over control for the four samples examined, 3 genes at 0.1 μ M DCVC increased namely, adenosine kinase, zinc finger protein X-linked and an enzyme with lyase activity. At 1 μ M DCVC two genes showed a >2-fold decrease, N-acetyltransferase 8 and complement factor H. At a lower stringency (1.5-fold change), a total of 63 probe sets were altered at 0.1 μ M DCVC and 45 at 1 μ M DCVC. Genes associated with stress, apoptosis, cell proliferation and repair and DCVC metabolism were altered as were a small number of genes that did not appear to be associated with the known mode of action of

DCVC. Some of these genes may serve as molecular markers of TCE exposure and effects in the human kidney. Supported by Department of Energy Cooperative Agreement DE-FC09-02CH11109.

1272 ROLE OF ORGANIC ANION TRANSPORTER 3 (OAT3) IN THE RENAL TRANSPORT OF GLUTATHIONE (GSH).

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The tripeptide GSH is important in maintenance of renal redox status and defense against reactive electrophiles and oxidants. Previous studies showed that GSH is transported across the basolateral plasma membrane (BLM) into the renal proximal tubule by both sodium-coupled and sodium-independent pathways. Substrate specificity and inhibitor studies in renal proximal tubular cells and BLM vesicles suggested the potential function of several carriers, among them the organic anion transporters 1 and 3 (Oat1 [Slc22a6] and Oat3 [Slc22a8]), in the uptake process. To investigate the potential role of Oat3 in GSH uptake, the cDNA for rat Oat3 was expressed as a His₆-tagged protein in *E. coli*, purified from inclusion bodies and by Ni²⁺-affinity chromatography, and reconstituted into proteoliposomes. Reconstituted Oat3 transported both GSH and the prototypical substrate *p*-aminohippurate (PAH) in exchange for 2-oxoglutarate (2-OG) and 2-OG and PAH in exchange for GSH, and PAH uptake was inhibited by both probenecid and furosemide, consistent with the function of Oat3. To investigate the function of Oat3 in GSH uptake in an intact cellular model, the rat proximal tubular cell line NRK-52E cells was assessed as a potential model. Analysis of mRNA expression by RT-PCR of Oat3 and other potential carriers in homogenates of both rat kidney cortex and NRK-52E cells revealed that whereas rat kidney cortex expressed all the potential GSH carriers (i.e., Oat1, Oat3, the sodium-dicarboxylate carrier 2 [NaC2; Slc13a3], organic anion transporting polypeptide 1a1 [Oatp1a1; Slco1a1], and multidrug resistance proteins 2 and 5 [Mrp2/5; Abcc2/5]), NRK-52E cells failed to show any expression. Analysis of GSH uptake across the BLM of NRK-52E cells showed little PAH- or 2-OG-stimulated GSH uptake. These results support the suggestion that Oat3 can function in GSH uptake and that NRK-52E cells possess a low background rate of GSH uptake and can thus be a good model for overexpression of specific, putative GSH carriers.

1273 THE ROLE OF iPLA₂ GAMMA IN OXIDANT-INDUCED ENDOPLASMIC RETICULUM LIPID PEROXIDATION AND RENAL CELL NECROSIS.

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We have previously demonstrated that inhibition of calcium-independent phospholipase A₂γ (iPLA₂γ), located in the endoplasmic reticulum (ER) and mitochondria of rabbit renal proximal tubular cells (RPTC), potentiates oxidant-induced lipid peroxidation and necrotic cell death. Because oxidant-induced ER lipid peroxidation may result in membrane disruption and lead to the release of ER Ca²⁺, an important trigger for RPTC necrosis, we investigated the role of ER-iPLA₂γ on oxidant-induced ER lipid peroxidation and the role of ER Ca²⁺ release in oxidant-induced necrosis. Using the fluorescent lipid, cis-parinaric acid, as a marker of lipid peroxidation, t-butylhydroperoxide (TBHP) treatment of rabbit kidney cortex microsomes produced lipid peroxidation and prior inhibition of iPLA₂γ with BEL potentiated the lipid peroxidation. TBHP-induced RPTC necrosis, measured by flow cytometry using propidium iodide (PI) staining, was potentiated by prior treatment with the iPLA₂ inhibitor BEL. Depletion of ER Ca²⁺ with thapsigargin (Tg) prior to TBHP exposure reduced RPTC necrosis and reduced the potentiation of TBHP-induced necrotic cell death by BEL (Control, 0.4%; TBHP, 29%; TBHP + BEL, 42%; TBHP + Tg, 17%; TBHP + BEL + Tg, 22%). These data reveal that 1) TBHP produces ER lipid peroxidation, 2) inhibition of ER-iPLA₂γ potentiates TBHP-induced ER lipid peroxidation, and 3) prior depletion of ER Ca²⁺ blocks the potentiation of TBHP-induced necrotic cell death produced by iPLA₂γ inhibition. We suggest that iPLA₂γ is cytoprotective against oxidant injury by blocking ER lipid peroxidation, which in turn, minimizes ER Ca²⁺ release and necrotic cell death in RPTC.

1274 ROLE OF iPLA₂γ IN RENAL CELL MITOCHONDRIAL PERMEABILITY TRANSITION.

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Our laboratory has previously demonstrated calcium-independent phospholipase A₂γ (iPLA₂γ) is localized to renal cell mitochondria and iPLA₂γ inhibition blocks caspase-mediated, cisplatin-induced renal proximal tubule cell (RPTC)

apoptosis. The mitochondrial permeability transition (MPT) is a key control point for apoptosis in many cell types. The goal of the present study was to determine the role of iPLA₂γ in renal cell MPT. MPT of isolated renal cortex mitochondria (RCM) was induced by Ca²⁺/PO₄¹⁻ and measured as the decrease in optical density at 540 nm. iPLA₂ activity was assessed by measuring the abundance of free fatty acids in isolated mitochondrial suspensions in the presence and absence of Ca²⁺ and the iPLA₂ inhibitor bromoenol lactone (BEL) by GC-MS. Ca²⁺/PO₄¹⁻ induced mitochondrial swelling was blocked by the MPT inhibitors, cyclosporine A (1 μM) and ATP (500 μM). The R-enantiomer of BEL, which specifically inhibits iPLA₂γ, inhibited Ca²⁺-induced RCM MPT, while S-BEL (negative control) had no effect. Ca²⁺ treatment resulted in a significant increase in free arachidonic acid (~70 μM) in the mitochondrial suspension that was blocked by pretreatment with BEL. No increases in free myristic, palmitic, stearic, oleic or linoleic acid were detected after Ca²⁺ treatment in this model. Addition of arachidonic acid (18 μM) to mitochondria, pretreated with BEL, restored Ca²⁺-induced MPT. Taken together these findings suggest that iPLA₂γ catalyzed arachidonic acid liberation mediates the MPT in renal cell mitochondria exposed to Ca²⁺/PO₄¹⁻.

1275 THE URINARY PROTEIN RESPONSE TO TOXICITY OF ETHYLENE GLYCOL IN KIDNEY TISSUE.

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Ethylene glycol (EG) exposure above threshold doses can lead to renal toxicity. EG is not toxic, but its metabolite—oxalic acid, which precipitates with calcium as calcium oxalate monohydrate (COM), produces the renal toxicity. In vivo studies show a greater accumulation of COM in the kidney of Wistar than in F-344 rats, which explains the higher sensitivity of the Wistars to EG. Urinary proteins can inhibit or promote crystal adhesion to renal tubular cells, which could alter the accumulation of COM in the tissue. Tamm-Horsfall protein (THP) and osteopontin (OPN) are two proteins that appear to inhibit COM adhesion. We hypothesize that the accumulation of COM crystals is regulated by the different expression of urinary proteins between the rat strains. To identify the role of these proteins, we treated F-344 and Wistar rats with 0.75% EG in drinking water for 4 weeks, and collected 24 hour urine samples from fasted rats before and after 1, 2, 3, and 4 weeks. Protein levels were identified in urine samples by Western blotting. COM crystal accumulation was observed in the kidneys of treated Wistar rats but not in F-344 rats. In Wistar rats, total THP levels increased over treatment time, but similarly in both EG-treated and control rats. THP levels in F-344 appeared higher than in Wistar before treatment, but did not change with treatment. A much higher OPN level was observed in F-344 than Wistar rats before treatment, but this decreased to the same levels as in Wistar after 1 week treatment. The lower levels of OPN and THP, as inhibitors of COM adhesion, in untreated Wistar rats could explain a higher degree of COM accumulation. However, the fact that the strain differences diminish in time after treatment with EG suggests that these two proteins may not be involved in the strain difference regarding COM accumulation after EG exposure. Supported in part by a research agreement with the American Chemistry Council.

1276 BINDING AND INTERNALIZATION OF OXALATE CRYSTALS BY PROXIMAL TUBULE CELLS FROM HUMANS AND RATS IS RELATED TO OXALATE CYTOTOXICITY.

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Ethylene glycol (EG) produces renal failure due to its metabolism to oxalate. Pathologic images have shown that accumulation of calcium oxalate monohydrate (COM) crystals in the kidney is associated with tubular cell necrosis. This mode of action is consistent between rats and humans, although there are pharmacokinetic and pharmacodynamic differences in sensitivity. Our previous studies have demonstrated that COM crystals produce greater cytotoxicity in proximal tubule (PT) cells from rats than from humans. The present studies compared the binding and internalization of [14C]-COM crystals to PT cells between human and Wistar and F-344 rats. Binding was determined by incubation of cells with COM at 40°C, followed by solubilization of cells. Internalization was determined by incubation of cells with COM at 37°C, then removal of bound COM with an EDTA incubation, followed by solubilization of cells. The results indicated that COM crystals bound to and were internalized by PT cells in concentration-dependent manners. Both the amounts of COM crystals bound to and internalized by rat PT cells were about five times more than by human cells, with no differences between Wistar and F-344 cells. Both binding and internalization of COM were highly correlated with the degree of cell death produced in cells (r = 0.97 and 0.92, respectively). These studies demonstrate that surface binding of COM to and internalization by PT cells plays a critical role in its cytotoxicity and explain why rat PT cells are more sensitive to

the cytotoxicity of COM crystals. At the same level of COM accumulation after EG exposure in vivo, rats would appear to be more susceptible than humans to COM-induced tissue damage. Supported in part by a research agreement with the American Chemistry Council.

1277 ROLE OF ORGANIC ION TRANSPORTERS AND ESTROGEN ON CISPLATIN [CDDP] TOXICITY IN HUMAN PROXIMAL TUBULAR CELLS.

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Organic cation- and anion-transporters (OCT, OAT) influence CDDP induced nephrotoxicity. Although OCT/OAT expression can be modulated by sex steroids in animal models, the role of OCT/OAT and estrogen on CDDP toxicity in human proximal tubular cells (hPTC) is unclear. In this study, we utilized an immortalized normal hPTC (HK-2) cell line to characterize functional role of OCT, OAT and 17 β -estradiol (17BE) on CDDP toxicity in-vitro. HK-2 cells (approx 2.0x10⁵) were seeded in 96-well plates and maintained for 24h in Dulbecco's Minimum Essential Media (10%FBS, 1% antibiotics) in a CO₂ (5%) incubator at 37°C. After 24h, cells were treated with fresh media containing 10 μ M, 20 μ M or 50 μ M (triplicate/treatment) of CDDP (dissolved in 0.9% sterile saline) for 24, 48, 72 & 96 hour respectively alone or in the presence of (100 μ M) prototypal OCT/OAT substrates, tetraethylammonium (TEA)/p-aminohippuric acid (PAH) respectively. To determine effect of 17BE, HK-2 cells were treated with (1, 5, 10, 20, 50 μ M) 17BE for 8h followed by treatment with CDDP (10, 20, 50 μ M) for 48 hours. Toxicity was determined using CellTiter Aqueous96 cell proliferation assay (Promega Corp, Madison, WI) and data expressed in terms of cell survival (Mean $\pm$ SD; n $\geq$ 4). HK-2 cells exhibited a dose- and time-dependent increase in susceptibility to CDDP, and was unchanged in presence of TEA or PAH. 17BE did not influence HK-2 cell survival nor exposure to 10 μ M CDDP. However, 1 & 5 μ M 17BE conferred significant (p < 0.05) protection against CDDP (20 & 50 μ M) concentrations, a phenomenon that was abrogated at > 5 μ M 17BE concentration. In conclusion, results demonstrate that in normal hPTC HK-2 cells, OCT/OAT substrates does not influence CDDP toxicity. However, low levels of 17BE confers protection against higher concentrations of CDDP. [Funds for this study was provided via MCPHS Faculty Seed Grant to SR, 2006].

1278 METALLOTHIONEIN ISOFORM 3 DEPENDENT REGULATION OF E-CADHERIN EXPRESSION IN HUMAN PROXIMAL TUBULE CELLS.

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Metallothioneins are metal-binding proteins that provide protection against the toxic affects of heavy metal including Cd²⁺ that induces nephrotoxicity. Previous studies from this laboratory have implicated the expression of the third isoform of metallothionein (MT-3) in the maintenance of proximal tubule vectorial active ion transport through an analysis of dome formation by human renal epithelial cell lines. It was shown that HK-2 cells have no expression of MT-3 and do not form domes in culture; whereas, the HPT cells and HK-2 cells stably transfected with MT-3 form these structures. Domes are a hallmark of cell cultures derived from transporting epithelium that retain the in situ property of vectorial active transport. In the present study, this association was further explored by determining the effect that the presence or absence of MT-3 had on the expression of the E-, P-, N-, K-, and KSP - cadherins in these cell lines. It was demonstrated that the HPT cells and HK-2(MT-3) cells had significant elevations in the expression of mRNA and protein for the E-, P-, K-, and KSP - cadherins compared to that of the HK-2 cells. In contrast, it was shown that the HK-2 cells had significantly elevated expression of N - cadherin compared to both the HPT and HK-2(MT-3) cell lines. These patterns of cadherin expression provide strong evidence that MT-3 might be involved in epithelial to mesenchymal transition (EMT) that is postulated to occur during disease states such as cancer or renal fibrosis and in the mesenchymal to epithelial transition (MET) that occurs during normal kidney morphogenesis.

1279 CHEMICALS AND GENDER DIFFERENCES.

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With reference to a government instruction, we have reviewed the scientific documentation on gender differences in the protection against harmful effects of chemicals. The tasks specified were:

- Review the state of art with respect to gender differences in uptake, disposition and health impact of chemicals during different stages in life.
- Describe if and how potential gender differences are considered in the design and evaluation of toxicity tests of chemicals and implementation of safety factors and guidance values.
- Propose actions so that both sexes are equally protected from harmful effects.

Gender differences in exposure, toxicokinetics, toxicodynamics, odor perception, sensory irritation and health effects of endocrine disruptors, metals (arsenic, lead, methyl mercury), carcinogens and urban air pollution, as well as testing procedures, use of safety factors and limit setting procedures were reviewed.

In summary, there is a considerable lack of knowledge about potential gender differences. Such differences have rarely been analysed, neither in experimental, nor epidemiological studies. Above all, knowledge about adverse effects of chemicals in women is lacking. Although available data suggest that gender differences in health effects are generally relatively small, examples of potentially significant differences in exposure, uptake, metabolism and sensitivity with respect to certain chemicals and environmental pollutants are found. The group of chemicals judged to have the biggest significance for gender-specific toxic effects are the endocrine disruptors. Although testing of chemicals includes both sexes, gender differences are seldom evaluated. Further, possible such differences are rarely taken into account in risk assessment and management.

In conclusion, there is a great need for research and compilations and analyses of existing data, particularly to identify research needs and how gender differences might be addressed in risk assessment and management.

1280 RISK ASSESSMENT ON VETERINARY DRUGS RESIDED IN LIVESTOCK PRODUCTS FOR RISK MANAGEMENT.

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Veterinary drugs are inevitably used for growth promotion and disease control of livestock animals. However, concerns on the safety of livestock products and prevalence of antimicrobial resistance have been increased. The present study was performed to develop risk assessment strategy apt to residual veterinary drugs in food and to rank the risk priority for risk management. We assessed the 269 veterinary drugs following to risk assessment steps as hazard identification, hazard characterization, exposure assessment and risk characterization. All scientific information of each drug were compiled and classified into direct and indirect hazardous factors, exposure factors and reference factors. The data on toxicity (T), antimicrobial resistance (AR), impact on human intestinal microflora, regulatory concerns for prohibition, acceptable daily intake, maximum residue limit (MRL) and residue violation rate for recent 5 years were allocated into hazardous factors and data on withdrawal period (WP), production quantity of each drug for five years (PQ) and food consumption amount were considered as exposure factors. The limit of information on residue violation rate (LI) was referred as reference factors. All factors were ranked into 0 to 5 and risk of each drug was scored according to the equation drawn by optimal weighing on toxicity, MRL and withdrawal period. The equation for risk scoring was: {residue violation rate (or estimated risk: 0.16667(3*MRL+2*WP+PQ))}x{AR+3*T}/4x{1+0.06667(LI-1)}. Among the 269 veterinary drugs assessed, furazolidon was the highest risky residual drug scored as 25.33 and followed by dimetridazole, carbadox, hygromycin B, avoparcin, olaquinoxid, metronidazole, chloramphenicol and enrofloxacin in order. Diaveridine, fenitrothion and fluralinate were allocated into the lowest risk grade. The highly ranked drugs in risk priority were assumed to have potent public health impact and should be controlled more carefully for the prevention of residue in food.

1281 COMMUNICATING CHEMICAL SAFETY, RISK PERCEPTION AND HAZARD TO LAY PUBLIC: THE GLOBAL HARMONIZED SYSTEM OF CLASSIFICATION AND LABELLING OF CHEMICALS IN THAILAND (GHS).

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The Global Harmonized System of Classification and Labelling of Chemicals or GHS is the result from the 1992 United Nations Conference on Environment and Development (UNCED) on developing a single but globally harmonized system to manage classification of chemicals and labels. The key message of GHS is a global system of hazard management. GHS is to be implemented as a national programme in Thailand in 2007. A practical and appropriate hazard communication is needed in order to protect people and the environment from chemical exposures, ensuring chemical safety use and to provide a comprehensive hazard communication system. A research project- GHS: Comprehensibility Testing is a part of GHS implementation, it is designed to find out the best-practice of risk communication. Target population covers all sectors; industrial, agriculture, consumer, transport, public health service and emergency responders.

GHS develops hazard communication tools to convey information that can be easily recognized and understandable, these tools include hazard symbols, hazard statements, safety data sheets and labels.

GHS hazard communication system is the source of information on total risk management and offers guidance on protective measures, hazard, avoidance of adverse effects, safety use measures, chemical storage/disposal, transporting, handling measures and emergency responding.

The result at this period covers two sectors; industrial and consumer group in Thailand. Findings show that each sector perceive hazard differently according to how high they are exposed to chemicals. Risk assessment and perception effects these two groups differently. It can be concluded that sufficient risk information effects risk-related behaviours and levels of self-protection. Information sufficiency is a potential factor in risk perception.

1282 CONCENTRATION/DURATION/EFFECT ($C \times T$) RELATIONSHIPS IN ASSESSMENTS OF HEALTH RISK FROM ACUTE INHALATION EXPOSURES – COMPARISON OF APPROACHES.

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Assessments of health risk from acute exposures (≤ 24 hours) often require extrapolation to a duration of interest. Traditionally, this extrapolation has been performed using Haber's rule where the product of the concentration multiplied by the duration results in a constant ($C \times T = k$). More recently, risk assessors have used the approach proposed by ten Berge et al. (1986) that the formula $C^n \times T = k$, with an exponent n on the concentration term, is more appropriate in defining this relationship. Categorical regression analysis also provides the capability for duration extrapolation. In this presentation, we compare the approaches that have been used in acute assessments for a few model compounds (acrolein, ethylene oxide, hexachlorocyclopentadiene [HCCPD], hydrogen sulfide, and phosgene), and explore some of the issues being addressed as the US EPA develops a method for performing assessments of health risk from acute inhalation exposures. The relevance of lethality to other less severe endpoints (e.g., irritation, neurotoxicity) when developing the value for the exponent n in the $C^n \times T$ formula are considered in the assessments of ethylene oxide and HCCPD. The assessment for acrolein was data limited, allowing extrapolation to durations no longer than 6-hours. The application of categorical regression analysis is investigated in the assessments of hydrogen sulfide and phosgene. General issues also discussed include dosimetry, estimating a time to recovery following an acute exposure, and toxicokinetic and toxicodynamic considerations when validating extrapolations from observed data. This comparative review leads to the conclusion that the appropriate approach is most often dependent on the available data, but requires additional toxicological judgment as to which data are most biologically relevant. [Disclaimer: The views expressed in this abstract are those of the authors and do not represent the policy of the U.S. Environmental Protection Agency.]

1283 LEAD (PB) EFFECT LEVELS AND TOLERABLE LEVELS OF EXPOSURE.

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Lead exposure causes permanent neurobehavioral deficits, particularly in children at relatively low exposure levels. Exposure is also associated with peripheral and central nervous system dysfunction and hematological changes, among others. Children, especially young children, show adverse health effects at lower blood lead levels and attain higher blood lead levels per μg ingested than adults because they absorb lead more readily and consume more food, and thus retain more lead on a body weight basis. While no safe level of lead exposure has been identified practical threshold levels have been developed. These have been used as screening tools to assess the impact of various sources of exposure on overall population body burdens. A steady state blood level of $10 \mu\text{g}/\text{dL}$ has been used as a lowest observed adverse effect level and was derived from studies in children. This blood level was used to identify a steady state provisional total tolerable intake level (PTTIL) of $6 \mu\text{g}/\text{day}$ in young children. Population studies of blood lead levels below $10 \mu\text{g}/\text{dL}$ have also identified adverse effects, but until recently these associations have not been clearly established. More recent studies have substantiated lead induced decrements in cognitive function in children below $10 \mu\text{g}/\text{dL}$. In one recent study blood levels as low as $2 \mu\text{g}/\text{dL}$ appear to be associated with the incidence of ADHA. A reduction in the PTTIL for young children of several-fold on the basis of effects noted below $10 \mu\text{g}/\text{dL}$ is warranted. A drawback of relying entirely on the PTTIL in assessing the risks of food borne lead is that it may not provide a clear basis for effective risk management strategies. As a complimentary approach a dose-response function with a slope ranging from 0 to 0.6 IQ points per $\mu\text{g}/\text{dL}$ was used to quantify effects that

may be expected from levels of lead in food that would result in blood lead levels below $10 \mu\text{g}/\text{dL}$. This approach will provide a robust scientific basis for weighing risk against the difficulty of avoiding or mitigating exposure

1284 LOWER SENSITIVITY OF HUMANS COMPARED TO RATS TO 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD), 2, 3, 4, 7, 8-PENTACHLORODIBENZOFURAN (PCDF) OR 2, 3, 7, 8-TETRACHLORODIBENZOFURAN (TCDF) -INDUCED CYP1A EXPRESSION.

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The relative toxicological potencies of dioxin-like compounds (DLCs) in a mixture are estimated using the Toxic Equivalency Factor (TEF) approach. The TEF for a given DLC is an estimate of its toxicity relative to TCDD. Almost without exception, data from animal or in vitro studies provide the underlying basis for the TEFs used in the risk assessment of DLCs. Human data for use in the development of TEFs, while desirable and preferred, are largely unavailable. In order to gain insight into species-specific differences in the biological activities of DLCs, we studied CYP1A mRNA expression and 7-ethoxyresorufin-O-deethylase (EROD) and phenacetin-O-deethylase (POD) activities in cultures of primary hepatocytes from adult human female donors and female Sprague-Dawley rats exposed to three different dioxin/furan congeners. Cells were exposed for 24 or 48 hours to TCDD, PeCDF or TCDF and full concentration-response (0.00001 - 100 nM) profiles were characterized. Cell viability and cytotoxicity was monitored by ATP quantitation and LDH-release as well as by visual inspection of cell morphology. TCDD demonstrated a clear threshold for gene and enzyme expression in both humans and rats with human cells being less sensitive for CYP1A1- and CYP1A2-induction. The rank order for potency was maintained between the species (TCDD>PeCDF>TCDF). The relative potency estimates varied depending on the species and endpoint. This study provides useful new information for addressing the TEF concept and certain questions related to human sensitivity to dioxins and furans.

1285 PREDICTIVE HUMAN HEALTH RISK ASSESSMENT FOR A NEW ALUMINUM SMELTER IN ICELAND.

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Alcoa is constructing the Fjarðal aluminum smelter in Reyðarfjörður, East Iceland. The smelter includes extensive design and operation features to reduce or eliminate environmental impacts. The Fjarðal facility will use imported anodes and spent pot liners will be exported rather than being placed in an onsite landfill. A predictive risk assessment was conducted to determine whether there was a consequential difference in human health risk related to air emissions from Fjarðal with and without the use of seawater scrubbers. Air dispersion modeling results for particulate matter (PM₁₀), sulfur dioxide, hydrogen fluoride, and PAHs were compared to the corresponding ambient air standards or guidelines from Icelandic or European directives, Norway, and U.S. EPA. Risk estimates were calculated for PAHs under current and future use scenarios for the following receptors: onsite outdoor worker, seagoing worker, hypothetical fenceline resident, future hypothetical resident, closest resident, residents in nearby villages, closest agricultural farmer and a visitor to the Holmanes Reserve. Use of seawater scrubbers decreased average SO₂ air concentration estimates in the short term; however, annual estimates were lower without seawater scrubbers. In the short term, both with and without seawater scrubbers, the number of exceedances of SO₂ predicted per year was well below the maximum number of allowed exceedances. Modeled concentrations of fluoride and PM₁₀ in air were well below health standards and risk based concentrations in soil. All risk estimates for carcinogenic PAHs were lower than the 10⁻⁶ risk, and hazard indices were well below the threshold of 1.0 for noncarcinogenic PAHs. The use of seawater scrubbers did not result in a consequential decrease in emissions, which were already within applicable guidelines and acceptable risk levels.

1286 HUMAN CELLS ARE LESS SENSITIVE THAN RAT CELLS TO PCB 126 AND AROCLOR 1254 ACROSS SEVERAL AHR-DEPENDENT GENES.

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In their evaluation of the EPA's 2003 dioxin reassessment draft, the National Academy of Sciences (NAS) stated that use of the toxic equivalency factor (TEF) methodology for estimation of non-cancer human health risks of dioxin-like com-

pounds (DLCs) was a reasonable approach (NAS, 2006). However, the NAS report emphasized that, "If significant differences in the REP (relative potency) of DLCs are found between humans and other species, then adjustments should be made in the TEFs." Our previous studies determined that human primary hepatocyte-derived REPs based on CYP1A activity and expression for PCB 126 and Aroclor 1254 are 10-1000 fold lower than those for rat primary hepatocytes. We extend these comparative studies by calculating species-specific REPs based on expression values for all genes responding to TCDD, PCB 126, and Aroclor 1254 using Affymetrix Genechips. Primary rat or human hepatocytes were treated with concentrations from -14 to -4 Log M. Human PCB 126 REPs for the well known aryl hydrocarbon receptor (AHR) battery genes Cyp1a1, Cyp1a2, Cyp1b1, and Aldh3a1 ranged from 10 – 1000 fold less than the WHO 2005 TEF value for PCB 126 of 0.1. However, rat PCB 126 REPs for these same genes failed to differ from the WHO TEF by more than 1 order of magnitude. The rat-specific Aroclor 1254 REP for Cyp1a1 induction was in line with the estimated Aroclor 1254 TEQ (based on WHO 2005 TEFs). At most, human cultures responded only at the highest concentration preventing calculations of human Aroclor REPs. We are extending these analyses to include other TCDD- and DLC-responsive genes. Overall, this study adds to the ever-growing body of scientific evidence demonstrating that humans are orders of magnitude less sensitive to DLC toxicity than rodents.

1287 ESTIMATION OF ATRAZINE IN STRATUM CORNEUM AND ITS TOXIC EFFECTS IN SKIN FOLLOWING TOPICAL APPLICATION.

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Sponsor: J. Riviere.

For risk assessment, a workable non invasive method for the estimation of atrazine in skin was needed. Moreover, the dermato-toxic effects of different concentrations of atrazine have not been studied so far. 15mg of 37% solution of atrazine in ethanol was topically applied to 6 different shaved sites on the back of rabbits (n=6). Each site was tape stripped with 10 consecutive adhesive tapes at 0.5, 1, 2, 3, 4, & 6 h of topical dosing to remove the stratum corneum (SC). Atrazine in tapes was extracted with methanol and analyzed with HPLC. In dermal toxicity studies, 500µl of 37%, 74% atrazine in ethanol and a commercial preparation (Atrazine® Ali Akbar Enterprises, Pakistan) was dosed on shaved backs of rabbits (n=4) for 4 days under occluded vs. non occluded conditions. Ethanol alone served as control. On 5th day, rabbits were euthanized and skin was scored for erythema and then examined microscopically. About 13.03 % of applied dose of atrazine was detected in SC of rabbits in 6 h. This finding warrants the dermal absorption of atrazine in persons exposed for longer duration. Significant differences ($P<0.05$) in erythema scores were observed with 74% atrazine and Atrazine® as compared to the control under occluded conditions. Similarly, significant differences in epidermal thickness & cell layers were observed in Atrazine® & 74% atrazine as compared to control in both dosing conditions. There were non significant differences in erythema, epidermal thickness or cell layers in occluded vs. non occluded regimen, indicating that atrazine is equally toxic regardless of its application procedures. Moreover, under non occluded application, no erythema was observed but microscopically significant epidermal hyperplasia was noticed. This indicates that even if there are no gross skin manifestations of atrazine yet this can produce significant damage to the epidermal barrier and hence can result in increase in penetration of self or other toxic substances.

1288 MOUSE SKIN INFLAMMATION BY LIPOPOLYSACCHARIDE: PRODUCTION OF HYDROXYL- AND LIPID-DERIVED FREE RADICALS REQUIRES INOS AND POSSIBLY XANTHINE OXIDASE.

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Free radical formation has been investigated in diverse experimental models of lipopolysaccharide (LPS)-induced inflammation. Here, using electron spin resonance (ESR) and the spin trap α -(4-pyridyl-1-oxide)-N-tert-butyl nitron (POBN), we have clearly detected an ESR spectrum of POBN radical adducts in the lipid extract of mouse skin treated with LPS for 6 h. The ESR spectrum was consistent with the trapping of a lipid-derived radical adduct. A hydroxyl radical detection technique with dimethyl sulfoxide demonstrated methyl radical formation, which implies of hydroxyl radical formation. The radical adduct formation was suppressed by aminoguanidine, and in inducible nitric oxide synthase knockout (iNOS $-/-$) mice, demonstrating the involvement of iNOS. As allopurinol suppressed the radical adduct formation, xanthine oxidase (XO) is presumably also involved in the free radical formation. NADPH oxidase was not required in the formation of these rad-

ical adducts, since the ESR signal intensity was increased by LPS treatment in NADPH oxidase knockout (gp91phox $-/-$) mice as much as it was in the wild type mouse. Nitric oxide end products were increased in LPS-treated skin at 6h. As expected, the nitric oxide end products were not suppressed by allopurinol but were by aminoguanidine. Interestingly, nitrotyrosine formation in LPS-treated skin was also suppressed by aminoguanidine and allopurinol independently. Our results imply that iNOS and XO, but not NADPH oxidase, work synergistically to form lipid radicals and nitrotyrosine in the LPS-treated skin.

1289 INDUCTION OF THE PGE2 BIOSYNTHETIC PATHWAY BY UVB LIGHT IS REGULATED BY THE P38 AND JNK MAP KINASES IN PRIMARY CULTURES OF MURINE KERATINOCYTES.

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Exposure to ultraviolet light, in particular the high-energy wavelengths of the ultraviolet B (UVB) spectra (290-320 nm), is a major causative factor in skin carcinogenesis. It is well recognized that an array of lipid mediators, including prostaglandins (PG), contribute to the carcinogenic process and that UVB light causes the release of arachidonic acid, a PG precursor. Prostaglandin E2 (PGE2), the primary PG in the skin, is synthesized through the sequential actions of cyclooxygenase (COX-2) and microsomal prostaglandin E synthases (mPGES) 1 and 2. Once formed, PGE2 binds to its receptors, EP1, EP2, EP3 and EP4, initiating biological activity. Numerous studies have shown that in many cancers, COX-2, mPGES1 and PGE2 as well as several of the PGE2 receptors are upregulated and are essential for tumor progression. In the present studies, we analyzed the effects of UVB on the expression of these proteins in undifferentiated and calcium-differentiated primary mouse keratinocytes. Using realtime PCR and western blotting, respectively, we found that UVB (2.5-25 mJ/cm²) caused dramatic dose-dependent increase in COX-2 mRNA (25-35 fold) and protein in both cell types. UVB also caused a 4-6 fold increase in mPGES mRNA expression. We found that the mRNA expression of only two of the four PGE2 receptors (EP1 and EP2) was induced by UVB (4-7 fold). In these cells, UVB was found to cause a dose-dependent activation of the p38 and JNK MAP kinases. Inhibition of these enzymes with SB203580, a p38 inhibitor, or SP600125, a JNK inhibitor, markedly inhibited mRNA expression of COX-2, mPGES1 and EP1. Taken together, these data demonstrate that UVB light effectively induces the PGE2 biosynthetic pathway in keratinocytes and that the activation of this pathway is regulated by MAP kinase activity. Supported by NIH grants ES05022, CA100994, CA093798 and NJ Commission on Cancer Research Fellowship 05-2413-CCR-E0.

1290 LYMPH NODES FROM TATTOOED MICE EXPOSED TO SIMULATED SOLAR LIGHT HAVE SUSTAINED PROTEIN EXPRESSION CHANGES.

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Approximately 24% of the US population has at least one tattoo. Although there are some reports of adverse reactions to tattoos, there is a paucity of data on tattoo pigment toxicity. We have examined inguinal and axillary lymph node protein expression in female SKH-1 hairless mice following tattooing and exposure to simulated sunlight. Groups consisted of no tattoo, tattooed with vehicle (10% aqueous glycerol), and tattooed with 20% w/v cadmium sulfide (CdS) or Pigment Red 22 (PR22) in vehicle. Mice were held for 2 weeks and exposed for 13 weeks to simulated solar light (1.4 SED/day). The mice (4/group) were sacrificed and the axillary lymph nodes removed, combined, frozen, and pulverized in liquid-nitrogen. Duplicate samples from each group were analyzed using polyacrylamide gel electrophoresis, the entire gel lane was excised into 30 bands, and each band subjected to in-gel trypsin digestion. The resulting peptides were analyzed using an automated nano-HPLC MS/MS system, and proteins were identified using the Mascot database search engine. ProteinTrack software was used to filter the protein results. In this first report of murine lymph node proteome more than 1700 proteins were identified. Hierarchical analysis of the total protein expression demonstrated that the non-tattooed and control (aqueous glycerol) proteomes were significantly different ($p<0.05$). Protein expression in mice tattooed with PR22 or CdS were different from each other and from the vehicle control. Analysis of the altered genes as a

result of tattooing with PR22 or CdS suggests there are changes in pathways involved in cell proliferation and inflammation. These results suggest (a) tattooing induces persistent changes in regional draining lymph node protein expression, (b) tattooing with PR22 and CdS induces changes above those detected with vehicle control.

1291 EXTRACELLULAR PROTON TRANSPORT BY SKIN KERATINOCYTES AS A TARGET FOR DERMATOTOXINS.

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The mammalian epidermis is a multilayered organ serving to control and protect the organism from external toxins. Basal keratinocytes, grow and differentiate, ultimately forming the cell layers above. In this process an extracellular pH gradient, in which the outermost layers are most acidic, is formed. Epithelial permeability, barrier function, and wound healing processes including matrix proteinase, glucocerebrosidase, and sphingomyelinase activities are all dependent on the acidification of the epidermis. In the current studies proton extrusion was examined in PAM212 and primary cultures of murine keratinocytes (MEK). The process was measured using a self-referencing H⁺ microsensor. We found a H⁺ gradient that decreased with increasing distance from the cells. H⁺ efflux properties were similar in both cell types. Amiloride 10-100 μ M did not alter the H⁺ flux, arguing against involvement of epithelial Na⁺ channels (ENaC) or the Na⁺/H⁺ antiporter (NHE). V-ATPase inhibitor bafilomycin A (1 μ M) and Na⁺/K⁺-ATPase inhibitor Ouabain (1 μ M) also had no effect on H⁺ efflux. While H⁺ extrusion was not altered by Ca²⁺ levels, it was diminished in the absence of Na⁺, and restored upon returning cells to Na⁺-containing buffer. Glucose addition caused a marked stimulation of H⁺ efflux, but only in the presence of Na⁺. We conclude that PAM212 and MEK dynamically acidify their extracellular environment, and that H⁺ efflux is not reliant upon Ca²⁺, or the NHE, ENaC, Na⁺/K⁺ ATPase, or V-ATPase transporters. Furthermore, we found that basal as well as glucose stimulated acidification is Na⁺ dependent. Supported by NIH grants 1U54AR055073-01, ES07148, CA100994, CA093798 and P41RR001395.

1292 INCREASED INCIDENCE OF CUTANEOUS MELANOMA IN TYR-HRAS+ INK4A/ARF+/- MICE CORRELATES WITH UV-INDUCED PHOTODAMAGE IN THE ANAGEN HAIR FOLLICLE BULBS OF NEONATES.

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Intermittent solar overexposure is associated with increased risk for cutaneous melanomas (CM) in humans. An animal model associating UV dosimetry with melanoma outcome is needed to test mechanisms of melanomagenesis and strategies for prevention. Our prior studies showed increased cyclopurimidine dimers (CPD) and photooxidative stress in whole skin samples collected from neonatal Tyr-HRAS+ Ink4a/Arf+/- transgenic mice exposed on postnatal days 3-8 to daily UV treatments (0.0, 1.0, or 2.0 J/cm²). Decreased latency for skin lesions was associated with increased CPD and oxidative stress in identically treated animals.

These skin lesions were evaluated histologically and classified as CM based on gene expression patterns (HRAS, Mitf, Trp-1, Trp-2, and 'silver') using laser microdissected tumor specimens and immunohistochemical detection of characteristic antigens (pan-RAS, S-100, Melan-A, neuron-specific enolase, and pan-keratins). Despite increased photooxidative stress and CPD detected in whole skin samples from both UV-treated dose groups, increased incidence of CM in photoexposed regions was observed in the 2.0 J/cm² dose group only.

The depth profile of CPD formation was then assessed by applying an object-oriented neural net-based image analysis algorithm (Feature Analyst; Visual Learning Systems, Missoula, Montana) running as an extension to ArcGIS (ESRI, Edmonds, CA) to detect and quantify immunolabeled CPD-bearing cells, within or outside of hair follicles, in large format (ca. 15 mm x 0.7 mm) digital image files. The incidence and latency of CM was closely associated with CPD detected in anagen hair follicle bulbs, the most mitotically active cutaneous melanocyte population in rodents. In contrast, weaker association was found between CPD detected in upper skin regions and CM.



1293 ADVANCES IN HEALTH ASSESSMENT IN U.S. EPA'S INTEGRATED RISK INFORMATION SYSTEM (IRIS) PROGRAM.

A. M. Kadry, K. Hammerstrom, L. Flowers, J. Vandenberg and P. Preuss. Office of Research and Development, National Center for Environmental Assessment, U.S. Environmental Protection Agency, Washington, DC.

The Integrated Risk Information System (IRIS) Program develops Environmental Protection Agency (EPA) scientific positions on potential adverse human health effects from exposure to various chemical substances found in the environment. The program follows EPA guidelines for health risk assessment and fosters consistent risk assessments across EPA program offices and regions. The IRIS database contains over 540 chemical-specific IRIS summaries, many with complete toxicological reviews or similar background documents. The assessments provide qualitative hazard identification, cancer weight of evidence characterizations, and quantitative toxicity values, i.e. oral reference doses and inhalation reference concentrations for noncancer effects and oral slope factors and inhalation unit risks for carcinogenic effects. IRIS users include EPA program and regional offices, other federal agencies, state and local agencies, international agencies, and the public. The IRIS web site receives over 21,000 hits per day.

The current process for developing an IRIS assessment consists of a public nomination process including announcement of substance selections, development of a draft assessment, internal Agency review, interagency review by interested federal agencies, external peer review with public comment period, final EPA approval and posting on the IRIS database. EPA is incorporating advances in the field of risk assessment in its assessment development process. These include greater use of mode-of-action data to inform hazard identification and cancer characterization and to identify appropriate low-dose extrapolation methods in cancer assessments, quantitative methods for expressing uncertainty, physiologically-based pharmacokinetic modeling, and approaches for assessing mixtures.

The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U. S. Environmental Protection Agency.



1294 THE USE OF MODE OF ACTION DATA IN INTEGRATED RISK INFORMATION SYSTEM (IRIS) HEALTH ASSESSMENTS.

L. Flowers, L. Cote, K. Hogan, C. Keshava and N. Keshava. NCEA, U.S. EPA, Washington, DC.

USEPA's Guidelines for Carcinogen Risk Assessment and Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure make greater use of the increased scientific understanding of the biological mechanisms leading to cancer following environmental exposures. The IRIS Program is considering mode of action (MOA) data in evaluating the carcinogenicity of environmental chemicals. Due to significant scientific advances in the field of both cancer and non-cancer effects, elucidation of a MOA for a particular response in animals or humans can inform human health risk assessments in a number of ways. The Guidelines suggest that MOA data, when available and of sufficient quality, can be useful for understanding whether findings in animals are relevant to humans, the potency of a chemical, its potential effects at low doses, and which populations or lifestages may be particularly susceptible. However, the increasing availability of MOA data raises several questions as to its application to risk assessment. For example, how can qualitative biological information on a MOA for chemical-induced carcinogenesis be linked with appropriate mathematical and statistical considerations for quantitative risk evaluations? What data are needed to determine whether a MOA supports particular approaches to low dose extrapolation? Other key implementation issues include a focus on the weight of evidence for a hypothesized MOA, lifestage issues related to carcinogenesis, and data needs for determining a MOA. This presentation will provide an overview of the understanding of MOA and its application to health assessment in the IRIS Program.

Disclaimer: The views expressed in this abstract are those of the author and do not represent the policy of the U.S. Environmental Protection Agency.



1295 EXTENDING IRIS TO INCLUDE LESS-THAN-LIFETIME REFERENCE VALUES.

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The EPA's Integrated Risk Information System (IRIS) Program is engaged in developing reference values [oral reference doses (RfDs) and inhalation reference concentrations (RfCs)] for less-than-lifetime exposures, including acute (less than or equal to 24 hours), short-term (up to 30 days), and subchronic (up to 10% of aver-

age lifespan) exposure durations. The intent is to include these less-than-lifetime reference values on the IRIS database, which has traditionally provided RfDs and RfCs for chronic exposure durations only. Pilot health assessments for 8 chemicals are in progress, 5 of which focus on the development of acute inhalation RfCs. In conducting these assessments, EPA has addressed a number of methodological issues, including endpoint selection, dose-response approaches (e.g., benchmark dose and categorical regression methods), duration extrapolation, physiologically-based pharmacokinetic (PBPK) modeling, and uncertainty factor application. As with chronic reference values, these guideline values are intended to be safe, i.e., likely to be without appreciable risk of adverse health effects to the human population, including sensitive subgroups, over a lifetime.

Case studies for 3 chemical assessments currently in progress – 1,1,1-trichloroethane, ethylene oxide, and dibutyl phthalate – are presented to illustrate issues encountered in developing less-than-lifetime reference values. Among the issues addressed in these case studies are the appropriateness of given study designs as the basis for a duration-specific reference value, considerations in endpoint selection, development of reference values based on either the same or different endpoints/modes of action for different exposure durations, and data extrapolation across exposure durations. Finally, the case studies illustrate considerations in the final review of a set of duration-specific reference values for a given chemical (e.g., Do values show a logical progression?).

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1296 ADVANCES IN CANCER RISK QUANTITATION.

K. A. Hogan¹, P. White², W. Chiu², J. Jinor² and L. Kopylev².

¹ORD/NCEA/IRIS, U.S. EPA, Washington, DC and ²ORD/NCEA-W, U.S. EPA, Washington, DC. Sponsor: A. Kadry.

This presentation will focus on recent advances in quantifying cancer risk and their uncertainties across animal and human carcinogenicity data. Issues requiring consideration when quantifying cancer risk include the contributions of pharmacokinetic models and biologically-based dose-response models, additivity to ongoing biological processes, and the combined impact of both mode-of-action information and population variability on dose-response relationships at low dose. In addition, there now is a greater need for broader characterization of uncertainty, integration of the results when multiple alternatives (models, data sets, modes of action, etc.) are being developed and explored, and enhanced summary presentation of toxicological databases. For instance, current work to better estimate point of departure uncertainty supports development of central estimates and bounds of risk. It is also anticipated that sensitivity analyses will be more routinely performed. All of these efforts contribute to a better quantitative characterization of cancer risk.

(Disclaimer: The views expressed in this abstract are those of the author and do not represent the policy of the U.S. Environmental Protection Agency).

1297 UPDATING THE IRIS HEALTH ASSESSMENT FOR DICHLOROMETHANE (METHYLENE CHLORIDE).

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¹National Center for Environmental Assessment, U.S. Environmental Protection Agency, Washington, DC and ²Syracuse Research Corporation, Syracuse, NY.

The IRIS Program is updating the dichloromethane (methylene chloride) health assessment. The current assessment on the IRIS database, completed in 1990, concluded that dichloromethane is a probable human carcinogen based on inadequate human data and sufficient evidence of carcinogenicity in animals. The reassessment will include evaluation of more recent human studies and an in-depth evaluation of issues regarding the mode of action of dichloromethane-induced carcinogenicity. Mutagenic activity has been demonstrated in short-term bacterial assays both with and without metabolic activation, although metabolic activation generally enhances dichloromethane mutagenicity. The results from in vitro genotoxicity assays with mammalian cells are equivocal (e.g., a dose-related increase in the occurrence of chromosome aberrations, but contradictory information concerning sister chromatid exchange). Evidence from in vivo studies indicates dichloromethane-induced clastogenicity, including chromosome aberrations, sister chromatid exchange, micronucleus, and DNA damage. Dichloromethane may undergo glutathione transferase-catalyzed bioactivation to yield S-(chloromethyl)glutathione as a reactive intermediate. This intermediate is likely to be involved in the carcinogenic mode of action. This presentation will summarize the available data and describe the challenges associated with interpretation of the data for human health risk assessment. (Disclaimer: The views expressed in this abstract are those of the authors and do not represent the policy of the U.S. Environmental Protection Agency).

1298 EPA TOXICOLOGICAL REVIEW OF ACRYLONITRILE.

D. Wong, A. Persad and L. Kopylev. ORD/NCEA, U.S. EPA, Washington, DC.

EPA is preparing an IRIS Toxicological Review of Acrylonitrile. The assessment is an update to the current assessment which has been on the IRIS database since the early 1990's. Voluminous new data from epidemiological and animal studies have since become available. In addition, new methodologies and guidelines have been developed and utilized by the Agency. The assessment evaluates both cancer and noncancer hazards from human and animal studies for derivation of a Reference Dose (RfD), Reference Concentration (RfC), oral slope factor and inhalation unit risk using new information.

This presentation will provide an up-to-date overview of the ongoing assessment. Specific areas that will be covered include epidemiology studies, animal studies, effects for derivation of RfC and RfD, mode of action for carcinogenicity, and cancer quantification.

(The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.)

1299 RELEVANCE AND FOLLOW-UP OF POSITIVE RESULTS FROM IN VITRO GENETIC TOXICITY TESTING.

B. Gollapudi¹ and M. P. Holsapple². ¹The Dow Chemical Company, Midland, MI and ²ILSI-Health and Environmental Sciences Institute, Washington, DC.

A battery of in vitro and in vivo genetic toxicity tests has been a critical component of the safety assessment of drugs, pesticides, and chemicals for many years. It is generally considered that results from in vitro studies demonstrate the intrinsic genotoxic properties of the test compounds. Accumulated knowledge has shown that the rate of positive in vitro tests, including the rate of positive findings not confirmed in vivo as well as false positives for non-carcinogens, has been high. There is a sense of urgency on the need to modify genotoxicity testing paradigms so as to generate useful information not only for hazard identification, but also for human risk assessment. The relevance of positive results obtained at concentrations that induce high levels of cytotoxicity, a requirement under current testing guidelines, is often questioned. A pertinent issue in this context is the necessity for testing in vitro at concentrations that far exceed any attainable in vivo exposures. Currently, there is a debate on the assumption that mutagenicity is equivalent to a mutagenic mode of carcinogenic action when positive results are obtained in both genotoxicity and carcinogenicity assays. A framework to establish whether or not genotoxicity is a key event in the observed carcinogenic response is needed to address this issue. In addition, it is suggested that the interpretation of dose response relationships for genotoxicity should be similar to that of other toxicity end points and non-linearity in dose-response should be applicable for both mutagenic and non-mutagenic carcinogens. The issue of applying uncertainty factors to an experimentally derived or modeled "no observed adverse effect level" or "benchmark dose" deserves further consideration for establishing human exposure levels for potential genotoxins. In addition, there is a need to develop follow-up testing strategies and weight of evidence approaches for the interpretation of positive in vitro genetic toxicity tests and their relevance to human health.

1300 RELEVANCE OF IN VITRO POSITIVE RESULTS IN GENETIC TOXICOLOGY: PLACE FOR IMPROVEMENT.

V. T. Thybaud. Drug Safety Evaluation, sanofi aventis, Vitry sur Seine, France. Sponsor: B. Gollapudi.

The in vitro genotoxicity tests are key components of the regulatory test battery for safety assessment. Data accumulated during the past years demonstrate a high rate of positive results, especially in the in vitro chromosome damage tests, for compounds found devoid of genotoxic or carcinogenic effects in animals. Several initiatives (e.g. International Workshop on Genotoxicity Testing, Health and Environmental Sciences Institute of International Life Sciences Institute) are on going to help consider how the relevance of in vitro positive findings could be improved. Several aspects of the assay performance and data interpretation are being considered for improvement. These include 1) the use of historical data in interpreting results from in vitro assays, 2) weight of evidence across assays, 3) mode of action (e.g. DNA reactive versus non-DNA reactive mechanisms) approaches to better define the level of concern for humans, 4) defining the limitations of the current in vitro models (e.g. human versus rodent cells, p53 status), 5) the impact of using extreme experimental conditions (e.g. criteria for the selection of the top concentration, cytotoxicity), and 6) further evaluation of the relationship between in vitro and in vivo potencies and of concentration/dose-response curves to move from qualitative to (semi) quantitative risk assessment. A collaborative work, involving scientists from regulatory authorities, academic laboratories and industries, will be needed to help advance the scientific basis for the interpretation of positive results and facilitate the development of follow-up testing strategies and criteria for determining the relevance of findings to human health.

1301 DON'T FORGET PARACELSUS: QUALITATIVE SCREENING ISN'T RISK ASSESSMENT.

J. T. MacGregor. *Toxicology Consulting Services, Arnold, MD.*

Paracelsus' insight that "the dose makes the poison" underpins the practice of toxicology, which is concerned in large part with determination of the biological effects expected under conditions of the exposure of interest based on knowledge of dose-response relationships. However, in the area of genetic toxicology regulatory practice frequently involves decision-making based on qualitative results from in vitro screening methods conducted under conditions designed to maximize exposure to active forms of the test article in cells with a reduced DNA repair capacity. Recent analyses have shown that qualitative categorization of results in such systems often over-predicts in vivo outcomes. It is clear that a re-evaluation of the history that led to current practices and default assumptions for regulatory decision-making is needed, and that regulatory practice should incorporate a risk-based approach that considers relevant in vivo exposure and response relationships. The historical basis of key assumptions about genetic testing strategies will be presented and, based on selected examples, current dogma regarding threshold vs. linear dose-response assumptions and possible steps toward an improved and economically feasible risk-based regulatory testing paradigm will be examined.

1302 CDER PERSPECTIVE ON POSITIVE RESULTS FROM IN VITRO GENETIC TOXICOLOGY ASSAYS.

D. Jacobson-Kram. *CDER, FDA, Silver Spring, MD.*

Results from carcinogenicity studies are generally not available for drugs until the time of approval. Many people, including healthy volunteers are often exposed to pharmacologically active doses of the drug before carcinogenicity results are available. FDA's Center for Drug Evaluation and Research (CDER) uses results of genetic toxicology studies as a surrogate for carcinogenicity during the drug development phase (clinical trials). A number of issues are considered in deciding whether drugs which give positive results in genetic toxicology studies can be given to subjects in clinical trials. These relate to the drug indication, the target population, duration of treatment and importance of the drug. For phase 1 studies in healthy subjects, no risk/benefit paradigm exists and for this population, risks must be minimal. ICH guidance recommends a test battery including a bacterial reverse mutation assay, an in vivo test for chromosome damage and an in vitro mammalian cell assay for cytogenetic damage or mutation at the thymidine kinase locus. Positive responses in the first two assay types are relatively rare; these assays have relatively high specificities and relatively low sensitivities. The in vitro mammalian assays have high sensitivity and low specificity. A review of the PDR indicates that 11 drugs with positive carcinogenicity results are uniquely identified by a positive in vitro mammalian cell assay, i.e. other assays are negative (personal communication, R. Snyder). Examination of this list of drugs suggests that factors other than genetic damage (e.g., exaggerated pharmacology) may be causally related to the carcinogenicity findings. These observations leave open the question of the contribution of mammalian in vitro assays in protecting the public health. CDER recently published guidance entitled Recommended Approaches for Integration of Genetic Toxicology Data. The guidance discusses various follow-up strategies to positive genotoxicity data. The strategies include assessing "weight of evidence" and "mode of action" as well as alternative follow-up testing.

1303 THE ROLE OF GENETIC TOXICOLOGY DATA IN THE EVALUATION OF MODE OF CARCINOGENIC ACTION FOR PESTICIDE CHEMICALS.

V. L. Dellarco. *Office of Pesticide Programs, U.S. EPA, Washington DC, DC.*

A pivotal focus of EPA's 2005 guidelines for cancer risk assessment is the carcinogenic mode of action (MOA) for a chemical (i.e., a biologically plausible sequence of key events that are obligatory and measurable steps leading to tumor development). This concept has permitted information on critical precursor events to be incorporated into the assessment of potential cancer risk and has led to a better scientific basis for the characterizing human relevance of laboratory animal responses and for informing quantitative estimates of risk. Similar MOA Frameworks have been developed by the International Programme on Chemical Safety and the U.S. EPA to guide transparent and structured analyses. The MOA Framework approach is based on Bradford Hill like criteria for establishing causality (i.e., a determination of whether a biological event is causal to the tumor response). Not only has the MOA framework become an increasingly valuable tool for organizing and analyzing data, but also for identifying data deficiencies. It is applicable to all MOAs, and has permitted the important distinction mutagenic and non-mutagenic MOAs. In

this presentation, case studies will be presented to illustrate the usefulness of the MOA Framework in evaluating mutagenic MOAs. The issues encountered with incorporating standard genetic toxicology data into the analysis will also be discussed.

1304 CONSULTANT IN TOXICOLOGY - A PERSPECTIVE.

R. A. Parent. *Consultox Ltd., Damariscotta, ME.*

INTRODUCTION

Although there are many retirees, academicians and governmental people who do consultations in Toxicology on a part time basis, there are more and more well qualified toxicologists who make a career out of doing consulting work in a variety of areas as is evidenced by close to one hundred registered toxicologists listed with the Roundtable of Toxicology Consultants.

Today, we present four highly successful consultants who have achieved a high level of success in attaining the height of their profession as Toxicology Consultants. They will share their insights into the tasks to be addressed in the various industries that they service.

1305 THE CHEMICAL SECTOR.

R. C. Kraska. *President, Kraska Consultants Inc., Aurora, OH.*

There are many consulting opportunities for toxicologists for both large and small companies in the Industrial Chemical Sector. Many large companies have fewer staff toxicologists due to employee down-sizing and attrition. Smaller companies who can not afford or justify a full time toxicologist need to turn to consultants when the need arises. It is common for major issues to be managed through trade associations who higher consultants and divide the cost among their members.

Usually opportunities arise from regulatory issues from the main federal laws which affect chemical products. EPA administers the Toxic Substances Control Act (TSCA) which provides authority over new chemicals coming on to the market and can require testing or controls of existing chemicals. OSHA administers the hazard Communication Standard (HCS) which requires company to provide MSDSs to customers and to label products with appropriate warnings. In addition, FDA issues arise with companies who supply the food packaging, food additive, cosmetic and medical device industries.

Some of the common tasks toxicology consultants have been involved in the past five years include:

- * Writing literature summaries and test plans for the High Production Volume (HPV) Chemicals Program under TSCA.
- * Guiding companies through HCS issues arising over the listing of new chemicals as carcinogens by IARC or NTP.
- * Assisting companies and trade associations comment on IARC and NTP nominations and ACGIH notices of intended changes for workplace threshold limit values.
- * On FDA issues, toxicologists can assist in the preparation of food or color additive petitions and food contact notifications or managing biocompatibility testing on materials supplied to the medical device industry.
- * Companies who export chemicals can have problems managing toxicological issues arising out of counterpart laws with different standards in Canada, the EU, Japan and Australia. China has been very active lately with new regulatory requirements for industrial chemicals.

1306 THE PHARMACEUTICAL INDUSTRY.

P. Frank. *Patricia Frank & Associates, Inc., Evansville, IL.*

Consultants to the pharmaceutical industry are a vital part of the preclinical development process, particularly in the small to medium size company. Pharmacodynamics, pharmacokinetics and metabolism and toxicology skills are all needed in order to bring a compound to the stage of clinical testing. Toxicology consultants with experience in developing drugs, including working with the FDA and comparable regulatory agencies world-wide, serve a vital function in companies without the internal skills or resources to define a development program based on the specific mechanism of action and proposed claim for the compound. These consultants work with the company to prepare briefing summaries and attend meetings with regulators. In addition, they place the pivotal toxicology studies in appropriate CROs, monitor those studies and prepare the summaries of the pre-

clinical work for regulatory submission. In many cases, the toxicology consultant becomes an integral part of the development team, participating in person or by conference call on a regular basis.

1307 THE MEDICAL DEVICE INDUSTRY.

S. C. Gad, *President, Gad Consulting Services, Cary, NC.*

While smaller in total sales than the pharmaceutical industry, the medical device industry (both domestic and International) is extensive and poorly equipped with internal resources to deal with questions as to the biocompatibility and safety of their products. Consulting practices in this area deal with both devices and the materials used in their construction, plus the growing field of drug/device combination products, and their evaluation under ISO and national standards. Few consultants are active in the field, and the opportunities and challenges will be presented and discussed from the perspective of 13 years experience as a toxicology and regulatory consultant in the field.

1308 LITIGATION SUPPORT.

R. A. Parent, *Consultox Ltd., Damariscotta, ME.*

Being involved in litigation involves continuing conflict. You are on one side of a case and the other side is trying to destroy you. In this kind of situation, your involvement must sit firmly on a solid base fact and truthfulness otherwise you will be destroyed. It's not enough to have the facts on your side, you must now present them in such a manner to survive pre-emptive motions and efforts toward summary judgments that are all laid out to destroy your credibility. That is still not enough. If you are lucky enough to get to a jury with a plaintiff's case, then your challenge is to break down these complex scientific issues into simple concepts that a lay jury off the street can understand. "Keep it simple stupid".

Of course, it goes without saying that your credentials must be accurate, extensive and impeccable. When I first became involved in litigation support, I asked a good friend for his insights and he told me that I had to choose sides and only work for either the plaintiff or defense. This, to my great satisfaction, is just not true. You can work for anyone who wants to hear the truth. Sometimes when you tell someone that they don't have a case, they are extremely grateful because you have saved them lots of money. Working for both sides also increases your credibility with judges and juries.

Litigation is not for the faint hearted. It is a challenge to remain cool when you are under attack. Most attorneys are quite civil and polite, but I have had some who have pounded on the table during a deposition. Of course, the deposition is the most trying time in a case. If you get to testify to a jury, everyone will treat you with respect because they don't want the jury to see their real personalities. It's a challenge

1309 BILIARY HYPERPLASIA AND CHOLESTASIS: RELEVANCE TO HUMAN RISK ASSESSMENT.

J. E. Klaunig¹ and M. J. Santostefano². ¹*Toxicology, Indiana University School of Medicine, Indianapolis, IN* and ²*Safety Assessment, GlaxoSmithKline, Research Triangle Park, NC.*

Drug-induced hepatic injury is the most frequent reason cited for the withdrawal of drugs from the marketplace and is quite common in non-clinical studies. Many mechanisms for liver injury exist. Although primary hepatocellular injury is most often emphasized, biliary and cholestatic liver injury is also noted. Biliary hyperplasia can result from cholestasis, hepatocellular injury, alterations in bile constituents, or through hormonal and vascular disruption. In preclinical species, depending on the underlying cause, biliary hyperplasia may resolve, stabilize or rarely progress to a more advanced proliferative lesion. While no data are readily available directly correlating non-clinical cholangiopathies with responses of patients in clinical studies, cholangiopathies also do cause morbidity and mortality in humans, and are a major cause of transplantation. Cholangiocarcinoma is a lethal disease, afflicting 3000 individuals in the US and many thousands worldwide. Cholangiocarcinomas are epithelial neoplasms that originate from cholangiocytes and may be found at any level of the biliary tree. The relationship of cholestatic biomarker to cholangiopathies is assumed for most cases and yet clear correlations between clinical markers and histological findings are not always apparent. This symposium will focus on anatomy and physiology of the biliary tree with emphasis on species differences that may impact human risk assessment, strengths and limitations of non-clinical and clinical monitoring paradigms, use of knockout mice in understanding mechanism, identification of novel biomarkers, and potential correlations or lack thereof between non-clinical and human findings.

1310 BILIARY EPITHELIAL HYPERPLASIA: ADVERSE, NON-ADVERSE, OR RELEVANT.

R. T. Miller and J. R. Hailey. *Safety Assessment, GlaxoSmithKline, Research Triangle Park, NC.* Sponsor: M. Santostefano.

Critical to the assessment of adversity and the relevance of the toxicity to humans is determination of what the lesion represents in the test species and whether the lesion that occurs in preclinical species may also occur in humans under similar conditions of exposure. Biliary hyperplasia, a relatively common finding in preclinical species, may be indicative of cholestasis, hepatobiliary injury, a static primary proliferative effect, or a precursor lesion to a more advanced proliferative lesion. In the absence of contrary information, the default assumption is that the lesion(s) may occur in humans. This presentation will focus on interspecies comparative pathophysiology responses in the liver. In addition, a summation of non-clinical data in rodents and potential correlation(s) in humans will be presented. Lastly, proposed approach and position relative to assessing the potential adversity of biliary epithelium hyperplasia in preclinical species will be discussed.

1311 BILE ACID BIOTRANSFORMATION AND TRANSPORT IN THE HEPATOBILIARY UNIT.

A. F. Hofmann. *Department of Medicine, University of California, San Diego, CA.* Sponsor: M. Santostefano.

Work over the past six decades in vitro and in vivo has elucidated principles of biotransformation and transport of bile acids relevant to the metabolism and toxicity of drugs. Bile acids consist of a steroid nucleus containing 1-4 hydroxy groups and an isopentanoic acid side chain. Both the pattern of nuclear substituents and the side chain length effect biotransformation and transport (PK), function (PD) and toxicity. Because of efficient intestinal conservation (enterohepatic circulation), most bile acids transported by the hepatocyte have their origin in intestinal absorption. Modification of bile acids by the intestinal bacteria converts 1° bile acids (formed in the hepatocyte) to 2° bile acids (formed by intestinal bacteria), with the result that the hepatocyte must transport a mixture of both 1° and 2° bile acids. After biosynthesis from cholesterol, bile acids are conjugated (N-acylamidated) with glycine or taurine; conjugates are impermeable to cell membranes enabling high (micellar concentrations of bile acids in the biliary tract and small intestine. Bile acids with a shortened side chain (nor- or dinor- bile acids) are not substrates for the enzymes of N-acyl amidation, and instead are metabolized by the hepatocyte as drugs - secreted into bile as such and/or hydroxylated and/or glucuronidated. Dihydroxy nor bile acids are absorbed passively by biliary epithelial cells, and return to the hepatocyte via the periductal capillary plexus, thus undergoing a cholehepatic circulation. Basolateral transporters involved in bile acid uptake from sinusoidal blood, and apical (canalicular) transporters involved in bile acid secretion into bile have been identified, as well as nuclear receptors that regulate bile acid biosynthesis and biotransformation. Bile acid toxicity occurs when there is excessive accumulation of toxic 2° bile acids or impaired canalicular secretion. An understanding of bile acid metabolism in the hepatobiliary unit appears useful for characterizing the handling of newly developed drugs.

1312 BILE DUCT OBSTRUCTION AND DESTRUCTIVE CHOLANGITIS IN MICE: INSIGHTS INTO PATHOGENESIS AND TREATMENT OF CHOLESTATIC LIVER DISEASES.

M. Trauner. *Department of Medicine, Medical University Graz, Graz, Austria.* Sponsor: M. Santostefano.

Cholestasis is an impairment of bile secretion which results from a functional defect in bile formation at the level of hepatocytes or obstruction/destruction of bile ducts. Animal models of cholestasis such as bile duct ligated mice and the Mdr2 (Abcb4) knockout mouse model of sclerosing cholangitis have permitted major insights into the molecular mechanisms of cholestasis. Exposure to cholestatic injury (results in reduced expression and function of hepatic uptake and excretory systems, changes which may maintain and contribute to cholestasis and jaundice. Recruitment of alternative efflux pumps and induction of phase I and II detoxifying enzymes may limit hepatic accumulation of potentially toxic biliary constituents in cholestasis by providing alternative metabolic and elimination routes. These molecular changes are mediated by bile acids, proinflammatory cytokines and hormones at a transcriptional and posttranscriptional level. Ligand-activated nuclear receptors (e.g., bile acid receptor FXR, xenobiotic sensors PXR and CAR) play a key role in mediating these changes and represent a worthwhile therapeutic target for cholestatic liver diseases. Sclerosing cholangitis in Mdr2 (Abcb4) knockout mice results from bile acid toxicity in the absence of mixed micelle formation with phospholipids. Modulation of bile composition and induction of bile acid detoxification and transport by side-chain shortened nor-ursodeoxycholic acid (norUDCA) heals

sclerosing cholangitis in this model, while conventional UDCA has no benefit. Future clinical studies will have to demonstrate whether norUDCA is suitable for the treatment of cholestatic liver diseases such as PSC and PBC in humans.



1313 VASCULAR ENDOTHELIAL GROWTH FACTOR REGULATION OF BILIARY GROWTH.

G. Alpini. *College of Medicine, Texas A&M University, College Station, TX.* Sponsor: M. Santostefano.

The proliferation of cholangiocytes (lining the biliary tree) is a typical hallmark of cholangiopathies, where it counteracts the loss/damage of intrahepatic bile ducts to compensate for the structural and functional derangement of the intrahepatic biliary tree. Proliferating cholangiocytes display enhanced secretory activity, which is important for maintaining a normal ductal secretion in spite of the loss of damaged bile ducts. Growth factors, neuropeptides and hormones modulate cholangiocyte proliferation. Vascular endothelial growth factor is secreted by several epithelia and modulates cellular functions by autocrine and paracrine mechanisms. The role of VEGF in the regulation of growth of cholangiocyte growth is unknown. We aim to: (i) evaluate the expression of VEGF-A and VEGF-C and their receptors in normal and proliferating cholangiocytes after extrahepatic bile duct obstruction (BDL); and (ii) determine the role and transduction mechanisms by which VEGF-A and VEGF-C regulate cholangiocyte proliferation.



1314 NON-CLINICAL AND CLINICAL CORRELATIONS OF HEPATOBILIARY TOXICANTS.

J. Cullen. *College of Veterinary Medicine, North Carolina State University, Raleigh, NC.* Sponsor: M. Santostefano.

The assessment of the safety of novel compounds is typically conducted in several animal species, including rats, mice, dogs and nonhuman primates. Currently, there is little information available on the concordance of the responses of the biliary tree in these species with the human response. Accordingly, this presentation will focus on the evaluation of a number of chemicals that produced histologic evidence of biliary injury in animals with respect to the serum biochemical responses indicative of biliary injury in exposed humans. In addition, a thorough review of non-clinical toxicity data for compounds that have been reported to cause cholestatic injury will be discussed.



1315 EPIGENETIC REGULATION IN REPRODUCTION, DEVELOPMENT AND DISEASE: IMPLICATIONS FOR TOXICOLOGY.

D. L. Shuey¹ and R. Watson². ¹Endo Pharmaceuticals Inc., Chadds Ford, PA and ²Mittek Systems Inc., Falls Church, VA.

Epigenetics is the study of events that regulate gene expression without change in DNA sequence. Evidence for the critical role of epigenetic events in normal and abnormal cellular function is rapidly expanding. DNA methylation, histone modification and RNA interference are important mechanisms by which gene expression is regulated. These events have been associated with control of cell proliferation, growth and differentiation, and alterations in epigenetic regulation have been associated with a variety of disease states. Genomic imprinting, which involves the monoallelic expression of a gene in a maternal or paternal specific fashion, also appears to be regulated by epigenetic mechanisms. Despite the clear, fundamental role of epigenetic mechanisms in controlling normal and abnormal cell function, the potential importance of epigenetic events as toxicologic mechanisms is just emerging. There are few examples of direct effects of toxicants on epigenetic regulation. The objective of this symposium is to provide an introduction to epigenetic regulation and the importance of epigenetic mechanisms in normal and abnormal cellular function in a variety of systems in order to stimulate thinking about these events as potential targets for toxicologic actions.



1316 INTRODUCTION TO EPIGENETIC MECHANISMS.

R. E. Watson. *Mittek Systems, Falls Church, VA.*

With the sequencing of the human genome, a great deal of attention has been paid to the significance of various polymorphisms and mutations, and which of these are likely to be linked to various phenotypes. Such analysis has indeed led to an improved understanding of which genes are involved in particular diseases, and how to better prevent or treat these diseases. However, the maintenance of normal patterns of genetic expression is a multidimensional process, and DNA sequence alone is only one of those dimensions. Another dimension involves epigenetic mechanisms

that regulate the level of genetic expression without altering the DNA sequence itself. Thus, the phenotype is changed while the genotype remains the same. DNA methylation, histone modifications, and RNA interference are examples of epigenetic mechanisms. This symposium will underscore the importance of epigenetics in a number of normal biological processes, including development, gene imprinting, and X-inactivation. Epigenetic aberrations have also been linked to various adverse conditions such as cancer, reproductive and developmental disorders, and neurological deficits. Overall, the study of epigenetics is an exciting, emerging field with great potential to enhance our understanding of biological phenomena and improve human health.



1317 EPIGENETICS AND CARCINOGENESIS: THE ROLE OF ALTERED DNA METHYLATION.

J. I. Goodman. *Pharmacology & Toxicology, Michigan State University, East Lansing, MI.*

The involvement of mutagenesis in carcinogenesis needs to be reconciled with the fact that not all carcinogens are mutagens and the view that nonmutagenic events play key roles in the transformation of a normal cell into a cancer cell. This apparent paradox can, in part, be resolved by considering the roles that altered DNA methylation, an epigenetic mechanism, play in carcinogenesis.

Inheritance occurs on two levels. Transmission of genes either in the somatic sense or from generation to generation is distinct from mechanisms involved in transmission of alternative states of gene activity. Epigenetics describes the latter and involves regulation of temporal and spatial control of gene activity, e.g., changes in gene expression during development, imprinting, segregation of gene activities such that daughters of a cell exhibit different patterns of gene expression, and mechanisms that permit the somatic inheritance of a specific set of active and quiescent genes. DNA methylation is an epigenetic mechanism controlling gene activity. Changes in DNA methylation are not mutations, i.e., 5MeC and cytosine base pair with guanine. Increased methylation may silence tumour suppressor genes (functionally equivalent to inactivation due to point mutation or allelic loss) and decreased methylation may up-regulate expression of oncogenes. Thus, altered DNA methylation can facilitate the aberrant gene expression underlying carcinogenesis. The role of DNA methylation in the regulation of gene expression will be juxtaposed with a discussion of how altered methylation is a fundamental, epigenetic mechanism involved in carcinogenesis. Data from my laboratory will illustrate both a novel PCR-based approach to assess methylation status in multiple regions of the genome simultaneously and progress towards our goal of identifying genes involved in transformation by virtue of altered methylation. Emphasis will be placed on showing how we test the hypothesis that susceptibility to carcinogenesis is related inversely to the capacity to maintain normal patterns of methylation, and implications for safety assessment will be noted.



1318 EPIGENETIC REGULATION OF OVARIAN FUNCTION.

H. A. LaVoie. *Cell and Dev. Biol. and Anat., University of South Carolina School of Medicine, Columbia, SC.* Sponsor: D. Shuey.

The ovary is a dynamic tissue. A subset of ovarian genes silent during the follicular phase is activated during the luteal phase, a time when many follicular phase genes are turned off. Changes in transcription are driven by hormonal cues, which lead to histone modifications facilitated by specific transcription factors. Transcription factors recruit their coactivators or corepressors to the 5'-regions of target genes. Many of these cofactors are histone acetyltransferases and deacetylases mediating the hyperacetylation and hypoacetylation of histones, respectively. Hyperacetylation of lysine (K) residues in the core histone tails (e.g., H3K9 and H3K14) promotes chromatin alterations that favor transcription, whereas hypoacetylation of histones promotes gene silencing or repression. Specific histone phosphorylations can also facilitate transcription. For example, phosphorylation of histone H3 serine 10 co-operates with acetylated K9 to promote transcription. Methylation of histones occurs at lysine and arginine residues and can be either permissive or repressive. For instance, methylation of histone H3K9 represses transcription, whereas trimethylation of H3K4 is associated with active transcription. Most studies of histone modifications in the ovary have evaluated the highly regulated steroidogenic acute regulatory protein (STAR) gene. STAR is silent in granulosa cells of the immature follicle and expressed robustly after the LH surge. In vivo studies of monkey and murine granulosa cells have shown that an ovulatory stimulus of hCG leads to increased acetylation of histone H3K9 or reductions in H3K9 methylation within the STAR gene promoter, respectively (1,2). In cultured porcine granulosa cells, FSH stimulates H3K9/K14 acetylation within the promoter coincident with STAR transcription and coculture with STAR transcriptional repressor EGF blocks the increase in H3 acetylation (3). Modification of histones may be a mechanism by which environmental stimuli can impact ovarian gene expression.

References:

- (1) J Biol Chem 276:27392-27399, 2001
- (2) Mol Endocrinol 18:791-806, 2004
- (3) Biol Reprod 72:862-871, 2005

**1319 EPIGENETIC PROGRAMMING BY MATERNAL BEHAVIOR.**

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Maternal care has long term effects on health outcomes and behavior, which last through life. How is maternal behavior, which is restricted to early childhood, memorized through life long after maternal behavior is gone? We will present a hypothesis, which is based in our work on maternal care in rats that epigenetic processes program critical genes in response to maternal care. Stress responses in the adult rat are programmed early in life by maternal care and are associated with epigenetic marking of the hippocampal exon 17 glucocorticoid receptor (GR) promoter. This stable epigenetic programming is nevertheless reversible in adult brain by treatment with either the HDAC inhibitor TSA or central infusion of the essential amino acid methionine a precursor to S-adenosyl-methionine (AdoMet) the donor of methyl-groups for DNA methylation. A molecular mechanism responsible for this dynamic equilibrium of the DNA methylation pattern is proposed here. High levels of maternal licking/grooming (LG) behavior over the first week of post-natal life results in induction of the transcription factor NGFI-A within the hippocampus of the offspring. Binding of NGFI-A to the exon 17 GR promoter within the 6-day-old offspring permanently marks the promoter sequence for histone acetylation and DNA demethylation. We will also present preliminary data from our human studies suggesting that epigenetic differences correlate with behavioral differences in humans as well. Our data illustrates a possible mechanism for how behavioral cues mediate epigenetic programming of genes within the brain. We propose that the epigenome mediates between the dynamic environment and our static genomes and provides a molecular link between nurture and nature. Supported by a grant from the CIHR, NIH to MS and MJM and NCIC to MS

**1320 VALPROATE: NOVEL ANALOGUES, APPLICATIONS AND MECHANISMS.**

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Valproate (Valproic Acid, VPA) was originally developed as antiepileptic agent, but was shown to be effective also for treatment of bipolar disorders, cancer, migraine prophylaxis, schizophrenia, as well as for neuroprotection in Alzheimer Disease and cerebral ischemia. However, VPA is teratogenic in animal models and the human, leading to neural tube defects (exencephaly in the mouse, Spina bifida in the human). We have now synthesized a number of analogous structurally related to VPA in order to study if the mechanisms of some VPA-induced effects are related. Furthermore, we developed analogues which are 10-40-fold more potent than VPA in vitro (e.g. differentiation of cancer cells such as leukaemia cells (Deubzer, 2006)), and up to 4-fold more potent in vivo (teratogenicity). VPA was shown to be a histone deacetylase inhibitor causing hyperacetylation of core histone proteins leading to an open chromatin conformation, derepression and transcriptional activation of a number of genes (Phiel, 2001; Göttlicher, 2001). We studied - with a large set of structurally diverse analogues of VPA - if HDAC-inhibition is related to the teratogenic effect. We demonstrate that only teratogenic analogues are able to induce differentiation in F9-teratocarcinoma cells, to activate PPARdelta, to increase NCAM/PSA in F9-cells, and to inhibit histone deacetylation. Latter effect was demonstrated by Western blotting of hyperacetylated core histone H4 and by a human HDAC enzyme test system. We also show a quantitative correlation between the IC50(HDAC) values and the teratogenic potency of these analogues which clearly points to HDACs as the previously described theoretical "teratogenic receptor" for this class of compounds (Bojic, 1998). In conclusion: We have developed VPA-analogues which are many-fold more potent than the lead substance VPA. Some of these compounds could be useful in cancer therapy, neuroprotection, cognitive enhancement and further applications. It is not clear if teratogenicity can be dissociated from these desired effects, as HDAC-inhibition is a crucial part of the molecular mechanisms of this compound class in multiple experimental models.

**1321 PATERNAL EXPOSURE TO CYCLOPHOSPHAMIDE (CPA) DISTURBS EPIGENETIC PROGRAMMING DURING EARLY EMBRYO DEVELOPMENT.**

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Paternal exposures may impact negatively on progeny outcome. Exposure of male rats to a male mediated developmental toxicant and anticancer alkylating agent, CPA, resulted in increased pre- and post-implantation loss, and malformations in

the offspring. CPA induced a stage specific stress response in male germ cells. The DNA damage induced by CPA was germ cell phase specific, with the greatest damage occurring during sperm chromatin remodeling. Drug induced insults to sperm chromatin may affect the function of the paternal genome in the zygote. Zygotes sired by drug-treated males displayed advanced developmental progression. In 1- and 2-cell embryos sired by CPA-treated males, the relative abundance of candidate gene transcripts was elevated significantly. We hypothesized that paternal CPA exposure disrupts epigenetic programming in the early embryo. To test this hypothesis, we examined the temporal patterns of histone H4 acetylation at lysine 5 and 5-methylcytosine immunostaining in zygotes sired by CPA-exposed and control males. Both the male and female pronuclei in embryos sired by CPA-treated males were significantly hyperacetylated, beginning in G1 and lasting into S-phase; in later stages of zygotic development, the extent of H4 acetylation did not differ. DNA methylation reprogramming was also dysregulated. In control zygotes, the male pronuclei underwent a gradual genome-wide demethylation, while the female pronuclei remained hypermethylated; in contrast, the male pronuclei in zygotes fertilized by drug-exposed sperm were dramatically hypomethylated. In 2-cell embryos sired by CPA-exposed sperm the number of micronuclei was substantially elevated. Thus, paternal drug exposure affected the epigenetic programming of not only the male, but also the female pronucleus, demonstrating the existence of pronuclear cross talk during zygotic development. Deregulation of epigenetic programming in the zygote may contribute to heritable instabilities later in development. Supported by CIHR.

**1322 MODELING A MIXTURE: PBPK/PD APPROACHES FOR PREDICTING CHEMICAL INTERACTIONS.**

V. C. Moser¹ and K. Krishnan². ¹NTD/NHEERL, U.S. EPA, Research Triangle Park, NC and ²SEST, University of Montréal, Montréal, QC, Canada.

Since environmental chemical exposures generally involve multiple chemicals, there are both regulatory and scientific drivers to develop methods to predict outcomes of these exposures. Even using efficient statistical and experimental designs, it is not possible to test in vivo all possible mixtures. Interactions could be based on kinetic (e.g., enzymatic, metabolic interactions) and/or dynamic (e.g., receptor interactions) factors. Recent advances in physiologically based pharmacokinetic/dynamic (PBPK/PD) modeling have confirmed the usefulness of these tools in predicting tissue levels of chemicals and biological responses. Mathematical predictions may be integrated with focused toxicology studies to improve the models, decreasing the amount of animal testing needed. With PBPK/PD modeling, the binary level interactions are interconnected within the mixture model, which can then be used to simulate the kinetics of chemicals in mixtures of higher levels of complexity. The unique ability of PBPK models to facilitate extrapolation of interactions from binary to more complex mixtures arises from the mechanistic basis of the methodology, i.e., linking all mixture components on the basis of interaction mechanisms. The methodology has been shown to be applicable to different mixtures of volatile organic chemicals. Furthermore, the modeling work with organophosphates illustrates how to address interactions in mixtures based on kinetic as well as dynamic factors. Progress in estimating interaction thresholds and employing different approaches for designing complex mixtures (e.g., chemical lumping) have extended the predictability of these models. Thus, the PBPK/PD modeling approach can be used in a range of experimental situations to predict the magnitude of chemical interactions and to assist in determining mechanisms for the interactions. Such information is critical to enhance the scientific basis of extrapolations (species, route, dose) essential for chemical mixture risk assessments. This is an abstract of a proposed presentation and does not necessarily reflect US EPA policy.

**1323 PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) AND PHYSIOLOGICALLY BASED PHARMACODYNAMIC (PBD) MODELING: TOOLS FOR EXAMINING INTERACTIONS BETWEEN CHEMICALS.**

M. Andersen. CIIT Centers for Health Research, Research Triangle Park, NC.

Chemical mixture toxicity has largely remained mired in exposure based approaches examining interactions in relatively high dose studies without sufficient attention to the influence of mixture exposures on delivery of active chemical to target tissues. Elaborate statistical models for mixture toxicity based solely on exposure levels, a common outcome goal of mixture research, are likely to be misleading about nature of interactions among chemicals when the study designs do not examine tissue doses separate from administered dose or do not provide model structures for in vitro-in vivo extrapolations. Interactions would better be described as consisting of two types - PK interactions where co-exposures alter the relationship between administered dose and tissue doses compared to the chemicals delivered individually. PD interactions would occur when tissue response to a unit dose to the

tissue is altered by exposure to the mixture compared to exposure to the single chemical. Physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) models simulate target tissue dose based on exposure levels (PBPK models) and simulate tissue responses based on target tissue doses (PBPD models). The PK models are much better developed than the PD models. Nonetheless, these two types of model structures describe the quantitative interrelationships among critical biological and physiological determinants regulating toxic responses. These models can account for PK and PD interactions and account for the changing nature of apparent interactions as doses escalate, for instance, the change from no interactions and additive toxicity at low doses to less than additive responses at higher doses. Furthermore, interactions examined in vitro can be used to generate predictions of in vivo responses using these PK/PD models. This talk details the role these models should play in studying mixtures and the value of PD model predictions in defining interactions among groups of chemicals.



1324 DEVELOPMENT OF PHYSIOLOGICALLY BASED PHARMACOKINETIC AND PHARMACODYNAMIC (PBPK/PD) MODELS TO DETERMINE DOSIMETRY AND CHOLINESTERASE INHIBITION FOLLOWING EXPOSURE TO BINARY MIXTURES OF ORGANOPHOSPHORUS INSECTICIDES.

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PBPK/PD models have been developed for the organophosphorus (OP) insecticides chlorpyrifos (CPF) and diazinon (DZN). These insecticides share common metabolic activation/detoxification pathways and a common mechanism of neurotoxicity associated with excessive cholinergic stimulation. To develop a binary OP PBPK/PD model, rats were orally administered CPF, DZN or a CPF/DZN mixture (0, 15, 30 or 60 mg/kg) and blood (plasma and RBC), brain and urine were collected for analysis. Chlorpyrifos, DZN and their respective metabolites, trichloropyridinol and isopropyl-methyl-hydroxypyrimidine were quantified in blood and/or urine and cholinesterase (ChE) inhibition was measured in brain, RBC, and plasma. Co-exposure to CPF/DZN at the low dose (15/15 mg/kg) did not alter the pharmacokinetics of CPF, DZN or their metabolites in blood. Whereas, a high binary dose (60/60 mg/kg) increased the C_{max} and AUC and decreased the clearance for both parent compounds, likely due to competition between CPF and DZN for CYP450 metabolism. At lower doses the pharmacokinetics appeared linear. A dose-dependent inhibition of ChE was noted in tissues for both the single and co-exposures, and the extent of inhibition was plasma > RBC ≥ brain. The overall relative potency for ChE inhibition was CPF > CPF/DZN > DZN. A comparison of the ChE response at the low binary dose (15/15 mg/kg), where there were no apparent pharmacokinetic interactions, suggested that the overall ChE response was additive. Based on these pharmacokinetic results a binary OP PBPK/PD model has been developed. This model facilitates understanding the mixture interactions and the potential for additivity, synergism or antagonism from multi-OP exposures. It is envisioned that this binary model will be useful for assessing exposure and health risk to a wide range of exposed individuals. (Supported by CDC/NIOSH grant 5 R01 OH003629-03)



1325 PBPK MODELING OF METABOLIC INTERACTIONS: EXTRAPOLATING FROM BINARY TO MORE COMPLEX MIXTURES.

K. Krishnan¹, S. Haddad², C. Emond¹ and R. Tardif¹. ¹SEST, University of Montréal, Montréal, QC, Canada and ²Dép. sciences biologiques, TOXEN, UQAM, Montréal, QC, Canada.

One of the challenges in risk assessment relates to the inability to make use of binary interaction data. The nature and magnitude of an interaction between two chemicals may be further altered by other chemicals in a mixture. This presentation will focus on the novel application of physiologically-based pharmacokinetic (PBPK) models to predict the kinetics of chemicals in mixtures of increasing complexity, solely on the basis of binary interaction data. In this methodology, individual chemical PBPK models are developed and interconnected on the basis of binary-level interactions. As such, with a single set of physiological parameters and multiple sets of chemical-specific parameters, the simulations of the kinetics of mixture components can be obtained. Binary connections between mixture components, where feasible, are established within the model on the basis of interaction mechanisms. Studies conducted so far have focused on the modeling of competition among CYP2E1 substrates for hepatic metabolism. Data collected in rats exposed to binary, ternary, quaternary and pentameric mixtures of toluene, m-xylene,

benzene, dichloromethane and ethyl benzene have been used to validate the predictions of the binary-based PBPK model. When all binary level metabolic interactions in a mixture can neither be identified nor characterized, the limits of metabolic interaction (0 – 100%) have been used to simulate the potential consequences of mixed exposures. Such an approach has been validated using several mixtures of volatile organic chemicals. The PBPK modeling approach has also been used to simulate the kinetics of mixtures of PCBs in rats, on the basis of metabolism constants that reflect the net effects of inhibition and induction. Overall, the PBPK modeling approach allows the prediction of the kinetics and internal dose of mixture components on the basis of binary level interactions as well as the net effect of processes affecting the rate of metabolism.



1326 DEVELOPMENT OF A PBPK MODEL FOR GASOLINE AND SEVERAL OF ITS COMPONENTS EXHIBITING METABOLIC INHIBITION.

J. E. Dennison. *Environmental Health, Colo State U, Ft. Collins, CO.*

Gasoline contains benzene and hundreds of other chemicals that are carcinogenic, neurotoxic, or systemically toxic. Exposure to gasoline mixtures reduces metabolism of individual components through metabolic inhibition. We explored this issue by conducting gas uptake pharmacokinetic (PK) experiments in F344 rats. Total metabolism of components was reduced, for example, by 4% – 27% during a six-hour exposure to 300 ppm gasoline. To develop a better quantitative understanding of this issue, a PBPK model was developed for gasoline PKs in the rat. Rather than attempting to develop inhibitory constant estimates for each component, the majority of the mixture was incorporated into the model as a lumped chemical with single parameter estimates for biochemical and partitioning processes. In addition, five individual chemicals (benzene, toluene, ethylbenzene, xylene, and n-hexane) were treated as single chemicals. Competitive inhibition was inferred from PK data and treated as occurring between each binary combination of chemicals within the mixture. Estimates of the inhibitory constant (K_i) for each non-lumped chemical were developed from initial PK experiments where one chemical was provided at very high levels and other chemicals were at low levels, to improve sensitivity. These constants were then used in the PBPK model without further refinement, and a constant for the lumped chemical was estimated from the whole gasoline studies. Finally, experiments and models were developed for lighter fractions of gasoline that would evaporate during a gasoline spill. The model provides an excellent description of gasoline PKs in the rat, and demonstrates the utility of the chemical lumping approach within the context of PBPK modeling. A model such as the one described can be used in risk assessment to determine the extent of metabolism of carcinogenic metabolites under various exposure conditions, and in other applications. [Supported in part by a Cooperative Agreement from ATSDR (U61/ATU 881475) and the NIEHS Quantitative Toxicology Training Grant T32 ES07321].



1327 MODELING THE INTERACTION THRESHOLD: THE BREAK-POINT BETWEEN ADDITIVITY AND NON-ADDITIVITY.

H. A. El-Masri. *NHEERL, U.S. EPA, Research Triangle Park, NC.*

Dose-dependent changes in toxicity mechanisms of single chemicals can possibly take place along the full dose-response spectrum. At high doses, the possibility exists for some steps in the principle mechanism of toxicity to shift to other mechanisms. The possibility of mechanism shifts for single chemicals can also be observed for interaction mechanism of chemical mixtures. For instance, interactions (synergism or antagonism) taking place at high individual doses of a mixture, may not be significant at low levels. One of the early experiments indicating a change of the mechanism of interaction with dose was conducted using chloral hydrate and ethanol. The boundary that separates the dose space into regions of interaction and additivity is called the interaction threshold boundary. The interest in defining this region is important because it signifies the need to include or exclude interactive effects in the calculations of health risks of the mixtures, specifically at low environmentally relevant doses. The interaction threshold boundary may take a variety of different shapes, and the shape of this boundary is not likely to be known. Hamm et al. (2005) developed a general procedure to accommodate various potential shapes for this boundary. Predictive modeling (such as PBPK/PD models) along with mode-designed experiments provides an efficient methodology for the identification of interaction thresholds. Based on mechanistic consideration of enzyme inhibitions, prediction of the presence of an interaction threshold between binary chemicals was performed using PBPK models for two different set of chemicals; volatiles (TCE and 1,1-DCE), and pesticides (chlorpyrifos and parathion).



1328 ADVANCES IN CAUSATION AND THERAPY OF PARKINSON AND PARKINSON-LIKE MOVEMENT DISORDERS: VIEWS FROM TOXICOLOGISTS AND CLINICIANS.

W. Zheng¹, G. W. Miller², E. Pira³, J. S. Rutchik⁴ and J. Zhang⁵. ¹Purdue University, West Lafayette, IN, ²Emory University, Atlanta, GA, ³University of Turin, Turin, Italy, ⁴UCSF, San Francisco, CA and ⁵UC Seattle, Seattle, WA.

Parkinson-like movement disorders, caused by environmental or occupational exposure to neurotoxins such as manganese or pesticides, are characterized by increased bradykinesia, rigidity, resting tremor, or posture difficulties. Key issues in clinical intervention include the lack of reliable means for diagnosis, as clinical signs and symptoms are not specific for each etiology, and the lack of therapeutic strategies. Thus, the translational research possesses the paramount importance for clinical management of neurotoxin-induced movement disorders. The purpose of this workshop is to address, mainly from the clinical point of view, the current understanding of causes, diagnosis, and therapeutic strategies in dealing with chemical-induced parkinsonian type of disorders, by using advanced clinical techniques and improved animal models. The workshop first discusses the challenge of identifying patients with idiopathic Parkinson's disease (PD) and manganism by an occupational neurologist, followed by discussion on the similarities and disparities of various toxicant-induced animal PD models and the possible uses of these models in searching for new treatment for PD. Recent advancement in developing biomarkers for IPD by proteomic technique is then presented. Cases of clinical application of magnetic resonance imaging (MRI) in diagnosis of asymptomatic manganism is also extensively discussed. Finally, the efficacy, application, and perspectives of metal chelating agents in clinical treatment of manganism patients with regard to the disease state is addressed. Not only does the workshop offer rationalized advices for researchers on diagnosis and treatment of chemical-induced Parkinson-like movement disorders, but more importantly it reveals theories underlying these advancements and thus suggests the future direction in environmental PD research. The workshop is of interest to those who are engaged in pesticide toxicology, metal toxicology, neuroscience, neurotoxicology, risk assessment, regulatory management, occupational health, and toxicology education.



1329 CLINICAL PRESENTATION OF PATIENTS WITH IDIOPATHIC PARKINSON'S DISEASE AND MANGANISM: SIMILARITIES AND DIFFERENCES.

J. S. Rutchik. *Occup & Env Medicine, UCSF, Mill Valley, CA*. Sponsor: W. Zheng.

Idiopathic Parkinson's disease (IPD) was initially described as a shaking palsy in 1817 by James Parkinson. The earliest report on Parkinson-like syndromes among manganese (Mn) ore crushers was described by Couper in 1837. Neurological sequelae from occupational exposure to Mn has been suspected to lead to an atypical parkinsonian syndrome, including bilateral simultaneous and symmetric low amplitude postural tremor with no rest tremor. Frequently, early dysarthria, gait and balance abnormalities, dystonia in limbs and face and action myoclonus and hyperreflexia are also observed in clinics. Most significantly, manganism displays early personality changes, emotional lability, visual and auditory hallucinations. The onset develops weeks and months after exposure to toxic metal, with poor or no response to levodopa without related psychiatric symptoms, initial rapid progression followed by stable course, and initial and transient elevation of Mn in blood, urine and hair. Recent MRI technology allows to detect bilateral hyperintensities in the globus pallidus and substantia nigra on T1 images which may be transient. In addition, pathological findings have revealed the degeneration and gliosis of the globus pallidus but without the presence of Lewy's bodies. Recently, some researchers based on diagnosis of welders with parkinsonism report that parkinsonian welders have a younger age of onset but otherwise no clinical differences from IPD. These investigators further report that PET scans of patients with manganism show a reduced uptake in the striatum. Thus, the question as to whether the clinical presentations of manganism and IPD are the same or different remains unsolved. This presentation discusses the research performed by a team consisting of a physician with American board certifications in neurology, occupational and environmental medicine, a U.S. neurotoxicologist, and two Chinese occupational medicine physicians to assess six workers with different exposure backgrounds in order to confirm a diagnosis of manganism. The current state of the art in the body of knowledge developed regarding the topic of manganism and parkinsonism is also discussed.



1330 TOXICANT-BASED MODELS OF PARKINSON'S DISEASE AND THEIR IMPACT ON CLINICAL CARE.

G. W. Miller. *Center for Neurodegenerative Disease, Emory University, Atlanta, GA*.

Exposure to various pesticides and other environmental chemicals has been suggested to be associated with the clinical occurrence of Parkinson's disease (PD). In animal models, these chemicals can reproduce certain neurobehavioral and neuro-

chemical changes typical of PD, but do not reproduce all of the clinical symptoms. Ideally, animal models used to identify causes, improved diagnosis, and treatments of the disorder should closely resemble the human condition. While several genetic abnormalities have been associated with early onset forms of PD, no clear genetic component has been associated to the more common late onset form. Given the substantial evidence that the environment participates in the pathogenesis of PD, our laboratory has focused on environmentally-relevant models of PD. The hope is that by using models that mimic the environmental component of the disease, we can improve our understanding of the disease process that can aid in the development of strategies to mitigate the adverse effects of the environmental toxicants. This presentation will evaluate the toxicant-based (MPTP, 6-OHDA, paraquat, rotenone, dieldrin) and genetic-based (knockout of alpha-synuclein, DJ-1, PINK1, Parkin, or transgenic over expressers of the aforementioned genes) models of PD. Particular attention will be given to the behavioral, biochemical, and neuropathological differences. While many of the genetic models for PD have provided useful information on disease pathogenesis, they consistently fail to show a substantial reduction in striatal dopamine, which is thought to be the major contributor to the bradykinesia observed in PD. Thus, an ideal model of Parkinson's disease must exhibit a progressive loss of striatal dopamine, in addition to the presence of the neuropathological hallmark Lewy bodies. Further model development and judicious selection of the most appropriate model should enhance our ability to develop novel diagnostic, preventive, and therapeutic strategies aimed at reducing the personal and societal burden of this disease.



1331 DEVELOPMENT OF PROTEIN BIOMARKERS WITH PROTEOMICS FOR DIAGNOSIS AND MONITORING THE PROGRESSION OF PARKINSON'S DISEASE.

J. Zhang. *Pathology, University of Washington, Seattle, WA*. Sponsor: W. Zheng.

Patients with Parkinson's disease (PD) typically lose significant neurons in the substantia nigra prior to development of significant symptoms for them to seek medical treatment. Conceivably, any therapy starting at clinically confirmed 'advanced stage' of PD might fail to stop the degenerative process. This presentation describes the latest findings on early diagnosis of PD by using recently developed high throughput proteomics to identify biomarkers in body fluids, particularly in human cerebrospinal fluid (CSF). More specifically, we have utilized a multiplex quantitative proteomics method, iTRAQ (isobaric Tagging for Relative and Absolute protein Quantification), in conjunction with multidimensional chromatography, followed by tandem mass spectrometry (MS/MS), to simultaneously measure relative changes in the CSF proteome of individuals with PD, Alzheimer's disease (AD), and dementia with Lewy body (DLB) as well as age-matched controls. The diagnosis of AD and DLB was confirmed by autopsy, whereas the diagnosis of PD was based on clinical criteria. The proteomic findings showed quantitative changes in PD, AD, and DLB as compared to controls; among more than 1,500 identified CSF proteins, 72, 136, and 101 of the proteins displayed quantitative changes unique to PD, AD, and DLB, respectively. Eight unique proteins were confirmed by Western blot analysis, and the sensitivity at 95% specificity was calculated for each marker alone and in combination. Several panels of unique markers were capable of distinguishing PD, AD and DLB patients from each other as well as from controls with high sensitivity at 95% specificity. Although these preliminary findings must be validated in a larger and different population of patients, they suggest that a roster of proteins may be generated and developed into specific biomarkers that could eventually assist in clinical diagnosis and monitoring disease progression of PD, AD and DLB. It is also expected that these markers may be useful in differentiating PD from parkinsonism caused by other etiology, including manganese induced neurotoxicity.



1332 CLINICAL APPLICATIONS OF MAGNETIC RESONANCE IMAGING (MRI) IN DIAGNOSIS OF ASYMPTOMATIC MANGANISM: DIFFERENCES FROM IDIOPATHIC PARKINSON'S DISEASE.

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Recent clinical reports have suggested that clinical and pathophysiological features of manganese-induced Parkinson-like movement disorder may, at times, overlap those of idiopathic Parkinson's disease (IPD). Clinical studies in humans and basic research on animal models have demonstrated that T1- and T1 MT-weighted MRI is able to detect abnormal deposits of this paramagnetic metal in the globus pallidus and substantia nigra. This presentation first discusses these characteristics among manganism patients in comparison to the MR imaging in IPD patients, where the MRI can evidence nigral changes in T2 and T2* signal associated with modifications in the iron deposition. The presentation then describes a series of clinical cases

where MRI has been used to investigate symptomatic Italian welders and asymptomatic Chinese miners. In former cases, the subjects had a well-documented manganese-induced Parkinson-like syndromes; application of MRI technique was able to allow for the setting up of a successful therapy programme in the early phase of the disease, which led to an ending in a clinical improvement along with the normalisation of the neuro-radiological parameters. In the latter cases, the possibility to identify significant MRI changes in pre-symptomatic subjects has been explored, allowing for development of preventive measures. In addition, the opportunity to make use of this technique in routine screening is discussed.



1333 EFFICACY AND SELECTIVITY OF METAL CHELATORS IN TREATMENT OF MANGANESE-INDUCED PARKINSON-LIKE MOVEMENT DISORDER.

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A major challenge in clinical aspects of manganese (Mn) toxicity study is the lack of effective therapeutic strategy for Mn-induced neurological impairment. Without treatment, the signs and symptoms progress and chances of recovery are usually poor. Treatment with levodopa has been used in most clinics for initial control of extrapyramidal symptoms or for intention to distinguish manganism from IPD. However, levodopa therapy generally exhibits limited benefits in improving clinical symptoms among manganism patients. Alternatively, chelation therapy has been recommended for the purpose of expelling Mn ions so to reduce the body burden. Based on our own experience and data available in literature for several known metal chelating agents, dimercaptosuccinic acid (DMSA) has no effect on manganism patients. EDTA treatment in manganism welders has shown an increased Mn excretion in urine and a decreased Mn concentrations in blood, although the clinical symptoms appear to be unaffected. Extracellular distribution and a less selective chelation spectrum may restrict the efficacy of EDTA in treatment of manganism. Recently, an antibacterial drug, sodium para-aminosalicylic acid (PAS), has emerged as an effective drug in treatment of Mn-induced Parkinson-like disorder. PAS is an FDA-approved drug for treatment of tuberculosis. Clinical trials with PAS on severe manganism cases show that the drug not only reverses extrapyramidal syndromes immediately after drug treatment, but it also has a stable prognosis long after therapy, suggesting a possible repair of damaged neurons. The exact mechanism(s) of PAS therapy remain unknown. Based on animal studies, PAS appears to chelate and therefore remove the toxic metal ions from their intracellular deposits. Noticeably, the aspirin moiety in PAS structure may render the drug more preferable in combating against inflammatory processes, which are known to participate in induction and progression of neurodegeneration in Parkinson's disease. (Supported in part by U.S. NIH/NIEHS ES08146 and China NSF#30070663)



1334 APPROACHES TO ASSESSING WHETHER A CARCINOGEN IS ACTING VIA A GENOTOXIC MODE OF ACTION.

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Genetic toxicology data are used worldwide in regulatory decision-making. For example, US EPA and FDA use genetic toxicology information, such as the International Conference on Harmonization (ICH) genotox battery and the EPA mutagenicity testing scheme, for regulatory decisions including setting standards for acceptable exposure levels in air, water, and food, as well as approval and registration of pesticides, pharmaceuticals, medical devices, and medical-use products. This workshop focuses on genotoxic carcinogens, although the role of epigenetic changes in the etiology of human cancers is also important. Due to the limitations of commonly used genotoxicity tests, it may be useful to develop approaches, such as toxicogenomics, computational structure-activity-relationship, and genetically-engineered mammalian cell tests and mouse models, which will provide better information as to whether specific chemicals are genotoxic carcinogens or epigenetic carcinogens. To determine if chemicals act via a genotoxic or epigenetic mode of action, a weight-of-the-evidence evaluation of the available data including an analysis based on the Hill criteria, i.e., temporal and dose-response relationship, biological plausibility, etc., is recommended.



1335 THE NEED TO IMPROVE THE PREDICTIVITY OF *IN VITRO* GENOTOXICITY TESTS IN TERMS OF CARCINOGENIC POTENTIAL.

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Analysis of nearly 1000 chemicals tested for carcinogenesis in rodents in terms of published genotoxicity results from the most commonly used *in vitro* tests has shown an acceptable sensitivity but a very poor specificity (Kirkland et al, 2005,

Mutation Research 584, 1-256). The mammalian cell tests (mouse lymphoma mutation, chromosomal aberrations in hamster cell lines etc.) were particularly prone to giving positive results with non-carcinogens. More than 50% of mammalian cell genotoxicity tests with non-carcinogens gave positive results. Analysis of *in vitro* genotoxicity tests submitted to regulatory agencies (e.g. German BfArM) in support of pharmaceutical safety has shown similar high frequencies of positive results in mammalian cells with non-carcinogens. As long as these *in vitro* tests are so inaccurate in predicting rodent carcinogenicity they are of no help in assessing whether a carcinogen is acting via a genotoxic mode of action. The potential mechanisms for false positive results need to be better understood and, in the long term, more reliable assays are needed. Several initiatives (from organisations such as IWGT, ECVAM, ILSI/HESI) are therefore underway to:

1. improve the interpretation of genotoxicity results from existing *in vitro* assays and protocols;
2. determine whether lower top concentrations (for non-toxic substances) and lower levels of toxicity (for toxic substances) can be used and yet still allow the detection of all important *in vivo* genotoxins and DNA-reactive mutagenic carcinogens;
3. investigate whether new approaches to *in vitro* genotoxicity testing with p53 and DNA repair competent cells that are genomically stable can be achieved.



1336 A NEW STRATEGY TO IDENTIFY CHEMICALS THAT ARE MUTAGENS AND CARCINOGENS BUT NOT NECESSARILY MUTAGENIC CARCINOGENS.

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Currently mutagenic chemicals are identified using a number of well-validated mutation or genetic toxicology tests. The routine test battery was selected to be a sensitive indicator of mutagenic potential and to have the ability to detect chemicals inducing point mutation and/or chromosomal alterations. The ability to detect the full spectrum of genetic damage is important for hazard identification. The routine battery is heavily focused on *in vitro* assays. It is widely recognized that, while these *in vitro* assays are very sensitive to genetic damage, they may not always reflect the ability of the test chemical to cause genetic damage *in vivo*. The *in vivo* component of the routine battery is generally conducted in bone marrow and measures chromosomal damage, in particular micronuclei. When one moves past hazard identification to assess the mode of action (MOA) for carcinogens, it is important to determine whether the chemical can, in fact, cause mutation in the cancer target tissue. We propose a new strategy to help determine whether carcinogenic chemicals assessed to be mutagens or genotoxins in the routine battery are, in fact, causing cancer via a mutagenic or non-mutagenic MOA. The approach involves using transgenic rodent mutation models, chronic treatment, assessing time to mutation, and performing a quantitative comparison between the mutation and tumor dose response curves in the target tissue.



1337 IDENTIFICATION OF PRESUMPTIVE HUMAN CARCINOGENS AND THEIR MODE OF ACTION USING TOXICOGENETICS.

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Humans are a genetically diverse population with a variety of age, environmental, and behavioral factors that affect their risk and potential for developing complex genetic and environmentally related diseases, such as asthma, cardiovascular disease, cancer, etc. Remarkably, genes and their functions that are known to be critical to the development of neoplasia are highly conserved and function similarly in humans and rodents. Significant genetic diversity is present between inbred (homozygous) laboratory and wild-derived mouse strains that captures much of the genetic diversity in humans. We are using genetically engineered mouse models (GEMM) on different genetic backgrounds to anchor an induced phenotype to tumor development with reduced latency as well as genetically diverse inbred mouse strains with dense single nucleotide polymorphism (SNP) maps (Perlegen-NIEHS SNP maps) for defining haplotype structure. Using a sufficient number of inbred mouse strains, we will identify genetic loci associated with toxicity to genotoxic or non-genotoxic human carcinogens associated with neoplasia by haplotype association analysis. By identifying haplotypes in common between mouse strains and humans that segregate with the observed phenotype, we aim to determine predictability of toxicity phenotypes and induction of neoplasia between rodents and humans. With this knowledge, we can employ reductive approaches to determine the gene-specific mechanistic basis associated with an environmentally related disease. With the sequencing of the human, mouse, and rat genomes and a significant increase in our understanding of their genomic structure and the diversity of environmentally-responsive genes with specific single nucleotide polymorphisms, new tools for inves-

rigating biology and toxicology at the systems level have been developed. We have the potential to significantly improve our analytic and predictive potential for identifying both genetic and epigenetic mechanisms relevant to cancer and other complex diseases.



1338 MUTAGENIC CARCINOGENS AND THE U.S. EPA CANCER RISK ASSESSMENT PROCESS.

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Sponsor: A. Jacobs.

Genotoxicity covers a broad range of endpoints including mutations, sister chromatid exchanges, DNA damage and DNA repair. In contrast, mutagenicity is restricted to gene mutations and chromosomal mutations. For cancer risk assessment, mutagenicity is the appropriate endpoint because tumors are formed by a multi-step process characterized by specific genetic alterations. Mutations can be induced by direct interaction of a chemical with DNA or by indirect action with DNA (e.g., through the production of reactive oxygen species). In general, extrapolation from a LOEL for tumors (rodent or human) is deemed to be linear, unless specific mechanistic data are available. The more we learn about tumor induction, the more unlikely it becomes that a single mode-of-action (MoA) will produce the multiple key events that are required to convert a normal cell into a transformed one. For example, mutations induced by environmental chemicals are almost exclusively the result of replication errors. Enhanced replication will increase mutation rate, such that cytotoxicity and regenerative cell proliferation are components of MoA for a so-called "mutagenic carcinogen". Similarly, the production of ROS can also be considered a key event for a mutagenic MoA. The impact of these types of key event on tumor dose response curve shape has to be considered. Additional data from molecular approaches will better define the key events involved in the induction of tumors (rodent and/or human) by environmental chemicals. The U.S. EPA cancer risk assessment framework provides a clear picture of the data that are required to move from a default, linear extrapolation for mutagenic chemicals. The significant components are definition of mode(s)-of-action by considering the key events for tumorigenicity, the human relevance of these MoAs and the dose-response characterization for key events. The bar is quite high, but through the use genomic and other molecular techniques to identify informative biomarkers of response for key events, it is certainly feasible to move to more science-based risk assessments. (This abstract does not necessarily reflect U.S. EPA policy).



1339 FDA PERSPECTIVE ON GENOTOXIC CARCINOGENS.

A. Jacobs. *CDER, U.S. FDA, Silver Spring, MD.*

For a pharmaceutical product, the risk/benefit from a nongenotoxic carcinogen differs greatly from that from a genotoxic carcinogen, all other factors being the same. Sometimes a pharmaceutical product is positive in genotoxicity assays but the mode of action is not direct DNA reactivity. Thus, being positive in assays in the International Conference on Harmonization (ICH) genotoxicity battery and giving positive results in a carcinogenicity study does not mean that the mode of action was direct genotoxicity or even any mechanism relevant to humans. Only by a determination of the mode of action of the carcinogenicity could such an evaluation be made. Approaches to elucidating the mode of action of drug-related carcinogenic effects in rodents have included an understanding of the pharmacologic effects, evaluation of formation of DNA adducts, and the use of transgenic mutation models and transgenic carcinogenicity models to aid in distinguishing genotoxic from nongenotoxic mechanisms.



1340 CAN SPECIES AND STRAIN DIFFERENCES IN DIOXIN SENSITIVITY BE EXPLAINED BY DIFFERENCES IN THE AHR RECEPTOR?

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There are large differences in species- and strain-dependent susceptibility to dioxin (TCDD)-induced toxic responses. Classic examples of species differences in experimental effects include the 1000 to 5000-fold difference in dioxin-induced lethality in sensitive guinea pigs and resistant hamsters and dioxin-induced cleft palate where mice are more than 200 times more sensitive than rats or humans or conversely dioxin-induced developmental toxicity where mice more resistant than rats or hamsters. The toxicity of dioxin and related compounds involves initial binding to and activation of the AHR receptor (AHR) and multiple mechanisms of AHR signaling have been identified, such as: (1) AHR/ARNT gene regulation through

DNA enhancer XRE sequences; (2) AHR/ARNT interactions with coregulatory proteins (e.g., coactivators, corepressors, mediators) and other transcription factors (e.g., Rb, NF- κ B) and receptors (estrogen and others), and; (3) modulation of protein kinase signaling and ligand-independent AHR activation via phosphorylation. In addition, a number of species and strain specific AHR polymorphisms have been identified, for example in the ligand binding domain (LBD) and transactivation domain (TAD) that can affect AHR function and genetic loci have been recently identified that can also affect AHR mediated toxicity. This workshop will discuss the differences in the structure and function of the AHR and non Ahr genetic loci in different animal species and strains and their relevance to dioxin sensitivity. Molecular aspects of AHR signaling are conserved across other nuclear receptor pathways and therefore the issues discussed may have relevance in explaining differences in species and strain sensitiveness for other receptor mediated toxins.



1341 COMPARATIVE ASPECTS OF AHR LIGAND BINDING AND LIGAND-DEPENDENT AHR ACTIVATION.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD, dioxin) and related dioxin-like compounds (DLCs) produce a variety of toxic and biological effects, the majority of which are mediated by the Ah receptor (AhR), a ligand-dependent transcription factor. While TCDD and related DLCs can produce a variety of species- and tissue-specific toxic and biological effects, significant species and strain differences exist in the sensitivity and susceptibility to these chemicals. This variation in species responsiveness to TCDD and related DLCs can result from differences in many distinct physiological and biochemical characteristics in these species. Although the AhR present in various species has very similar characteristics, significant differences exist in AhR ligand binding affinity and specificity and ligand-dependent AhR transformation and DNA binding. Homology modeling and site-directed mutagenesis of the AhR ligand binding domain (LBD) provides a plausible explanation to account for some of this variation, particularly that of the lower binding affinity of human AhR and mouse AhRd alleles (which contain a naturally occurring polymorphism at Ala375 in the LBD). Additional amino differences exist between the AhR LBD of various species that may contribute to some of the observed variation in ligand binding and ligand-dependent AhR activation. Although DNA binding analysis reveals quantitative differences in TCDD-dependent transformation and DNA binding of hepatic AhR from various species, the nucleotide specificity of DNA binding of these TCDD:AhR complexes are similar. The relative transformation efficiency of AhR from various species appears to be both dependent upon differences in the AhR itself as well as regulatory actors, such as a small molecular weight heat-stable negative modulatory factor(s) that we have identified in cytosol. While multiple mechanisms certainly contribute to species differences in responsiveness to DLCs, some of these differences reside at the level of the AhR.



1342 COMPARATIVE TOXICOGENOMICS OF TCDD-MEDIATED HEPATIC RESPONSES: EVIDENCE FOR SPECIES-SPECIFIC AHR REGULONS.

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TCDD and related compounds elicit a broad spectrum of significant age, gender, strain and species-specific toxic and biochemical effects. Most, if not all, of these effects are due to alterations in gene expression mediated by the AhR. Although the importance of the AhR in TCDD and related compound toxicity is well established, the roles of differential gene expression in eliciting species-specific effects have yet to be elucidated. In order to further investigate differential gene expression in TCDD elicited hepatotoxicity, comparative temporal and dose-dependent toxicogenomic studies were conducted in human HepG2, mouse Hepa1c1c7 and rat H4IIE hepatoma cell lines and in hepatic tissue from Sprague Dawley rats, and C57BL/6 mice which were phenotypically anchored to histopathology, clinical chemistry and hepatic lipid accumulation. Data comparisons were facilitated through the use of comparable study designs, cDNA microarray platforms, and data analysis methods. Although TCDD induced similar increases in liver weight, significant differences in histopathology, clinical chemistry and hepatic lipid content were observed between Sprague Dawley rats and C57BL/6 mice, consistent with the marginal overlap of differentially expressed orthologous genes. Comparison of TCDD mediated gene expression responses between HepG2, Hepa1c1c7 and H4IIE cells also identified minimal conservation between orthologs, consistent with results from comparative computational DRE searches. Therefore, despite significant conservation in human, mouse and rat AhR biology, these data indicate that differences in TCDD elicited hepatotoxicity are mediated by different gene expression profiles suggesting the existence of species-specific AhR regulons. Funded by R01 ES12245 and Superfund P42 ES04911.

1343 VARIATION IN AH RECEPTOR STRUCTURE IN MOUSE, RAT AND HUMAN IN RELATION TO DIOXIN TOXICITY.

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Major toxic effects of dioxins are mediated by the AH receptor (AHR). Therefore variation in AHR structure/function can have substantial impact on dioxin susceptibility. In mice, an AHR polymorphism in the ligand-binding domain reduces binding affinity for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) about 10-fold in the DBA/2 strain vs the C57BL/6 strain; consequently, dose-response curves for numerous biochemical & toxic effects are shifted about one log to the right in DBA/2 mice. In the Han/Wistar(Kuopio) rat strain a polymorphism that deletes 38 or 43 amino acids from the AHR transactivation domain (TAD) is associated with a >1000-fold resistance to TCDD lethality. Genes in the conventional AH gene battery remain TCDD-responsive in H/W rats despite the large deletion in the TAD. The TAD deletion may selectively alter the AHR's ability to dysregulate particular genes that are key to dioxin toxicity. We use gene expression arrays in H/W rats vs dioxin-sensitive strains to identify genes whose dysregulation by TCDD underlie major dioxin toxicities. In human cells/tissues we found that the AHR's binding affinity for TCDD generally is lower than in cells/tissues of most standard laboratory rodent species & strains. Lower affinity for TCDD in humans may partially account for the apparent lower sensitivity of humans to the predominant types of toxicity observed in rodents. However, affinity varies more than 10-fold within the human population. Individual human samples that exhibit the highest affinity for TCDD have affinity similar to that in C57BL/6 mice. No polymorphisms have yet been identified in human AHR that explain the basis for variation in affinity. The effect of varied affinity on individual human susceptibility to dioxin toxicity also is unknown. The extant polymorphisms in the human AHR gene predominantly lie in the TAD but none of these seems to exert a substantial effect on receptor function assessed *in vitro*. However, it is possible, as in the H/W rat model, that structural variation in the human AHR may selectively affect only a limited set of specific responses.

1344 A NEW KNOCK-IN MOUSE MODEL FOR MECHANISTIC STUDIES OF DIOXIN ACTION.

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Han/Wistar (Kuopio; H/W) rats are extremely resistant to acute lethality of TCDD with an LD50 value of over 10,000 µg/kg. However, this resistance is response-dependent. Some biochemical and toxic effects of TCDD (dubbed type I effects) such as CYP1A1 induction, thymus atrophy and fetotoxicity are fully manifested also in H/W rats. Conversely, type II effects parallel acute lethality and display prominent interstrain differences; they include liver toxicity, wasting syndrome and tumor promotion. Breeding studies indicate that the peculiar selective responsiveness of H/W rats to TCDD emanates from two genes, by far the most important of which is the AH receptor (AHR). Wildtype rat AHR is 106 kDa in molecular mass but the H/W AHR is only ca. 98 kDa due to a point mutation in the first nucleotide of intron 10, which disrupts the exon/intron junction and results in 3 altered mRNA species that give rise to 2 different AHR proteins, both restructured at the transactivation domain. The deletion variant lacks 43 amino acids near the C-terminus, while the insertion variant (AHRsiv) is devoid of the last 38 amino acids but has 7 novel amino acids inserted at its C-terminal end. To gain further insight into the roles played by the AHR variants in TCDD responsiveness, we expressed wildtype and variant rat AHRs in mice. Their cDNAs were subcloned into modified pTransgene vectors carrying human elongation factor-1α promoter, chicken β-globin core insulators, 95 proximal nucleotides of the mouse AHR 5' leader and about 700 nucleotides from authentic rat AHR 3' UTR. The constructs were microinjected into C57BL/6J pronuclei, and construct-positive mice were crossed with AHR-deficient mice to eliminate the mouse innate AHR. Liver biopsies revealed a high expression level for all three constructs. Our pilot studies show that the transgenic constructs are functional *in vivo* and indicate that mice with the AHRsiv are resistant to acute lethality of TCDD. These studies provide important new evidence in support of the AHR's primary structure as a major determinant of susceptibility to dioxin toxicity.

1345 LOCI THAT INFLUENCE DIOXIN TERATOLOGY IN MICE.

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In an effort to understand how genes other than *Ahr* can influence individual sensitivity to environmentally induced disease, we performed a linkage analysis to identify murine loci that influence the incidence of cleft palate and hydronephrosis

in developing mice exposed to the pollutant 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin). Administration of 64 µg/kg dioxin to C57BL/6J (B6) dams at embryonic day 9 (E9) led to palatal clefting and hydronephrosis in nearly 100% of embryos by E17. In contrast, similar exposure of CBA/J (CBA) dams led to cleft palate in only 8% and hydronephrosis in 69% of embryos. To determine the genetic basis for this strain dependent sensitivity, linkage analyses on the progeny of a B6CBAF1 intercross and a CBAB6CBAF1 backcross were performed. The incidences of cleft palate and hydronephrosis were assessed and genomic DNA from embryos was analyzed at informative simple sequence length polymorphism (SSLP) markers. One locus segregating with dioxin-induced cleft palate was identified ($p < 0.01$) and designated as chemically mediated teratogenesis number 1 (Cmt1). The Cmt1 locus is located on chromosome 3.

1346 CURRENT CONSIDERATIONS IN SETTING EXPOSURE GUIDANCE FOR SENSORY AND RESPIRATORY TRACT IRRITATION.

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A fundamental aspect of worker and public health protection is the development of exposure guidance levels that protect exposed groups from critical adverse health effects. For many occupational exposures, exposures in emergency situations due to chemical releases, and for other acute exposures situations, inhalation is a primary exposure route of concern. There are numerous organizations and agencies that establish guidance levels for acute inhalation, and among the guidance levels that are developed sensory irritation is the single most common critical end point. Acute sensory irritation has been a central issue in setting occupational exposure guidance since the inception of the field. In addition, issues related to potential chemical exposure due to accidents, terrorism, and natural disasters has increased the emphasis on setting acute exposure levels for public health protection. Thus, health guidance related to irritation impacts risk management decisions for most workplaces, chemical emergency response events, and acute chemical releases to communities. Despite the importance of judgments about human irritation for public health protection, uniform guidance on many of the key evaluation and interpretation issues related to irritant response is lacking. The goal of this workshop is to present and discuss new developments in the fields of chemosensory irritation and risk assessment to inform methods for setting exposure guidance for irritants. Current and evolving drivers for exposure limit development and risk assessment practices for assessing irritant response are described. Several areas are highlighted where the science continues to evolve, including the developing methods for evaluating and interpreting human olfaction and irritant responses, approaches and research tools for interpreting animal findings, and application of new risk assessment techniques for setting human exposure guidance levels.

1347 ISSUES AND APPROACHES FOR SETTING HEALTH-BASED EXPOSURE LIMITS FOR IRRITANTS.

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Sensory irritation is a common effect on which to base exposure guidelines. Key issues addressed by the risk assessor for setting exposure guidance for irritants, include: whether the irritation should be considered an adverse effect; the application of animal test methods, such as the RD₅₀, to guidance setting; the extrapolation of irritation effects across time; the application of new dose-response methodologies, such as benchmark dose (BMD) modeling, to irritant evaluation; and the size of the uncertainty factor to apply in risk assessment, especially to protect sensitive subpopulations such as asthmatics. Strategies for standard setting based on irritation may depend on whether the effect is considered adverse in the traditional sense. Occupational and emergency standards strive to minimize but not necessarily eliminate irritation in their guidance levels. In contrast, ambient standards or reference levels are more likely to treat sensory irritation as an undesirable effect, if not an adverse effect. The RD₅₀ method has been shown to be correlated with occupational exposure levels and has been used as the basis for setting a number of standards. We have found that the RD₅₀ correlates with human exposure levels reporting minimal irritation, thus supporting its use in occupational and public standard setting. Extrapolation of adverse effects across time is often based on a function of concentration and time. However, recent analyses suggest the irritant effects are best described as a function of concentration, irrespective of time, after the first few minutes. Application of BMD modeling can provide insight on the protectiveness of the standard as well as the size of UFs to use in the analysis. Concentrations producing irritation in human and animal studies may be adjusted using uncertainty factors (UFs) such as 3 or 10. The UF of 3 is more of an average value, and may not be protective of the full population distribution, particularly if the point of departure is derived from animal data.

**1348 UNDERSTANDING THE MECHANISTIC BASES OF CHEMOSENSATION: OLFACTION AND SENSORY IRRITATION.**P. Dalton. *Monell Chemical Senses Center, Philadelphia, PA.* Sponsor: A. Maier.

Sensory irritation associated with the manufacture and use of volatile materials has been an occupational health concern for many years. Many organizations that establish exposure guidelines distinguish between olfaction and irritant responses. Because odor properties can often be detected at much lower concentrations than those capable of eliciting upper respiratory tract irritation, confusion between odor and irritation coupled with variability in odor sensitivity and response can produce significant obstacles for evaluating the potential for adverse effects or annoyance from worker or environmental exposures. Although rigorous research methods have been developed to accurately quantify sensory irritation in human evaluations, many studies fail to incorporate key elements necessary to adequately distinguish between olfactory vs. irritant responses. Specifically, research studies evaluating sensory irritation from volatile materials should be capable of (1) distinguishing between the annoyance or concern elicited by odor sensation and that elicited by true sensory irritation, (2) evaluating exposure-related factors that affect odor or irritancy responses, and (3) separating physiologically-mediated symptoms from psychogenically-mediated symptoms. This presentation will describe the state-of-the-science in our understanding of the biological bases for distinguishing between odor and irritation perception and the current measurement methods and techniques used for assessing each endpoint for the purpose of defining human response thresholds. Objective measures of ocular and upper respiratory tract irritation onset obtained in conjunction with subjective reports can lend valuable input to the decision process for determining occupational, community or emergency exposure limits. Distinguishing between the exposures that elicit local effects of sensory irritation in the eyes and upper respiratory tract and the exposures that elicit self-reports of irritation due to cognitive or emotional factors is a key component in establishing guidance protective of exposed individuals.

**1349 CONVERGENCE OF PSYCHOPHYSICAL AND PHYSIOLOGICAL METRICS FOR ASSESSMENT OF HUMAN SENSORY IRRITATION.**W. S. Cain. *Surgery (Otolaryngology), UCSD, La Jolla, CA.* Sponsor: A. Maier.

When exposure to an airborne substance reaches a point where a person can feel sensory irritation, some local physiological responses often accompany the sensation. For example, a brief burst of just threshold irritating vapor can cause a reflex response in an ocular muscle. The magnitude of the response, quantifiable by the movement of tiny targets on the skin, varies with the magnitude of stimulation and can therefore serve as a metric for it. Exposure may also cause the nose to run. A sponge placed for some seconds on the septum can absorb mucus commensurate with level of stimulation. The biochemical composition of the mucus can also represent the presence of irritation. An electrode placed on the septum can record a receptor potential with sensitivity almost identical with that measured psychophysically. Nasal resistance can increase with presence of an irritating vapor. The level of nitric oxide emitted from the nose may increase with exposure to an irritating vapor. The pattern of breathing may also change. All such effects offer a second view, beyond the perceptual response, but each presumably grants a different view. Some likely serve best as an index for just brief stimulation and some for longer stimulation. This presentation will illustrate the effects and highlight their potential usefulness as "tracks" for sensory irritation. Suitable indices should help us understand vulnerabilities to low-level irritating, or chemesthetic, effects of vapors, such as whether sensitivity varies with age, sex, previous exposure to contaminants, and existing condition of the airways, e.g., chronic inflammation. Their adoption in studies of exposure may help us converge on protocols that may bring some uniformity to decision-making about permissible levels of sensory irritation in the workplace and the environment.

**1350 INTEGRATING PATHOLOGY AND DOSIMETRY TO ASSESS THE TOXICITY OF INHALED IRRITANTS.**J. R. Harkema, S. A. Carey and J. G. Wagner. *Pathobiology, Michigan State University, East Lansing, MI.*

The objectives of this presentation are to 1) provide a brief overview of the comparative anatomy and physiology of the respiratory tract in laboratory animals and humans, and 2) discuss various methods used by the pathologist to assess injury and remodeling of the upper and lower conducting airways caused by exposure to airborne irritants. Examples of assessment will include methods at the macroscopic, microscopic and molecular levels of analysis. While the general organization of respiratory system is fundamentally the same in all mammals, there is sufficient species variation in the structure of the upper and lower respiratory tract and its cellular

makeup to produce considerable differences in response to inhaled irritants. The nature, severity and distribution of airway lesions caused by inhaled irritants are due to an integration of several factors including physicochemical characteristics of the inhaled irritant, exposure regimen, local airway dosimetry, and cellular susceptibility. Airway lesions induced by inhaled irritants are site specific and range from death of surface epithelial cells with concurrent acute inflammation, to remodeling of the airway wall due to intramural fibrosis and epithelial cell proliferation. A variety of state-of-the-art imaging techniques, such as magnetic resonance imaging, for visualizing macroscopic changes in airways, along with light, electron, and confocal microscopy for assessing cellular injury and tissue remodeling may be used by the pathologist. Examples will be provided on how computational models of geometrically correct airways are being used along with standard morphometric techniques to better define dose-response relationships along the respiratory tract of laboratory animals, and to estimate the risk of an inhaled irritant to human health.

**1351 EVALUATING IRRITANT RESPONSES FOR LIMIT SETTING IN EUROPE.**G. Johanson. *Work Environment Toxicology, Inst. of Environ Med., Karolinska Institutet, Stockholm, Sweden.*

For many airborne chemicals, and about one third of the occupational exposure limits (OELs), the critical effect is irritation. According to the guidance document developed by the Scientific Committee on Occupational Exposure Limits (SCOEL) of the European Commission, responses to irritants may be viewed as a continuum, ranging from no effects to serious health effects. In proposing health-based OELs SCOEL considers adversity/nuisance to occur somewhere between "very slight effects/awareness of exposure" and "slight irritant effects or nuisance/easily tolerable". The application of these criteria is often complicated by lack of data and/or different types of data for different chemicals. When available, SCOEL always considers reports of controlled human exposures, including objective (e.g. blink frequency, tear film stability, nasal swelling, inflammatory markers in nasal lavage) as well as subjective (symptom questionnaires, visual analog scales) measurements. As such studies have several limitations (e.g. costs, ethics, few subjects, selection bias, reporting bias), there is a need for additional, more easily obtained data, such as RD50s and lateralization (as presented by other speakers at this workshop). Although occasionally included in the SCOEL evaluations (e.g. RD50 data on formaldehyde, methyl isocyanate and phenol), such data have not yet been directly translated into health-based OELs.

The globally harmonized system for classification and labelling of chemicals (GHS) also has a number of qualitative criteria for respiratory irritation, according to which chemical may be classified as Category 3 (transient target organ effects). Efforts are now underway to integrate the GHS into the European legislation. Respiratory irritation is not an issue in the REACH legislation but may be included at a later stage.

New data on acute effects of ammonia, acetic acid and n-hexanal obtained in controlled chamber exposures of volunteers will be presented as case studies to illustrate how substances might be handled within the SCOEL and GHS frameworks.

1352 TRANSCRIPTOMIC ANALYSIS OF CARDIAC ENLARGEMENT IN FEMALE SD RATS TREATED WITH INSULIN SENSITIZER DRUGS.B. A. Rosenzweig, A. Knapton, P. Espandari, E. Herman and K. Thompson. *CDER, U.S. FDA, Silver Spring, MD.*

Thiazolidinedione (TZD) drugs for lowering insulin resistance induce a potential dose-limiting cardiac hypertrophy in patients. Similar effects can be observed in animal models. This study was initiated to identify gene expression markers of cardiotoxicity induced by the TZD rosiglitazone (ROSI) and the isoxazolidinedione PNU-182716 (PNU) in female Sprague-Dawley rats. Groups of 10 week old rats received daily doses of ROSI (80 mg/kg), PNU (800 mg/kg), 1% carboxymethyl cellulose, or water by gavage for up to 14 d. Heart, liver, and kidney tissues were collected for gene expression analysis. Affymetrix array analysis of the 7 and 14 d samples was performed to identify differentially expressed genes (DEGs). Treatment-induced changes in the expression of a subset of DEGs at 1, 3, 7, and 14 d were assayed using qRT-PCR. ROSI-treated rats had a statistically significant elevation in heart weight at 14 d that was not seen in PNU-treated groups, or at earlier time points. Although cardiac histopathology was normal in all groups, cytometric analysis showed evidence of cardiac hypertrophy in ROSI-treated rats. ROSI induced a greater effect on the phenotype of brown and white adipose tissue than PNU, which could be observed after 3 d of treatment and increased with dosing. mRNA levels of the cardiac hypertrophy markers ANF and BNP were significantly increased by ROSI, with maximal levels observed by 7 d. Little or no effect on these 2 genes was induced by PNU. ROSI and PNU both induced similar levels of

change in the cardiac expression of the PPAR target genes FABP4, RBP7, aquaporin 7, and monoglyceride lipase in rats dosed for 7 or 14 d. The induction in FABP4 expression was near maximal after 1 d of treatment with ROSI or PNU and remained relatively constant for the duration of the study. These results are in concordance with other lines of evidence that support an indirect mode of action in the induction of cardiac enlargement by TZD drugs and suggest that natriuretic peptides might be useful as biomarkers to detect the onset of the toxicity.

1353 EVALUATION OF CARDIOTOXICITY POTENTIAL OF THE CANCER THERAPEUTIC AGENT GLEEVEC BASED ON GENE EXPRESSION DATA IN RAT.

A. Vladimirova, D. N. Halbert and M. Fielden. *Iconix Biosciences, Mountain View, CA.*

Gleevec (Imatinib mesylate) is a small molecule protein tyrosine kinase inhibitor that inhibits the Bcr-Abl fusion protein implicated in chronic myeloid leukemia (CML). Recently this anti-cancer drug was reported to promote heart failure in some patients with no previous history of heart disease. A study in mice (Kerkela et al. *Nature Medicine*. 2006;12:908) determined that Gleevec led to significant deterioration of contractile function and ventricular dilation after 3-4 weeks of treatment. We administered Gleevec at 15 and 150 mpk for 1, 3 and 5 days in male SD rats and studied the patterns of gene expression changes in heart relative to controls and reference compounds. Gene expression data were analyzed using DrugMatrix™, a large toxicogenomic reference database that includes over 200 compounds profiled in heart in short term repeat-dose studies. Histopathological profiles of Gleevec-treated animals did not reveal myocyte degeneration or fibrosis, yet gene expression profiles reveal perturbations of stress-responsive genes as well as muscle contraction and development processes. The stress-inducible gene ATF3 was consistently induced over 10-fold relative to controls. Over-expression of ATF3 in hearts of transgenic mice has been shown to cause hypertrophy, reduced contractility and abnormal conduction, suggesting a role of ATF3 in Gleevec-induced cardiotoxicity. Pathway analysis also indicates modulation of genes involved in cardiac hypertrophy, cAMP and Ca++ signaling, and cardiac muscle contraction. Gleevec treatment induced MEF2, known to be activated by stress signaling and during pathological cardiac hypertrophy. We will also present findings that may implicate regulation of histone acetylation in the development of heart pathology. The pattern of gene induction is highly similar to that of idarubicin and epirubicin, known to be cardiotoxic. These results lead to hypotheses to explain Gleevec-induced heart disease and demonstrate that gene expression data may provide early insight into mechanism of toxicity in the absence of histopathological changes.

1354 TIME COURSE OF TRANSCRIPTIONAL CHANGES PRODUCED BY RETINOIC ACID IN THE ZEBRAFISH EMBRYONIC HEART.

J. Chen¹, S. A. Carney², R. E. Peterson¹ and W. Heideman¹. ¹Pharmaceutical Sciences Division, University of Wisconsin - Madison, Madison, WI and ²Skin Science Research, P&TD Technology, Kimberly-Clark Inc., Madison, WI.

All-trans retinoic acid (RA) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) both cause cardiac toxicity in developing zebrafish. Both agents produce functional and morphological heart defects that are ultimately associated with circulatory collapse. Furthermore, both RA and TCDD are thought to work by activating transcription factors. We were interested in determining whether the genes affected by RA overlap those affected by TCDD from the time of exposure to the point of heart failure. We characterized the effects of RA (1μM) exposure on embryonic heart morphology and function beginning at 72 hours post fertilization (hpf), and measured changes in heart mRNA abundance using AffyMetrix GeneChips. Cardiac toxicity was assessed at 2, 8, 12, 24, 30, and 48 hr after exposure and heart tissues were collected for microarray analysis at 1, 2, 4, and 12 hr after exposure. Self-Organizing Map clustering of the RA microarray results revealed 5 clusters of genes with distinct expression patterns over time, showing groups of genes up or down regulated immediately after RA exposure as well as groups that changed expression later in the course of toxicity. Early responses were consistent with homeostatic responses aimed at maintaining retinoid metabolism, as well as regulation of known retinoic acid receptor targets. Interestingly, RA and TCDD did not produce similar transcriptional responses at early time points, suggesting that they induce heart failure through distinct mechanisms. However, at late stages the RA and TCDD expression profiles revealed a common cluster of genes involved with chromosome assembly, cell cycle control, and DNA replication. The reduced expression of this common gene cluster may contribute to the impaired cardiomyocyte proliferation seen in both RA and TCDD treated embryos. We speculate that this may be a common response to agents that damage the developing heart. (Supported by NIH RO1 ES012716 and UW Sea Grants R/BT17, R/BT20 and R/BT22).

1355 ZEBRAFISH AS A MODEL FOR ENVIRONMENTAL TOXIN-INDUCED HYPOPLASTIC LEFT HEART SYNDROME.

A. C. Grimes^{2,1}, H. A. Stadt¹, P. Zhang¹, G. Hunter¹, K. Erwin¹, L. L. Satterwhite¹ and M. L. Kirby^{1,2}. ¹Duke University, Durham, NC and ²MUSC, Charleston, SC. Sponsor: E. Levin.

Hypoplastic left heart syndrome (HLHS) is a devastating congenital cardiovascular malformation of unknown etiology. In a recent epidemiological study, a cluster of HLHS occurring in the 1980's in the Baltimore-Washington area has been linked to the environmental release of dioxins and polychlorinated biphenyls (PCBs). These compounds are known to act through the aryl hydrocarbon receptor (AhR), but the mechanisms by which the AhR pathway elicits its toxic effects are poorly understood. Having previously shown evolutionary conservation in the cardiac out-flow tract of the zebrafish, we are utilizing this species and a model PCB (PCB126), to investigate mechanisms of toxicity of such compounds. We have found that cardiac tissues are specifically targeted in early development, leading to a phenotype we describe as 'stringy heart'. Prior to 24 hours post-fertilization (hpf), cardiac markers appear unaffected by PCB126 exposure, but by 72hpf, exposed fish exhibit extreme pericardial and yolk-sac edema, jaw malformations, a stretched, 'stringy' heart, a severely reduced bulbus arteriosus and diminished or absent blood circulation. By performing cell counts, we have found that, similar to cases of HLHS, PCB126 specifically affects ventricular, but not atrial, myocardium. Between 36 and 72hpf, there is a marked reduction in proliferation in affected tissues, as shown by BrdU incorporation, and the lack of a corresponding increase in apoptosis suggests cell-cycle arrest. This led us to hypothesize that the tumor suppressor protein, p53, acts downstream of the AhR to induce cell-cycle arrest and senescence. We have subsequently shown that inhibition of p53 activity, using a chemical suppressor, pifithrin-α, rescues the 'stringy heart' phenotype. Furthermore, we have found that levels of both p53 protein and mRNA are elevated following PCB126 exposure. This work validates the use of zebrafish for the study of environmental toxin-induced HLHS and is the first report of a potential mechanism by which activation of the AhR may lead to cardiac-specific toxicity.

1356 ASSESSMENT OF DRUG EFFECTS ON CARDIAC REPOLARIZATION IN ADULT ZEBRAFISH BY ECG.

C. Parng, W. L. Seng and P. McGrath. *Phylonix Pharmaceuticals, Inc., Cambridge, MA.* Sponsor: C. Zhang.

In this study, we assessed drug effects on cardiac repolarization by conventional ECG techniques adapted for performance on adult zebrafish. To validate this method, 10 well characterized antiarrhythmics from different classes (Class 1A: procainamide, disopyramide; Class 1B: lidocaine, and mexiletine; 1C: flecainide, and propafenone; Class II: propranolol, and metoprolol; Class III: dofetilide, and Class IV: verapamil) and two vessel dilators (clonidine and terfenadine) were tested. μ-conotoxin GIIIB (CTX), which has been shown not to affect zebrafish heart rate, was used to control zebrafish movement. Baseline heart rate before drug treatment was recorded and served as an internal control for each compound. The length of the PR interval, QT interval, QRS complex and ST segment were analyzed and compared. Normally distributed values were displayed as mean ± standard deviation (n=4). The mean of normally distributed continuous variables were compared using unpaired two-tailed t-tests. P values of 0.05 or less were considered significant. QT intervals were plotted against the associated RR intervals and fit to the equation $QT = QT_c * RR^\alpha$ by the method of least squares. The resulting equation was used to correct the observed QT interval for variations in heart rate. Our results showed that zebrafish ECG is highly reproducible. Zebrafish resting heart rate and conduction intervals are similar to those observed in adult humans. Drugs including procainamide, disopyramide, dofetilide, and terfenadine which cause QT prolongation in humans also resulted in QT prolongation in zebrafish. Lidocaine and mexiletine, causing QT shortening in human resulted in QT shortening in zebrafish. Propranolol and metoprolol, causing prolonged PR intervals in human, resulted in abnormal PR intervals in zebrafish. These results indicate that zebrafish can be a predictive model for drug induced cardiotoxicity in humans.

1357 HERG-LITE®: THE COMPLETE SCREEN FOR HERG RISK.

B. Wible, P. Hawryluk, Y. Kuryshv and A. Brown. *ChanTest, Inc., Cleveland, OH.*

Direct block of the hERG potassium channel by non-antiarrhythmic drugs (NARDs) is a major cause of QT prolongation and torsades de pointes (TdP). Recently, we showed that certain NARDs may produce hERG risk by inhibition of channel trafficking. HERG-Lite® (HL) is the only high-throughput, comprehensive assay for hERG risk that predicts whether compounds are hERG blockers, trafficking inhibitors, or both. This antibody-based chemiluminescent assay uses two cell lines to monitor hERG surface expression: hERG-WT (wild-type) is a biosensor for trafficking inhibitors while hERG-SM (single mutant) detects channel

blockers. To determine the accuracy with which HERG-Lite predicts hERG risk, we are screening an 1120 compound library from Prestwick Chemical Company in HERG-Lite and FASTPatch (FP), our automated patch-clamp system. To date, 611 compounds have been tested in both assays: HL at 1, 10 and 30 μ M and FP at 10 μ M. There is 90% agreement between the hERG assays with respect to hERG risk prediction. Of the 611 compounds screened, 338 (~55%) are null in both assays while 273 (~45%) were positive in one or both of the assays. Of those 273, 131 were predicted to be blockers in HL and have been confirmed in FP, 62 were identified in HL and FP as dual risk compounds, i.e. both direct block and trafficking inhibition, and 17 were shown to be trafficking inhibitors without block. Importantly, 29% of hERG blockers are also trafficking inhibitors. Overall the incidence of hERG trafficking inhibition in the Prestwick library is 12.9% making this an important consideration in hERG risk assessment. In this first round of screening, the incidence of HL false negatives for blockers was only 3.3% as 20 compounds showed greater than 15% hERG current inhibition in FP but no significant rescue of hERG-SM. Forty-three compounds (~7% of the total) rescued hERG-SM but were not detected as blockers in FP. These may be FP false negatives or they may be rescuing hERG-SM without block. If the latter possibility is correct, these compounds may be useful hERG "agonists" by increasing channel surface expression.

1358 BRADYARRHYTHMIA INDUCED BY OXIDANT ATMOSPHERE INHALATION IN CONSCIOUS RATS. A TELEMETRY STUDY IN HEALTHY AND MYOCARDIAL INFARCTED (MI) RATS.

J. Morin¹, S. Lioriot¹, D. Preterre², J. Henry¹ and F. Dionnet². ¹U644, INSERM, ROUEN, France and ²CERTAM, ROUEN, France. Sponsor: R. FORSTER.

Increasing evidences of cardiorespiratory responses to urban atmospheric pollutant have been published during the past few years. The oxidant potential of Diesel emissions has increased due to oxidation catalysis setup. This can be probed by the NO₂/NO_x ratio which correlates with the amount of reactive oxygen species to be generated during the test atmosphere contact with aqueous media containing CPH as a spin probe and assayed by electron spin resonance (ESR). We assess the impact of inhalation for 3 hours of diluted untreated (UDE) (low oxidant) and Oxycat treated (ODE) (high oxidant) Euro3 Diesel engine emissions and of Ozone on Heart rate variability (RMSSD) and arrhythmia occurrence. Beat to beat interval (RR) time series were obtained using the ECG-Auto (EMKA Paris France) software from continuous telemetry recordings (DSI international). Automated analysis procedure of arrhythmia identification and quantification have been developed using a dynamic symbol encoding process application on RR time series. UDE proved to decrease RMSSD in both healthy and MI rats, and to induce extrasystoles and tachyarrhythmia in MI rats only. ODE, and ozone induced a marked bradycardia (increased RR values), and bradyarrhythmia, with a latent period of 1-2 hours. These bradycardia and bradyarrhythmia events which were never observed in unexposed healthy nor MI rats led to increased RMSSD and Shannon entropy values signing most probable impacts on heart autonomic control. While no adaptative process was evidenced upon repeated exposure to UDE, with ODE and Ozone, maximal impacts where seen at first exposure and reduced impacts where seen after 3 daily exposures when increased lung and heart SOD and GPx activity levels were observed signing activation of antioxidant defenses.

In conclusion, inhalation of atmospheres containing oxidant pollutants induce numerous arrhythmias in both healthy and MI rats. These arrhythmia may be part of the mechanisms contributing to the growing evidences of atmospheric pollutant induced cardiac injuries.

1359 A NOVEL MURINE MODEL OF OCCUPATIONAL RHINITIS FOLLOWING INHALATION OF TOLUENE DIISOCYANATE.

V. J. Johnson, B. Yucsoy and M. I. Luster. *Toxicology and Molecular Biology Branch, NIOSH/CDC, Morgantown, WV.*

Diisocyanates are low molecular weight chemicals that have been identified as the leading cause of occupational asthma. Epidemiological evidence suggests that diisocyanate-induced rhinitis is a comorbid and often preceding condition in patients with occupational asthma. Understanding the pathogenesis of occupational rhinitis and its biological and chronological relationship with asthma will be critical for disease prevention. In this regard, we have developed a murine model of toluene diisocyanate (TDI)-induced rhinitis. Female C57BL/6 mice were exposed to TDI vapor via inhalation for 4 hours/day for 12 days with or without a 2 week rest period and TDI challenge. The nasal mucosa was examined after each experimental stage for alterations in cytokine expression and pathology. Mice exposed to TDI vapor for 2 weeks showed elevated expression of IL-4, IL-5, IL-13, IL-10 and IFN γ suggesting a mixed Th1/Th2 immune response. Expression of proinflammatory cytokines (TNF α and IL-1 β) and adhesion molecules (VCAM-1 and ICAM-1) was also up-regulated. These cytokine changes corresponded to a marked influx of inflama-

tory cells into the nasal mucosa, eosinophils being the predominant cell type. Removal from exposure for 2 weeks resulted in a reduction in cytokine expression and pathology to control levels. Subsequent challenge with TDI vapor resulted in robust upregulation of the same cytokine genes as well as eosinophilic inflammation. The present model shows that TDI inhalation induces allergic rhinitis displaying many of the cardinal features of human disease. Future work will use this model to define the immune mechanisms and also examine the temporal/dose relationship between TDI-induced rhinitis and asthma.

The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy.

1360 LEAD SKEWS THE DEVELOPMENT AND FUNCTION OF BONE MARROW-DERIVED DENDRITIC CELLS TOWARD THE TYPE-2 IMMUNE PHENOTYPE.

D. A. Lawrence, D. Gao and T. K. Mondal. *Wadsworth Center, Albany, NY.*

Although lead (Pb) has significant effects on the development and function of macrophages, B cells, and T cells and has been suggested to promote allergic asthma in mice and humans, Pb modulation of bone marrow (BM)-derived dendritic cells (BM-DC) and the resultant DC effects on Th1 and Th2 development had not been examined. Thus, we developed BM-DCs with murine granulocyte macrophage-colony stimulatory factor (mGM-CSF) $\pm$ PbCl₂ to evaluate any phenotypic changes of BM-DC derived in the presence of Pb (Pb-DC). Pb-DCs after ten days of development in vitro had significantly lower expression of CD11c. Although fewer CD11c+ DCs (% and MFI) were generated with Pb, Pb-DCs had significantly greater MHC class II (I-A) expression than the day-10 non-Pb-exposed DCs (DCs). However, these differences diminished upon LPS stimulation for two days. After LPS stimulation, CD80, CD86, CD40, CD54, and MHC-II were all up-regulated on both Pb-DCs and DCs, but Pb-DCs expressed significantly less CD80 than DCs. The CD86:CD80 ratio suggests a PB-DC potential for Th2 cell development and enhanced immunity, since CD80 has preferential interaction with CTLA-4, which affects immunosuppression. After LPS stimulation, IL-6, IL-10, IL-12 and TNF α levels significantly increased with both Pb-DCs and DCs, although Pb-DCs produced significantly less cytokines than DCs, except for IL-10, which further supports preferential skewing toward type-2 immunity by Pb-DCs. In vitro studies confirm that Pb-DCs have the ability to polarize antigen-specific T cells to Th2 responses. Pb-DCs also enhanced allogeneic and autologous T cell proliferation in vitro, and in vivo studies suggest that Pb-DCs inhibited Th1 effects on humoral immunity. The Pb effect was mainly on DC and not T cells, and its modification of DC function appears to be the main cause of its promotion of type-2 related immunity, which may relate to its enhanced activation of the Erk/MAP kinase pathway.

1361 CONTACT AND RESPIRATORY CHEMICAL ALLERGENS INDUCE DIFFERENTIAL CUTANEOUS CYTOKINE SECRETION PATTERNS.

M. Cumberbatch¹, L. Schneider¹, R. J. Dearman¹, I. Kimber¹ and C. E. Griffiths². ¹Syngenta CTL, Macclesfield, CHESHIRE, United Kingdom and ²Dermatology Centre, Manchester University, Manchester, United Kingdom.

Prolonged topical exposure of BALB/c strain mice to the contact allergen dinitrochlorobenzene (DNCB), or to the respiratory allergen trimellitic anhydride (TMA), promotes the selective development of type 1 and type 2 immune responses, respectively. The early events which may play a role in the initiation of polarized immune responses to different classes of chemical allergen are of particular interest. In the current experiments we have examined the kinetics of cutaneous cytokine expression induced following exposure of BALB/c mice to 1% DNCB or to 25% TMA. Expression of the proinflammatory cytokines tumor necrosis factor α (TNF- α) and interleukin (IL)-1 β and the anti-inflammatory cytokine IL-10 has been analyzed by cytokine protein array. Groups of mice (n=10) were exposed on the dorsum of both ears to chemical 30 min, 2h, 4h, 24h, 48h and 72h prior to preparation of dorsal ear explants for culture on medium for 16h. Exposure to DNCB was associated with a rapid increase in TNF- α secretion 2h following exposure that had declined by 4h, and a subsequent up-regulation of IL-1 β expression that was sustained from 4h to 72h. In contrast, treatment with TMA was associated with a very rapid induction of IL-10 (within 30 min) which did not return to basal levels until after 24h of exposure. At the later time points (24 to 72h), TMA stimulated increased expression of TNF- α and IL-1 β . Taken together with previous observations on the tempo and vigor of epidermal Langerhans' cell (LC) migration by chemical allergens, this suggests that treatment with DNCB provokes the cytokine milieu (TNF- α and IL-1 β) necessary for rapid and vigorous LC migration. In contrast, the early expression of IL-10 induced by exposure to TMA results in delayed kinetics of LC migration until later time points when the cytokine balance is in

favor of proinflammatory cytokine expression. These data suggest suggests that epidermal LC are important targets for the polarization of immune responses to chemical allergens.

1362 CHEMICAL RESPIRATORY ALLERGENS UP-REGULATE GRANZYME B GENE EXPRESSION.

H. T. Caddick, C. J. Betts, R. J. Dearman and I. Kimber. *Syngenta CTL, Macclesfield, CHESHIRE, United Kingdom.*

Selective T helper (Th) 1 and Th2 type responses are induced following repeated topical exposure of BALB/c strain mice to chemical contact and respiratory allergens, respectively. In previous experiments, gene expression profiles following exposure of mice to the chemical respiratory allergen trimellitic anhydride (TMA) have been analyzed using oligonucleotide microarrays. Mice received a single topical exposure of 10% TMA on the dorsum of each ear and auricular lymph node tissue was isolated 24 h to 120 h later. Of the 34,000 probe sets represented on the array, 21,000 were detected in lymph node tissue, but only 46 genes with statistically significant allergen-specific alterations were identified. The most strongly up-regulated gene was the T cell cytolytic molecule granzyme B. In the current experiments, changes in granzyme B expression in tissue isolated from mice treated with TMA, or with the contact allergen 2,4-dinitrochlorobenzene (DNCB), have been measured using real time PCR. Mice were exposed to 10% TMA, to 1% DNCB or to vehicle alone on the dorsum of both ears. Draining lymph nodes were excised 24 to 120h later, total RNA prepared and changes in gene expression quantified by real time PCR. Levels of granzyme B were normalized against the housekeeping gene HRPT and expressed as fold changes relative to naïve (untreated) controls. Treatment with TMA resulted in a marked increase in granzyme B expression (fold changes of 5.7+/-2.4, 20.7+/-1.9 and 9.0+/-0.9 induced 48, 72 and 96h after exposure, respectively, n=3). Less marked changes were observed for DNCB-activated cells (fold changes of 3.51+/-0.1, 5.6+/-0.7 and 3.11+/-1.0 induced 48, 72 and 96h after exposure, respectively). Less than 2fold changes were observed throughout the time course for tissue derived from vehicle-treated animals. Selective up-regulation of granzyme B gene expression has been observed following exposure to the respiratory chemical allergen TMA, consistent with recent observations that granzyme B may be the preferential mechanism for Th2 cell death.

1363 ASSESSMENT OF PROTEIN ALLERGENIC POTENTIAL IN MICE : RESPONSES TO ARA H 1, ARA H 2 AND POTATO LECTIN.

I. Kimber and R. J. Dearman. *Syngenta CTL, Macclesfield, CHESHIRE, United Kingdom.*

Assessment of the potential allergenicity of novel proteins is an important issue, particularly for those derived from genetically modified plants. We have shown previously that the measurement of specific IgE antibody production induced by systemic (intraperitoneal) exposure of BALB/c strain mice to a range of proteins correlates with allergenic potential. In the current investigations, immune responses to the purified peanut allergens ara h 1 and ara h 2 and to potato agglutinin, a material considered to lack allergenicity, have been compared. Specific IgE antibody was measured by homologous passive cutaneous anaphylaxis assay and specific IgG antibody measured by enzyme-linked immunosorbent assay. Intraperitoneal administration of peanut agglutinin (1%w/v) induced relatively vigorous IgG antibody production but failed to cause detectable IgE antibody production. Treatment with 1% w/v ara h 1 stimulated a comparatively weak IgG antibody response but nonetheless IgE antibody production was detected. In contrast, exposure to ara h 2 under the same conditions failed to induce either detectable IgG or IgE antibody. The relatively poor immunogenicity of both peanut proteins in the BALB/c strain mouse may be a reflection of prior dietary exposure to cross reactive soy proteins. Further experience with a wider range of proteins is required, but results to date are encouraging that this approach may constitute a method for the prospective identification of protein allergens, provided that lack of allergenicity (IgE) is only considered a secure negative when robust immunogenicity (IgG) is demonstrated.

1364 THE SENSITIZER NISO4 TRIGGERS THE PRODUCTION OF THE BIO-ACTIVE FORM OF INTERLEUKIN-12 BY HUMAN DENDRITIC CELLS.

D. Antonios, S. Kerdine-Römer and M. Pallardy. *Toxicologie, Faculté Pharmacie, University Paris-Sud, Chatenay-Malabry, France.*

Dendritic cells (DCs) are professional antigen presenting cells which capture and process antigens such as invading bacteria, viruses and haptens. After antigen uptake, DC undergo a maturation process leading to the upregulation of surface markers and production of cytokines. We and others have already shown that NiSO₄, a metallic hapten, induces the maturation of human DCs, in vitro.

In this work, we address the question whether NiSO₄ by itself or in synergy with other signals can induce the secretion of Interleukin-12 (IL-12) by human DCs derived from CD34+ progenitor cells (CD34-DC) or from human monocytes (Mo-DC).

In CD34-DC, NiSO₄ alone induces the secretion of IL-12p40 but not the production of IL-12p70, the bio-active form of IL-12. The production of IL-12p70 induced by NiSO₄ in CD34-DC needs a complementary signal provided by IFN-gamma. IL-1 beta, a proinflammatory cytokine, synergizes with both signals to augment the production of IL-12p70. We then analysed the expression of IL-12p35 mRNA using RT-PCR and found that IL-12p35 mRNA was expressed only in the presence of NiSO₄ and IFN-gamma and further enhanced with the addition of IL-1 beta.

Mo-DC activated with NiSO₄ alone produce a high level of IL-12p40. The presence of IFN-gamma is sufficient for a strong induction of IL-12p70 showing differences with CD34-DC. We also show that the bacterial lipopolysaccharide (LPS) can act in synergy with NiSO₄ to induce the production of IL-12p70.

These results suggest first that NiSO₄ alone is able to induce the production of high levels of IL-12p40 in human DCs but no detectable amounts of IL-12p70 are measured. Second, IL-12p70 production induced by NiSO₄ in both models needs the presence of IFN-gamma. Finally, a bacterial product such as LPS in combination with NiSO₄ produce high levels of IL-12p70 in Mo-DC. Signalling pathways induced by these different combinations are still to identify.

1365 PHARMACOLOGICAL DIFFERENTIATION OF EARLY AND LATE PHASE ASTHMA-LIKE RESPONSE IN TRIMELLITIC ANHYDRIDE (TMA) SENSITIZED AND CHALLENGED BROWN NORWAY RATS.

X. D. Zhang and P. D. Siegel. *HELD, NIOSH/CDC, Morgantown, WV.*

Trimellitic anhydride (TMA) is a low molecular weight chemical that may induce specific-IgE and occupational asthma. We have previously demonstrated that dermal exposure to dry TMA powder with subsequent TMA challenge produces asthma-like early (EAR) and late airway responses (LAR) in Brown Norway rats (BNR), as indicated by Penh (an index of airway resistance). Potential mediators involved in LAR or EAR were examined, using selective pharmacological inhibitors, in the present study. Eight BNR were sensitized by 4, weekly, 4 hr dermal exposures to 40 mg dry TMA powder. Two weeks following the final dermal exposure, BNR were given a 10 min, 40 mg/m³ TMA nose-only aerosol challenge. Pharmacological agents were administered either prior to and/or 1 hr after EAR, and Penh was recorded every 0.5 min for 16-20 hrs. Results: Dexamethasone (2 mg/kg, i.p.) inhibited TMA induced EAR or LAR in TMA sensitized and challenged BNR. Intranasal steroid, fluticasone, (10µl/nostril, daily for 3 days before airway challenge) did not inhibit EAR and LAR, suggesting that the Penh changes were not due primarily to upper airway responses. An aerosol of 1% Salbutamol, a β₂ agonist neither prevented or reversed the EAR or LAR. Both Ketanserin, (10 mg/kg, i.p.) a 5-HT₂-receptor antagonist, and Cetirizin, a H1-blocker, (30 mg/kg, i.p.) administered 1 hr after TMA challenge inhibited the subsequent LAR. Montelukast (10 mg/kg, i.p.), a leukotriene receptor antagonist, inhibited EAR, but not LAR, when administered either before or 1 hr after challenge. The adenosine antagonist, aminophylline (30 mg/kg, i.p.) also inhibited LAR in some, but not all treated/challenged rats. Selective pharmacological inhibition of mediators demonstrates different pathophysiological mechanisms of EAR and LAR for the asthma-like responses in the BNR TMA model.

The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent agency determination or policy.

1366 OPTIMIZATION OF THE HUMAN CD34+-DENDRITIC CELLS MODEL FOR DETECTION OF CHEMICAL SENSITIZERS.

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Allergic contact dermatitis is a delayed-type hypersensitivity reaction caused by exposure to contact allergens. Animal assays are currently used to identify the sensitizing potential of new chemicals. However, such studies must be replaced by alternative methods in a near future due to public concern for using animal testing. Our laboratory is part of a national project launched by AFSSAPS (French Medicinal Products Agency) to develop in vitro models for allergy prediction. Due to the variability of different dendritic cell (DC) and langerhans cell (LC) models described in the literature, our initial goal is to characterise and optimize the CD34+-DC model to obtain a robust test system. Another laboratory will optimize the monocytes-DC system in view of comparing both models. CD34+-DCs were cultured in RPMI medium supplemented with 10% of foetal bovine serum (FBS) in the presence of GM CSF (200 U/ml) and TNF-alpha (50 U/ml), as well as Flt3-L (50 ng/ml)

and/or SCF (50 ng/ml), two cytokines needed for cell proliferation. Our results show that the association of Flt3-L and SCF versus Flt3-L or SCF alone increases by two-fold the number of DCs at day 7. The addition of GM-CSF and TNF- α at day 4 and 7 was tested and the phenotype was determined (CD1a, CD14, CD34, langerin, CD83, e-cadherin, CLA, HLA-DR, CD40, CD86). These DCs do not express langerin and e-cadherin. On the other hand, CD34⁺-DC cultured with TGF- β (10 ng/ml) added at day 0 and 5 express langerin, e-cadherin and CLA, different markers of the LC. Moreover, the effect of substituting serum AB (SAB) to FBS in culture was tested. Culturing DC in SAB did not improve differentiation especially with TGF- β since there was an increase in CD14⁺ cells, and a decrease in langerin, e cadherin, and CLA expressions.

Finally, the two models (DCs and LCs) have been tested with prototype haptens such as nickel sulfate and dinitrochlorobenzene to induce maturation, and their functionality has been evaluated using a mixed lymphocyte reaction and allogeneic T-lymphocytes.

1367 CYP1B1 AND EPHX1 ARE ASSOCIATED WITH P53 ACTIVATION AND SUPPRESSION OF HUMORAL IMMUNITY BY 7, 12-DIMETHYLBENZ(A)ANTHRACENE(DMBA) IN C57BL/6N MICE.

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7,12-Dimethylbenz(a)anthracene (DMBA) is a potent carcinogen that induces immunosuppression of both humoral and cell-mediated immunity in mice and other species. It is widely accepted that DMBA is metabolically activated by phase I enzymes to reactive intermediates. Our previous studies have shown that P450 CYP1B1 and EPHX1 (microsomal epoxide hydrolase, mEH) appear to be important enzymes in the metabolic activation of DMBA. Certain metabolites of DMBA may cause DNA damage leading to immunosuppression. The tumor suppressor protein p53 plays a pivotal role in the cellular response to the DNA damage and in the maintenance of genomic stability. Previous studies have shown that DMBA-induced bone marrow toxicity is dependent on the activation of p53 (Page et al. *Tox Sci* 74, 85-92, 2003). Therefore, the purpose of the present studies was to determine whether p53 activation is associated with DMBA-induced suppression of humoral immunity. Female C57BL/6N wild-type (WT), CYP1B1 ^{-/-}, EPHX1 ^{-/-}, and AhR ^{-/-} mice were treated with 0, 17, 50, or 150 mg/kg (cumulative dose) DMBA in corn oil by oral gavage once a day for 5 days. Western blot and flow cytometry assays were used to assess p53 levels in mouse splenocytes. Following 5 days of DMBA treatment, total p53 levels were significantly increased in the nuclear portion of spleen cell lysates in all of the DMBA treated WT mice in a dose-dependent manner, as compared to control mice. Western blots showed that p53 was activated by phosphorylation at Ser15. In contrast, the total p53 and phospho-p53 (ser15) levels in both CYP1B1 ^{-/-} and EPHX1 ^{-/-} mice showed no significant p53 activation. AhR ^{-/-} mice demonstrated the same response as WT mice; therefore, AhR does not appear to play a role in DMBA-induced p53 activation. In summary, metabolic activation of DMBA by CYP1B1 and EPHX1 appears to be important in p53 signaling and subsequent immunosuppression. These effects are AhR-independent. This work was supported in part by NIH RO1 ES05495.

1368 RESVERATROL (TRANS-3, 5, 4'-TRIHYDROXYSTILBENE) PROTECTS T CELLS FROM 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)-INDUCED APOPTOSIS *IN VITRO* AND REDUCES TCDD-INDUCED THYMIC APOPTOSIS *IN VIVO*.

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Resveratrol, a compound found in red grapes, acts as an antagonist of aryl hydrocarbon receptor (AhR) and blocks AhR-mediated signaling. In the present study, we examined whether resveratrol would prevent TCDD-induced immunotoxicity *in vitro* and *in vivo*. For *in vitro* study, we cultured primary T cells from C57BL/6 mice in the presence of ConA for 24 hrs, treated with TCDD (100-1000 nM) in the absence or presence of resveratrol for 24 hrs, and determined apoptosis. Similar studies were also performed with ConA-activated EL-4 T cells. We observed significant reduction in TCDD-induced apoptosis in both primary T cells and EL4 cells in the presence of resveratrol *in vitro*. Next, we examined whether resveratrol would neutralize the effects of TCDD *in vivo*. We treated C57BL/6 mice with TCDD (10 mg/kg body weight) before or after resveratrol treatments. On day 4-5, thymuses of treated mice were characterized. We observed significant reduction in thymus size and weight loss in mice treated with TCDD when compared to mice that were treated first with resveratrol and then TCDD, demonstrating effectiveness of resveratrol in neutralizing TCDD-induced thymic toxicity. We also observed TCDD-induced upregulation of CYP1A1 expression in thymic cells but significantly reduced

CYP1A1 expression in the presence of resveratrol. Furthermore, upon examination of apoptosis in thymic cells, we observed significant TCDD-induced apoptosis but reduced apoptosis in the presence of resveratrol. Resveratrol was also effective in neutralizing TCDD-induced thymic toxicity in mice that were first exposed to TCDD and then treated with resveratrol. Taken together, our data demonstrate that resveratrol being a natural product can be used as a prophylactic agent to neutralize the toxic effects of TCDD (This work was supported in part by NIH grants R01AI053703, R01ES09098, R01 AI058300, R01DA016545, R01HL058641).

1369 EXAMINING THE GENETIC SUSCEPTIBILITY TO THE IMMUNOTOXICITY OF A TRICHLOROETHYLENE METABOLITE.

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MRL ^{+/+} mice exposed to occupationally relevant doses of the environmental contaminant trichloroethylene (TCE) in drinking water had been shown to develop autoimmune disease associated with the expansion of activated apoptosis-resistant CD4⁺ T cells. Subsequent experiments tested whether the effect of TCE on CD4⁺ T cells was mediated by its oxidative metabolite, trichloroacetaldehyde hydrate (TCAH). The genetic susceptibility to TCE/TCAH was also examined by comparing the response of autoimmune-prone MRL ^{+/+} mice to genetically-related, but not autoimmune-prone, C3H/HeJ mice.

Female MRL ^{+/+} mice exposed to TCAH for 40 weeks in drinking water developed skin inflammation and hair loss similar to autoimmune alopecia. CD4⁺ T cells in the TCAH-treated MRL ^{+/+} mice were resistant to activation-induced apoptosis, and expressed decreased levels of FasL, a cell surface molecule that mediates apoptosis. A matrix metalloproteinase that cleaves FasL from the cell surface, MMP-7, was increased in these mice.

Unlike MRL ^{+/+} mice, C3H/HeJ mice treated with TCAH for 40 weeks did not demonstrate alopecia or increased levels of MMP-7. Correspondingly, the CD4⁺ T cells from the TCAH-treated C3H/HeJ mice were not resistant to activation-induced apoptosis and expressed normal levels of FasL.

These results suggest that TCE works via its metabolite TCAH to inhibit activation-induced apoptosis in the CD4⁺ T cells of susceptible mouse strains. This effect may promote autoimmunity by suppressing the deletion of activated self-reactive T cells. Genetic susceptibility to the immunotoxicity of TCE/TCAH may reflect a propensity to produce metalloproteinases. This work was supported by the EPA and the Arkansas Biosciences Institute.

1370 EVIDENCE FOR IMMUNE DYSREGULATION AND POSTNATAL AUTOIMMUNE DISEASE FOLLOWING DEVELOPMENTAL EXPOSURE TO TCDD.

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Immune development is altered following perinatal exposure to the environmental immunotoxic contaminant 2,3,7,8-tetrachlorodibenzo-dioxin (TCDD). Furthermore, the effects from such exposure may be more dramatic or persistent than following exposure into adulthood. Whether perinatal TCDD exposure predisposes or enhances postnatal autoimmune disease is unknown. To address this, we conducted a study to evaluate the influence of perinatal TCDD exposure on 24 week-old offspring of high affinity AhR C57BL/6 mice and low AhR affinity autoimmune-prone NZB X SWR (SNF1) mice. Time-pregnant C57BL/6 (10 dams/treatment) and time-pregnant NZB X SWR (10 dams/treatment) female mice were gavaged on gestational day (GD) 12 with a single dose of 0, 5 or 10 μ g/kg and 0, 40 or 80 μ g/kg, respectively. Examination of the splenic phenotypes showed that perinatal exposure to TCDD caused marked increase in the percentage of CD8⁺ T cells in C57BL/6 splenocytes compared to controls. TCDD perinatal exposure also generated a marked increase in the percentages and total cellularity of autoreactive V β 3⁺ and V β 17⁺ T cells in C57BL/6 spleen. However, no significant changes in autoreactive V β 3⁺ and V β 17⁺ T cells were observed in SNF1 spleens. Interestingly, a high percentage of autoreactive V β 3⁺ and V β 17⁺ T cells were seen in the SNF1 control lymph nodes and even higher concentrations observed in the TCDD-exposed SNF1 mice. Moreover, splenocytes from C57BL/6 mice exposed perinatally to TCDD showed elevated levels of marginal zone B cells characterized by an increase in expression of CD1high/CD23neg-low marker while no significant phenotypic changes were observed in SNF1 splenic B cells. Furthermore, both strains showed no significant changes in the phenotypic expression of CD4 and

CD8 markers in thymocytes when compared to controls. These results suggest that perinatal exposure to TCDD may lead to immune dysregulation and postnatal expression of autoimmunity.

1371 THE EFFECTS OF TCDD ON THE FATE OF NAÏVE DENDRITIC CELLS.

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Exposure to the environmental contaminant TCDD (dioxin) causes suppressive effects on the immune system, presumably via activation of the Aryl hydrocarbon receptor (AhR). Dendritic cells (DCs), professional antigen-presenting cells in the immune system, express AhR although the level of expression depends on the maturational state of the DCs. We hypothesize that TCDD-induced AhR activation causes a loss in DC function and/or survival in unimmunized animals. C57BL/6 mice were gavaged with either vehicle or TCDD (15 µg/kg). Immune cells from the spleens, and popliteal and brachial lymph nodes (PBLNs) were harvested 7 days post-treatment, and analyzed for phenotypic biomarkers. Two distinct subpopulations of splenic DCs, the CD11c high (mature) and CD11c low (immature) cells, were identified by flow cytometry. The frequency and number of splenic CD11c high DCs was decreased in TCDD-exposed mice when compared to vehicle-treated controls. TCDD increased the expression of CD40 and CD86 on the CD11c high DCs in the spleen while their expression of CD11a and MHC class II was decreased. No significant TCDD effects were observed for the CD11c low DCs in the spleen. Loss of splenic DCs was apparently independent of Fas- or TNF-mediated apoptosis, as mice deficient for Fas, FasL or TNF receptors also exhibited a significant loss of splenic CD11c high DCs after TCDD exposure. In contrast, an increase in the number of CD11c+ cells was observed in PBLNs of TCDD-treated C57BL/6 mice. This study demonstrates the presence of a TCDD-sensitive (CD11c high) DC subpopulation in the splenic tissue of naïve mice. Studies are underway to determine if DC loss in the spleen following TCDD-exposure is attributable to enhanced apoptosis or migration. Collectively, these results suggest that TCDD may induce immune suppression in mice by disrupting DC homeostasis. Supported by NIH grants RR17670 and ES013784.

1372 ARYL HYDROCARBON RECEPTOR (AHR) IS REQUIRED FOR OPTIMAL ANTI-LISTERIA RESISTANCE.

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Listeriosis, caused by *Listeria monocytogenes*, is an important food-borne disease that causes considerable morbidity and a relative high mortality rate. *L. monocytogenes* is a prototypic intracellular pathogen that can multiply to great numbers within infected cells (hepatocytes and macrophages). Both innate and adaptive immune responses are important in controlling listerial infection. The aryl hydrocarbon receptor (AhR) is part of a powerful signaling system in response to xenobiotic agents such as polychlorinated hydrocarbons (PCHs) and polycyclic aromatic hydrocarbons (PAHs). There is evidence that it also regulates normal developmental and immune responses. For example, AhR activation can lead to release of TNF-α, whose beneficial role in resistance to listeriosis has been shown previously. In this study, we tested two hypotheses: (1) constitutive expression of AhR is important for resistance to listeriosis and (2) activation of AhR can enhance the killing of intracellular *L. monocytogenes*. Our data demonstrate that AhR null mice are more susceptible to listeriosis than AhR heterozygous mice when inoculated i.v. with log-phase *L. monocytogenes*. In the AhR responsive TIB73 murine hepatocyte cell line, activation of AhR by β-naphthoflavone (BNF) significantly reduced the intracellular burden of *L. monocytogenes* in infected cells. We are in the process of using siRNA for AhR to knock down AhR mRNA in TIB73 cells to further explore the role of AhR in restricting intracellular *L. monocytogenes*. The relationship of TNF-α, reactive oxygen species, and nitric oxide to AhR-mediated anti-*Listeria* resistance in vivo, and inhibition of intracellular growth of *L. monocytogenes* in vitro, are also being explored. Taken together, these data suggest an important role for the AhR in innate immunity to *L. monocytogenes* infection.

1373 GW7845, A PPAR AGONIST, INDUCES MITOCHONDRIA-DEPENDENT APOPTOSIS IN PRO/PRE-B CELLS.

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Apoptosis is a critical event in the deletion of lymphocytes prior to their migration to the periphery; thus pro/pre B cells also may be particularly susceptible to inappropriate apoptotic stimuli. Both malignant and normal B cells are highly susceptible

to apoptosis induced by structurally diverse peroxisome proliferator-activated receptor γ (PPARγ) agonists, including GW7845. Previously, we have shown that treatment of cultured pro/pre-B cells (BU-11) with GW7845 (40 µM) results in cytochrome c release. Here, we investigated the role of mitochondria in GW7845-induced apoptosis and the mechanisms leading to mitochondrial membrane alterations. The essential role of the intrinsic apoptotic pathway in GW7845-induced apoptosis is supported by the fact that this apoptosis was reduced significantly in APAF1 null primary pro-B cells. The effect of several permeability transition pore inhibitors on GW7845-induced mitochondrial membrane potential loss and apoptosis was tested. While bongkreic acid and atractyloside had no effect on these parameters, cyclosporin A (CsA) modestly reduced the loss of membrane potential and formation of a sub-G0/G1 population. However, CsA did not consistently prevent release of cytochrome c. Calcium played a significant role in the loss of mitochondrial membrane potential, as BAPTA, a calcium chelator that inhibits GW7845-induced apoptosis, reduced the loss to nearly background. However KN-93, an inhibitor of CaMKII that also inhibits GW7845-induced death, effected only a modest reduction in membrane potential loss. These results suggest that calcium may be initiating two separate pathways that influence the mitochondria. Finally, initial results suggest that activation and translocation of Bax is the primary mediator of cytochrome c release and the initiator of the intrinsic apoptosis pathway. Considering that PPARγ agonists are being investigated for potential chemotherapeutic use, it is important to understand the mechanisms by which they induce apoptosis in normal cells to minimize adverse effects.

1374 PLUMBAGIN-INDUCED APOPTOSIS IN LYMPHOCYTES IS MEDIATED THROUGH INCREASED REACTIVE OXYGEN SPECIES PRODUCTION AND ACTIVATION OF THE CASPASE CASCADE.

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Impure extracts from plants containing plumbagin (PLB) have been shown to suppress the immune response. However, most of these claims are supported only by anecdotal evidence with few scientific reports describing the mechanism of action or the efficacy of plumbagin in the suppression of the immune response. In the current study, we tested the hypothesis that plumbagin-induced suppression of the immune response was mediated through the induction of apoptosis. Splenocytes from C57BL/6 mice cultured in the presence of 0.25 µM or greater concentrations of PLB showed significantly reduced proliferative responses to mitogens, including anti-CD3 mAbs, Concanavalin A, lipopolysaccharide and SEB in vitro. Exposure of naïve and activated splenocytes to > 0.25 µM PLB led to a significant increase in the levels of apoptosis. PLB treatment led to a significant increase in the levels of reactive oxygen species (ROS) in naïve and activated splenocytes. Furthermore, treatment with the ROS scavenger, N-acetylcysteine (NAC), prevented PLB-induced apoptosis, suggesting a role of ROS in PLB-induced apoptosis. PLB exposure led to activation of caspase-8, and caspase-9, as well as loss of mitochondrial membrane potential. PLB activation of the caspase cascade was dependent on ROS generation as treatment with NAC prevented the activation of caspase-8 and caspase-9. Finally, treatment of mice with PLB (5mg/kg) led to thymic and splenic atrophy as well as a significant suppression of the response to SEB and DNFB in vivo. Together, these results suggest that plumbagin has significant immunosuppressive properties which are mediated by the induction of apoptosis.

1375 BLOOD GENE EXPRESSION SIGNATURES PREDICT EXPOSURE LEVELS.

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To respond to potential adverse exposures properly, health care providers need accurate indicators of exposure levels. This is particularly important in the case of acetaminophen (APAP) intoxication, the leading cause of non-viral liver failure in the U.S.

We hypothesized that gene expression patterns derived from blood cells would provide useful indicators of acute exposure levels. To test this hypothesis, we used a blood gene expression data set from rats exposed to APAP to train classifiers in two prediction algorithms and to extract patterns for prediction using a profiling algorithm. Prediction accuracy was tested on a blinded, independent rat blood test data set and ranged from 88.9 to 95.8 %. Genomic markers outperformed predictions based on traditional clinical parameters. The expression profiles of the predictor

genes from the patterns extracted from the blood exhibited remarkable (97% accuracy) trans-tissue APAP exposure prediction when liver gene expression data was used as a test set. Analysis of human samples revealed separation of APAP-intoxicated patients from control individuals based on blood expression levels of human orthologs of the rat discriminatory genes. The major biological signal in the discriminating genes was activation of an inflammatory response after exposure to toxic doses of APAP. These data and results support the hypothesis that gene expression data from peripheral blood cells can provide valuable information about exposure levels, well before liver damage is detected by classical parameters. It also supports the potential use of genomic markers in the blood as surrogates for clinical markers of potential acute liver damage.

1376 GENOMIC ANALYSIS SUGGESTS HIGHER SUSCEPTIBILITY OF CHILDREN TO AIR POLLUTION.

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Exposure to environmental pollutants early in life is of increasing concern because of the risks it poses for development of e.g. cancer. Differences in response to and health effects of hazardous airborne exposure between children and adults have been reported, with children apparently being more susceptible. The aim of the study was to improve our understanding of differences in susceptibility to air pollution between children and adults by comparing genome-wide gene expression profiles in healthy children and their parents. Human oligonucleotide microarrays representing sequences for more than 20,000 genes were used to analyze gene expression in blood samples from 47 children and their parents inhabiting two areas in the Czech Republic with different levels of ambient air pollution. Overrepresentation of biological pathways, processes and functions was analyzed by EASE and the new tool T-profiler using either pre-selected lists of significantly differentially expressed genes or using the total dataset without pre-selection to gain a genome-wide overview of the transcriptomic response. As a phenotypic endpoint of genotoxic effects micronuclei frequencies were analyzed. Gene expressions were investigated for correlation with micronuclei frequencies in order to distract effects on pathways relevant for environmental carcinogenesis, from the whole genome response. All analysis identified defined lists of genes and/or significantly overrepresented biological pathways. More genes and pathways were responsive in children than in parents and significance of specific pathways in children but not in adults suggest differences in susceptibility to air pollution at the transcriptome level.

1377 GENE EXPRESSION PROFILING OF HUMAN BRONCHIAL EPITHELIAL CELLS EXPOSED TO SINGLE-WALLED CARBON NANOTUBES.

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Single-walled carbon nanotubes (SWNT) are nano-scale tubes with impressive electrical and thermal properties, which make them attractive for numerous applications. Early reports of SWNT toxicity by dermal absorption and inhalation in several mammalian species are not conclusive. In an effort to explore the potential harm of SWNT to humans, a combined systems biology and toxicogenomics approach was applied. Normal human bronchial epithelial (NHBE) cells cultures were treated *in vitro* with SWNT. Gene expression profiles were obtained at 0 and 24 hours after treatment and compared to those obtained with silica (SiO₂) and carbonyl iron (CI) as positive and negative controls respectively. Cell pellets were snap-frozen and stored at -80°C. Biotinylated cRNA probes were synthesized from total RNA isolated from the cell pellets and hybridized onto CodeLink™ microarrays containing oligomers from 19,912 unique human genes. Statistics showed high reproducibility among triplicate arrays (maximum CV=7%). Hierarchical agglomerative clustering performed from the top 100 significantly-expressed genes, as well as Principal Components Analysis (PCA), showed that SWNT and CI gene expression profiles were similar to each other but different to SiO₂. Furthermore, Integrated Bayesian Inference System (IBIS) identified several genes as potential biomarkers for exposure to SiO₂ and SWNT. Pathway and network analysis showed that SiO₂ causes perturbation of extracellular matrix remodeling and cell adhesion pathways, which included down-regulation of matrix metalloproteinases (MMP), collagen proteins, integrins, tissue plasminogen activators (PLAT), plasminogen activator inhibitors, and laminin; whereas IL-8 was up-regulated. SWNT caused down-regulation of MMP-1 and PLAT. Understanding the molecular processes leading to toxicity requires a global toxicogenomic approach, miRNA and proteomic profiling are also being investigated.

1378 EVALUATION OF THE EFFECTS OF SERIAL PHLEBOTOMY ON THE TRANSCRIPTOME OF MAJOR TISSUES AND ON THE RESPONSE TO TOXICANTS IN RATS.

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Toxicokinetic (TK) parameters are essential for the proper toxicologic assessment of experimental compounds. Early TK studies are typically conducted in rats before any robust *in vivo* toxicologic evaluation. These studies involve collecting small volumes of blood (less than 0.5 ml) at several time points over a short period of time (typically 24 hrs). While the amount of blood collected and the timing of the collections are designed to minimize any effect on the physiological status, it is unknown how they affect sensitivity to toxicity or the transcriptome of tissues. In this study, our objective was to evaluate the impact of serial bleeding on the transcriptome of various major tissues (kidney, heart, liver, spleen) and their response to 2 well-characterized molecules, doxorubicin and cisplatin. Male Sprague-Dawley rats (n=6/group) received single intravenous injections of saline, doxorubicin (8 mg/kg) or cisplatin (4 mg/kg). In each group, 3/6 rats were serially bled by tail vein 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12 and 24 hrs after treatment. Rats were then sacrificed. Blood was collected for hematology and serum chemistry evaluation. Heart, liver, kidney, spleen, and heart were collected for histopathology and gene expression profiling with Affymetrix RAE230A arrays. Serial bleeding was associated with slight decreases of red blood cell parameters and serum total proteins, but did not result in histopathological changes or in increased sensitivity to doxorubicin and cisplatin toxicity. In addition, serial bleeding did not induce significant gene expression changes in either vehicle- or compound-treated rats when compared to their respective control groups. Overall, these results suggest that the serial bleeding procedure used in TK studies minimally affect tissue response to toxicants, and support the feasibility of gene expression studies using tissues collected from these studies.

1379 "PERCELLOME" TOXICOGENOMICS PROJECT FOR THE MECHANISM-BASED TOXICOLOGY.

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The whole genome sequence for human and mouse, and technology for monitoring whole genome expression enabled us to launch the Percellome Toxicogenomics Project for the development of the comprehensive gene cascade database/informatics by the high-precision transcriptome data.

For this project, we put a normalization method designated as "Percellome" into practical use (BMC Genomics 7:64, 2006). It generates mRNA expression values as "copy numbers per one cell" from DNA microarrays and Quantitative PCR. Thus, transcriptome data are plotted on to a common linear scale, and directly compared among experimental groups, studies, different organs, and furthermore, different platforms that adopt Percellome method (Agilent and CodeLink custom arrays).

Up to now, the time- and dose-dependent alteration of gene expression induced by a single oral exposure in mouse liver (4 time points x 4 dose levels, triplicate, 48 samples per chemical) were studied on 90 chemicals including drugs, food-related chemicals, receptor ligands, pesticides, industrial products, etc. The data is expressed in 3-D graph (X=time, Y=dose, Z=copies per cell) made of 45,000 layers of surfaces that correspond to the probe sets of the Affymetrix MOE430v2 GeneChip ("MilleFeuille" data, see <http://toxicomics.nihs.go.jp/db/> for selected data). MF data allow biologists to check the biologically plausible alterations.

Percellome Project now includes subprojects for repeated dosages, multi-organ linkages, inhalation toxicogenomics that covers exposure levels of sick building syndrome and fetus toxicogenomics for developmental toxicology as well as development of data analysis tools including unsupervised clustering method. New findings from each case and from the database will be demonstrated.

Eventually, this approach should lead to our ultimate goal of creating "virtual mouse" *in silico* in the future.

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1380 DOSE REPOSE RELATIONSHIP ASSESSED BY STANDARD AND OMIC TOOLS IN A 28-DAY RAT TOXICITY STUDY WITH THE ANTIANDROGEN FLUTAMIDE.

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Since the development of the omic tools their sensitivity compared to that of more traditional tools based on gravimetry, microscopic examination or hormonal analyses has been debated. Therefore, to contribute to this discussion we have assessed

using omic and traditional tools the effects of a wide range of dose levels of the antiandrogen flutamide (FM) on male rats. Flutamide was administered to Sprague-Dawley rats at the doses of 0, 0.04, 0.2, 1, 6 and 30 mg/kg/day for 28 days. At necropsy, blood samples were collected for luteinizing hormone and testosterone measurements. The testes were collected for histopathological assessment in addition to the evaluation of gene expression changes either using full genome DNA chip or qPCR analyses. Results showed that the hormonal changes (increase in testosterone levels) and testicular lesions (Leydig cell hyperplasia) were detected from 6 mg/kg/day. The omic data (transcripts profiling) also showed changes from 6 mg/kg/day. A comparison of sensitivity and dose response effects observed between the two different approaches will be presented. Overall, these data show that, using a standard 28-day toxicity study and a well characterized adverse effect, the NOEL based on transcript profiling analyses is similar to the NOEL based on histopathological examinations.

1381 ENHANCED HEPATIC GENE EXPRESSION OF STRESS-RESPONSE FACTORS IN RATS WITH DICLOFENAC-INDUCED HEPATOTOXICITY: ROLE OF GI BACTERIAL ENDOTOXIN.

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Diclofenac (DCLF) is a widely used non-steroidal anti-inflammatory drug that has been associated with idiosyncratic hepatotoxicity. Previous studies have suggested a link between bacterial endotoxin and DCLF-induced hepatotoxicity. Liver injury from DCLF is associated with GI injury, which can increase translocation of bacterial products into the blood. To examine the relationship between gut bacteria and DCLF hepatotoxicity, male Sprague-Dawley rats were pretreated for 4 days with vehicle or with polymyxin B (150 mg/kg) and neomycin (450 mg/kg) to sterilize the gut. Rats were then given a hepatotoxic dose of DCLF (100 mg/kg) or vehicle. After 6 hrs, the livers were collected for histopathology and mRNA profiling. In contrast to the naïve gut sterilized rats, DCLF treatment induced a large number of gene expression changes and increased serum ALT activity. Gut sterilization prior to DCLF administration resulted in lowered ALT activity and decreased the number of expression changes. Transcripts uniquely regulated in rats with ALT elevations were related to cell cycle regulation, molecular chaperone activity, stress response, and DNA replication. In DCLF-treated rats, hepatic neutrophil accumulation and the regulation of several neutrophil chemotaxis genes suggested involvement of neutrophils in the mechanism of injury. The MAPK pathway, along with stress-response factors such as GADD45, HYU1, and HSP40, were over-expressed in livers from DCLF-treated rats relative to gut-sterilized, DCLF-treated rats. Interestingly, these changes are similar to those seen in livers from endotoxin-treated rats. Overall, these results are consistent with the hypothesis that intestinal translocation of bacterial endotoxin contributes to the pathogenesis of DCLF-induced hepatotoxicity. (Supported by Abbott Laboratories and NIH Grant DK061315).

1382 LONGITUDINAL TOXICOGENOMIC ANALYSIS OF TRICHLOROETHENE EXPOSURE IN LIVERS OF MRL +/- MICE.

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Trichloroethene (TCE) is an industrial chemical and environmental pollutant that is associated with liver and kidney toxicity, and autoimmune diseases. Its hepatotoxic effects are mainly due to its toxic metabolites chloral hydrate, trichloroacetic acid, and dichloroacetic acid. To elucidate the mechanisms by which TCE asserts its hepato- and immunotoxic effects, we chronically exposed female MRL +/- mice, which are susceptible to develop autoimmune disorders, to a low dose of TCE in the drinking water. After 11, 24, 36, and 48 weeks, livers were excised from exposed mice and age-matched controls, and RNA was isolated. Liver gene expression was measured following hybridization of biotin-labeled antisense cRNA fragments to Affymetrix GeneChip® arrays. Data were analyzed using multiple microarray analysis software packages for computational hierarchical cluster analysis and principal component analysis. Furthermore, signaling and metabolic pathways affected by treatment with TCE were identified using Ingenuity™ Pathways Analysis and the Ingenuity™ Pathways Knowledge Database (Ingenuity, Mountain View, CA). A global functional analysis revealed that during the earlier times, changes in the cell cycle (at 12 weeks) and metabolic functions (at 12 and 24 weeks) predominated, whereas at the later times (at 36 and 48 weeks), expression of genes involved in the regulation of cancer, cell death, and the immune system homeostasis were more prominently affected. In addition, changes in cell-to-cell signaling and liver

disease occurred after prolonged TCE exposure. The gene expression data correlate well with the observed liver pathology. These results provide a mechanism for TCE-induced pathology and suggest that prolonged exposure to low doses of TCE, which are common in polluted drinking water sources, can result in hepatotoxicity and liver cancer. Our model may be important for understanding risks associated with TCE exposure in susceptible individuals.

1383 EARLY LIFE-STAGE TOXICITY OF NANOPARTICLES IN FISH.

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We have studied the dose- and particle type-dependent early life-stage toxicity of nanoparticles in tomcod. Tomcod eggs were fertilized and exposed to 0.4 to 50 µg/ml carbon nanoparticles or 0.08 to 10 µg/ml carbon nanotubes. In one experiment, tomcod embryos were treated starting on day 17 after fertilization, and in the second experiment, larval-stage tomcod were treated beginning on day 3 after hatching. The test nanoparticles included carbon toner particles and the raw combustion soot material and the sludge byproduct that were obtained during the processing and purification of carbon fullerenes with metal catalysts. The surfaces of the carbon nanotubes were polyethylene glycol derivatized. Clear toxicity was observed at the highest concentrations of some but not all nanoparticles types. Significant increases in embryonic death were observed with 50 µg/ml soot and sludge nanoparticles. In comparison, neither the carbon nanotubes nor toner nanoparticles had a significant effect on embryo survival. Significant effects on survival were also observed in embryos treated with 10 µg/ml sludge, a manufacturing process waste product which would have the greatest chance of entering the ecosystem. Similar concentration-dependent effects on survival were observed in the second experiment in which 3 day-old larval stage tomcod were treated with nanoparticles. Abnormal behavior, as seen by decreased activity, was observed at concentrations as low as 2 µg/ml for the sludge nanoparticles. Other endpoints demonstrated that embryonic toxicity occurred at the lowest concentration of nanoparticles used in our experiments. A significant delay in hatching occurred in embryos treated with 0.4 µg/ml of the 3 types of carbon nanoparticles. Thus, naïve young life-stages of tomcod have proven to be sensitive monitors of chemical contamination for a variety of toxicological endpoints.

1384 AQUATIC FATE AND TOXICITY OF NANOPARTICLES: AGGLOMERATION, SEDIMENTATION AND EFFECTS ON DAPHNIA MAGNA.

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The nanoparticulate inorganic pigments titanium dioxide (TiO₂) and zinc oxide (ZnO) are used in sunscreens to protect the human skin against UVB light. It has been demonstrated that these nanoparticles do not penetrate human skin. However, the possible impact on the environment and its aquatic organisms has not been well examined.

We analysed the agglomeration and sedimentation and tested the acute aquatic toxicity of TiO₂ and ZnO particles. The influence of the test medium, the dispersion method and the pH on the fate and the effects of the particles were tested. Additionally, the characteristics of the nano-scaled materials were compared to those of the micron-scaled materials.

The particle size distributions in the test dispersions were characterized by laser scattering and dynamic light scattering analysis, and sedimentation of particles was visually observed at different time points. The mass of non-sedimented particles was determined. Acute aquatic toxicity to *Daphnia magna* was tested considering the OECD Test Guideline 202.

Dispersion of the nano-powders generally leads to suspensions with agglomerated particles and sedimentation. However, agglomeration and sedimentation varied significantly depending on the choice of test media.

The acute aquatic effect of nano-TiO₂ was low and did not significantly differ from that of the micron-sized material.

The aquatic toxicity of ZnO particles largely depended on the solubilisation of Zn rather than on the particles themselves. Therefore the pH of the test medium is critical. Microscopy of the *Daphnids* revealed particles in the intestine, the filtering chamber and inside the carapax as well as adsorption to the body surface. Adsorbed and ingested particles were agglomerates rather than primary nano-scaled particles.

1385 DISTRIBUTION OF NANOPARTICLES IN THE SEE-THROUGH MEDAKA (ORYZIAS LATIPES).

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Water-suspended fluorescent nanoparticles (solid-latex solution) were used to investigate the distribution of nanoparticles in the eggs and bodies of see-through medaka (*Oryzias latipes*) because the environmental fate of manufactured nanopar-

ticles is considered an emerging environmental concern. Particles 39.4 nm to 42,000 nm in diameter were adsorbed to the chorion of medaka eggs and accumulated in the oil droplets, and 474-nm particles had the highest bioavailability to eggs. Particles 39.4 nm in diameter shifted into the yolk and gallbladder during embryonic development. Adult medaka accumulated 39.4-nm nanoparticles mainly in the gills and intestine when exposed to a 10-mg/L nanoparticle solution. Nanoparticles were also detected in the brain, testis, liver, and blood. Concentrations of nanoparticles in the blood of male and female medaka were 16.5 and 10.5 ng/mg blood protein, respectively. These results suggest that nanoparticles are capable of penetrating the blood-brain barrier and eventually will reach the brain. Salinity-dependent acute toxicity was observed in medaka eggs exposed to nanoparticles for 24 h. The bioavailability and toxicity of nanoparticles depends on environmental factors and multiple physicochemical properties. Further studies on the toxic effects of nanoparticles used in commercial products, and their environmental relevance, need to be done to define the risks and benefit of nanomaterial applications.

1386 TOXICOLOGY OF TITANIUM DIOXIDE (TiO₂) NANOPARTICLES: 1. CHARACTERIZATION AND TISSUE DISTRIBUTION IN SUBCUTANEOUSLY AND INTRAVENOUSLY INJECTED MICE.

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Medical products are beginning to use nanotechnology but efforts to ensure their safety are lagging behind. We studied TiO₂ nanoparticles (average size=4.7 nm, W740x, Degussa) in vivo. Particle sizes in media suspensions were measured by dynamic light scattering, and assessed for aggregation by light microscopy. Particles resuspended in sterile saline were injected into Balb/c female mice on days 1 and 2. Mice received either 4 subcutaneous (SC) injections in the inguinal and axillary area, or 2 intravenous (IV) injections in the tail vein. The total doses were 5600 mg/kg (SC) or 560 mg/kg (IV). Large doses were used to ensure detectability of biological responses. IV injections were well tolerated, while SC injected mice showed clinical and hematological signs of systemic inflammatory response. Necropsies were performed on day 3 and 5. Brain, liver, spleen, lymph nodes, lungs and kidney were examined macroscopically and microscopically. In the SC injected mice, most of the injected particles remained at the injection site as a solid aggregation. In the IV injected mice, lungs, liver and spleen were noticeably whitish in color, indicating presence of TiO₂ deposits. Macroscopically, all other tissues appeared normal. Histological analysis showed accumulation of TiO₂ aggregates inside macrophages in the liver, spleen and most markedly in the lungs of IV injected mice. In SC injected mice, very few or no macrophages with TiO₂ were observed in those tissues. However, hepatic vacuole formation observed in those mice may suggest hepatocellular degeneration. These results indicate that nanoparticles of TiO₂ probably aggregate immediately after SC injection inducing acute inflammation. In IV injected mice they are rapidly cleared from the circulation accumulating in lung, liver, and spleen indicating potential organ specific toxicity of TiO₂ nanoparticles.

1387 NANOSIZE TiO₂ STIMULATES REACTIVE OXYGEN SPECIES IN BRAIN MICROGLIA AND DAMAGES NEURONS, *IN VITRO*.

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This study reports that a commercially available, widely used nanosize titanium dioxide (TiO₂) product (Degussa P25), damages cultured neurons through microglia-generated reactive oxygen species (ROS) and oxidative stress (OS). Immortalized mouse brain microglia (BV2) were exposed to physicochemically characterized P25 and their release of ROS examined with fluorescent and chemiluminescent probes. Affymetrix microarray analysis on P25-exposed microglia indicated that numerous apoptotic and OS pathways were up-regulated. Immortalized rat dopaminergic (DA) neurons (N27), exposed to 2.5–40 ppm P25 for (1–72 hr), responded with early (1–48 h) increases in intracellular ATP followed by significant reductions at all tested concentrations (72 h). Initiation of apoptosis in the N27 neuron was suggested by significant increases in Caspase 3/7 activity after 24 hr exposure to P25 (>40 ppm). Direct damage to N27 neurons was indicated by significant reductions of Hoechst 33342 nuclear stain after 24 and 48 hr exposure to 20 and 40 ppm. Primary cultures, dissociated from embryonic rat striatum (containing a predominance of DA neurons), were exposed to (5–40 ppm) P25 and exam-

ined after 6, 24, and 48 hr exposure. Significant increases of nitrite, an index of reactive nitrogen species, occurred at >20 ppm exposure after 1 h (>10 ppm after 3 h). The number of immunohistochemically stained neurons in these cultures was reduced by ~32% and ~40% in response to 5 ppm after 6 and 24 hr exposure. Microscopic evidence of apoptosis was also documented. Together, these data indicate that P25 damages neurons directly, but more rapidly and at lower concentrations in complex cultures containing microglia, plausibly through OS mediated pathways.

1388 EFFECTS OF COPPER NANOPARTICLES ON VIABILITY AND FUNCTION OF PC-12 CELLS.

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This investigation was designed to determine whether different nano-sized copper particles would induce dopamine (DA) depletion in a cultured neuronal phenotype, PC-12 cells. Cells were exposed to different sizes of copper nanomaterials Cu-40, 60 & 80 nm) for 24 h. The Cu particles were obtained in powder form and in sizes of 40, 60, and 80 nm particles from Nanotechnologies Inc., Austin, TX. Characterization of these particles was performed using light microscopy, electron microscopy, and photon correlation spectroscopy. Using the high illumination CytoViva system, the particles agglomerate to ~1–5 µm when dispersed in solution, which was also shown by photon correlation spectroscopy. The electron microscopy images show a non-spherical structure that is closer to a crystalline form. MTT assays and LDH assays were performed to assess cell viability and cell membrane integrity and leakage, respectively. The MTT results show marked toxicity above concentrations of 5 µg/mL for Cu 40 and Cu 60 (EC50: 8.3 µg/mL and 7.7 µg/mL, respectively) while Cu 80 showed toxicity above 10 µg/mL (EC50: 18.2 µg/mL). The LDH assay showed Cu 40 had significant leakage at concentrations of 10 µg/mL, Cu 60 had significant leakage at 7.5 µg/mL, and Cu 80 had significant leakage at 25 µg/mL. The results derived from the dopamine (DA) and its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) assays suggest a significant dose-dependent decrease in the levels of DA and its metabolites as a result of exposure to 5 to 50 µg/ml concentrations of Cu nanoparticles compared to control cells. Further study is required to evaluate the specific intracellular distribution of copper nanoparticles, metal dissolution rates in cells, and cellular matrices, and relevance to their size, surface area, and other physicochemical parameters.

1389 EFFECT OF ORGANIC SURFACE FUNCTIONALIZATION AND SONICATION ON SILVER NANOPARTICLE CYTOTOXICITY IN NEUROBLASTOMA CELLS.

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The immense nanoparticle surface area per unit weight is an important feature for novel product development. In this study, unfunctionalized silver (Ag) NPs (Ag 25nm) and organically functionalized Ag NPs (Ag 25–30nm F) were examined with or without dispersion sonication for their effect on toxicity in mouse N2A neuroblastoma cells. The NP sizes, shapes, and completeness of dispersion in solution were characterized with light microscopy, TEM, and UV-visible spectroscopy. Using light microscopy, large agglomerates of unfunctionalized Ag 25nm NPs were clearly evident whereas organically functionalized Ag NPs were found less agglomerated. Sonication of either particle type improved overall NP dispersion. Increasing concentrations of unfunctionalized Ag 25nm led to significant reductions in cell viability at concentrations between 25–100 µg/ml. This effect was concurrent with a loss of neurite extensions, cell shrinkage, and detachment from the surface. Unfunctionalized Ag NPs sonicated for 60 s demonstrated toxicity at concentrations as low as 10 µg/ml, suggesting a size-dependent effect of Ag NPs. In contrast, N2A cells incubated with functionalized Ag25–30F NPs retained similar morphologies to control cells at concentrations from 5–50 µg/ml. Mitochondrial membrane permeability assessed by fluorescent staining was increased in cells incubated with unfunctionalized Ag 25nm. Ag25–30F NPs had little or no effect on membrane permeability. These findings were consistent with the morphological observations and cell toxicity results. Our findings demonstrate that organic surface functionalization can drastically reduce the toxic effect of Ag NPs, an effect that can remain despite sonication perturbation commonly used for NP dispersion.

1390 NEUROINFLAMMATORY RESPONSES FOLLOWING EXPOSURE TO ENGINEERED NANOMATERIALS.

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Multi-walled carbon nanotubes (MWCNT) and fine crystalline silica are largely used in the manufacture of composites and as blasting abrasives. While the widespread application and economic benefits of manufacturing such materials are highly promising, their adverse effects on the nervous system have not been fully investigated. Populations at greatest risk for exposure are industrial workers involved in the manufacturing, handling or utilization of such materials. Here, we evaluated the potential neurotoxic effects of exposure to MWCNT (67nm diameter, 5-13µm length) and silica (<2µm) in a murine model. Exposure (by pharyngeal aspiration) of mice to MWCNT (10, 20 or 40µg/mouse) or silica (1mg/mouse) elicited neuroinflammation in discrete brain areas. Specifically, the expression of CCL2, TNFα and IL6 increased (2 to 10-fold; P<0.05) in the olfactory bulb, hippocampus and frontal cortex, within 24h following exposure. This inflammatory outburst is a consequence of microglial activation, in response to perturbation of the neuronal microenvironment and function. Silica also induced the expression of IL1α, OSM, TLR2 and FGF2, effects not observed with MWCNT at the doses tested. In addition, both MWCNT and silica induced region-selective expression of metallothioneins I and II (>2-fold, P<0.05), metal binding proteins that are regulated by inflammatory stress signals and afford protection against oxidative injury. Whether the region-specific neurotoxicity observed following pharyngeal aspiration is due to systemic responses or possible redistribution of these materials to the brain remains enigmatic. In summary, our findings indicate that exposure to engineered nano- and fine particles can elicit neuroinflammatory changes.

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1391 TOXICOLOGY OF TITANIUM DIOXIDE (TiO₂) NANOPARTICLES: 2. IMMUNOLOGICAL EFFECTS IN SUBCUTANEOUSLY AND INTRAVENOUSLY INJECTED MICE.

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Nanoparticles that are internalized are very likely to interact with the reticuloendothelial system (RES). This will be more important with therapeutic nanoparticles that may require multiple injections in significant quantities. We evaluated effects of TiO₂ particles injected either subcutaneously (SC) or intravenously (IV) on several standard measures of the immune response. Suspensions of ~ 4.7 nm uncoated TiO₂ (W470X, Degussa) particles were injected at total doses of 5600 mg/kg (SC) or 560 mg/kg (IV) over two days. At +1 day, there was no significant change in any cell population in peripheral blood except CD8+ in SC mice. In contrast, at day +5 there was a dramatic increase in granulocytes in circulation in SC as well as proportional decreases in circulating lymphocyte percentages. Macrophage percentages were not affected. No changes were observed in any cell population in draining lymph nodes. In SC mice, there was an increase in granulocyte number in the spleen, mirroring the increase in peripheral blood. In the spleen of IV injected mice a number of macrophages containing TiO₂ aggregates were observed in the marginal zone of the white pulp, suggesting a pathway for the interaction of TiO₂ with T cells. After 3 days of culture with Con-A, an apparent lack of T-cell proliferation was seen in lymph nodes from SC but not IV injected animals. This was seen by both direct measurement of proliferation and from B-cell:T-cell ratios after culture. Cytokine analyses of the mitogen stimulated spleen and lymph node cells indicated increased production of TNF-α, IL-6 and gamma-interferon. These results indicate that the acute inflammatory response seen in SC injected mice may well have secondary effects on the immune response. These findings, along with earlier results with polystyrene nanoparticles suggest that the immune system may be especially sensitive to effects of nanoparticles.

1392 SIZE AND SURFACE COATING DIFFERENCES IN SILVER NANOPARTICLE TOXICITY AND INFLAMMATORY RESPONSE IN JURKAT CELLS.

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The use of silver nanoparticles (Ag NP) in many commercially available products for their strong antibacterial and antiviral properties has led to questions about their toxicity to mammalian cells. Our studies show that their toxicity is dependent

upon their size, concentration, and chemical surface composition. First, we examined the effect of toxicity measures due to size for uncoated Ag NPs (10, 25, and 80 nm, Nanotechnologies, Inc.) and surface coated Ag NPs (15, 30, 80nm, Clarkson University). The primary NP sizes and morphologies were visualized and characterized with scanning and transmission electron microscopy. Previous studies show a size and concentration-dependent toxicity. However, in the current studies, the coated Ag NPs were in considerably less toxic than the uncoated Ag NPs. The MTT assay of cell viability shows that surface coated functionalized particles are not toxic while unfunctionalized particles are toxic at the exposure levels and time course studied. The initiating, irritation inflammatory response using IL-2 and TNF-α production showed significant increase in non-functionalized silver particles while only marginal induction in functional particles. The results indicate that toxicity depends on the surface based on coating deactivation rather than nominal size of the nanomaterial. Other characteristics including overall dimension, surface dissolution, etc. require further studies.

1393 MANUFACTURED NANO-SIZED ALUMINA PARTICLES INDUCE ENDOTHELIAL CELL DYSFUNCTION: IMPLICATIONS IN VASCULAR DISEASE.

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Manufactured nanoparticles (MNP) display unique physicochemical characteristics due in part to their small size and large surface area that also make them highly desirable for a number of applications. Alumina or aluminum oxide is the most abundantly produced nanosized particle. Due to the extreme potential of MNP and their increased usage, occupational and public exposure will dramatically increase in the future. Human exposure via inhalation and ingestion can lead to increased blood concentrations. The vascular endothelium is uniquely susceptible to such xenobiotics circulating through the bloodstream. Inflammation or dysfunction of the endothelial layer has been shown to be an initiating event for the development of vascular diseases. It is hypothesized that MNP will lead to increased endothelial cell dysfunction, evidenced by increased inflammatory processes. Human umbilical vein endothelial cells and primary porcine pulmonary artery endothelial cells were treated with nano-sized (~10 nm) alpha-gamma alumina particles (10-100 µg/mL). It was found that alumina significantly increased mRNA expression of vascular cellular adhesion molecule-1 (VCAM-1) and E-selectin (ELAM-1). Alumina treatment also increased VCAM-1, ELAM-1, and intracellular adhesion molecule-1 (ICAM-1) protein determined by Western blot and immunofluorescence analysis. The protein induction of VCAM-1 was inhibited by co-treatment with an NF-κB inhibitor (BAY 11-7085), suggesting that the NF-κB signaling pathway may be responsible for VCAM-1 induction. These results suggest that nano-alumina particles can lead to endothelial cell activation and dysfunction by the induction of a number of pro-atherogenic inflammatory events and that these events may be modulated through the NF-κB pathway.

1394 THE PREFERENTIAL BINDING OF HUMAN SERUM PROTEINS TO NI, AL AND DIAMOND NANOPARTICLES.

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In a physiological environment, proteins rapidly accumulate on particulate material that has a charged or hydrophobic surface. A growing body of research has demonstrated that these surface-bound proteins facilitate the detection of particulate material by phagocytic cells and help determine the distribution and clearance of these materials as well as the severity of secondary immune or inflammatory responses. Identifying these bound proteins and understanding how surface properties control protein binding is essential for predicting particle induced toxicity. As a first step in this process, the identity and relative proportion of proteins bound to the surface of nickel (nominal size 50 nm), aluminum (nominal size 51 nm) and diamond (nominal size 75 nm) nanoparticles following their exposure to human serum were determined. Particle bound proteins were isolated, assayed for total protein and labeled with ¹³C containing tags using an Applied Biosystems ICAT system. Each bound protein sample was combined with an equal amount of free serum proteins that had been labeled with analogous ¹²C containing tags and these combined samples were separated on a 1-D SDS PAGE gel. Protein bands were excised, subjected to in-gel trypsin digestion and analyzed by LC MS/MS following avidin purification. By quantifying the number of equivalent peptide sequences containing ¹³C and ¹²C tags, the relative proportion of proteins bound to each particle and present in free serum was determined. Although some serum proteins such as β-2-glycoprotein and α-2-HS-glycoprotein readily accumulated on all particles, each mate-

rial displayed a characteristic ability to preferentially bind various proteins. Differential binding of proteins, however, could not be explained simply by the surface charge of the nanoparticles which underscores the need for expanded studies to identify the relevant surface properties.

1395 QUALITATIVE AND QUANTITATIVE UPTAKE OF SiO₂-COATED FLUOROPHORE NANOPARTICLES.

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In this research, the uptake of nanoparticle-based luminescent probes was examined for biological and environmental imaging and detection applications. Organic fluorophores currently used in cell imaging decompose during imaging, resulting in photobleaching. However, rare earth ions doped inside of silica nanoparticles are stable and do not photobleach, still emitting fluorescence with intensities comparable to single organic fluorophores. Silica-coated FITC (SiO₂-FITC) and rhodamine (SiO₂-RB) nanoparticles were measured using transmission electron microscopy (TEM). Both nanoparticles are close to 100 nm in diameter and possess similar spherical morphology. Cell viability was determined using MTT assay, and results showed that the SiO₂-FITC particles significantly decrease cell viability at 100 µg/mL, while SiO₂-RB nanoparticles are not toxic at the concentrations tested (0-100 µg/mL). Fluorescence microscope images show particles agglomerated within cells and verify that washing procedures are successful in removing particles not taken up into cells. A protocol to quantify the uptake of particles into cells using this washing procedure was developed using a fluorescence microplate reader. This method allows for the determination of the critical concentration of particle uptake into the cell, in order to relate this information to cell size and toxicity.

1396 PHOSPHATIDYL SERINE ENHANCES RECOGNITION AND UPTAKE OF SINGLE-WALLED CARBON NANOTUBES BY RAT BRAIN MICROGLIAL CELLS.

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Single-walled carbon nanotubes (SWCNT) are a class of nanosized materials with wide applications in industry and medicine. Inhaled SWCNT have been shown to cause a robust inflammatory and oxidative stress response in the lung and cardiovascular system. Interactions with phagocytosing cells determine SWCNT elimination or spreading into different tissues. The challenge is how to make nanotubes delivered specifically to the target site. We established that macrophages do not effectively recognize non-functionalized SWCNT but readily engulf SWCNT coated with phosphatidylserine (PS), an "eat-me" signal for phagocytes. In the brain, important immunological functions are associated with the ability of microglial cells to exert phagocytic activity. As inhaled nanoparticles have been detected in the brain we were interested to study interactions of SWCNT with microglial cells. Microglial cells were isolated from postnatal rat brains and identified with CD11 antibody. Primary cultures were obtained with 70% microglial cell population which could be activated by lipopolysaccharide (LPS) to produce NO revealed by labeling of cells with a specific fluorogenic reagent, DAF-2DA. Further we studied phagocytosis of SWCNT by microglial cells. Cells were incubated with SWCNT coated with either fluorescently labeled nitrobenzoxadiazole (NBD)-PS or NBD-phosphatidylcholine (PC) for 2h. Fluorescent microscopy was applied and revealed that NBD-PS-SWCNT were a more preferred target of recognition and uptake by microglial cells than NBD-PC-SWCNT. Control experiments were performed to confirm that NBD-phospholipids remained bound to SWCNT. Thus, similar to macrophages, microglial cells utilize PS as a signal for effective recognition and engulfment of SWCNT. Supported by NIOSH OH008282, NIH HL70755, HL70809, AHA0535365N, Human Frontier Science Program.

1397 EVALUATION OF *IN VITRO* PENETRATION OF QUANTUM DOT NANOPARTICLES INTO HUMAN SKIN.

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Topical exposure to nanoparticles is becoming more of an issue due to the increased use of nanosized ingredients in consumer products and because of possible occupational and environmental exposure. Because of their small size, there is concern

about the ability of these particles to penetrate through the barrier layer of skin. *In vitro* skin absorption studies were conducted using cadmium selenide quantum dots (Qdots) as a model nanoparticle because of their relative stability and intense fluorescence. Qdots (PEG coating, maximum emission 621 nm, hydrodynamic diameter 37 nm) were synthesized and formulated into an oil-in-water emulsion, and applied to human cadaver skin assembled in diffusion cells. Initial absorption studies were conducted for 24 hr followed by examination of sections from paraffin embedded skin using a fluorescence microscope. Fluorescence from Qdots appeared to be on or in the surface layer of skin (the stratum corneum), in the opening of hair follicles or in the folds (furrows) of the skin surface. Subsequent studies with Qdot emulsion formulations were conducted for 3, 8, and 24 hr. Frozen skin sections were evaluated for location of fluorescence with a laser scanning confocal microscope (LSCM). Fluorescence in the skin appeared to be found predominately in the stratum corneum with small amounts possibly penetrating further into the upper region of the viable epidermis. Spectral analysis of the fluorescence detected by LSCM showed that most fluorescence observed in the viable epidermis and all fluorescence in the dermis was due to autofluorescence. No significant elevation of cadmium (Qdots) was observed in receptor fluid samples taken beneath the skin. It therefore appears that absorbed Qdots were found predominately in the stratum corneum and they did not penetrate through the skin.

1398 ACCUMULATION OF NANOSCALE QUANTUM DOTS IN MULTIPLE ORGANS *IN VIVO*, FOLLOWING DERMAL EXPOSURE TO DERMABRADED, BUT NOT TAPE STRIPPED OR INTACT SKH-1 MOUSE SKIN.

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There are currently many cosmetics and sunscreens that contain nanoscale materials such as titanium dioxide and zinc oxide. To date the possible long-term toxicological effects of percutaneous absorption of nanoparticles have not been determined. We used nanoscale polyethylene glycol coated cadmium selenide (CdSe) core quantum dots (QD) as surrogate nanoparticles, and evaluated (a) tissue Cd distribution following intradermal administration and (b) penetration into intact, tape stripped or dermabrased in mouse skin. Female SKH-1 mice were intradermally injected with 5 µL of 19 µM QD, and following sacrifice at 24 hr, tissues were removed and analyzed for Cd content using inductively coupled plasma mass spectrometry (ICP-MS). Approximately 6% and 1% of the administered Cd dose were found in the liver and regional lymph nodes, respectively, with lesser amounts detected in the kidney, spleen, and heart. QD were visualized in the skin and lymph nodes using fluorescence microscopy. These results support the use of liver and regional draining lymph nodes as sentinel organs for assessment of the distribution after dermal exposure of QD. QD were suspended in an oil-in-water emulsion at approximately 9 µM and applied at 2 mg/cm² to the dorsal skin of mice pretreated as follows: untreated; tape-stripped to remove the stratum corneum; dermabrased. Cd was not detected by ICP-MS in sentinel organs of untreated or tape-stripped mice 24-48 hr post application; however, in dermabrased mice, approximately 1% and 0.01% of the applied QD were detected in liver and lymph nodes, respectively. These results suggest that percutaneous absorption of nanoscale materials depends on skin barrier integrity, and hence the potential hazard of topically applied nanoscale materials needs further evaluation.

1399 CELLULAR MECHANISMS OF QUANTUM DOT NANOPARTICLE UPTAKE IN PRIMARY HUMAN EPIDERMAL KERATINOCYTES.

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The pathway of nanomaterial uptake into cells may be an important determinant of cytotoxic potential and effects. Quantum dots (QD) 655 with cationic (PEG-amine, NH₂), anionic (carboxylic acid, COOH), and neutral (PEG) surface coatings are non-selectively taken up by primary neonatal human epidermal keratinocytes (HEK) at low concentrations, but the mechanisms of cellular uptake are unknown. The global hypothesis tested herein is that QD surface coating is the primary determinant of the magnitude and mechanisms of cellular uptake by HEK. A fluorescence microplate screen was used to quantitatively determine differences in the magnitude of uptake of 10 nM QD 655 by HEK after 24 h at 37°C. Statistical comparison revealed significant differences among all coatings, with COOH > NH₂ > PEG. Also, we investigated the temperature dependence of QD uptake by performing similar experiments at a temperature of 4°C. Uptake of all three QD

types was significantly lower at 4°C than at 37°C. A commercially available ATP-dependent bioluminescence assay confirmed that cellular ATP levels were significantly decreased at 4°C compared to 37°C for QD of all three coatings. Taken together, these data indicated that uptake for QD of all three surface coatings is energy dependent and requires active, and not passive, mechanisms of cellular transport. Co-localization of QD 655 of all three coatings with a fluorescent dextran and immunofluorescently labeled clathrin and caveolin were used in conjunction with biochemical inhibitors of clathrin assembly and caveolae formation to identify the contribution of macro- and micropinocytotic mechanisms of uptake. These results showed a clear dependence of QD uptake on surface coating, as well as the involvement of active transport mechanisms in all three cases. This is of importance for understanding mechanisms of nanoparticle cytotoxicity and cellular fate, as well as for risk assessment. (Supported by EPA-STAR Program Grant RD-83171501.)

1400 EFFECTS OF MECHANICAL FLEXION ON THE PENETRATION OF AMINO ACID-DERIVATIZED NANOPARTICLES IN SKIN.

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In the occupational setting, activities that involve repetitive motion can intensify mechanical loads that are applied to the skin. Processes, such as constant hand movements, can alter the permeability barrier of skin and thus increase risks associated with exposure to nanoparticles. In order to investigate the relationship between mechanical stressors and nanoparticle exposure, a flexing apparatus was designed to simulate repetitive wrist motion. Dermatomed porcine skin (400µm; n=3/time point) was fixed to the flexing device and topically dosed with 33.5 mg/mL of a fullerene substituted phenylalanine derivative (Baa-(FITC)-NLS). Skin was flexed for 60 or 90 min or left unflexed (control). Percutaneous absorption was assessed using a flow-through diffusion cell system for 8 h and 24 h. After each time point, samples were bisected and either frozen or processed for transmission electron microscopy (TEM). Frozen tissues were sectioned at 20µm to visualize Baa-(FITC)-NLS penetration by confocal microscopy. TEM was used to detect the fullerene within skin at the ultrastructural level. Skin flexed for 60 and 90 min depicted dermal penetration at 8 h, whereas Baa-(FITC)-NLS did not penetrate into the dermis of unflexed skin until 24 h. TEM analysis revealed nanoparticle localization within the intercellular spaces of the stratum granulosum, suggesting that penetration of the derivatized fullerene occurs through intercellular lipid moieties. These results suggest that external factors, such as a repetitive flexing motion and the associated mechanical stressors, can influence interactions that occur between nanoparticles and skin. These results are important for risk assessment of nanoparticles because they indicate that skin is an important route of exposure. (Supported by US EPA-STAR Program Grant RD-83171501 and National Academies Keck Futures Initiative.)

1401 PHARMACOKINETICS OF SKIN DISTRIBUTION OF QUANTUM DOT NANOPARTICLES IN ISOLATED PERFUSED SKIN.

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The disposition and pharmacokinetics of nanoparticles in tissues are crucial parameters for targeting nanotechnology-based drug delivery systems as well as defining their toxicological profile. Quantum dots (QD) are an excellent model nanomaterial since they can be synthesized with varying surface coatings that modulate disposition, as well as being amenable to quantification in tissues due to their fluorescent intensity. QD consisted of a CdSe core (8.40nm x 5.78nm) surrounded by a polyethylene glycol (PEG) coating to yield a hydrodynamic size of approximately 57nm. The maximum emission wavelength of the QD is 621nm, with a quantum yield of 54%. QD were mixed with 300 ml of media and intra-arterially infused into the isolated perfused porcine skin flap (IPPSF) for 4 hrs (dose phase). QD media was replaced with fresh media and the IPPSF perfused for an additional 4 hrs (wash-out phase). The arterial and venous perfusate was sampled and the fluorescence quantitated. A physiological-based pharmacokinetic model of QD uptake and release from skin, using continuously monitored arterial and venous QD concentrations as input, was developed based upon a model previously used to quantitate platinum distribution (J Pharm Sci 78: 550-555, 1989) in the same experimental system.

Clear arterial-venous QD extraction was observed at all doses. A dose dependent uptake of QD into skin with delayed washout was observed at higher doses. Future studies will assess the effect of differential QD surface coatings on uptake and cutaneous distribution using model kinetic parameters as endpoints. These data begin to define nanomaterial characteristics that correlate to tissue uptake and persistence. (Supported by US EPA-STAR Program Grant RD-83171501.)

1402 DISTRIBUTION OF QUANTUM DOT NANOPARTICLES IN ISOLATED PERFUSED SKIN.

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The disposition of nanoparticles in tissues is important for developing nanotechnology-based drug delivery systems as well as defining their toxicological profile. Quantum dots (QD), nanomaterials that naturally fluoresce, can be synthesized with surface coatings that modulate disposition and are amenable to localization in skin and other tissues due to intense fluorescence. QD were synthesized with a 8.40nm x 5.78nm CdSe core and polyethylene glycol (PEG) coating. The QD have a maximum emission wavelength of 621nm and a hydrodynamic size (in water) of approximately 57nm. Flow-through diffusion cells were used to assess QD penetration through porcine skin. The isolated perfused porcine skin flap (IPPSF) was used to determine whether intra-arterially infused QD would distribute to the skin or localize in the surrounding vasculature or tissue. QD were infused into the IPPSF (6.67nM, 3.33nM, 1.67nM, or 0.83nM) for 4 hr (dose phase), followed by an additional 4 hr perfusion (wash-out phase). Upon termination of the perfusion, the IPPSF was cut into 6 segments, flash-frozen in liquid nitrogen, cryosectioned at 25µm, and imaged by confocal microscopy. The images revealed epidermal blistering caused by QD aggregation and localization within capillaries, muscle, and subcutaneous tissue. The frequency of the QD decreased with a decrease in QD concentration and from the proximal to the distal end of each IPPSF. These results indicate aggregation of infused QD in the skin vasculature with concomitant blistering of the skin. This is important for risk assessment and drug delivery, since it shows the QD can aggregate in multiple tissues and cause pathological effects. Future studies will assess the effect of differential QD surface coatings on uptake and the cutaneous distribution. (Supported by US EPA-STAR Program Grant RD-83171501.)

1403 BIOLOGICAL INTERACTION OF FUNCTIONALIZED SINGLE-WALL CARBON NANOTUBES IN HUMAN KERATINOCYTES.

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Carbon nanotube-based nano-vectors, especially functionalized nanotubes, have shown potential for therapeutic drug delivery. Derivatized single-wall carbon nanotubes 6-aminohexanoic acid (AHA-SWNT), are soluble in aqueous stock solutions over a wide range of physiologically relevant conditions, however, their interactions with cells and their biological compatibility of AHA-SWNT has not been explored. Human epidermal keratinocytes (HEK) were dosed with AHA-SWNT ranging in concentration from 0.0000005-0.05 mg/mL. MTT cell viability decreased significantly (p< 0.05) from 0.00005 mg/mL to 0.05 mg/mL after 24 hr. Proinflammatory cytokines IL-6, IL-8, TNF-α, IL-10 and IL-1β were assessed in HEK. Cytokine analysis did not show a significant increase in IL-6 and IL-8 in the media containing 0.00005 mg/mL of AHA-SWNT from 1 hr to 48 hr. IL-6 tended to increase in cells treated with 0.05 mg/mL of AHA-SWNT from 1 to 48 hr, while IL-8 showed a significant increase at 24 hr and 48 hr. No significant difference (p< 0.05) in TNF-α, IL-10 and IL-1β expression was noted at any time point. Transmission electron microscopy of HEK treated with 0.05 mg/mL AHA-SWNT for 24 hr depicted AHA-SWNT localized within intracytoplasmic vacuoles in HEK. Treatment with the surfactant 1% Pluronic®F127 caused dispersion of the AHA-SWNT aggregates in the culture medium leading to less toxicity. These data clearly showed that the lower concentration of 0.00005 mg/mL of AHA-SWNT maintains cell viability and induces a mild cytotoxicity, while the 0.05 mg/mL of AHA-SWNT can initiate an irritation response that was demonstrated by the IL-8 release. (Supported by US EPA-STAR Program Grant RD-83171501 and the National Academies Keck Futures Initiative.)

1404 SIZE- AND DOSE-DEPENDENT TOXICITY OF SiO₂ NANOPARTICLES IN KERATINOCYTES.

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Although manufactured nanomaterials (NM) are becoming widely used for advancing product technology, there is a serious lack of information concerning impact on human health and the environment as large scale production, pervasive product incorporation and probable release of NM become increasingly eminent. Regarding their possible effect on cytotoxicity, and from this NM implication on human health, our main focus was to investigate eukaryotic cellular toxicity resulting from nanoparticle (NP) exposure. This study examined the cytotoxic effects of well-dispersed amorphous silica (SiO₂) in mouse keratinocytes (MK). MK were exposed over a range of dose and average size distributions with homogeneous suspension of either 35, 51, 110 and 420 nm SiO₂ at 0, 10, 50, 100 and 200 µg/mL for 24 h. Medium lactate dehydrogenase (LDH) leakage was dose- and size-dependent with the two smallest NP. Exposure of 35 and 51 nm at 200 µg/mL at 24 h resulted in 70% and 40% of LDH leakage, respectively. However, no changes were observed for both 110 and 420 nm NP for any concentration. Tetrazolium salt (MTT) reduction assays studying cell mitochondrial viability/function showed for 35 and 51 nm at high concentrations (200 µg/mL) produced significant toxicity compare to the larger 110 and 420 nm particles. Additional studies were carried out to investigate if redox potential of cells such (GSH/GSSG ratio) and mitochondria membrane potential as mechanism of SiO₂ toxicity. GSH levels of 35 nm SiO₂ at concentrations of 50, 100 and 200 µg/mL were 90, 80, and 65%, respectively. Silica nanoparticles larger than 35 nm showed no changes in GSH levels when compared with controls. Based on the results, silica NP show size- and dose-dependent toxicity.

1405 PHYSICAL CHARACTERIZATION AND DERMAL IRRITATION IN RABBITS OF TITANIUM DIOXIDE NANOPARTICLES AND NANOFIBERS.

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The field of nanotechnology has increased significantly in recent years, with a variety of applications ranging from enhanced paint pigmentation, to quantum dot semiconductors, to controlled release pharmaceuticals. With this rapid growth has also come the need to better understand the safety of nanomaterials, including nanoparticles (NP) and nanofibers (NF). The first objective of this study was to characterize submicron titanium dioxide (TiO₂) particles and fiber samples produced using a novel approach by LNK Chemsolutions. The TiO₂ NP and NF were characterized using energy-dispersive x-ray spectroscopy (EDS) and scanning/transmission electron microscopy (SEM/TEM). SEM/TEM analyses revealed that the NP were 1-2 µm diameter agglomerations of 10-100 nm spheres. SEM analysis showed the NF to be 0.5-5 µm lengths of 50-100 nm diameter cylindrical rods. The second objective of this study was to evaluate the local tolerance of TiO₂ NP and NF. New Zealand white rabbits (6/group) were administered 1 mL of TiO₂ NP and NF at 7.5 mg/mL, or 20 mg/mL for 6 hours/day for 14 days to a shaved dose site approximating 10% of the body surface area. A concurrent control group was administered the vehicle alone. Body weight, food consumption and clinical observations were performed throughout the study and an assessment of the dose site was performed daily. At the conclusion of the study the animals were euthanized, underwent a gross necropsy, and a sample of the dermal dose site was collected for histopathologic evaluation. There were no effects on body weight or food consumption and no edema or erythema was observed at any time during the study with either the NP or NF at either concentration. Additionally, there were no significant pathology findings of the dermal dose site. Based on these results, the nanomaterials were well tolerated over the 14-day study, at up to 20 mg/cm², the highest dose tested.

1406 SINGLE-WALLED CARBON NANOTUBES INDUCE OXIDATIVE STRESS AND INFLAMMATION IN SKIN.

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Single-walled carbon nanotubes (SWCNT) are a novel material with unique electronic and mechanical properties. A variety of different techniques are available for the production of SWCNT; however, the most common is via the disproportionation of gaseous carbon molecules supported on catalytic iron particles (HiPco). The

SWCNT produced by this method usually contain significant amounts of iron that may act as a catalyst of oxidative stress. The skin is a prime target for SWCNT toxicity as topical exposure can result during technologic processing and use. We hypothesized that SWCNT are toxic to the skin and the toxicity is dependent on their content of iron. The major toxicity mechanisms include induction of an inflammatory response, and oxidative stress exacerbated by iron. To test this hypothesis, the effects of SWCNT were assessed *in vitro* and *in vivo*. Exposure of human keratinocytes (HaCaT) revealed cytotoxicity in cells exposed to SWCNT; partially-purified SWCNT (0.25 weight % iron) exerted lower toxicity than unpurified SWCNT (40 weight % iron). Murine epidermal cells (JB6 P+) revealed a significant dose-dependent activation of AP-1 following exposure to unpurified SWCNT while partially-purified SWCNT did not activate AP-1. NF-κB was dose-dependently activated by both unpurified and partially-purified SWCNT. *In vivo* experiments evaluated the skin of SKH-1 mice following 5 days of unpurified SWCNT exposure (2, 4, or 8 mg/kg). Depletion of glutathione, increased myeloperoxidase activity, and an increase in IL-6 levels were observed following exposure to unpurified SWCNT. Histological evaluation of the skin following SWCNT exposure revealed an increased number of mast cells and polymorphonuclear leukocytes around hair follicles of the mouse skin. These data indicate that dermal exposure to SWCNT, particularly unpurified SWCNT, can result in inflammation, oxidative stress and dermal toxicity during occupational exposures.

1407 ANTAGONISM OF THE ARYL HYDROCARBON RECEPTOR.

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The aryl hydrocarbon receptor (AHR) binds to a variety of ligands and subsequently controls transcription of target genes, which mediate the toxic or carcinogenic effects of these compounds. Many AHR agonists and partial agonists are known to induce transcription; however, a pure antagonist of the AHR has not been discovered. This ideal pure antagonist would competitively inhibit the effects of a potent AHR agonist, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Previous reports indicate that 3'-methoxy-4'-nitroflavone (MNF), 3,3'-diindolylmethane (DIM), 6-methyl-1,3,8-trichlorodibenzofuran (MCDF), α-naphthoflavone (ANF), salicylamide (SAL), and resveratrol (RES) are potential antagonists. To measure the antagonistic properties of these compounds, we performed dose-response experiments with TCDD and the potential antagonists in H1G1 mouse hepatoma cells, which stably express green fluorescent protein regulated by AHR-responsive elements. We also tested these compounds' induction of endogenous CYP1A1 and CYP1B1 mRNA expression in H1G1 cells. ANF and RES induced some expression of both the reporter and endogenous genes and did not effectively antagonize TCDD's induction of the reporter or endogenous genes. MCDF and MNF acted as partial agonists by inducing some expression and inhibiting TCDD's induction of the reporter and endogenous genes. While both DIM and SAL were effective antagonists of the reporter gene, SAL did not effectively antagonize TCDD's induction of the endogenous genes. Only DIM potentially antagonized TCDD's induction of the endogenous genes. Next, we measured the ligands' regulation of CYP1A1 and CYP1B1 mRNA expression in MCF-7 breast cancer cells. The efficacy of the compounds in the two cell types differed, suggesting the efficacy of AHR ligands could be species- and/or tissue- specific. Further research should investigate the mechanisms of the differences between the induction of endogenous and reporter genes in H1G1 cells and between the induction of the endogenous genes in H1G1 and MCF-7 cells.

1408 REGULATION OF GENE EXPRESSION BY THE ARYL HYDROCARBON RECEPTOR IN BREAST TUMOR CELL LINES.

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Previous reports have shown differences between estrogen receptor (ER)-positive and ER-negative breast cancer cells in regulation of gene expression by the aryl hydrocarbon receptor (AHR). We compared immortal (MCF-10F) and transformed (BP-1) ER-negative cells as well as ER-positive MCF-7 cells. While previous studies have found that AHR agonists do not induce cytochrome P450 1A1 (CYP1A1) expression in ER-negative cell lines, we found that expression of CYP1A1 and cytochrome P450 1B1 (CYP1B1) mRNAs is induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in MCF-10F cells, BP-1 cells, and MCF-7 cells. In contrast, 3'-methoxy-4'-nitroflavone (MNF), an AHR antagonist, does not increase mRNA expression of CYP1A1 or CYP1B1 in MCF-10F or MCF-7 cells, but it does increase CYP1A1 and CYP1B1 expression in BP-1 cells. Chromatin immunoprecipitation assays showed increased occupancy of CYP1A1 and CYP1B1 regulatory regions by AHR in both MCF-10F and BP-1 cells treated with TCDD. This increased promoter occupancy was not found in MCF-10F cells treated with MNF. We conclude that gene expression and promoter occupancy in response to AHR ligands are dependent upon differences among breast cell phenotypes that are not limited to ER status.

1409 THE ROLE OF THE ARYL HYDROCARBON RECEPTOR IN DIOXIN-INDUCED PIGMENTATION.

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Exposure of humans to dioxins is often manifested by alterations in the skin, mainly the development of epidermal cell hyperplasia (chloracne) and hyperpigmentation. Dioxin mediates most of its toxic effects by binding to the aryl hydrocarbon receptor (AhR), a ligand dependent transcription factor. In this study we investigated the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on human melanocytes, the melanin producing cells of the skin. We used normal human melanocytes as an *in vitro* model system to study the effects of TCDD on pigmentation. RT-PCR revealed that melanocytes express both AhR and aryl hydrocarbon receptor nuclear translocator (ARNT). The functionality of this pathway in melanocytes was determined by studies of the expression of AhR-regulated genes. Exposure of melanocytes to TCDD resulted in a dose-dependent increase in CYP1B1 mRNA levels. Interestingly, an increase in CYP1A1 mRNA levels was not observed, although induction of CYP1A1 mRNA levels is a common response to TCDD in most cells. Melanocytes responded to TCDD (1-10nM) exposure with the activation of tyrosinase, a key enzyme in the melanogenic pathway. Tyrosinase activity was measured in an absorbance based enzyme activity assay by the formation of dopachrome after the addition of L-dopa. The tryptophan photoproduct 6-formylindolo[3,2-b]carbazole (FICZ), another high affinity ligand for the AhR, seems also to increase tyrosinase enzyme activity. AhR antagonists, such as 3'-methoxy-4'-nitroflavone and alpha-naphthoflavone, abolished the effects of TCDD on tyrosinase activation suggesting an involvement of the AhR in mediating dioxin-induced pigmentation.

In summary, the results of this study show that normal human melanocytes *in culture* respond to TCDD exposure with activation of the melanogenic enzyme tyrosinase. The activation of the melanogenic pathway by TCDD is most likely mediated via the AhR receptor.

1410 ADDRESSING AHR ACTIVATION BY PHARMACEUTICALS.

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Activation of the aryl hydrocarbon receptor (AhR) is a liability to be avoided for pharmaceuticals. The concern is to establish a threshold for toxicity for AhR activators. AhR is a transcription factor that activates several genes the most commonly studied of which is CYP1A and whose induction is used as a marker of AhR activation. The challenge for discovery toxicologists is how to assess the risk associated with AhR activation for small molecules. The aim of these studies was: (1) develop & validate *in vitro* models to detect AhR activation and (2) establish a correlation between AhR activation and toxicity *in vivo*. HepG2 cells transiently transfected with a DRE-luciferase reporter construct were used to screen > 400 pharmaceuticals. The EC50s and efficacy, expressed as % of TCDD, for benchmark compounds in this system were: TCDD (0.42-1nM, 100%), β -naphthoflavone (10 μ M, 117%), 3-methylcholanthrene (0.42 nM, 172%), and Benzantracene (6 μ M, 160%). The EC50s and efficacy for the pharmaceuticals ranged from 500nM to 10 μ M and 10 to 150% of TCDD. In addition, the small molecules that activated AhR also induced CYP1A1 mRNA in rat hepatocytes. The AhR antagonist 4-methoxy-3-nitroflavone mediated a dose-dependent inhibition of AhR activation confirming the small molecules mediate their effects via the AhR. To establish a correlation between the *in vitro* endpoints and *in vivo* toxicity, several compounds with AhR activities ranging from 10 to 102% of TCDD were dosed to rats and resulted in dose-dependent increases in CYP1A up to 75-fold after a single dose. In 2 week studies (> 7 compounds), toxicity consistent with AhR activation was observed. The compound with the highest *in vitro* efficacy (102%) had the most severe effects (CYP1A induction of 1124-fold, steatosis in the liver, elevated TSH). CYP1A induction in rat, dog, and human hepatocytes was evaluated to establish risk assessment to man. The same pattern of CYP1A induction was observed in all species demonstrating that preclinical data could be used for human risk assessment.

1411 A HIGH THROUGHPUT FLUORESCENT IMAGING BASED ASSAY FOR INDUCTION OF CYP1A1/2 - APPLICATION IN DRUG DISCOVERY.

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Early identification of new chemical entities (NCE) with the potential to activate the Aromatic Hydrocarbon Receptor (AhR) is important for the reduction of compound attrition in the late stages of the drug discovery process. The activation of

the aryl hydrocarbon receptor (AhR), the key transcriptional regulator of CYP1A1/2 expression, occurs through a diverse set of chemical structures, therefore CYP1A1/2 inducers cannot easily be predicted by their chemical properties alone. Here we describe the development of an imaging based assay to assess potential of NCE to activate the AhR by quantification of CYP1A1/2 expression in the rat hepatoma cell line H4-II-E-C3 using specific antibodies. High assay throughput was achieved by the use of both 96 and 384 well culture systems, the IN Cell 3000 confocal imaging platform and automated analysis of fluorescent images using specific bespoke algorithms. Treatment of H4-II-E-C3 cells, cultured in either 96 or 384 well plates, with the prototypic CYP1A1 inducer TCDD produced a significant increase in both the total intensity of CYP1A1 expression and the number of cells within the population showing fluorescence above the basal level. This assay is able to demonstrate the efficacy and potency of AhR agonists, showing good correlation with both the activation of the AhR, as measured by receptor translocation, and transcriptional upregulation of CYP1A1 mRNA. To conclude, we have developed an automated high throughput imaging assay to allow screening of compound collections for potential CYP1A1/2 protein induction.

1412 DIFFERENTIAL RECOGNITION OF LXXLL PEPTIDE MOTIFS BY THE Q-RICH SUB-DOMAIN OF THE MOUSE AND HUMAN ARYL HYDROCARBON RECEPTOR.

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A number of cofactors have been shown to bind to the human and mouse aryl hydrocarbon receptor transactivation domains (TAD) including the steroid receptor coactivators 1 (SRC-1), nuclear coactivators 2 (NcoA2, SRC-2), p300/CBP co-integrator protein (p/CIP) and CREB binding protein (CBP). In addition to interspecies differences in receptor-ligand binding affinity, the human and mouse AhR receptor C-terminal TADs share only 58% amino acid sequence identity. The aryl hydrocarbon receptor (AHR) has a complex transactivation domain comprising proline/serine/threonine-rich (PST) glutamine (Q-rich) and acidic amino acid residue sub-domains. We wanted to test whether the mouse AHR (mAHR) and the human AHR (hAHR) Q-rich sub-domains differentially recruit LXXLL-motif coactivators. Using Gal4 DNA binding domain (Gal4DBD) LXXLL-motif fused peptides we were able to map the critical LXXLL binding sequence of the hAHR to amino acid residues 663-688. Interestingly, increasing amounts of expressed LXXLL-motif fused Gal4DBD-peptides dramatically differed in their ability to influence the DRE-driven transactivation potential of the mAHR and hAHR in Cos-1 cells. Co-expressing increasing amounts of coactivators also differentially altered Gal4DBD mAHR Q-rich and hAHR Q-rich fusion protein activity as measured in Gal4-driven reporter assays. The Gal4DBD hAHR Q-rich fusion protein also showed relatively higher activity than the cognate mAHR sub-domain. Therefore the hAHR Q-rich domain may prove to be a more significant component in the overall transactivational activity of the human receptor compared to the same sub-domain in the mAHR. These findings suggest that the human and mouse AHR may both display differential recruitment of coactivators and hence potentially exhibit divergent regulation of specific target genes.

1413 DEVELOPMENT OF AN AHR ANTAGONIST USING PROTACS APPROACH: A POTENTIAL APPROACH FOR CHEMOPREVENTION.

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Activation of the aryl hydrocarbon receptor (AHR) leads to many adverse biological effects including tumor promotion, epithelial hyperplasia, endocrine disruption and immunodeficiency. Emerging evidence also links activation of the AHR to the development of type II diabetes. The AHR is thought to mediate its biological effects via its upregulation of a number of target genes. Agonists of the AHR includes dioxin (2, 3, 7, 8 tetrachlorodibenzo-p-dioxin) and polyhalogenated aromatic hydrocarbons found in cigarette smoke and other air pollutants. Our hypothesis is that blocking the actions of the AHR via appropriate AHR antagonists is a valid approach to be used for treatment of a number of human disease states. In the current project we have focused on the development of an AHR antagonist using the PROTACS (PROteolysis Targeting Chimeric molecules) approach. PROTACS is a novel approach of tagging small recognition sequences of a specific E3 ubiquitin ligase complex on to a known ligand for the receptor of interest (AHR) for targeting its degradation via the 26S proteasomal pathway. PROTACS contain a ligand for the AHR, a recognition sequence for the pVHL E3 ubiquitin ligase complex and a linker moiety. The initial, parent compound that serves as the AHR ligand in these studies is apigenin. Here, we demonstrate the design, efficacy and specificity of apigenin bound PROTAC molecules. The design of the apigenin-Protacs was initiated by synthesizing apigenin derivatives that retained binding to the AHR and antagonistic activity. The Apigenin-PROTACS proved to be capable of degrading

the AHR in both HepG2 (human hepatoma) and NHK (Primary human keratinocytes) cells in a time-dependent and concentration-dependent manner. These initial experiments demonstrate that the PROTAC approach can be effective in inhibiting the actions of the AHR blocking AHR-mediated effects.

1414 EFFECTS OF 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON SKIN PIGMENTATION AND ADIPOSE TISSUE HOMEOSTASIS.

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The overall aim of our current research is to advance the knowledge of the endogenous function of the aryl hydrocarbon receptor (AhR) and to clarify the mechanisms behind TCDD toxicity. We use in vivo and in vitro models and activate the AhR with TCDD and the suggested endogenous ligand, the tryptophan photo-product 6-formylindolo[3,2-b]carbazole (FICZ). Our hypothesis defines functions for the AhR in responses to light (adaptation and stress-responses) and in feed-back systems that regulate levels of ligands via induction of metabolizing enzymes. TCDD, because of its high lipid solubility, is concentrated in fat and in the keratinized compartment of epidermis. In humans, epidermal hyperplasia and hyperpigmentation of skin areas are the most well established dioxin related effects. In addition, disturbed lipid metabolism has been reported. We have detected that TCDD and FICZ both activate melanogenesis in primary human melanocytes via activation of the melanin producing enzyme tyrosinase. The results point to a possible function of the AhR in mechanisms that give protection against light. We also study stress-related acute effects in rat adipose tissue and primary adipocytes. Treatment of Sprague-Dawley rats with a dose of 50 µg TCDD per kg body weight led to significantly increased plasma corticosterone levels already one day post treatment. In epididymal fat from the exposed rats pronounced effects on the expression of several adipose specific genes such as leptin, adiponectin and C/EBPα were observed five days post TCDD treatment. These effects, which may be explained by reduced preadipocyte maturation, coincided with reduced plasma triglyceride and leptin levels. It must be kept in mind that this response to a high dose of TCDD on lipid metabolism contrasts with the increased triglyceride levels seen in humans with chronic low dose exposures. In conclusion, our data show that the effects seen after dioxin exposure in humans can be studied experimentally and may be related to physiologic roles of the AhR.

1415 ARYL HYDROCARBON RECEPTOR ANTAGONISTS INCREASE UV-INDUCED APOPTOSIS IN HUMAN ORAL KERATINOCYTES.

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The aryl hydrocarbon receptor (AHR) is a ligand activated transcription factor that not only regulates xenobiotic metabolism, but also cell fate decisions. Our overall goal is to determine whether modulation of the AHR signaling pathway may be useful for therapeutic interventions. We have previously shown that mouse hepatoma cells lacking expression of the AHR (LA1) were more sensitive to UV-induced apoptosis than their wild-type counterpart (Hepa1c17). These results implied that the AHR may play a protective role towards apoptosis mediated by intrinsic stimuli. In this report, we have used human oral keratinocytes to: 1) determine whether the protective role of AHR is cell-type specific, 2) compare the effectiveness of different AHR antagonists in their ability to increase apoptosis susceptibility in human oral keratinocytes and 3) determine whether human oral squamous cell carcinomas may vary in their AHR expression levels. Our results indicate that AHR plays a role in conferring apoptosis susceptibility in human oral keratinocytes that is similar to that described in mouse hepatoma cells. Further, like the AHR antagonist, 3'-methoxy-4'-nitroflavone, apigenin and kaempferol are effective in increasing UV light induced apoptosis in oral keratinocytes. Finally human oral squamous cell carcinomas vary dramatically in AHR expression levels. Future experiments will determine whether the apoptotic response varies in these carcinoma cell lines in a manner that corresponds to their AHR levels and whether it is modulated upon treatment with AHR antagonists.

1416 MODIFICATION OF ARYL HYDROCARBON RECEPTOR CONTROLLED CYP1A1 TRANSCRIPTION BY PHORBOL-12-MYRISTATE-13-ACETATE.

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The aryl hydrocarbon receptor (AHR) is a ligand-inducible transcription factor that regulates the expression of a battery of metabolic genes. The protein kinase C (PKC) family of serine/threonine kinases consists of a least ten members, and these proteins collectively are involved in regulating numerous biological processes. Several reports have concluded that PKC plays a role in the regulation of AHR signal transduction. Additionally, AHR transcriptional activity is modulated by the phorbol ester phorbol-12-myristate-13-acetate (PMA). The ability of PMA to en-

hance AHR activity using luciferase reporter systems is believed to be attributed to its PKC activating properties, and has been termed the "PMA effect". In contrast to reporter gene systems which exhibit enhanced expression of an AHR-driven CYP1A1 luciferase construct, we demonstrate here that ligand-induced endogenous CYP1A1 and CYP1B1 mRNA levels are greatly reduced in HepG2, HeLa, and MCF-7 cell lines when PMA (81 nM) is co-administered with the AHR ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) for short time periods (2-4 h). However, when cells are exposed to TCDD for longer periods of time (24 h) CYP1A1 levels are elevated in the presence of PMA. In order to determine if the PMA-mediated decrease in CYP1A1 expression results from alterations in chromatin remodeling chromatin immunoprecipitation experiments were performed in MCF-7 cells. These studies showed that upon a 1 h co-treatment of PMA and TCDD the amount of acetylated histone-4 and RNA polymerase II is decreased at the CYP1A1 enhancer and promoter, respectively. Therefore, these studies show that the PMA-mediated decrease of AHR transcriptional activity results from the reduced amount of chromatin remodeling in response to ligand activation of the AHR. This data shows that PMA treatment causes a rapid response that inhibits TCDD-induced pre-transcriptional chromatin remodeling on the AHR-responsive gene, CYP1A1.

1417 CHARACTERIZATION OF ARNT (ARYL HYDROCARBON RECEPTOR TRANSLOCATOR) ISOFORMS FROM MYA ARENARIA.

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The aryl hydrocarbon receptor nuclear translocator (ARNT) is a member of the bHLH-PAS family of transcription factors. Member proteins contain well-conserved DNA binding and heterodimerization domains, although the transactivation domains show considerable sequence divergence. Vertebrate ARNT is constitutively expressed and plays a role in regulating gene expression in response to changes in cellular environment. For example, exposure to aryl hydrocarbons results in binding to another bHLH protein and ARNT dimerization partner, the AHR. A previous study revealed that some invertebrate species, including *M. arenaria*, *D. melanogaster* and *C. elegans*, have AHR homologs that apparently do not bind prototypical exogenous ligands. A single AHR gene and four distinct forms of ARNT have been identified in *M. arenaria*. All four ARNTs contain intact PAS A domains, but two have truncated PAS B domains which may influence ARNT interaction with AHR and other dimerization partners. As part of the characterization of these ARNT forms, a yeast two-hybrid system was used to test protein interaction between clam AHR and each form of ARNT. Preliminary data suggest clam AHR interacts with all forms of clam ARNT and one form interacts with human AHR. To better understand their function, tissue expression in the clam was assessed by Western blot with a pan-ARNT polyclonal antibody. Western blots indicate ARNT-A1 (72 kDa) is the predominantly expressed ARNT protein and is present in all tissues tested. ARNT-D3 protein (42 kDa) is seen in both gill and gonad tissue and ARNT-C2 (54 kDa) protein is expressed primarily in gill. ARNT-2B2 protein (45 kDa) is rarely expressed and only in gonad. Preliminary analysis has not identified a correlation between ARNT expression and gender, season or stage of gametogenesis. Since ARNT is known to be involved several vertebrate important signaling pathways, further characterization of ARNT expression may provide additional understanding of its role.

1418 INHIBITION OF ESTROGEN-MEDIATED GENE EXPRESSION RESPONSES BY DIOXIN IN THE UTERI OF C57BL/6 MICE.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) exhibits diverse antiestrogenic properties including the inhibition of estrogen-induced uterine growth and proliferation. One proposed mechanism involves the inhibition of estrogen-mediated gene expression through ER/AhR crosstalk. However, only a limited number of inhibited responses have been identified which are unlikely to fully account for all of the uterine antiestrogenic effects of TCDD. Therefore, the effects of TCDD on ethynyl estradiol (EE) mediated gene expression were investigated in the immature ovariectomized C57BL/6 uterus using cDNA microarrays with complementary physiological and histological phenotypic anchoring. Mice were gavaged with either vehicle, 3 daily doses of 10 µg/kg EE, a single dose of 30 µg/kg TCDD or a combination of EE and TCDD and sacrificed after 4, 12, 24 and 72 hours. TCDD cotreatment inhibited EE-mediated increases in uterine wet weight by 37, 23 and 45% at 12, 24 and 72 hrs, respectively. In addition, TCDD reduced EE-mediated stromal edema, hypertrophy and hyperplasia and induced marked luminal epithelial cell apoptosis. A 2 × 2 factorial microarray design was used to identify EE- and TCDD-specific gene expression responses as well as their interactive effects. Of the

2,753 uterine genes regulated by EE, only 133 were significantly modulated upon cotreatment with TCDD, indicating a gene-specific inhibitory response to TCDD. The EE-mediated induction of many genes, including trefoil factor 1 and keratin 14, were inhibited by greater than 90% upon cotreatment with TCDD. Functional annotation of these inhibited responses was associated with cell proliferation, water and ion transport, and maintenance of cellular structure and integrity. These responses correlate with the observed histological alterations and may collectively contribute to the antiestrogenic effects of TCDD on the uterus. Funded by NIH R01 ES12245

1419 SCREENING FOR ESTROGEN RECEPTOR (ER ALPHA) LIGANDS USING HIGH CONTENT NUCLEAR TRANSLOCATION ANALYSIS.

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The discovery of new drugs targeting the estrogen receptor (ER α) which would exhibit reduced incidence of adverse side effects acting selectively in different tissues is crucial for the treatment of breast cancer and for hormone replacement therapy. ER α undergoes nucleo-cytoplasmic shuttling, yet the nuclear compartment is strongly favored even in the absence of hormonal ligands. Our goal was to design an ER α whose cellular distribution would be responsive to estradiol-like ligands and to use this receptor to carry out drug screens based on nuclear translocation. To accomplish this, the N-terminus, DNA binding domain, hinge and partial ligand binding domain regions of the GR (containing the nuclear localization signal) was fused with most of the ER ligand binding domain and tagged with the green fluorescent protein (GFP). A high content imaging system was used for image acquisition and analysis of translocation dynamics. Compounds that elicited a translocation value of 85 or greater were identified as hits. We have screened 13,868 synthetic compounds and pure natural products, which produced 390 primary hit compounds, and the primary hit number was reduced to 41 compounds after filtering the hits for cytotoxic false positive results, which generated a hit rate of 0.3% for the screen. Cherry picking experiments were used to eliminate false positive fluorescent DNA-binding compounds, which further reduced the hit rate to 0.058% resulting in 8 primary hit compounds. These compounds will be further tested for ER α activity in secondary assays by the principal investigator's laboratory.

1420 CROSS-TALK BETWEEN ARYL HYDROCARBON RECEPTOR AND PREGNANE X RECEPTOR PATHWAYS.

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Pregnane X receptor (PXR) and aryl hydrocarbon receptor (AhR) are two ligand-dependent transcription factors that regulate the expressions of distinct sets of genes. Many genes regulated by PXR and AhR are important for phase I, II and III metabolic detoxification. Research on PXR-regulated gene expression has been facilitated by the identification of the regulatory module (XREM) around -7 kb of cyp3a4 genes which is mainly regulated by PXR. We have developed a HepG2 cell-based PXR-driven luciferase assay in order to analyze cyp3a4 inducers in cell culture. Interestingly, we found that rifampicin and TCDD show synergistic induction of the PXR/XREM-driven luciferase reporter gene expression. The cyp3a4 mRNA was also synergistically induced by cotreatment with TCDD and PXR ligand (rifampicin), suggesting cross-talk between PXR and AhR regulated pathways. Overexpression of AhR enhanced the synergistic activation, while small interfering RNA targeting AhR diminished the magnitude of synergistic activation in reporter gene assay. Using GST-pulldown and coimmunoprecipitation assays, we found a direct interaction between the AhR and PXR. Consistent with domain-specific interaction between AhR and PXR, AhR expression plasmids with different domain deletions showed differential trans-activities in reporter gene assay with PXR-driven CYP3A4-luc gene. These data suggested that AhR and PXR pathways crosstalk through direct interaction between AhR and PXR to regulate mutual transcription activity (Supported in part by NIEHS Grants ES09859).

1421 THE PREGNANE X RECEPTOR AND THE MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II GENES.

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Several studies have shown that the Pregnane X Receptor (PXR) acts as a sensor to a broad range of natural and synthetic xenobiotics mediated through the induction of the detoxification enzyme CYP3A and other drug metabolizing enzymes.

Adding to this classical response, we have shown that PCN (a PXR activator in rodents) induces transcription and protein levels of Major Histocompatibility Complex class II (MHC-II) genes in primary rat hepatocytes. These findings triggered further research on the role of PXR and its activators on the regulation of genes involved in the immune response. The interest in MHC-II gene expression in disease state has recently increased within the medical community. There is data suggesting that MHC-II in non-Antigen Presenting Cells such as tumor cells can boost the immune response against itself, however, the mechanism involved in its ectopic expression is still unknown. To investigate further the molecular mechanism by which PXR and its activator rifampicin induces transcription of MHC-II genes, we performed a series of time course and dose response experiments in various cell lines. Human fibrosarcoma (HT-1080), bronchial epithelial cells (BEAS-2B) and hepatoma cells (HepG2) were treated with rifampicin and MHC-II mRNA levels evaluated. Preliminary results showed induction of HLA-DR α (2-fold) and HLA-DQB1 (5-fold) mRNAs in HT-1080 cells. The presence of human PXR1 mRNA in HT-1080 fibrosarcoma treated with rifampicin was confirmed by means of RT-PCR. HLA-DR α mRNA expression was also found to be induced in HepG2 cells treated with rifampicin. These findings support the idea of a potential involvement of PXR by enhancing transcriptional induction of MHC-II genes. This research adds a new dimension to a synchronized relationship of immune and detoxification regulation through PXR function as means of a xeno-protective mechanism.

1422 PPARB/D PROTECTS AGAINST EXPERIMENTAL COLITIS THROUGH A LIGAND-INDEPENDENT MECHANISM.

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PPAR γ and PPAR β/δ have overlapping roles in the negative regulation of genes involved in inflammatory responses. Recent reports suggest that ligand activation of PPAR γ in the epithelium of the colon protects against experimentally induced colitis in mice. Since PPAR β/δ can negatively regulate inflammation in a number of cell types, and is highly expressed in the epithelial cells of the colon, PPAR β/δ may also have a role in experimental colitis. In these studies, colitis was induced by administration of dextran sodium sulfate (DSS) to wild-type and PPAR β/δ -null mice, and a cohort of mice were treated with the PPAR β/δ specific ligand GW0742. Symptoms of disease and expression of inflammatory cytokines were examined to determine whether the absence of receptor expression and/or ligand activation of the receptor influenced the development of experimental colitis. PPAR β/δ -null mice exhibited an increased sensitivity to DSS-induced colitis, as shown by marked differences in body weight loss, colon length, colonic morphology, and increased mRNAs encoding the inflammatory markers IFN- γ , TNF- α , and IL-6 levels as compared to similarly treated wild-type mice. Although diarrhea score, bleeding score, disease activity index, and myeloperoxidase activity were not significantly increased in PPAR β/δ -null mice, there was a trend in this direction. Interestingly, these differences were not affected by PPAR β/δ ligand activation in either genotype. These studies demonstrate that PPAR β/δ expression in the colonic epithelium inhibits inflammation and protects against DSS-induced colitis via a ligand-independent mechanism. (Supported by CA89607, CA97999 and CA124533)

1423 LIGAND ACTIVATION OF PPAR-BETA INHIBITS SKIN CARCINOGENESIS.

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PPAR β -null mice exhibit enhanced tumorigenesis in a two stage bioassay model as compared to wild-type mice, which is likely due in part to enhanced epidermal hyperplasia and decreased apoptosis following treatment with phorbol ester. Previous work also showed that ligand activation of PPAR β induces terminal differentiation and inhibits proliferation in primary keratinocytes, and this effect does not occur in the absence of PPAR β expression. In the present studies, the effect of ligand activation of PPAR β on skin carcinogenesis was examined using both in vivo and in vitro skin carcinogenesis models. Inhibition of skin cancer was observed in wild-type mice administered GW0742, and this effect was likely the result of ligand induced

terminal differentiation. These effects were not found in similarly treated PPAR β -null mice. Ligand activation of PPAR β also inhibited cell proliferation and increased expression of mRNAs encoding terminal differentiation markers in keratinocyte cancer cell lines that represent different stages of skin carcinogenesis. These studies suggest that topical administration of PPAR β ligands is chemopreventive against chemically-induced skin cancer. (Supported by CA9607, CA97999 and CA124533)

1424 ACTIVATION OF MOUSE AND HUMAN PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR ALPHA (PPAR α) BY PERFLUOROALKYL ACIDS (PFAAS) INVESTIGATED USING A TRANS-ACTIVATION MODEL IN COS-1 CELLS.

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The purpose of this work was to evaluate the interaction of PFAAs with PPAR α and determine the potential for activation or antagonism of the pathway. Activation of mouse and human PPAR α by perfluorobutane sulfonate (PFBS; C4), perfluorohexane sulfonate (PFHS; C6), and perfluorononanoic (PFNA; C9) was examined using a transient transfection cell assay. Cos-1 cells were transfected with either mouse or human PPAR α receptor luciferase reporter plasmid. Transfected cells were exposed to negative controls (0.1-0.8% DMSO and water), PPAR α agonist (WY14643 [10 μ M]), PPAR α specific antagonist (MK886 [20 μ M]), and freshly prepared solutions of PFBS, PFHS, PFNA (5-100 μ M). All treatments were tested both alone and in competition with MK886. Treatment induced changes in luciferase activity were measured after 24 hrs incubation. PFBS did not significantly activate mouse or human PPAR α , when compared to vehicle controls. PFHS did not significantly induce luciferase activity of the mouse PPAR α vector, but 30 μ M PFHS did significantly increase activation of the human PPAR α , although this activity was not inhibited by MK886. PFNA significantly induced mouse and human PPAR α -mediated luciferase activity at concentrations of 40-100 and 5-100 μ M PFNA, respectively, however this activity was not significantly reduced by MK886. In summary, PFNA induced activity with both mouse and human receptors while PFHS only induced activity through human PPAR α . If PFHS and PFNA induced these effects by binding to the ligand binding site of the PPARs, one would hypothesize that MK886 would compete for binding and reduce the activity. Since this did not occur, it implies another mechanism for the induction by these PFAAs. This abstract does not necessarily reflect U.S. EPA policy.

1425 PPAR β ATTENUATES HEPATOTOXICITY BY SUPPRESSING ACTIVATION OF STELLATE CELLS AND EXPRESSION OF PRO-INFLAMMATORY GENES.

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Peroxisome proliferator-activated receptors (PPAR) are transcription factors that belong to the nuclear hormone receptor superfamily. PPARs can modulate target gene expression by both upregulation and repression of transcription. While the roles of PPAR α and PPAR γ are well defined, the functional role(s) of PPAR β remain largely uncertain. Previous work has shown that PPAR β is protective against carbon tetrachloride (CCl₄)-induced hepatotoxicity as indicated by elevated serum ALT and hepatic TNF α levels in the absence of PPAR β . In this study, hepatic stellate cells (HSCs) were isolated from wild-type and PPAR β -null mice and cultured in vitro. Expression of mRNA encoding PDGF β , PDGF receptor β , SMA α and collagen type1 α 1 were markedly higher in the PPAR β -null HSCs as compared to their wild-type counterparts. Western blotting confirmed the overexpression of SMA α protein in PPAR β -null HSCs. In a separate study, gene expression profiles were examined using microarray analysis of mRNA from wild-type and PPAR β -null mouse livers treated with CCl₄ revealing that a number of genes with diverse functions were differentially regulated between the two genotypes in response to CCl₄. In particular, expression of TWEAK-receptor that is regulated by TNF α and leads to NF κ B activation was greatly enhanced in PPAR β -null mice as compared to similarly treated wild-type mice. These results support the hypothesis that PPAR β protects against chemically-induced hepatotoxicity, possibly via the inhibition of hepatic stellate cell activation and repression of pro-inflammatory responses. (Supported by CA9607, CA97999 and CA124533)

1426 ROLE OF MU-OPIOID RECEPTOR IN TRAMADOL-MEDIATED ANALGESIC ACTIONS IN THE MOUSE TAIL-FLICK TEST.

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The mu opioid receptor plays an important role in mediating analgesic actions of a class of opioids including morphine and codeine. Tramadol, a centrally acting analgesic structurally related to codeine and morphine, consists of two enantiomers, both of which contribute to analgesic activity via different mechanisms. Tramadol has been proposed to induce analgesia via both opioid and non-opioid pathways. However, details of the mechanisms remain unclear. The purpose of this study was to determine the role of mu-opioid receptor in tramadol-induced analgesic actions using mu-opioid receptor knockout mice. Analgesic responses in the tail-flick test are expressed as the percentage maximum possible effect (% MPE) at the time point at which the greatest analgesic responses were observed for tramadol. Acute intraperitoneal administration of tramadol showed a clear dose-analgesic response relationship in the wild-type female mice with the ED50 of 69.5 mg/kg (66.4 – 72.8 mg/kg, 95% confidence intervals). However, no tramadol dose-response relationship was demonstrated in mu-opioid receptor knockout mice. A tramadol dose of 144 mg/kg, which is more than double of ED50 dose in wild-type mice, produced only % MPE value of 14.5 $\pm$ 0.7 in mu-opioid receptor knockout mice. Higher doses of tramadol caused animal death without increase in analgesic effects. The result from present study clearly indicated that mu-opioid receptor plays a critical role in tramadol-mediating analgesic actions, at least in the tail-flick test mouse model. (Supported in part by NIH grant RR-P20 RR17701)

1427 STRUCTURE ACTIVITY RELATIONSHIPS BETWEEN CAPSAICINOID ANALOGUES AND TRPV1 ACTIVATION.

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Capsaicinoids elicit inflammatory cytokine production and cell death in cells that express the vanilloid receptor 1 (TRPV1). Cell surface and intracellular sub-populations of TRPV1 differentially regulate these responses. Activation of the intracellular pool of TRPV1 causes calcium efflux from the endoplasmic reticulum (ER). Disruption of calcium concentrations in the ER triggers a homeostatic mechanism known as the ER stress response. ER stress responses are intended to compensate for stress and promote cell survival, although prolonged stimulation of ER stress signaling causes cell death. A critical ER stress response pathway involves eukaryotic translation initiation factor 2 α kinase 3 (EIF2AK3 or PERK). This particular ER stress pathway appears to be responsible for cell death caused by ER stress-inducing agents. Induction of the pro-apoptotic gene product DNA damage inducible transcript 3 (DDIT3) is believed to cause cell death. We used synthetic variants of capsaicin and its analogue nonivamide to correlate TRPV1 receptor activation with DDIT3 expression and cell death. Capsaicinoids are composed of a 3-MeO-4-OH-benzylamide ring moiety and a hydrocarbon tail. Structural modifications to the vanilloid ring drastically reduced the ability of the capsaicinoids to stimulate TRPV1, DDIT3 gene expression, and to produce cell death. Specifically, removal, methylation or repositioning of the 4-OH group reduced DDIT3 induction and toxicity. Demethylation of the 3-MeOH group reduced toxicity in a TRPV1-over-expressing cell line, but increased toxicity in the BEAS-2B cell line suggesting a change in the mechanism of toxicity, independent of TRPV1 activation, but not DDIT3 induction. Collectively, a more thorough understanding of capsaicinoid-TRPV1 structure activity relationships will more clearly define how TRPV1 functions, how capsaicinoid variants and metabolites alter cellular function, and elucidate novel mechanisms of capsaicinoid action in cells of diverse origin.

1428 MODULATION OF EPITHELIAL CELL TUMOR NECROSIS FACTOR- α RECEPTOR RELEASE BY ANTIOXIDANTS, TNF- α AND INTERLEUKIN -1 β

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Tumor necrosis factor- α (TNF- α) is a pleiotropic cytokine implicated in the pathogenesis of numerous inflammatory and fibrotic lung diseases. The two receptors for the cytokine, TNFR1 and TNFR2, exist in both membrane-bound and soluble forms, both forms capable of binding TNF- α . Our lab has investigated the solubilization or shedding patterns of the TNFRs, specifically from murine, lung type II-like, epithelial cells (MLE-15), in response to TNF- α or to another pro-inflammatory cytokine, interleukin-1 β (IL-1 β). Our results demonstrate differential

modulation of TNFR shedding by TNF- α and IL-1 β . Specifically, we observed that TNF- α inhibited while IL-1 β stimulated shedding of the TNFR1. Conversely, both cytokines enhanced the release of TNFR2. However, the mechanisms regulating this generation of soluble TNF receptors remain unclear. It is well known that both cytokines induce the formation of oxidants. To test our hypothesis that reactive oxygen species (ROS) modulate ligand-induced TNFR shedding, MLE-15 cells were exposed to TNF- α or IL-1 β in the presence or absence of the antioxidant dimethyl sulfoxide (DMSO). Effects of hydrogen peroxide and phorbol myristate acetate (PMA) were also examined as positive controls. PMA stimulated the release of both TNFRs whereas exposure of MLE-15 cells to hydrogen peroxide had minimal effects. Pretreatment with DMSO dose dependently reduced basal shedding as well as stimulant-induced release of both receptors. This reduction by DMSO was not accounted for by enhanced cell death as measured by lactate dehydrogenase assay. Further studies utilizing other antioxidants such as N-acetylcysteine (NAC) and trolox will explore the role of oxidants in TNFR shedding as our data suggests that basal, and stimulant-induced, TNFR shedding from murine lung epithelial cells involve activation of sheddases by oxidants.

1429 FENRETINIDE-INDUCED APOPTOSIS OF HUH-7 HUMAN HEPATOMA CELL IS ASSOCIATED WITH INDUCTION OF THE RAR BETA GENE.

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Retinoids are a family of compounds related to vitamin A, which play crucial roles in vision, embryogenesis, differentiation, and antiproliferation. The goals of this study are to examine the effect and mechanism for retinoid-induced apoptosis in human hepatoma cells. A panel of 13 retinoids was screened in human hepatoma cell line Huh-7, HepG2, and Hep3B for their apoptotic effect. As a result, fenretinide [N-(4-hydroxyphenyl) retinamide], a synthetic retinoid and structure analogue of all-trans retinoic acid, showed significant apoptotic effect in Huh-7, but not in HepG2 and Hep3B, by decreasing cell number by 75% and inducing caspase-3 cleavage/activation. Further analysis using TUNEL assay revealed 20-fold increase in DNA double-strand break positive cells in fenretinide-treated Huh-7 cells. Phosphatidylserine translocation, another hallmark of apoptosis measured by flow cytometry, showed 17-fold increase in fenretinide-treated Huh-7 cells. Retinoids exert their biological effect by binding to nuclear receptors. To determine the mechanism responsible for fenretinide-induced apoptosis, mRNA level of 11 nuclear receptors, including retinoic acid receptor (RAR) alpha, beta, gamma; retinoid x receptor (RXR) alpha, beta, gamma; orphan nuclear receptors Nur77, Nurr1, Nor1; and xenobiotic sensors CAR and PXR, was quantified by real-time PCR before and after fenretinide treatment. Huh-7 cells have the highest basal mRNA level of RAR beta, RXR alpha, and Nurr1 among the three cell lines. A correlation between the induction of RAR beta mRNA level and apoptosis was noted after fenretinide treatment. At as early as 2 hours after fenretinide treatment, RAR beta mRNA level was increased, reaching 13- and 34-fold induction at 24 hours and 48 hours, respectively. In conclusion, fenretinide induces cell-type specific apoptosis in human hepatoma cells, which is associated with up-regulation of RAR beta gene expression. (NIH CA 53596 and AA14147)

1430 TRACE ELEMENTS IN COMMERCIAL INFANT FOOD.

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High growth rate and development of organ systems in childhood requires a balanced diet rich in nutrients. Low intake or low bioavailability of minerals may lead to deficiencies which may cause impairment of normal development of important body functions. The diet of many infants is based on industrial produced food and it is therefore important that the food represent an appropriate supply of trace elements.

Our study included 76 different infant products e.g. breast-milk substitute, porridges, fruit purée and dinners from Norwegian manufacturers. Samples were drawn from homogenized food product and the total concentration of major minerals, trace elements and toxic compounds were determined by High Resolution Inductively Coupled Plasma Mass Spectrometry, HR-ICP-MS. The estimated total daily intake of the minerals calcium, magnesium, sodium, zinc, iron and copper was two times or more higher than the recommended daily intake. The total daily intake of iron and zinc was higher than the tolerable upper intake level (WHO), but not considered as hazardous. None of the products contained toxic elements such as mercury, cadmium, arsenic and lead in amounts which can be considered as a health hazard for the consumer. Dinners containing fish had the highest mercury and arsenic levels, 110 $\mu\text{gAs}/100\text{g}$ and 0.60 $\mu\text{gHg}/100\text{g}$. The highest cadmium concentration was 102 $\mu\text{g}/100\text{g}$ and found in porridge.

1431 FSIS NATIONAL RESIDUE PROGRAM.

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The National Residue Program (NRP) is a multi-component analytical testing program for residues present in domestic and imported meat, poultry and egg products. The NRP is managed by the Food Safety and Inspection Service (FSIS), a public health regulatory agency within USDA that protects consumer health by regulating meat, poultry, and egg products. The NRP protects consumer health by detecting, identifying, and removing meat, poultry and egg products adulterated with veterinary drugs, pesticides and other chemical contaminants.

The Zoonotic Diseases and Residue Surveillance Division annually publishes the National Residue Program Sampling Plan and the Data from the testing results. In 2005, FSIS sampled and tested 17 compound classes of veterinary drugs, pesticides and environmental contaminants, comprising approximately 80 residue compounds. Under the Domestic Scheduled Sampling Plan (preliminary results) 21,472 samples were collected and analyzed. From these, sixty-two (62) chemical residue violations were found and consisted of 31 antibiotics, 10 avermectins/milbemycins, 9 sulfonamides, 6 florfenicol, 1 nitrofurantoin and 5 chlorinated hydrocarbon/chlorinated organophosphate. No residue violations were found in testing for arsenic, carbadox, nitroimidazoles, phenylbutazone, racetopamine, thyrostats, trenbolone, zeranol, chloramphenicol, beta-agonists and melengestrol acetate. The FSIS NRP, in conjunction with the EPA and FDA, plays a pivotal role in protecting consumers from violative levels of chemical residues in meat, poultry, and egg products. Furthermore, FSIS works cooperatively with state meat and poultry inspection programs to achieve mutual objectives in residue prevention and control.

1432 THE NORWEGIAN NATIONAL REPORTING SYSTEM AND REGISTER OF SEVERE ALLERGIC REACTIONS TO FOOD.

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Background: The Norwegian National Reporting System and Register of Severe Allergic Reactions to Food (The Food Allergy Register) is a voluntary reporting system for medical doctors, started with Government funding in 2000. Norway has about 4 million inhabitants. The reporting system covers all first-line doctors (family and specialist practices, emergency wards, some relevant hospital departments). The aim is to collect information about the incidence of food allergy reactions, risk groups and risk situations, offending foods and follow-up by the health care system. Also, food allergen surveillance is a major goal. The Food Allergy Register: One-page reporting forms and informed consent forms are mailed to all relevant doctors at least once a year. Serum samples may be submitted for specific IgE analysis, and food samples for allergen analysis.

Results: By July 1, 2006 the number of reports was 485, with a slightly increasing trend. Two marked age peaks of food allergic reaction incidence are observed, one as expected among small children, the other among young adults age 20-35 (35% of cases). A female/male ratio of 60/40 has been found among the adult cases. Common incriminating foods are hazelnut, peanut, and shellfish, while fish allergy appears to be rare. No deaths have been reported. A number of cases with unexpected presence of allergens in commercial foods have been discovered.

Discussion: Underreporting is a problem, and the true incidence of severe allergic reactions certainly is higher than the number of reports. However, the reporting system has provided valuable information on risk groups and the most important incriminating allergens, and a number of cases of unexpected allergens in commercial foods have been detected. The value of the register increases as more cases are reported.

1433 AVOIDING PERCHLORATE EXPOSURES FROM FRESH FRUITS AND VEGETABLES: AT WHAT COSTS?

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Perchlorate residues have been detected in fruits and vegetables grown in the Colorado River Basin and in water and milk samples collected from multiple regions in the US, raising concerns about the safety of the food supply. At high doses, perchlorate can interfere with iodide uptake by the thyroid gland and disrupt its physiological functions in the body. Excessive exposures of expectant mothers may affect the fetus and newborn and result in effects such as delayed development and decreased learning capability. Since the thyroid plays a major role in proper development in addition to metabolism in children, their exposure to perchlorate is also

of potential concern. While avoidance of these foods could result in decreased perchlorate exposures, these foods are major sources of calcium, vitamins A and D, antioxidants and water-soluble vitamins. We use food consumption data from surveys conducted by USDA and CDC to compare the nutrient profile of consumers of foods that may be contaminated with perchlorate to that of non-consumers of these foods. We assess the nutritional benefits associated with consuming these foods and risk associated with avoiding them, and compare these risks to the risks of exposure to perchlorate.

1434 BALANCING AND COMMUNICATING RISKS AND BENEFITS ASSOCIATED WITH EGG CONSUMPTION – A RELATIVE RISK STUDY.

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The American Heart Association (AHA) and the National Cholesterol Education Program (NCEP) recommend restricting egg consumption based on the hypothesis that egg intakes will result in an increase in blood cholesterol levels, which, in turn, is associated with increased cardiovascular disease (CVD) risks. However several studies have shown that dietary cholesterol from eggs has little if any effect on plasma cholesterol or the risk for heart diseases after adjustment for other potential risk factors. Further, eggs are known to be a good source of high quality protein, B vitamins and folate, fat-soluble vitamins such as A, D, and E, and essential minerals (iodine, zinc, calcium, and iron). Eggs also contain lutein and zeaxanthin, which might be involved in the prevention of cataracts and age-related macular degeneration, and rich in choline, which may have a role in normal development of memory. Hence, wide-sweeping recommendations to restrict egg consumption to avoid CVD risk may be misguided when nutritional contribution from eggs is not considered. A relative risk study is conducted to evaluate and quantify the relative risks of CVD associated with dietary cholesterol and eggs compared to other factors including BMI, dietary, lifestyle, or drugs, etc. on cardiovascular disease and the health benefits of egg consumption. Estimates of egg contribution to dietary cholesterol and nutrition are developed based on consumption data from nationwide food consumption survey. A report card to communicate the relative risks of egg cholesterol and the nutritional benefits is explored in this paper.

1435 STRATEGIC CONSIDERATIONS IN THE DESIGN AND CONDUCT OF ANIMAL MODELS FOR ASSESSMENT OF FOOD PROTEIN ALLERGENICITY.

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There is a recognized need for a relevant animal model for the assessment of allergenic potential of novel foods, in particular those derived from genetically modified plants. There remains some debate about the most appropriate design for such a model, and we propose here one approach based on practical and theoretical considerations. The preferred strategy is predicated on the identification of novel proteins that have allergenic properties (as judged by the ability to induce both IgG and IgE antibody), and to distinguish these from other proteins that despite being immunogenic lack allergenic potential (the ability to induce IgG, but not IgE, antibody). The recommended experimental approach is configured to provide maximum sensitivity for the purposes of hazard identification. To this end BALB/c mice are used; this strain having an atopic-like phenotype and being predisposed to mount vigorous IgE antibody responses. In order to maximise opportunity for elicitation of immune and allergic responses, and to avoid the possibility of tolerance induction associated with oral administration, exposure is systemic via intraperitoneal injection. In order to reflect accurately the inherent immunologic properties of test proteins exposure is effected in the absence of adjuvant. At various times after the initiation of exposure IgG antibody responses are measured using enzyme-linked immunosorbent assay and IgE antibody detected using passive cutaneous anaphylaxis. A detailed rationale for this experimental design, together with an analysis of performance characteristics and strengths and limitations with respect to hazard identification will be described.

1436 SAFETY EVALUATION OF A SALACIA OBLONGA EXTRACT FOR USE IN FUNCTIONAL FOODS.

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Salacia oblonga holds potential as a natural method to mitigate the blood glucose response for people with diabetes by inhibiting the activity of intestinal α -glucosidases. As part of a safety evaluation of novel ingredients for use in blood glucose control, the toxicity of a Salacia oblonga root extract (SOE) was evaluated in a 92-

93 day feeding study in rats using doses of 250, 1250, and 2500 mg/kg/day via oral gavage. An in vivo-in vitro rat peripheral blood lymphocyte chromosomal aberrations assay was added at termination of the subchronic rat study to examine cultured lymphocytes for possible chromosomal aberration induction. This was conducted due to a previous weak; although reproducible, positive chromosomal aberrations response in cultured peripheral blood human lymphocytes after acute in vitro treatment with SOE. No noteworthy signs of toxicity were noted in feeding or body weight, functional observational battery or motor activity. No treatment related changes were considered to be of toxicological significance. The present study results indicate that SOE was negative for the induction of chromosomal aberrations in cultured rat peripheral blood lymphocytes after 90 consecutive days of treatment with SOE. The No Observable Adverse Effect Level was determined to be 2500 mg/kg/day following oral gavage to rats for 92-93 days.

1437 NEONATAL MICE AS MODELS FOR PREMATURE INFANTS INFECTED WITH *E. SAKAZAKII*-CONTAMINATED INFANT FORMULA.

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Premature or very-low-birth-weight human infants exposed to *Enterobacter sakazakii* (*E. sakazakii*) in reconstituted powdered infant formula may develop infections resulting in septicemia, necrotizing enterocolitis, meningitis, hydrocephalus, or death. Animal models are needed to estimate and understand the infectivity of *E. sakazakii* in human infants. Due to their underdeveloped central nervous system at birth, the infection of neonatal mice with *E. sakazakii* may mimic that of premature human infants. Our objective was to compare the susceptibilities of three mouse strains to *E. sakazakii* strain MNW2 by observing mortality and infectivity. Timed-pregnant dams of the CD-1, BALB/C and C57BL/6 strains were obtained, acclimatized, and allowed to give birth naturally. At postnatal day (PND) 3 or 4, the pups were orally gaged with a single dose of vehicle or 10^4 - 10^{12} colony-forming units (CFU) *E. sakazakii* strain MNW2 per ml reconstituted powdered infant formula. All pups surviving to PND 10 or 11 were sacrificed and brains, livers, and ceca excised and analyzed for the presence of *E. sakazakii*. *E. sakazakii* was isolated from 66.7%, 38.5% and 36.4% of brains, 60.0%, 30.8% and 45.4% of livers, and 26.7%, 23.1% and 18.2% of ceca from treated CD-1, BALB/C and C57BL/6 litters, respectively. No deaths occurred in any of the control groups for any mouse strain. Among the three strains, CD-1 appears to be the most sensitive demonstrating a dose-dependent response in mortality (10^{11} CFU resulted in 34.8% mortality). In C57BL/6 mice, mortality occurred only at the highest dose administered (4.2% at 10^{12} CFU). Although BALB/C mice had 19% mortality at 10^7 CFU, it was not dose-dependent. The results of this experiment suggest that the CD-1 mouse strain is the most susceptible to *E. sakazakii* infection and may serve as a potential animal model for the infection in human infants. (Funded by ILSI North America Technical Committee on Food Microbiology to MAS).

1438 SUBCHRONIC RAT FEEDING STUDY WITH GENETICALLY-MODIFIED MAIZE GRAIN FROM INSECT-PROTECTED, HERBICIDE-TOLERANT PLANTS.

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We evaluated health effects of daily consumption of a diet formulated with the combined-trait maize product DAS-Ø15Ø7-1xDAS-59122-7. 15Ø7x59122 maize provides in-plant protection from CRW and ECB larval feeding damage via expression of *Bt*-derived Cry34Ab1, Cry35Ab1, and Cry1F insecticidal proteins, and the *Streptomyces viridochromogenes* PAT protein confers tolerance to the herbicidal active ingredient glufosinate-ammonium. Diets conforming to Purina Mills Certified Rodent LabDiet[®] 5002 were formulated by substitution of transgenic (TG) test (15Ø7x59122), non-TG near-isogenic control, or 3 non-TG commercial reference maize grain sources at 34% (w/w). We verified TG protein presence, stability, and biological activity in the TG test diet. Young adult Crl:CD(SD) rats (12/sex/group) ate the diets for 92-94 days. Compared with rats fed the non-TG control diet, no toxicologically significant differences were observed in rats fed the 15Ø7x59122 diet with respect to body weight/gain, food consumption/efficiency, mortality, clinical signs of toxicity, or ophthalmological observations. No test diet-related effects were observed for neurobehavioral assessments, clinical pathology, organ weights, or anatomic pathology. Response variable values for animals in the test diet group fell within the range of natural variation observed for animals in the reference diet groups. Under the conditions of this study, daily consumption of a rodent diet formulated with 15Ø7x59122 maize grain caused no adverse health effects in young adult rats. We conclude that grain from the combined-trait maize product DAS-Ø15Ø7-1xDAS-59122-7 is nutritionally equivalent to and as safe as grain from non-transgenic maize.

1439 TOXICOLOGICAL ASSESSMENT OF TRANSGENIC SALMON IN A RODENT DEVELOPMENTAL STUDY.

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In recent years, concerns about the safety of foods derived from the application of biotechnological advances (known as genetically modified or GM foods) have re-introduced the issue of how best to determine the safety of whole foods. The current study was conducted to identify any adverse or beneficial effects due to growth-enhanced genetically modified salmon in the diet of rodents. Three modified AIN93G diets were prepared containing 20% casein protein (Diet1), 20% wild-type salmon protein (Diet2) or 20% transgenic salmon protein (Diet3). All diets were balanced for lipids (7.8%) and mineral and vitamin content. Pregnant Sprague Dawley dams (day 7 gestation; 10 per group) were brought in and immediately put on diet. The dams and subsequent pups were monitored for weight and food consumption on a weekly basis. Pups were born at day 22-23 of gestation. Pups were weaned at postnatal day (PND) 21 and maintained on the same diet as their dams until PND 28 or 80. Dams were euthanized upon weaning of the pups. No changes in dams' gestation weights or food consumption were observed between any of the diets. During lactation, dam weights were significantly greater on PND 7, 14 and 21 in Diet3 compared with Diet1. A significant increase in pup body weights was observed during the lactation period in Diet3 compared with Diet2 in male pups from PND 7 to 21 and in female pups at PND 14 and 21. No body weight differences were noted in the pups from these groups from PND 28 to PND 80, although female body weights from Diet2 and male body weights from Diet3 were significantly increased compared with Diet1 over this interval. No changes were observed in preputial separation in males, but vaginal opening in female pups was advanced by a day in both Diet2 and Diet3 compared with Diet1. The only difference between groups fed Diet3 compared with those fed Diet2 was increased body weight gain during the lactation period. These effects may be due to subtle differences in lipids and/or proteins of the fish which have yet to be determined.

1440 28-DAY ORAL (GAVAGE) TOXICITY STUDIES IN RATS OF GREEN TEA CATECHINS PREPARED FOR BEVERAGES.

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Studies have been done that suggest that tea catechins have beneficial effects when taken as a food supplement. The current studies evaluated the possible toxic effects to rats of two green tea catechin preparations for use in beverages which have different catechin monomer compositions and also a decaffeinated preparation in order to ascertain if the effects observed were due to the presence of caffeine. These green tea catechin preparations were administered orally by gavage to rats once daily at dosage levels up to 2000 mg/kg/day for 28 days. All groups consisted of 5 male and 5 female Crl:CD(SD)IGS BR rats. There were no deaths attributed to any of the green tea preparations. The clinical condition of the animals, functional observational battery and motor activity, hematology and urinalysis evaluations, organ weights, and gross necropsy findings were unaffected by any of the tea preparations. No adverse effects on serum chemistry parameters were attributed to any of the test articles. There were no effects on body weights or microscopic findings attributed to dosing with the decaffeinated preparation, whereas lower body weights were observed throughout the study in the 1000 and 2000 mg/kg/day males administered one of the catechin preparations which has a high caffeine content. The no-observed-adverse-effect level (NOAEL) for localized gastric effects was 1000 mg/kg/day based on gastric erosions at 2000 mg/kg/day. No other target organs were identified. Thus, the NOAEL for systemic toxicity following oral administration was 2000 mg/kg/day (the highest level tested) under the conditions of these studies. The changes in body weight and gastric findings observed were attributed to caffeine, as no similar findings were observed in rats dosed with the decaffeinated catechin preparation.

1441 GENOTOXICITY STUDIES ON GREEN TEA CATECHIN.

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Tea catechins have been demonstrated to give beneficial effects on health. A recent investigation indicates that ingestion of tea beverage enriched with catechins is effective on the prevention of body fat accumulation. We evaluated the genotoxic po-

tential of tea catechin preparations, namely green tea catechin, using standard genotoxicity tests. Bacterial reverse mutation assay (Ames test), the chromosomal aberration assay in cultured Chinese hamster lung cells (CHL/IU), the mouse lymphoma L5178Y/tk assay (MLA), and a bone marrow micronucleus assay in ICR CD mice were employed in the present study. Our data shows that there were no significant increases in the revertant colonies were found in the Ames test, but positive responses were observed in the in vitro chromosomal aberration assay and in vitro mouse lymphoma L5178/tk assay. However, the in vivo study demonstrated no significant increase in micronucleated erythrocytes in the bone marrow of ICR CD mice administered a high dose of the test substances up to 2,000 mg/kg. These results were comparable with those of other genotoxicity studies of tea catechins that demonstrated minimal genotoxic concern. Combined with favorable information of chemopreventive effect of tea catechins on mutagenesis and/or carcinogenesis, we conclude that there is no substantial genotoxic concern of green tea catechin under the conditions for anticipated use.

1442 EIGHT WEEKS EXPOSURE EFFECTS OF A NOVEL DIETARY SUPPLEMENT-THERMOPLUS ON SERUM BIOCHEMISTRY AND HISTOPATHOLOGY OF VITAL TARGET ORGANS OF FISHER RATS.

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We have recently shown antitoxic properties (Mol. Cell. Biochem., 2006) and the safety of a caffeine and ephedra containing metabolic nutrition system (MNS-orange) in an in vivo animal model (Arch.Toxicol.79:330-340, 2005). ThermoPlus, a new dietary supplement primarily composed of Salvia officinalis, Camellia sinensis, Paullinia cupana, thiamine, and niacin was designed to work in conjunction with metabolic nutrition systems. The present study evaluated the safety of this novel dietary supplement-ThermoPlus (TP; AdvoCare®) on serum biochemistry and organ histopathology in Fisher 344 rats. TP is a unique combination of phytochemicals/nutraceuticals designed to provide nutritional support that helps stimulate metabolism of the body ultimately translating into more effective caloric consumption. In addition, the sage and oolong tea components are known to suppress appetite. Fisher 344 rats (adult male and female) were fed control (-TP) or TP containing (1X, 7X and 14X, 1X=daily human dose) diets and sacrificed after 2 months. Serum chemistry analysis and histopathological examination of liver, kidney, heart, brain, lung, spleen, and duodenum sections revealed that TP exposure did not adversely influence serum chemistry (proteins, lipids and carbohydrate profiles), genomic integrity of the liver and tissue architecture. Specific enzyme markers (ALT/AST: liver; CPK: heart; BUN/Creatinine for kidney) of the target organs disclosed no TP-induced toxicity. TP-exposed livers were also subjected to Western blot analysis to study its influence on the expression of the pro- and anti-cell death genes (bcl-XL and p53). Overall, TP-exposed rats were more active, and the results suggest that TP exposure produces normal serum chemistry in rats fed up to fourteen times the normal human dose and does not adversely influence any of the vital target organs [Supp. by AdvoCare®, Carrollton, TX].

1443 EFFECTS OF SYNEPHRINE AND BITTER ORANGE ON CARDIOVASCULAR AND PHYSIOLOGICAL VARIABLES IN FEMALE RATS.

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Little is known about the adverse health effects of the dietary supplement bitter orange (B) which contains 6% synephrine (S). The purpose of this study was to determine acute physiological and cardiovascular effects of B and S in rats. Telemetric transmitters monitored temperature, motor activity, heart rate, blood pressure, and electrocardiography for 5 days in young female Sprague-Dawley rats that were gavaged once daily with the vehicle (0.25% methyl cellulose). Subsequently, rats (12/dose level) received a daily dose of B (0, 10, 50, or 100 mg synephrine/kg) or 90% S (100 mg synephrine/kg) for 6 weeks. Data were collected prior to dosing and at 0.25, 0.5, 1, 2, 4, and 6 hours after dosing with B or S. There were significant linear dose and dose*day effects for diastolic, systolic, mean, and pulse blood pressure, heart rate, activity, and QA interval at 2 or more of the time points after dosing. For all dose groups, B and S had the greatest effect on blood pressure. Compared to controls, systolic and mean blood pressure were significantly increased in the 50 and 100 mg/kg B dose groups at all time points, and pulse and diastolic pressure were increased at 4 or more time points. Pulse, mean, and systolic pressure were increased in the 10 mg/kg group at 2 or more time intervals. S caused a smaller increase in blood pressure than B at 100 mg/kg. Additionally, B decreased the QA interval in all dose groups. Both B and S had more profound effects on blood pressure than on temperature, activity, or heart rate. B significantly increased the corrected QT interval. B and S had no effect on body weight or food consump-

tion. The mortality rate for each of the three B dose groups was 8.3% with contributing factors being hypothermia, ataxia, prolonged QT intervals, and partially inverted T waves. These results suggest that both B and S may have adverse physiological effects and that B may have more significant effects on blood pressure than an equivalent dose of S.

1444 D-RIBOSE HAS NON-SIGNIFICANT TOXICITY IN BOTH SHORT AND LONG TERM SUPPLEMENTATION.

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D-Ribose (DR), a pentose carbohydrate, has repeatedly demonstrated its beneficial properties in replenishing cellular high energy phosphate compounds with an accompanying functional improvement following ischemic or hypoxic stressful situations without obvious significant toxicity. Food ingredients and dietary supplements (including DR) must clearly show safety-in-use. To that end, DR has been tested for toxicity in both animal and human investigations. Adult female rodent experiments analyzing both embryotoxicity and teratogenicity revealed no effects at an oral DR dose at 5% of the diet (a daily dose of ~4 gms/kg body weight/day). Findings revealed no developmental defects in the fetus, including visceral malformations or anomalies. When adult male and female rodents were fed 5% DR in the diet for 13 weeks (3.6 and 4.4 gms/kg body weight/day) in a standardized OECD Guideline 408 protocol, no abnormal measured hematological or biochemical parameters were found. Human studies have also reflected similar findings. In an acute evaluation, using escalating single oral doses of DR (0, 2, 5, 10 gms/dose) in young and older adult humans, there were no significant biochemical or purine abnormalities except for mild asymptomatic, hypoglycemia and a slight elevation in uric acid concentrations. When providing DR (20 gm/dose) for 14 days to healthy adult subjects, similar findings were also observed. There were no long-term significant changes in hematological or biochemical parameters; however, an acute elevation in uric acid and aspartate aminotransferase levels occurred at day 7 with normalization to baseline in both measurements by day 14. A clinically, asymptomatic hypoglycemia was found throughout the 14 day trial. The data from these investigations give further support for the safety of DR in both an acute and chronic setting.

1445 GENETIC TOXICOLOGY ASSESSMENT OF A NOVEL OXYGEN-COORDINATED NIACIN-BOUND CHROMIUM(III) (NBC) COMPLEX.

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Chromium (III) is a novel micronutrient essential for normal protein, fat and carbohydrate metabolism. NBC is a unique form of bioavailable chromium. We have previously assessed the acute oral toxicity, acute dermal toxicity, primary dermal and eye irritation, Ames' bacterial reverse mutation assay, mouse lymphoma test, and 90-day subchronic toxicity of NBC, and demonstrated its safety [J. Inorg. Biochem. 99 (2005) 2161-2183]. Recently, we demonstrated the long term (52-weeks) safety of NBC by administering either 0 or 25 ppm [1,000 mg elemental Cr(III) HED] doses of NBC in diet for 52 consecutive weeks to Sprague-Dawley rats (Eurotox 2006). In this study, we examined the genetic toxicology profile of NBC in a mouse micronucleus (MN) assay model, using young, healthy adult male and female Cr1:CD1(ICR) mice. NBC was administered to mice in corn oil by single dose oral gavage at doses of 1000, 2000 and 3000 mg NBC/kg body weight. Cyclophosphamide (70 mg/kg body weight) was used as a positive control. Mice were sacrificed at 24 and 48-hr post-treatment, and marrow aspirated from both femurs of each animal into a tube of fetal bovine serum. Following centrifugation, the resulting pellets were resuspended in serum. The slides were prepared from the suspensions and stained with Giemsa. A minimum of 2000 polychromatic erythrocytes (PCEs) per animal or 10,000 per treatment group were scored for micronuclei using 1000X brightfield microscopy. The number of PCEs among 500 total erythrocytes (expressed as the PCE fraction) was determined for each animal. No adverse clinical events or reduction in body weight gain were observed in the NBC treated animals. Numbers of MN-PCEs were not increased in any group treated with NBC compared to the corresponding negative control groups, while the positive control groups displayed strong increase in MN-PCEs. The results of the micronucleus test are in agreement with and further extend our previous findings with regards to the safety of NBC.

1446 ASSESSMENT OF POTENTIAL HUMAN HEALTH RISKS POSED BY BENZENE IN A COMMERCIAL BEVERAGE.

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A recent study by the Center for Food Safety and Nutrition (CFSAN) provided limited data regarding the concentration of benzene in a selection of commercial beverages. A reaction between sodium benzoate and vitamin C appears to give rise

to benzene in some drinks under certain conditions. CFSAN reported that several beverages contained levels of benzene which exceeded the EPA drinking water standard of 5 ppb. One of the beverages, Crystal Light Sunrise Classic Orange (CLSCO), was reported to have concentrations as high as 87.9 ppb, though a newer formulation registered levels <1 ppb in CFSAN testing. Due to limited testing by CFSAN, a more robust study was conducted to better characterize the levels of benzene in CLSCO. Samples of the beverage were obtained from retail outlets in the Houston Texas area, following a statistically-based sampling strategy. Three formulations were obtained during sampling: 16 oz old and new formulations, and 32 oz. formulation. Analysis of the samples revealed mean benzene concentrations of 90 ppb in 16 oz old formulation bottles, and undetectable levels in the 16 oz new formulation product. The 64 oz product was determined to contain a mean concentration of 3.38 ppb benzene, though sodium benzoate is not listed as an ingredient. Upper bound point estimates of cancer risk for exposure to these two products were calculated as 5.22×10^{-6} for teens and 2.76×10^{-7} for adults. Hazard indices for teens and adults were calculated at 0.277 and 0.0002 respectively. Monte Carlo analysis, utilizing distributions for consumption rate, benzene concentration, and body weight, resulted in a 95th percentile excess cancer risk of 7.94×10^{-6} for teens and 3.52×10^{-7} for adults. Non-cancer risk for teens and adults at the 95th percentile was estimated at 0.575 and 0.0022 respectively. These calculations indicate that cancer and non-cancer risks due to exposure to benzene in CLSCO are below levels of concern.

1447 CYTOTOXICITY OF PYRROCIDINES IN HEPG2 HEPATOCYTES AND PK15 RENAL CELLS.

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Pyrrocidines are newly reported polyketide-amino acid-derived antibiotics produced by *Acremonium zeae*, a prevalent seed-borne endophyte of corn. Pyrrocidines exhibits potent activity against Gram-positive bacteria, including drug resistant strains, and displayed significant activity against *Candida albicans*, as well as fumonisin and aflatoxin producing fungi. In this study, we evaluated the effect of pyrrocidines in two mammalian cell lines, HepG2 cells (a cell line derived from a human hepatocellular carcinoma) and PK15 cells (a cell line derived from a normal pig kidney). The HepG2 and PK15 cells were incubated overnight to form monolayers and treated with 0 to 100 μ M of pyrrocidine A and B for 24 hours. Cytotoxicity was evaluated by the MTT assay. Pyrrocidine A and B were cytotoxic to both HepG2 and PK15 cells after 24 hours of treatment. ED₅₀ of pyrrocidine A to HepG2 cells was $0.66 \pm 0.16 \mu$ g/mL and to PK15 cells was $1.03 \pm 0.37 \mu$ g/mL. Pyrrocidine B was less potent than pyrrocidine A. ED₅₀ of pyrrocidine B to HepG2 was $15.37 \pm 4.47 \mu$ g/mL and to PK15 was $16.63 \pm 5.47 \mu$ g/mL. The cytotoxicity of pyrrocidine A to HepG2 cells was more potent than other known mycotoxins, e.g. deoxynivalenol ED₅₀ = 8.36μ g/mL, fumonisin B₁ ED₅₀ > 100 μ g/mL, zearalenone ED₅₀ > 100 μ g/mL and moniliformin ED₅₀ = 3.5 μ g/mL. A sequential morphological study using time-lapse motion photography suggested that the cell death in PK15 cells induced by pyrrocidine A was consistent with apoptosis. This is the first report of toxicity in a mammalian system. *In vivo* study are essential for risk assessment.

1448 IMMUNOTOXICITY OF NIVALENOL LACKING XENOBIOTIC METABOLISM AFTER ORAL ADMINISTRATION FOR 90-DAY IN RATS.

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Nivalenol (NIV), one of the trichothecene mycotoxins produced by *Fusarium* species, is an important contaminant of wheat and barley showing worldwide distribution. In the present study, we examined exposure effect of NIV through diets at doses of 0, 6.25, 25, and 100 ppm for 90 days on immune function and activities of detoxifying enzymes in the liver of male F344 rats. Examination of serum immunoglobulin levels revealed a slight increase of IgM at 100 ppm, while IgM at lower doses and IgG or IgA at any doses did not fluctuate. Flow cytometric analysis of splenic cells revealed increases of B-cell population from 25 ppm and decrease of T-cell population at 100 ppm associated with an elevation in the ratio of helper/cytotoxic T lymphocytes at 100 ppm. On the other hand, increases of

splenic NK activity against Yac-1 cells were observed in all treatment groups, while the level at 100 ppm was lower than that observed at 25 ppm. At 100 ppm, the reduction of the NK-R-P1A+ splenic cell counts was apparent. With regard to the activities of detoxifying enzymes, i.e., cytochrome P450 3A2 and 1A2, and glutathione-S-transferase isozymes, in the liver, NIV did not induce any dose-related change. Furthermore, de-epoxy form of NIV, a bacterially metabolized NIV, could not be detected in the feces or sera of administered animals. In summary, immunotoxicity of NIV was apparent from 25 ppm judging from the increase of splenic B-cell population, while increase of NK activity was apparent from 6.25 ppm as a signature of beneficial host-defense responses. Alterations in immune function by NIV were not accompanied with xenobiotic reaction in the liver as well as enterobacterial metabolism.

1449 EFFECTS OF FUSARIUM MYCOTOXINS AND THEIR MIXTURES ON CACO-2 CELLS DEATH: IMPLICATION OF P53, P21 GENES AND CASPASE-3.

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We determined the implication of the p53 and p21 genes and caspase-3 activation in cell death caused either by deoxynivalenol (DON), zearalenone (ZEA), and fumonisin B1 (FB1) individually or by mixtures in the human intestinal cell line, Caco-2. The following endpoints were used: malonaldehyde production, inhibition of protein and DNA syntheses, DNA bases modifications, DNA fragmentation, and cell viability as measured by the Neutral Red (NR) and MTT tests, activity of caspase-3 and transcriptional levels of p53 and p21 genes. The reduction of cell viability results from the balance between cell proliferation and cell death triggered by either apoptosis or necrosis. In caco-2 cells which bear a mutated p53 gene, FB1 induces apoptosis in approximately 30% of cells triggered by a mechanism p53-independent and caspase-3 and p21 independent. DON and ZEA induce apoptosis in Caco-2 cells through a mechanism likely caspase-3 and p21 dependent at transcriptional level, with modulation of p53 gene without its implication in the cell death. The mixtures of mycotoxins induce caspase-3 activity in increasing order: [FB1+ZEA] < [ZEA+DON] < [FB1+DON+ZEA] < [FB1+DON] after 24h. The caspase-3 activity is increased with the mixtures of mycotoxins even though the number of cells undergoing apoptosis did not increase due to concomitant cell proliferation.

The ability of the toxins to inhibit DNA synthesis is 70%, 43% and 45% for 10 μ M of ZEA, DON, and FB1, respectively. Their binary mixtures (at 10 μ M each), inhibit DNA synthesis by 35%, 62%, and 65%, far less than additive effects. Surprisingly, the tertiary mixture (10 μ M each) only inhibits DNA synthesis by 25% leading to cell proliferation and higher survival rate. Altogether these data suggest a potential promoter effect.

1450 INCREASED ACCUMULATION OF FUMONISIN B1, SPHINGOID BASES AND SPHINGOID BASE 1-PHOSPHATES: EXPLAINING THE DIFFERENTIAL SENSITIVITY OF RAT KIDNEY AND LIVER TO FUMONISIN TOXICITY.

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Fumonisin is a mycotoxin in maize and inhibitors of ceramide synthase a key enzyme in the de novo sphingolipid biosynthesis pathway. In liver and kidney inhibition of ceramide synthase results in a marked increase in the ceramide precursor sphinganine. This study was conducted to investigate the differential changes in sphinganine, sphingosine, sphinganine-1-phosphate and sphingosine-1-phosphate in kidney, liver, serum and heart of male Sprague-Dawley rats fed diets containing fumonisins B1, B2 and B3 at a ratio of 1.00:0.45:0.10, respectively. The total fumonisins in the diets were 1.1, 13.5 and 88.6 μ g/g. Animals were killed after 1, 3, 5 and 10 days of feeding. The tissues were analyzed for sphingoid bases, sphingoid base 1-phosphates, and fumonisins B1, B2, and B3, and were microscopically examined for the presence and severity of lesions. Fumonisin B1, but not B2 or B3, was easily detected in liver, kidney and serum. There was a time- and dose-dependent increase in sphinganine in both liver and kidney that was closely correlated with the tissue concentration of fumonisin B1 and histopathologic findings. However, the sphinganine and sphingosine in kidney, but not liver, was quickly metabolized to sphinganine and sphingosine-1-phosphate. The concentration of fumonisin B1 in liver and kidney that first elicited an increase in sphinganine was similar in both tissues, however, over time, the kidney accumulated significantly more fumonisin B1 (10X) and total sphinganine (sphinganine plus sphinganine-1-phosphate).

Thus, the relative sensitivity of male Sprague-Dawley rat kidney and liver is most likely a consequence of differences in the mechanisms responsible for both fumonisin B1 uptake/clearance and sphinganine metabolism via the enzymes sphinganine kinase and sphinganine-1-phosphate lyase.

1451 FOOD CO-CONTAMINATION AND HUMAN DIETARY EXPOSURE OF AFLATOXIN B1 AND FUMONISIN B1 IN CHINA.

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Co-contamination of aflatoxin B1 (AFB1) and fumonisin B1 (FB1) in human dietary components was widely reported. To investigate current co-contamination status of these two mycotoxins in food and potential human dietary exposure, a cross-sectional study including food frequency surveys and food sampling were carried out in residents from 3 different areas in China. Daily food consumptions were recorded in 113 residents from different households. A total of 208 food samples, consisting of 107 corn, 29 rice, 16 wheat-flour, 17 peanut, and 39 vegetable oil were measured for AFB1 and FB1 by the ELISA. The median AFB1 levels (μ g/kg) were 2.3 in corn (range, 0.4-136.8), 0.6 in rice (0.1-1.4), 0.4 in wheat-flour (0.1-0.9), 0.2 in peanut (0-0.7), and 9.1 in vegetable oil (0.2-114.4), respectively. Seventeen out of 107 (16%) corn samples had AFB1 level higher than 20 μ g/kg. The median FB1 levels (mg/kg) were 1.1 in corn (range, 0-37.0), 0.2 in rice (0-0.5), and 0.2 in wheat-flour (0-0.4), respectively, but FB1 was not detectable in peanut and vegetable oil. Thirty eight out of 107 (36%) corn samples had FB1 levels higher than 2 mg/Kg. Furthermore, the high co-existence rates of AFB1 and FB1 were found in 93% corn (99/107), 93% rice (27/29), and 81% wheat-flour (13/16), respectively. Nine out of 107 (8%) corn samples had AFB1 and FB1 higher than 20 μ g/kg and 2 mg/kg. Based on the dietary consumption data, the median daily intakes for AFB1 and FB1 were 1.4 (range, 0.2-49.8) μ g and 187.2 (range, 30.0-10,541.6) μ g. The different dietary patterns among these 3 areas resulted in the different contamination levels of AFB1 and FB1, and great variations were found in samples collected from different areas. Taken together, this study demonstrates that the high co-contamination of AFB1 and FB1 existed in food samples daily consumed by the residents in China and the potential health effect for chronic exposure to these two mycotoxins needs to be further studied. (Supported by CA94683 from NCI)

1452 THREE-MONTH INTERVENTION TRIAL WITH NOVASIL CLAY IN GHANAIS: STUDY DESIGN AND CLINICAL OUTCOMES.

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A double-blinded, placebo controlled clinical trial was conducted to investigate the safety and tolerance of dietary NovaSil (NS) in human populations in Ghana over a period of three-months. Volunteers (507 subjects, ages from 18 – 58 yrs) were clinically screened to evaluate their general health, pregnancy status, and levels of blood AFB1-Albumin adducts. Of these subjects, 177 (101 males, 76 females) were randomly assigned to 3 groups: high-dose (HD), low-dose (LD) and placebo-control (PL) groups receiving 3.0, 1.5 and 0 g NS/day in capsules. These were administered to each participant under the supervision of trained study monitors prior to meals. Blood and urine samples were collected for laboratory analysis. Physical examinations were performed monthly and follow-up was taken to record any side effects daily. Ninety-four percent of the participants (166 of 177) completed the study and compliance rate was 97.4%, 96.4%, and 98.5% for HD, LD, and PL, respectively. Mild to moderate side effects were recorded in some participants. Throughout the study duration, the total reported side effects with respect to possible complaints were 0.66%, 0.68%, and 0.48% for HD, LD, and PL, respectively. Symptoms included nausea, vomiting, diarrhea, flatulence, abdominal discomfort, heartburn and dizziness. These side effects were neither dose-dependent nor statistically significant among the three groups. Also, some participants (in all groups) reported having increased appetite in the course of the study. Overall, 99.4% of person time during the study reported no side effects. No significant differences were shown in hematology, liver and kidney function, electrolytes, and vital signs in the three groups. These findings demonstrate that NS clay for the protection of humans at high risk for aflatoxicosis is practical and economically feasible. (Supported by USAID LAG-G-00-96-90013-00).

1453 THREE-MONTH CLINICAL INTERVENTION TRIAL WITH NOVASIL CLAY IN GHANAIS: REDUCTION OF URINARY AFLATOXIN M₁ LEVELS.

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NovaSil clay (NS) has been found to prevent aflatoxicosis in multiple animal species by decreasing toxin bioavailability from the gastrointestinal tract. A randomized, double-blinded, placebo controlled phase IIa clinical trial was conducted in 177 healthy Ghanaian volunteers who received NS clay capsules either in 1.5 g/day (low dose), 3.0 g/day (high dose), or placebo for a period of 3 months. Our objectives were to evaluate the efficacy of NS for reducing AFM₁ biomarkers of exposure in more than 600 urine samples. AFM₁ was measured by immunoaffinity-HPLC-fluorescence methods. The cleanup procedure involved acidification of urine with ammonium formate followed by dilution with deionized H₂O. Samples were passed through preparative monoclonal antibody columns followed by sequential washing steps with PBS and deionized H₂O. AF metabolites were eluted from the column with 80% MeOH then evaporated to dryness and resuspended in MeOH and ammonium formate. Fluorescence intensity was measured using HPLC. The protocol was validated, and the structure of AFM₁ was confirmed by mass spectrometry. Levels of AFM₁ in urine samples collected at baseline and 1-month after intervention did not show a statistically significant difference between the placebo group and low dose group or between the placebo group and high dose group. In urine samples collected after 3-months of the intervention, no statistically significant difference was found between the placebo group and low dose group; however, a significant decrease (up to 58%) in the median level of AFM₁ was found in the high dose group as compared to the median level in the placebo group (P=0.0391). These results suggest that intervention with NS can considerably reduce urine levels of AFM₁. (Supported by USAID LAG-G-00-96-90013-00).

1454 THREE-MONTH CLINICAL INTERVENTION TRIAL WITH NOVASIL CLAY IN GHANAIS: REDUCTION OF SERUM AFLATOXIN-ALBUMIN ADDUCT LEVELS.

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NovaSil clay (NS) provides significant protection from the adverse effects of aflatoxins (AF) in multiple animal species by decreasing bioavailability from the gastrointestinal tract. It is postulated that NS clay can be safely added to human diets to diminish exposure and health risks from AF contaminated food. To determine the safety and efficacy of NS in humans, a randomized, double-blinded, and placebo controlled phase IIa clinical trial was conducted in 177 healthy Ghanaian volunteers who received NS clay capsules either in 1.5 g/day (low dose), 3.0 g/day (high dose), or placebo for a period of 3 months. The efficacy of NS for reducing AF exposure was evaluated by measuring serum AF-albumin adducts in more than 700 serum samples collected at baseline, 1, 3, and 4 months using radioimmunoassay. Levels of serum AF-albumin adduct at baseline were comparable for the 3 treatment arms with the average 1.563±0.315, 1.505±0.322, and 1.493±0.375 pmol AF/mg albumin for the low dose, high dose, and the placebo groups, respectively. No statistical significance (P=0.345) was found among groups of the low dose (1.214±0.215 pmol AF/mg albumin), the high dose (1.168±0.244 pmol AF/mg albumin), and the placebo (1.253±0.335 pmol AF/mg albumin) in samples collected at 1 month of the trial. However, the levels of serum AF-albumin adduct in samples collected at 3 months of the trial were significantly decreased in both low dose group (0.894±0.155 pmol AF/mg albumin, P<0.0001) and high dose group (0.900±0.156 pmol AF/mg albumin, P<0.0001) compared to the levels of the placebo group (1.195±0.216 pmol AF/mg albumin). Mixed effect model analysis revealed significant dose/time interactions (P<0.001). These results suggest that the three-month intervention trial with NovaSil significantly decreased serum levels of AF-albumin adduct, a long-term biomarker for AF exposure.

1455 THREE-MONTH CLINICAL INTERVENTION TRIAL WITH NOVASIL CLAY IN GHANAIS: NO INFLUENCE ON UTILIZATION OF VITAMINS A AND E.

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Dietary exposure to aflatoxins (AF) decreases serum and tissue vitamins (Vit) A and E levels in addition to causing liver damage. NovaSil clay (NS) provides significant protection from the adverse effects of AF in animals. To further evaluate the influ-

ence of NS on utilization of these two vitamins in humans, levels of Vit A and E were measured by HPLC methods in 655 serum samples collected at 0, 1, and 3 months from a phase 2a clinical trial carried out in 177 healthy Ghanaian volunteers who either received NS 1.5 g/day (low dose), 3.0 g/day (high dose), or placebo for 3 months. The baseline serum levels of Vit A were comparable (P=0.8702) for groups of the placebo (2.09±0.56 µmol/L), the low dose (2.15±0.74 µmol/L), and the high dose (2.14±0.92 µmol/L). No significance was found among groups of low dose (2.28±0.85 µmol/L), high dose (2.21±0.95 µmol/L) and the placebo (2.21±0.57 µmol/L) in 1 month samples (P=0.7324). No significance was found among groups of low dose (2.22±0.87 µmol/L), high dose (2.30±0.71 µmol/L), and the placebo (2.32±0.83 µmol/L) in 3 months samples (P=0.7305). The baseline serum levels of Vit E were also comparable (P=0.6798) for groups of the placebo (16.07±4.74 µmol/L), the low dose (16.40±5.08 µmol/L), and the high dose (15.64±4.97 µmol/L). No significance was found among groups of low dose (17.9±4.99 µmol/L), high dose (17.08±4.83 µmol/L) and the placebo (17.32±4.97 µmol/L) in 1 month samples (P=0.5868). No significance was found among groups of low dose (17.03±4.71 µmol/L), high dose (17.33±4.21 µmol/L), and the placebo (18.01±6.29 µmol/L) in 3 months samples (P=0.7618). Furthermore, levels of Vit A (P=0.3665) and Vit E (P=0.1543) were not statistically significant among the treated groups and the placebo group in samples collected at 1 month post the trial. These results suggest that NS does not affect utilization of Vit A and E in humans.

1456 DETECTION OF ZERANOL AND METABOLIC ANALOGS IN MOLDY FOOD MATERIALS BY LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION CONTAINING SCREEN-PLATED DISPOSABLE ELECTRODES.

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Zeranol (Z) is a semisynthetic growth promoter with estrogenic activities and a naturally occurring mycotoxin metabolite. The presence of low but biologically active levels of Z and its metabolic analogs (Zs) in the diets presents a potential health risk to human consumers. Therefore, it is imperative to be able to determine trace levels of Z and Zs in the food resources. Differentiation of Z and Zs is also advantageous in distinguishing environmental contamination from Z abuse. A simple and rapid high performance liquid chromatography method with electrochemical detection using disposable carbon-plated electrodes for simultaneous determination of Z and 4 major Zs (α -zearalenol, β -zearalenol, β -zearalanol and zearalenone) was developed. With optimal amperometric conditions, the 5 compounds could be separated in 8 minutes using ammonium acetate buffer (pH 7.0) with detection limits in the 10 ppb range. Samples subjected to intentional or unintentional contamination of Z-related compounds, such as beef, pork, chicken, feed, cereal, soybean, rice and beer were analyzed after a single methanol extraction step that required no sample cleanup or derivatization process. The simplicity and reproducibility of this method has made a routine screening possible. Micrograms of Z, α -zearalenol, β -zearalenol, and/or β -zearalanol were discovered from moldy feed, cereal, soybean and rice extracts at a per gram basis, indicating high yield of these compounds from natural environment. Our results also indicated different profiles in food-derived Zs in Taiwan and suggested that rice and soybean consumption in Taiwan may be an unforeseen risk factor for diseases associated with estrogenic activities of Z and Zs.

1457 SORPTION OF ARSENIC BY FERRIHYDRITE AND MAGNETITE AND PREVENTION OF TOXICITY IN HYDRA.

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Arsenic is a toxic trace element found in groundwater due to natural and industrial processes. Exposure has been linked to cancers of the bladder, lungs, skin, kidneys, nasal passages, liver, and the prostate. Inorganic arsenic is usually found in two forms, arsenate (H₂AsO₄⁻ and HAsO₄²⁻) and arsenite (H₂AsO₃⁻ and ₂AsO₃⁻), with both forms often found together. The purpose of this research was to utilize the properties of clay minerals and iron oxides to develop enhanced composite materials for the sorption of arsenic. A variety of clay minerals and synthesized iron oxides were screened for their ability to bind As(III), as sodium arsenite, and As(V), as sodium arsenate. From this initial screening, As(III) adsorption isotherms were performed on ferrihydrite and magnetite; whereas As(V) isotherms were conducted on ferrihydrite and CP-LPHM. All isotherm data were fit to the Langmuir equation to determine adsorption capacity (Q_{max}). Ferrihydrite bound 95.93% of As(V) and 97.23% of As(III) in the screening studies and had a Q_{max} of 1.288 mol/kg for As(III) and 0.744 mol/kg for As(V). Magnetite exhibited a Q_{max} of 0.481 mol/kg

for As(III). Ferrihydrite and magnetite were combined with chitosan to make composite gel beads. Ferrihydrite was found to be a highly effective sorbent for As(III) and As(V), and magnetite was effective for As(III) removal from water. Ferrihydrite protected adult hydra at levels up to 100 times the minimal effective concentration for As(III) and could potentially be used as a dietary intervention to protect animals from As toxicity. Studies are ongoing to assess the arsenic binding ability of iron-amended-chitosan gel beads and composites. Supported by NIEHS P42 ES04917.

1458 CARRY-OVER OF FEED CONTAMINANTS TO FOOD OF ANIMAL ORIGIN: APPLICATION OF MECHANISTICALLY BASED KINETIC MODELING.

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Animal feed which is accidentally poisoned or structurally polluted with environmental contaminants may lead to unwanted transfer of contaminants to domestic animals and, hence, to food of animal origin. In this context accumulation of contaminants after variable exposure scenarios, back-tracing of contaminant sources from animal food data and the determination of withdrawal periods are crucial points in maintaining food safety.

To address these issues various kinetic transfer models were developed. These kinetic models describe the transfer of dioxins and pesticides from feed/grass/soil to eggs of laying hens and milk of lactating cows, aflatoxin B1 from feed to milk of lactating cows and metals such as cadmium from feed/grass/soil to tissues of sheep and lactating cows.

Model applications comprise the back-tracing of the exposure period, the extend of internal exposure and the withdrawal period of lactating cows fed dioxin contaminated clay, the calculation of the long-term excretion of dioxins in cow's milk originating from local soil/grass intake in a heavy industrialised area, the determination of bioavailability of dioxins from soil, inclusive long-term transfer to eggs of laying hens and the determination of the exposure period needed to exceed current EU cadmium standards for the liver and the kidney in cows.

Moreover, the models served the comparison of standards in feed and animal products. For example it turned out that the current EU limit of 0.75 ng TEQ/kg feed of laying hens and the current EU residue level of 3 pg TEQ/g egg fat do not comply.

1459 MECHANISM OF ARSENITE-INDUCED CELL DEATH IS DISTINCT FROM BOTH NOCODAZOLE AND TAXOL.

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Arsenite, a known mitotic disruptor, causes cell cycle arrest and cell death at anaphase. The mechanism causing mitotic arrest is highly disputed. We compared arsenite to the spindle poisons nocodazole and Taxol. Response was distinct to each drug. In TR9-7 cells, mitotic index analysis indicated an earlier peak in mitotic index during nocodazole and Taxol treatments vs. arsenite treatment. Arsenite and nocodazole displayed distinct mitotic catastrophe DNA morphology while Taxol-treated cells exhibited fragmented nuclei during cell death. Immunocytochemical analysis of α -tubulin in mitotic cells showed spindle formation in arsenite- and Taxol-treated cells but not in nocodazole-treated cells. Spindle formation in arsenite-treated cells appeared irregular and multi-polar. γ -tubulin staining show that Taxol- and nocodazole-treated mitotic cells always contained two centrosomes. In contrast, many arsenite-treated mitotic cells contained more than two centrosomes. We tested additional cell lines to examine whether arsenite sensitivity correlated with that of Taxol and nocodazole. HeLa, A375 (melanoma), and A2780 (ovarian carcinoma) cells were sensitive to all three drugs. CP70 cells, a cisplatin-resistant derivative of A2780 cells, were as sensitive to mitotic arrest inducing drugs as their parental line. A549 (lung adenocarcinoma) cells were sensitive to nocodazole and Taxol, but were resistant to arsenite. Co-treatment with BSO, a glutathione synthase inhibitor, and MK571, an MRP export pump inhibitor, sensitized A549 cells to arsenite but did not alter nocodazole or Taxol sensitivity. Carmustine, a glutathione reductase inhibitor and DNA alkylating agent, increased A549 arsenite sensitivity, but decreased both nocodazole and Taxol sensitivity suggesting that arsenite may be useful as co-treatment in order to enhance DNA damage induced apoptosis despite cells being resistant to arsenite alone. In conclusion, arsenite-induced mitotic arrest, cell death, and resistance appear to be independent of effects on tubulin polymerization. (Supported by PHS grants ES011314 and F30 ES013372.)

1460 ARSENIC TRIOXIDE INDUCES PROGRAMMED CELL DEATH IN HUMAN LEUKEMIA (HL-60) CELLS.

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Abstract: Although arsenic is a poison and a carcinogen, it has been used for as a medicine for thousands of years, dating back to the ancient Greeks and Romans. Recently, arsenic trioxide has been successfully used to treat all-trans retinoic acid (ATRA) resistant relapsing acute promyelocytic leukemia. Previous studies in our laboratory have showed that arsenic trioxide significantly induces cell death in HL-60 cells. However, its molecular mechanisms of action are poorly understood. In the present study, we used human leukemia (HL-60) cells as a model to investigate the in vitro effect of arsenic trioxide on cell growth inhibition and cell death mechanisms. To achieve this goal, we performed the flow cytometric analysis using annexin-V and caspase-3 kits, and DNA fragmentation. The flow cytometric assessment showed a strong dose-response relationship between arsenic trioxide exposure and early and late stage apoptosis in HL-60 cells. Upon 24 hrs of exposure, the results of annexin V/PI Staining showed that the percentages of early apoptotic cells were $1 \pm 0\%$, $11.5 \pm 0.7\%$, $19 \pm 5.7\%$, and $18 \pm 0\%$ in 0, 2, 4, 6, 8 $\mu\text{g/mL}$ of arsenic trioxide, respectively. The result of caspase-3 activity also indicated a significant increase ($p < 0.05$) of caspase 3 positive cells undergoing late apoptosis in arsenic trioxide-treated HL-60 cells. These results were further confirmed by the result of DNA laddering assay showing a clear evidence of DNA fragmentation in arsenic trioxide-treated HL-60 cells. In summary, these studies indicate that arsenic trioxide represents an apoptosis-inducing agent in HL-60 promyelocytic leukemia cells and suggest that this agent may be more broadly used for treatment of leukemias other than APL. Findings from these data indicate that the molecular mechanisms of arsenic trioxide is mediated via phosphatidylserine externalization, caspase-3 activation, and morphology changes as demonstrated by the results of flow cytometric analysis, and DNA laddering assay.

Key words: Arsenic trioxide, HL-60 cells, DNA fragmentation, annexin V, and caspase 3.

1461 ENHANCED EXPRESSION OF METALLOTHIONEIN ISOFORM 3 (MT-3) PROTEIN IN TUMOR HETEROTRANSPLANTS DERIVED FROM As^{+3} AND Cd^{+2} TRANSFORMED HUMAN UROTHELIAL CELLS.

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The heavy metals arsenite and (As^{+3}) and cadmium (Cd^{+2}) are known human carcinogens and both of them have been associated with the development of bladder cancer. Previous studies from this laboratory have shown that both As^{+3} and Cd^{+2} can malignantly transform normal human urothelial cells. Furthermore, this laboratory has also demonstrated that the third isoform of the metal binding protein metallothionein (MT-3) is not expressed in the normal human bladder but its expression increases in bladder cancer. In this study, this laboratory has proposed that MT-3 might be a biomarker for the development of human bladder cancer. Immunohistochemical staining of MT-3 on archival diagnostic specimens showed that only 2 of 63 (3.17%) benign bladder specimens had even weak reactivity for the MT-3 protein. In contrast, 103 of 107 (96.26%) high grade urothelial cancers and 17 of 17 (100%) specimens of carcinoma in situ stained positive for the MT-3 protein. For low grade bladder cancer it was shown that 30 of 48 specimens (62.5%) expressed the MT-3 protein. Using a cell culture model (UROtsa), it was demonstrated that expression of the MT-3 protein was not required for malignant transformation of urothelial cells by either Cd^{+2} or As^{+3} . In contrast, it was shown that the cells transformed by Cd^{+2} and As^{+3} that did not express the MT-3 gene in cell culture, gained expression of MT-3 when grown as heterotransplants in nude mice. The gain in MT-3 expression when cells were grown as heterotransplants was also shown to occur for the MCF-7, T-47D, Hs 578t, MDA-MB-231 breast cancer and the PC-3 prostate cancer cell lines. An analysis of MT-3 mRNA and protein expression suggested that a post-transcriptional mechanism was responsible for accumulation of the MT-3 protein. The results provide strong evidence that MT-3 could be a biomarker for the development of high grade bladder cancer and that the expression of the MT-3 gene is not involved in the in vitro malignant transformation of UROtsa cells by Cd^{+2} and As^{+3} .

1462 TRANSFORMATION OF HUMAN UROTHELIAL CELLS (UROtsa) BY As^{+3} AND Cd^{+2} INDUCES THE EXPRESSION OF KERATIN 6A.

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This laboratory has demonstrated that both Cd^{+2} and As^{+3} can directly malignantly transform the UROtsa cell line and that the tumor heterotransplants produced from these transformed cells had histologic features consistent with human bladder

cancer. Microarray analysis of total RNA from the parental and transformed cells suggested that keratin 6a was overexpressed as a result of cell transformation. This preliminary analysis was confirmed in the present study. It was shown that the Cd²⁺ and As³⁺ transformed cells grown on serum-containing growth medium, and the derived tumor heterotransplants, all overexpressed keratin 6a mRNA and protein compared to UROtsa cells grown on serum-containing growth medium. Immunostaining of keratin 6a in tumor heterotransplants showed focal staining of the tumor cells that was localized to the cytoplasm. Focal immunostaining of keratin 6a was also found in some archival patient specimens of high grade bladder cancer, confirming translation of the results to human bladder cancer. The level of keratin 6a mRNA and protein was also found to be increased in UROtsa cells grown of serum-free growth medium and that increased expression was mediated by the epidermal growth factor (EGF) and insulin in the growth formulation. It is suggested that keratin 6a expression may be a biomarker for malignant urothelial cells that possess an activated EGF pathway that enhances cell proliferation.

1463 GENE EXPRESSION PROFILING OF HYPERKERATOTIC SKIN FROM INNER MONGOLIANS EXPOSED TO ARSENIC.

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Millions of people worldwide have been chronically exposed to arsenic levels in drinking water that greatly exceed the current World Health Organization's recommended limit of 10 µg/ml. The skin is a major target of arsenic toxicity, and some of the first clinical signs of chronic arsenicosis are skin abnormalities including abnormal pigmentation and hyperkeratoses (HK), which pose a high risk for cancer development. The Bayingnormen (Ba Men) region of Inner Mongolia has over 300,000 residents that have been exposed to high levels of arsenic in the drinking water for >20 years. Many of these residents have clinical symptoms of chronic arsenic exposure. To further our understanding of the mechanisms of arsenic toxicity, we examined the gene expression of arsenic-exposure-related HK from 7 Ba Men individuals (exposed to 212-950 µg/ml arsenic in drinking water) and non-lesioned skin from 4 control subjects from a nearby area (exposed to 7 µg/ml arsenic in drinking water). Gene expression profiling using Affymetrix® GeneChip Human U133 Plus 2.0 arrays was performed on RNA isolated from these samples. Over 4000 genes had differential expression in HK compared to normal skin (1-way ANOVA, p<0.05; ≥1.5-fold change in expression). The HK samples had keratin gene expression patterns characteristic of hyperproliferative skin. In addition, the altered genes in HK are consistent with proposed mechanisms of arsenic carcinogenesis. Genes that are altered in response to oxidative stress (HMOX-1, CAT, NQO1, GPX2), DNA damage (GADD45A, XPC, XRCC5, DDB2) and involved in apoptosis and cell cycle regulation were all changed in HK skin. These results suggest that arsenic-related gene expression changes in human samples are mechanistically consistent with previously observed laboratory studies of arsenic carcinogenesis and may identify useful biomarkers of arsenic exposure. [This abstract does not necessarily reflect EPA policy.]

1464 TELOMERASE AND CHRONIC ARSENIC EXPOSURE IN HUMANS.

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Arsenic exposure has been associated with increased risk of skin, lung and bladder cancer in humans. The mechanisms of carcinogenesis are not well understood. Telomerase, a ribonucleoprotein containing human telomerase reverse transcriptase (hTERT), can extend telomeres of eukaryotic chromosomes, delay senescence and enhance cell proliferation. Telomerase activation is shown to promote tumor growth and maintain genome instability and has been suggested as biomarker for assessing cancer risk. The aim of this study is to investigate arsenic effects on hTERT gene expression. Ba Men residents in Inner Mongolia are chronically exposed to arsenic via well water and showed associated health effects, including cancer. The 324 study subjects, including equal number of males and females, have been exposed to arsenic for more than 10 years. Most subjects (76%) have been exposed to arsenic at the concentration of 0.34 to 200 µg/L and the remaining subjects exposed up to 826 µg/L. Water and whole blood samples were collected from the study subjects. RNA was isolated from the blood samples and cDNA was synthesized using reverse transcriptase. hTERT mRNA was quantified using real-time PCR and were linked to arsenic concentrations in drinking water. The results showed that levels of hTERT expression were positively associated with the concen-

trations of arsenic concentrations in drinking water (p<0.0001) after adjusting for age, sex, smoking and pesticide exposure. Among these factors, only age showed significance in affecting hTERT expression. This study provided a linkage between chronic arsenic exposure and telomerase expression in this human population and provided some insight into the mechanisms of arsenic carcinogenesis. The expression of hTERT may potentially be as a useful biomarker for assessing health effects from chronic arsenic exposure. (This abstract does not necessarily reflect U.S. EPA policy.)

1465 OXIDATIVE DNA DAMAGE INDUCED BY INORGANIC ARSENITE EXPOSURE DETECTED USING THE IMMUNO-SPIN TRAPPING METHOD.

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Inorganic arsenicals are known to be toxic and carcinogenic in humans. Chronic arsenic poisoning has occurred as a result of the consumption of high levels of inorganic arsenic-contaminated well-water. Prior work has indicated that inorganic arsenite exposure is associated with 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG) formation, a biomarker of oxidative DNA damage, possibly as a prelude to arsenite-induced genotoxicity. However, the standard method of detecting 8-oxo-dG has been questioned because of high background rates and because it cannot directly measure DNA radicals, but may instead adventitiously measure oxidation during DNA extraction and sample preparation. Thus, in this study, we investigated the ability of inorganic arsenite to induce oxidative DNA damage using immuno-spin trapping (IST). IST is based on the direct trapping of radicals with 5,5-dimethyl 1-pyrroline N-oxide (DMPO), forming stable nitron adducts, and combines the simplicity, reliability, specificity and sensitivity of spin trapping for the detection of DNA radicals. Rat epithelial liver TRL1215 cells were exposed to micromolar levels of arsenite for 24 h. Arsenite exposure induced DNA radicals in a dose-dependent manner. The values in arsenite-exposed cells were 1.5 to 2.0-fold higher than those in control cells. The addition of L-buthionine (S,R)-sulfoximine (BSO), a specific glutathione synthase inhibitor, or hydrogen peroxide (H₂O₂), as an oxidant, both increased the generation of DNA radicals induced by the arsenite exposure. DNA radical production was 40-fold higher in the presence of BSO and 3-fold higher in the presence of H₂O₂ compared to arsenite alone. These results indicate that cellular GSH plays an important role in the protection against arsenite genotoxicity and H₂O₂ may enhance this genotoxicity. In addition, long-term exposure (> 4 weeks) to submicromolar levels of arsenite caused a delayed increase in DNA radicals in a dose-dependent manner. This work may have implications in chronic arsenic poisoning in humans.

1466 POSSIBLE INTERACTIONS BETWEEN CELLULAR METHYL METABOLISM AND ADAPTIVE EFFLUX DURING CHRONIC ARSENIC EXPOSURE IN HUMAN CELLS.

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Inorganic arsenic is a human carcinogen that targets potentially the prostate. In this work, we studied the early stages (< 16 weeks) of chronic arsenite exposure (5 µM) in the normal human prostate epithelial cell line, RWPE-1. These cells will undergo malignant transformation with 30 weeks of continuous arsenic exposure. These transformants exhibit genomic DNA hypomethylation, adaptation to arsenic toxicity and glutathione increase. Arsenic is effluxed as a triglutathione complex by the ATP Binding Cassette C1 protein (ABCC1) and increases in glutathione likely increase efflux capacity. Thus, we investigated methyl and glutathione metabolism during arsenic adaptation. The early chronic arsenic-exposed cells showed a time-dependent increase in homocysteine (Hcy) levels (1.5-fold) and in expression of S-adenosylhomocysteine hydrolase (2.5-fold) combined during the early phases, with a decrease in methionine adenosyltransferase 2A (45% reduction). Thus, it appears that S-adenosylmethionine (SAM) recycling is reduced in favor of over-production of Hcy. The expression of cystathionine β-synthase, a key enzyme in the transsulfuration pathway where cysteine is synthesized from Hcy, was markedly upregulated (1.5-fold) in arsenic exposed cells. An arsenic exposure duration-dependent increase also occurred in reduced glutathione, which was associated with an increase in expression of the genes involved in production of glutathione from cysteine, glutamate cysteine-ligase (8.0-fold) and glutathione synthetase (2.0-fold). Expression of the transport protein ABCC1 was markedly increased. These data indicate arsenic reduced SAM recycling leading to an accumulation of Hcy, possibly for increased glutathione production through the transsulfuration pathway. In combination with increased ABCC1 expression, this ultimately could lead to an increase in efflux capacity as an adaptation to arsenic intoxication. Therefore, arsenic adaptation may alter significantly cellular methyl metabolism.

1467 IN UTERO ARSENIC EXPOSURE ENHANCES SKIN SQUAMOUS CELL CARCINOMA FORMATION AND INDUCES PROLIFERATIVE LESIONS OF THE URINARY BLADDER, UTERUS AND ADRENAL IN Tg.AC MICE.

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The carcinogenic effects of *in utero* arsenic exposure were studied in Tg.AC v-Ha-ras transgenic (Tg.AC) mice, considered a genetically pre-initiated model for skin carcinogenesis. Pregnant Tg.AC mice received drinking water containing sodium arsenite at 0 (control), 42.5 or 85 ppm arsenic from day 8 to 18 of gestation. Dams delivered normally. At weaning (4 weeks) offspring were grouped (n = 25) according to maternal dose, and topically exposed to the skin tumor promoter 12-O-tetradecanoyl phorbol-13-acetate (TPA; 2 µg/0.1 ml acetone, twice/week) from weaning until sacrifice at 40 weeks of age. Compared to control, arsenic alone did not induce skin tumors, while TPA alone produced papillomas and squamous cell carcinomas (SCC) in excess. However, combined prenatal arsenic and postnatal TPA markedly increased the tumor multiplicity (tumors/mouse) of cutaneous SCC in males (2.7-fold) and females (2.3-fold), compared to TPA alone. Prenatal arsenic prior to TPA also markedly increased (up to 10-fold) the incidence of mice with two or more skin SCC in a dose related fashion. *In utero* arsenic also induced adrenal cortical tumors in males (maximum incidence 29%) and females (26%) compared to control (0% in both) irrespective of TPA. *In utero* arsenic exposure induced urinary bladder (UB) transitional cell hyperplasia in both females (maximum incidence 33%) and males (28%), compared to control (0% in both), that appeared largely independent of TPA exposure. Two UB papillomas also occurred in arsenic treated females. Uterine cystic hyperplasia was common with early life arsenic exposure (42% maximum) compared to control (4%), and three uterine tumors (1 adenoma and 2 leiomyoma) occurred in arsenic treated females. Thus, transplacental inorganic arsenic enhanced skin SCC formation when combined with subsequent TPA in Tg.AC mice. *In utero* arsenic exposure also induced adrenal tumors and proliferative lesions of the UB and uterus.

1468 THE NITRIC OXIDE PRODRUG, V-PYRRO/NO, PROTECTS AGAINST ARSENIC TOXICITY AND APOPTOSIS AT THE CELLULAR LEVEL.

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The liver is an important target tissue of arsenic. The compound O2-vinyl 1-(pyrrolidin-1-yl)diazene-1-ium-1,2 diolate (V-PYRRO/NO) is a liver-selective nitric oxide (NO) producing prodrug that is metabolized by hepatic P450 enzymes to release NO in hepatocytes. V-PYRRO/NO can protect against the toxicity of various organic hepatotoxicants *in vivo* and *in vitro* but any role in arsenic toxicity is undefined. Thus, we studied the effects of V-PYRRO/NO pretreatment on arsenic-induced toxicity and apoptosis in cultured rat liver epithelial (TRL 1215) cells. TRL 1215 cells acted upon the prodrug to release NO, producing nitrite levels in the extracellular media after 24 hours of exposure to 500 or 1000 µM V-PYRRO/NO measured at 79.0 ± 5.6 and 313.0 ± 12.9 µM, respectively, compared to control levels of 7.50 ± 0.38 µM. Cells were pretreated with V-PYRRO/NO at 500 or 1000 µM for up to 24 hours, then exposed to arsenic (as sodium arsenite) for additional 24 hours and cytotoxicity was measured. Arsenic was significantly less toxic in V-PYRRO/NO (1000 µM) pretreated cells (LC50 = 30.3 ± 2.9 µM) compared to control cells (LC50 = 20.1 ± 1.9 µM). V-PYRRO/NO alone produced a small increase in metallothionein, a metal-binding protein. However, V-PYRRO/NO pretreatment greatly enhanced arsenic induction of metallothionein. V-PYRRO/NO pretreatment markedly reduced apoptotic cell death induced by arsenic (15 µM) as measured by DNA fragmentation ELISA analysis. Activation of the c-Jun N-terminal kinase (JNK) pathway can be critical to apoptotic cell death and pretreatment of cells with V-PYRRO/NO suppressed JNK activation after arsenic exposure. Thus, the prodrug, V-PYRRO/NO, protects against the adverse effects of arsenic in liver cells in culture, including apoptotic cell death and the concurrent activation of the JNK pathway, apparently through generation of NO. The role of NO in preventing arsenic hepatotoxicity *in vivo* deserves further study.

1469 METABOLISM OF PENTAVALENT AND TRIVALENT DIMETHYLATED ARSENIC IN THE MOUSE.

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Dimethylarsinic acid (DMA(V)) is a rat bladder carcinogen after its chronic exposure in either drinking water or the diet. DMA(V) is also a major urinary metabolite of mammals exposed to inorganic arsenic. In mice, iv and po administration of

[14C]-DMA(V) results in rapid urinary excretion of unspecified [14C]. This study assessed the chemical form of the arsenicals excreted in urine by mice after exposure to DMA(V) or dimethylarsinous acid (DMA(III), iodide form), the putative reduced metabolite of DMA(V). Adult female B6C3F1 mice were administered po DMA(V) (0.6 or 60 mg As/kg) or DMA(III) (0.6 mg As/kg). Control mice were administered water. After treatment, the mice were housed for 24 h in metabolism cages for collection of urine. Two methods were used for urinary arsenic analysis. Hydride-generation atomic absorption spectrometry (HG-AAS) was used to detect for DMA(V), DMA(III) and trimethylarsine oxide (TMAO). HPLC-ICP-MS was used to analyze for dimethylthioarsinic acid (DMTA) and trimethylarsine sulfide (TMAS). All five arsenicals were detected in the urine of control and treated mice; levels of each arsenical in urine of treated mice were five times or more higher than in urine of control mice. Urinary levels of DMA(V), TMAO and DMA(III) of treated mice were in the ppm range, except for DMA(III) in the low dose DMA(V) group, which was in the ppb range. Urinary levels of DMTA and TMAS of treated mice were in the ppb range. The rank order of arsenicals detected by HG-AAS in urine of DMA(V)-treated mice was DMA(V) > TMAO > DMA(III). In urine of DMA(III)-treated and control mice, the rank order of arsenicals detected by HG-AAS was TMAO > DMA(V) > DMA(III). Similar values of DMTA and TMAS were detected in urine of each treatment group. TMAO is the predominant metabolite of pentavalent and trivalent dimethylated arsenic excreted in urine of mice, which supports the hypothesis of oxidative methylation of arsenic. (This abstract does not represent U.S. EPA policy.)

1470 CHRONIC EXPOSURE TO ARSENITE IN DRINKING WATER IMPAIRS GLUCOSE TOLERANCE IN C57BL/6 MICE.

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Chronic exposures to inorganic arsenic (iAs) have been associated with an increased prevalence of type 2 diabetes mellitus. We have shown that trivalent arsenicals inhibit insulin-stimulated glucose uptake by cultured adipocytes. The current study examines *in vivo* diabetogenic effects of iAs, in an animal model. Here, weanling male C57BL/6 mice consumed deionized water containing iAs(III) (25 or 50 ppm) or water without iAs(III) for 8 weeks. Body weight and water consumption were monitored throughout the study. At 8 weeks, intraperitoneal glucose tolerance tests were administered to all mice. Skeletal muscle, pancreas, liver, and adipose tissue were collected and analyzed for arsenic species. Mice in the 25 and 50 ppm groups consumed significantly less water than did mice in the control group. However, body weights did not differ among treatment groups. Exposure to iAs(III) resulted in impaired glucose tolerance in mice of the 50 ppm, but not 25 ppm, group. The range of concentrations of arsenic in tissues of control mice (.03 to 4.9 ng/g) were significantly lower than the range of concentrations in tissues of mice in 25 ppm (36.9 to 422.8 ng/g) or 50 ppm (120.1 to 1165.4 ng/g) groups. In general, the highest concentrations of arsenic were found in the liver, followed by skeletal muscle, pancreas, and adipose tissue. Dimethylarsenic was the predominant form of arsenic found in tissues of both control and iAs(III)-treated mice. Notably, the average total concentration of arsenic species in livers from mice in the 50 ppm group was comparable to the highest concentration of total arsenic reported in livers of residents of Bangladesh who had consumed water with much lower levels of iAs (0.22 to 2 ppm). These data suggest that the lower susceptibility of mice than humans to the diabetogenic effects of iAs reflect higher rates of clearance of iAs and its metabolites from target tissues. (This abstract does not reflect EPA policies.)

1471 IDENTIFYING CRITICAL CYSTEINE RESIDUES IN ARSENIC (+3 OXIDATION STATE) METHYLTRANSFERASE.

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Arsenic (+3 oxidation state) methyltransferase (AS3MT) catalyzes methylation of inorganic arsenic to mono, di, and trimethylated arsenicals. Orthologous AS3MT genes in genomes ranging from simple echinoderm to human predict a protein with five conserved cysteine (C) residues. In human and rat, these are C32, C61, C85, C156, and C206. Roles of the highly conserved residues in catalysis were tested by site-directed mutagenesis in which each C was changed to a serine (S) residue. Studies with rat AS3MT (rAS3MT) found that either C156S or C206S was catalytically inactive and that C61S preferentially catalyzed monomethylation of arsenic. In contrast, human AS3MT (hAS3MT) with C61S, C156S or C206S was catalytically active, suggesting hAS3MT compensated functionally for loss of C

residues critical in rAS3MT. The hypothesis that other C residues in hAS3MT maintained activity in single mutants was tested using hAS3MT with double C to S mutants. Double mutants of conserved C residues (C61S/C156S, C61S/C206, C156S/C206S) were catalytically inactive. Double mutants of a conserved C and a non-conserved C (C156S/C334S, C206S/C334S) were inactive. Preservation of catalytic activity in single C to S mutants of hAS3MT and its loss in double C to S mutants suggests a non-conserved C (e.g., C334) in hAS3MT can provide redundancy that is lacking in rAS3MT. Redundant C residues in hAS3MT might preserve catalysis by allowing formation of disulfide bonds needed for proper protein folding or by interactions with trivalent arsenicals which are substrates for this enzyme. (This abstract does not reflect US EPA policy.)

1472 ARSENITE AND MONOMETHYLARSONOUS ACID-INDUCED SIGNAL TRANSDUCTION IN HUMAN BLADDER CELLS VIA THE PRODUCTION OF REACTIVE OXYGEN SPECIES.

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Arsenicals have commonly been seen to induce ROS which can lead to DNA damage, oxidative stress, and carcinogenesis. UROtsa cells (human urothelium) were used to study the effects of arsenicals on the human bladder. Arsenite [As^{III}] and monomethylarsonous acid [MMA^{III}] induce oxidative stress, form 8-hydroxyl-2'-deoxyguanosine, and increase stress proteins in UROtsa cells after exposure to concentrations as low as 1 μM and 50 nM, respectively. This evidence implicates ROS as a plausible mechanism for the signaling alterations seen in UROtsa cells after acute arsenical exposure. MMA^{III} has been shown to increase phosphorylation of ERK 2 in the MAPK signaling cascade downstream of ErbB2. Cyclooxygenase-2 (COX-2) is induced after exposure to 50 nM MMA^{III} and 1 μM As^{III} . To determine importance of ROS in the MAPK cascade and its downstream induction of COX-2, specific ROS inhibitors were used in co-treatment for 4 hr with MMA^{III} or As^{III} and COX-2 protein levels were assessed. For 10 μM As^{III} treatment, COX-2 protein was decreased after co-treatment with DTPA (Fe chelator), superoxide dismutase (O_2^-), sodium azide (NaN_3 , $^1\text{O}_2$), and catalase (H_2O_2). For 500 nM MMA^{III} treatment, COX-2 protein was decreased after co-treatment with potassium iodide ($\bullet\text{OH}$), NaN_3 , or SOD. To determine the importance of ROS in the toxicity of arsenicals, the inhibitors were used in co-treatment with As^{III} or MMA^{III} and cell viability was assessed. The toxicity of As^{III} to UROtsa cells decreased 20% with the addition of NaN_3 , showing the importance of $^1\text{O}_2$ in arsenic-mediated signaling and toxicity. Toxicity of MMA^{III} was ameliorated by the addition of KI or NaN_3 supporting previous evidence that MMA^{III} does induce formation of $\bullet\text{OH}$ which can lead to increased levels of 8-oxo-dG. These findings support that low-level As^{III} or MMA^{III} cause cellular changes via production of specific ROS and by inhibiting ROS during treatment with arsenicals, COX-2 induction and toxicity of arsenicals can be decreased. (Supported by NIH grants ES04940, ES06694, ES07091)

1473 IN UTERO AND POSTNATAL EXPOSURE TO ARSENIC ALTERS EXTRACELLULAR MATRIX GENE EXPRESSION IN THE LUNG.

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Growth and development requires the temporal and spatial coordinated expression of genes and gene products. During this critical time, *in utero* and early postnatal exposure to toxicants has the potential to affect gene expression, altering organ structure and physiological function. However, only limited attention has been paid to the effects of environmentally relevant exposures to toxicants during these critical periods of development. In this study female C57Bl/6 mice were exposed to arsenic in drinking water (0, 5, 10, 50 or 100 ppb) prior to mating. Arsenic exposure to the pregnant mothers was continued through birth of pups and until the pups were weaned. Postnatal pups were sacrificed on days 1, 7, 12 and 28 after birth and mRNA from the lungs was extracted for gene expression analysis. Gene expression levels for collagen 1a1, 1a2 and 3a1 and for elastin were determined using real time PCR. In addition, pulmonary response to methacholine challenge was carried out on 28 day old animals. Results showed a dose dependent increase in expression of collagen 1a2 on postnatal day 7 and a dose dependent increase in expression of collagen 3a1 on postnatal day 12. Arsenic-induced alterations in elastin expression were both age and dose dependent. Arsenic at these exposures did not produce any changes in expression of collagen 1a1. Pulmonary function indicated a dose dependent increase in responsiveness to methacholine challenge when expo-

sure was 10 ppb and above. These data indicate that exposure to environmentally relevant levels of arsenic during critical developmental times can lead to alterations in structural genes and in lung function.

This study is supported by NIH Center Grant P30ES006694 and SBRP Grant P42ES004940.

1474 TRANSCRIPTIONAL MECHANISMS INVOLVED IN THE MODULATION OF NQO1 GENE EXPRESSION BY ARSENITE, CADMIUM, AND CHROMIUM.

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NAD(P)H:quinone oxidoreductase (Nqo1)-mediated detoxification of toxic quinones is involved in the prevention of carcinogenesis. Heavy metals alter the carcinogenicity of AhR ligands by modulating the induction of Nqo1, but the mechanism(s) remain unresolved. In this study, to decipher the molecular mechanisms involved in the alteration of Nqo1 expression, we analyzed the effect of the heavy metals As^{3+} (5 μM), Cd^{2+} (5 μM), and Cr^{6+} (25 μM) on the transcriptional activation of this gene and post-transcriptional modifications of its gene products in Hepa 1c1c7 cells. As^{3+} and Cd^{2+} induced constitutive Nqo1 activity and potentiated the induction by increasing concentrations of the AhR ligand TCDD (0.1-10 nM). On the other hand, Cr^{6+} inhibited inducible Nqo1 activity. In parallel, both As^{3+} and Cd^{2+} , but not Cr^{6+} , induced Nqo1 mRNA in a time-dependent manner and potentiated TCDD-induced Nqo1 mRNA with all concentrations of TCDD tested. Cr^{6+} , on the other hand, had no effect on the time-dependent induction of Nqo1 mRNA and completely inhibited the induction of Nqo1 mRNA with all concentrations of TCDD. The induction of Nqo1 mRNA by the heavy metals was completely inhibited with the DNA transcription inhibitor actinomycin-D, indicating a requirement for *de novo* mRNA synthesis for the induction of Nqo1 mRNA. Furthermore, the protein synthesis inhibitor, cycloheximide decreased Nqo1 mRNA induction, an effect that was partially reversed with the proteasome inhibitor MG-132, thus suggesting a role for a labile protein in the transcriptional induction of Nqo1 by heavy metals. Interestingly, the heavy metals increased Nrf2 protein expression within 0.5h after treatment, suggesting a role for this labile protein in the induction of Nqo1 expression. Surprisingly, all three metals decreased Nqo1 mRNA stability. The three metals did not alter the Nqo1 protein half-life, relinquishing the role of post-translational effects. Thus, it is apparent that heavy metals alter Nqo1 expression at the transcriptional level, possibly through a Nrf2-mediated pathway.

1475 SODIUM ARSENITE-INDUCED CELL CYCLE ALTERATIONS AND APOPTOSIS IN MELANOMA CELL LINES.

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Arsenic trioxide is an efficacious chemotherapeutic used to treat acute promyelocytic leukemia. Arsenic is also undergoing clinical trials for treatment of other hematological malignancies and solid tumors. We are examining arsenic as a potential drug for the treatment of melanoma. A375 and SK-Mel-2 melanoma cell lines were sensitive to clinically achievable concentrations of arsenite. SK-Mel-3 and SK-Mel-28 cell lines were resistant to arsenite, requiring supra-physiological concentrations for toxicity. However, both resistant cell lines were sensitized to arsenite by pretreatment with buthionine sulfoximine (BSO), a glutathione synthesis inhibitor. Resistance to arsenite was also overcome by co-treatment with MK571, an inhibitor of the MRP family of transport proteins that extrude arsenic from the cell. Arsenite alone or combined with BSO or MK571 induced caspase 3 and poly(ADP-ribose) polymerase (PARP) cleavage suggesting apoptotic cell death. Apoptosis in arsenite-sensitive cell lines was associated with mitotic arrest. In A375 cells, active caspase 3 co-localized with the mitotic marker phospho-histone H3. Mitotic arrest was characterized by cyclin B stabilization. As cyclin B degradation is required for exit from mitosis, the ability of roscovitine, an inhibitor of the cyclin B/CDK1 complex, to promote mitotic exit was tested. Roscovitine decreased the mitotic index of mitotic arsenite-treated cells and abrogated PARP and caspase 3 cleavage. These data suggest that mitotic arrest or exit from an aberrant mitosis caused apoptosis in arsenite-sensitive A375 cells. In contrast, no mitotic arrest was observed in arsenite-resistant SK-Mel-3 and SK-Mel-28 cell lines treated in the presence or absence of BSO or MK571. However, GADD45 α induction suggests G2 arrest prevented mitotic arrest in the SK-Mel-3 and SK-Mel-28 cells. Although the pathway leading to cell death may differ between melanoma cell lines, the ability of arsenite to induce apoptosis in these cells merits further study of its potential use in the treatment of melanoma. (Supported by PHS grants R01ES011314 and T32ES011564.)

1476 SHIFT IN FTIR PATTERNS IN ARSENITE-EXPOSED RAT SPLENOCYTES RELATED TO RHO KINASE AND MAPK SIGNALING PATHWAY.

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Arsenite has long been considered a toxic metal that can cause cancer in many organs. From our previous report, we reported that arsenite can cause vinculin and actin cytoskeleton disruption in mouse fibroblasts in a dose-dependent manner and this effect can be blocked by EGFR and PI3K inhibitors. In this study, we found that arsenite can also cause FTIR spectra pattern shift of amide bands in arsenite-exposed rat splenocytes in dose-dependent manner but this toxic effect can be completely blocked by Rho kinase and MAPK inhibitors. The toxic responses induced by arsenite related to the Rho kinase-MAPK signaling and actin cytoskeleton disruption should be further investigated.

1477 ARSENIC STIMULATES HEPATIC CAPILLARIZATION AND INCREASES SINUSOIDAL ENDOTHELIAL MEMBRANE BOUND RAC1-GTPASE *IN VIVO* AND *EX VIVO*.

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Arsenic (AsIII) is a naturally occurring environmental toxicant that causes a wide range of organ specific diseases and cancers in millions of people worldwide. Epidemiological evidence demonstrates that drinking water contaminated with arsenic enhances vascular remodeling in humans and contributes to the pathogenesis of liver diseases through poorly defined mechanisms. To investigate the hypothesis that AsIII targets the hepatic endothelium to initiate pathogenic change, mice were exposed to 0 or 250 ppb of AsIII in their drinking water for one, two, or five weeks. AsIII exposure did not affect the overall health of the animals, the general structure of the liver, or hepatocyte morphology. There was no change in the total tissue arsenic levels, indicating that arsenic does not accumulate in the liver at this level of exposure. However, arsenic stimulated increased sinusoidal endothelial cell (SEC) capillarization, a process by which SECs lose fenestrae and develop a basement membrane. In addition to ultrastructural demonstration of SEC defenestration and capillarization, quantitative immunofluorescence revealed increased sinusoidal PECAM-1 and laminin-1 protein expression suggesting gain of adherens junctions and a basement membrane. These effects are recapitulated in isolated SECs exposed to AsIII *ex vivo*. AsIII stimulated Rac1-GTPase localization to SEC plasma membranes *in vivo* and inhibiting Rac1 in isolated cells prevented AsIII-induced surface PECAM expression *ex vivo*. In conclusion, these data demonstrate that As(III) exposure causes functional changes SEC cell signaling for sinusoidal capillarization that may be initial events in pathogenic liver changes promoted by arsenic in drinking water. This work was supported by NIEHS grant E07373 (AB), DS (NCI CA76541) and EPA STAR Fellowship FP-91654201 (AS).

1478 SYNERGIES IN VASCULAR CELL SIGNALING STIMULATED BY CO-EXPOSURE TO ARSENIC AND ETHANOL.

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Arsenic in the drinking water contributes to vascular diseases in millions of people worldwide through unresolved mechanisms. Further, little is known of the combined effects of co-exposures to other environmental toxicants that affect the vasculature, such as alcohol. Humans consuming alcohol may also be exposed to AsIII and these two agents share common dose-dependent angiogenic vascular effects. To investigate potential interactive mechanisms between these two agents, porcine aortic smooth muscle (SMC) or human microvascular endothelial (HMVEC) cells were exposed to a non-cytotoxic concentration of arsenite (5 μ M) in the presence or absence of 0.1% ethanol for 15 min, 1 h or 4h. Co-exposure, but not exposure to either agent alone, caused rapid increases in serine phosphorylation of PKC δ and tyrosine phosphorylation of 109 kDa protein in both SMC and HMVEC. This 109 kDa protein was identified as PYK2, which is known to regulate integrin signaling in vascular cells. Signaling synergy was also shown to be cell specific, since co-exposure increased activation of a 47 kDa Src family kinase member in HMVEC, but not SMC. In comparison, both agents equally increased membrane localization of PLC γ 1 and pro-oxidant Rac1-GTPase in both cell types with no further increase

following co-exposures. Arsenic-induced expression of the angiogenic ligand, vascular endothelial cell growth factor (VEGF) requires PKC δ activation (Soucy *et al Chem Res Toxicol* 17:555-563, 2003). Exposure of the SMC to arsenic or ethanol alone did not significantly induce VEGF, but co-exposure to the agents caused a 5-6 fold induction of VEGF mRNA levels. Thus, the effects of arsenic and ethanol on vascular cell signaling provided synergy in induction of an important vascular mitogen and remodeling factor with potential implications for pathogenic interactions between these two prevalent environmental toxicants at the level of angiogenesis.

1479 ARSENATE REDUCTION BY GLYCOGEN PHOSPHORYLASE. II. EFFECT OF ENZYME INHIBITORS.

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Some enzymes that catalyze phosphorolytic (or arsenolytic) cleavage of substrates can reduce arsenate (AsV) to the more toxic arsenite (AsIII) in a thiol-dependent fashion. We have shown this for purine nucleoside phosphorylase (PNP; Toxicol Sci 70:13, 2002), glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Toxicol Sci 85:859, 2005) and glycogen phosphorylase (a GP; poster of Nemeti and Gregus). As inhibitors of PNP and GAPDH antagonize AsV reduction catalyzed by PNP and GAPDH, respectively, it was of interest to know whether GP inhibitors (GPI) counter reduction of AsV by GP. Therefore, we examined if GP-catalyzed AsV reduction is impaired by SH reagents as well as three groups of compounds with known GP inhibitory actions: endogenous compounds (glucose, ATP, ADP), non-specific GPIs (caffeine, quercetin), and specific GPIs (DAB, BAY U6751). The effect of these agents was tested on reduction of AsV by rabbit muscle GP in presence of glycogen (substrate), AMP (activator) and glutathione. AsIII formed in the assays from AsV was quantified by HPLC-hydride generation-atomic fluorescence spectrometry. Experiments with N-ethylmaleimide and p-chloromercuribenzoate indicated that the AsV reductase activity of GP was moderately sensitive to thiol reagents. Glucose above 5 mM and ADP or ATP at physiological levels inhibited GP-catalyzed AsV reduction. 1,4-dideoxy-1,4-iminoarabinitol (DAB; an active site GPI), BAY U6751 (an AMP site GPI), caffeine (a nucleoside site GPI) and quercetin (an unclassified GPI) all diminished reduction of AsV by GP in a concentration dependent manner. AMP at high concentration alleviated the inhibitory effect of BAY U6751 probably because BAY U6751 uses the AMP binding site. Glucose that potentiates the inhibitory effects of caffeine and BAY U6751 on the glycogenolytic activity of GP also enhanced the inhibitory effects of these GPIs on GP-catalyzed AsV reduction. Thus, GPIs inhibit not only the glycogenolytic activity of GP but also its glutathione-dependent AsV reductase activity, suggesting that the latter is coupled to glycogenolysis. (Supported by OTKA and the Hungarian Ministry of Health)

1480 REDUCTION OF ARSENATE BY GLYCOGEN PHOSPHORYLASE. I. INGREDIENTS OF THE REACTION.

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The environmentally prevalent arsenate (AsV) is reduced in the body to the much more toxic arsenite (AsIII). We have shown that two enzymes catalyzing phosphorolytic cleavage of their substrates, namely purine nucleoside phosphorylase (Toxicol Sci 70:13, 2002) and glyceraldehyde-3-phosphate dehydrogenase (Toxicol Sci 85:859, 2005), can reduce AsV. Another phosphorolytic enzyme that may also carry out this process is glycogen phosphorylase (GP). With inorganic phosphate (Pi), GP catalyzes the breakdown of glycogen to glucose-1-phosphate (G-1-P), but it also accepts AsV. To test the hypothesis that GP can reduce AsV, we incubated AsV with GP (the phosphorylated form) or GPb (the dephosphorylated form) purified from rabbit muscle. AsIII formed was quantified by HPLC-hydride generation-atomic fluorescence spectrometry. In the presence of AMP, glycogen, and glutathione (GSH), both GP forms reduced AsV, and AsIII formation rates linearly correlated with the enzyme and AsV concentrations. The AsV reductase activity of GP was 10-fold higher than that of GPb. However, incubation of GPb with GP kinase and ATP (that converts GPb to GP) increased AsV reduction by phosphorylase up to the rate produced by GP when incubated with ATP and GP kinase. AsV reduction by GP (like AsV reduction in rats) required the presence of GSH. It also required glycogen (a substrate for GP), and was greatly stimulated by AMP (an allosteric activator of GP) even at low micromolar concentrations. Pi, substrate for GP competing with AsV, inhibited AsIII formation moderately at physiological concentrations. G-1-P, the product of GP-catalyzed glycogenolysis, also decreased AsV reduction. In summary, GP is the third phosphorolytic enzyme identified capable of reducing AsV *in vitro* to AsIII. For AsV reductase activity of GP, the presence of GSH and glycogen is indispensable, suggesting that the reduction is linked to glycogenolysis. While its *in vivo* significance remains to be tested, further characterization of the GP-catalyzed AsV reduction is presented in the accompanying poster (Gregus and Nemeti). (Supported by OTKA and Hungarian Ministry of Health)

1481 BEHAVIORAL ASSESSMENTS OF LONG-EVANS RATS FOLLOWING A 13-WEEK SUBCHRONIC TOLUENE EXPOSURE.

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Whereas the acute effects of volatile organic compounds (VOCs) are relatively well understood, there is some controversy regarding the potential for persistent effects following long-term exposure. The current study sought to develop an animal model of subchronic exposure to VOCs which may be used to predict effects of long-term exposure to these agents on public health. Adult, male Long-Evans rats were exposed to toluene (0, 10, 100, or 1000 ppm) 6 hr/day, 5 days/week for 13 weeks. Three cohorts of animals (N=40 per cohort) were assessed in a variety of behavioral tasks beginning 2 days after the exposure ended. Cohort 1 was tested on the elevated plus maze (EPM) and showed no differences in anxiety measures between the toluene groups. Cohort 2 was assessed for motor activity on the Figure-8 maze followed by an assessment of anxiety on the EPM. No differences in total motor activity or anxiety were seen between the toluene groups. However, indices of anxiety were significantly different from Cohort 1, presumably as a result of prior Figure-8 maze testing. Cohort 3 was tested on a trace fear conditioning task and showed no toluene-induced differences in cue learning. Conditioning to context was marginally enhanced by toluene, but with no clear dose-dependency. Training to perform a visual signal detection task to assess learning and sustained attention began in Cohort 2 12 days after the end of exposure. Acquisition of a lever-press response for food (autoshaping) was delayed in all toluene-exposed groups; all rats eventually learned the response. Learning a subsequent visual discrimination task was impaired in the 100 and 1000 ppm groups. These results indicate that subchronic exposure to toluene may cause persistent impairments in behavior. [This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.]

1482 VISUAL FUNCTION CHANGES AFTER SUBCHRONIC TOLUENE INHALATION IN LONG-EVANS RATS.

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Chronic exposure to volatile organic compounds, including toluene, has been associated with visual deficits such as reduced visual contrast sensitivity or impaired color discrimination in studies of occupational or residential exposure. These reports remain controversial, however, in part due to a lack of confirmation from controlled laboratory experiments. This study examined rats following exposure to 0, 10, 100 or 1000 ppm toluene by inhalation (6 hr/d, 5 d/wk, 13 wk) as described in Bushnell et al., (SOT, 2007). The week following exposure, rats (n=10/dose group) were surgically implanted with indwelling electrodes over visual cortex. The next week, pattern elicited visual evoked potentials (VEP) were recorded over a range of visual pattern contrasts. Four weeks after exposure, rats were anesthetized, evaluated ophthalmologically, and electroretinograms (ERG) were recorded. No significant changes were observed in VEP amplitudes or contrast thresholds derived from VEP contrast-amplitude functions. There were no dose-related ophthalmologic findings. The latencies of the ERG A- and B-waves were unchanged by toluene treatment. There was, however, a significant dose x intensity interaction for the amplitudes of A- and B-waves. The amplitudes of B-waves were reduced only at stimulus luminance values higher than the apparent rod/cone break in rats treated with 1000 ppm toluene. The ERG results are consistent with a selective deficit in cone photoreceptor function following subchronic toluene exposure. These data provide experimental confirmation that repeated exposure to toluene may lead to subtle changes in visual function.

This abstract does not reflect EPA policy.

1483 SUBCHRONIC TOXICITY OF INHALED TOLUENE IN RATS: IMMUNOLOGY, CARDIAC GENE EXPRESSION, AND MARKERS OF OXIDATIVE STRESS.

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The health effects of long-term exposure to volatile organic compounds (VOCs) are poorly understood, due primarily to insufficient human exposure data and inconsistent animal models. To develop a rodent model of long-term exposure to VOCs, a sub-chronic inhalation study with multiple endpoints was conducted in rats exposed to toluene, a prevalent VOC. We report here that toluene did not affect immune function or gene expression of ion channel proteins, heme oxygenase (HO-1), or markers of oxidative stress in cardiac tissue. Effects on nervous system functions appear in separate reports. Adult male Long-Evans rats were exposed to toluene vapor at 0 ± 0, 10 ± 1.4, 97 ± 7, or 995 ± 43 ppm (mean ± SD) for 6h/d,

5 d/wk for 13 weeks. During the final week of exposure, subgroups of rats from each concentration were immunized with sheep red blood cells (SRBCs) to assess antibody synthesis; separate groups were sensitized to bovine serum albumin (BSA) to elicit delayed-type hypersensitivity (DTH) responses. Cardiac gene expression was assayed using real time PCR for HO-1, L-type calcium channel (L-Ca), KV1, connexin-40 and sodium channel protein-6 (SCP6). Activities of enzymes sensitive to inhibition by oxidative stress were also analyzed in mitochondrial and cytosolic fractions. Titers of SRBC-specific IgM and IgG antibodies and DTH responses to BSA were not affected by toluene. No consistent exposure-related changes were noted in cardiac gene expression or activity of enzymes associated with oxidative stress. These results indicate that sub-chronic inhalation of toluene in rats did not affect humoral or cellular immunity, did not change the expression of a number of genes coding for important cardiac proteins, and did not induce oxidative stress in cardiac tissue. These findings contrast with significant changes in CNS function observed in other rats in this study. This report does not reflect EPA policy.

1484 AGE-RELATED EFFECTS OF TOLUENE ON THE MOTOR ACTIVITY OF BROWN NORWAY RATS.

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Senescence raises many uncertainties regarding susceptibility to environmental exposures. Compromises in reserve and repair mechanisms, and alterations in metabolic capacity, may make the aging population more susceptible to environmental contaminants. Additionally, increased variability between individuals might complicate accurate assessment of susceptibility in the aging population. This study was designed to assess the relative sensitivity of aging Brown Norway (BN) rats to the behavioral effects of toluene. BN rats have been used extensively in aging research, although little is known about how they respond to environmental chemicals. BN rats were supplied by the National Institute on Aging at 4, 12 and 24 months of age (N=8 per age). The rats were tested weekly in a commercial photocell device for measuring motor activity during 30-min sessions. Toluene was administered in corn oil by gavage, 30-min prior to testing, in doses of 0, 300, 650 and 1000 mg/kg. Weekly dosing and testing continued until all rats received each treatment. Following vehicle, horizontal activity was highest in the 4-month rats and lowest in the older rats; vertical activity also decreased with age. When expressed relative to vehicle-control values, toluene produced dose-related increases in horizontal activity and decreases vertical activity. Toluene effects were most pronounced in the oldest rats. The oldest rats were, however, the heaviest and therefore received a larger amount of toluene (as mg/kg) than the younger, lighter rats. To evaluate this potential confound, all rats were given the same amount of toluene regardless of body weight. Age-related differences in sensitivity were still obtained. These results indicate advancing age may increase susceptibility to toluene.

This is an abstract of a proposed presentation; the information does not necessarily reflect Agency policy.

1485 CIRCADIAN CONTROL OF THE RAPID DEVELOPMENT OF TOLERANCE TO THE ANALGESIC ACTION OF MORPHINE AND ETHANOL IN THE RAT.

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Sprague-Dawley rats weighing 180-220 g, were maintained in light of 12 hr light-dark cycle (lights on at 07:00 hr) and temperature condition of 21±1°C. In the first experiment, groups of 8 animals were injected i.p. with morphine sulfate 5 mg/kg, or saline at 06:00, 12:00, 18:00, and 24:00 hr for three consecutive days, and the degree of analgesia was assessed 30 minutes post administration by four different procedures. Pain sensitivity was measured through the use of analgesia-meter front paw and hind paw pressure base procedure, tail flick, and the hotplate methods (maintained at 55°C). The peak pain sensitivity was observed in control animals during the light phase while the minimum was observed during the dark phase. Meanwhile, there was a circadian variation in the degree of analgesia produced using each procedure. Maximum analgesia was observed during the light phase (18:00 hr) and minimum analgesia at 12:00 hr. Repeated administration of morphine during the light phase resulted in tolerance development in animals using the hot plate and tail flick methods. Animals developed tolerance using the front paw procedure at each specific hour of treatment. In the second experiment, group of 8 Sprague-Dawley rats weighing 250-350 g were injected i.p. with ethanol (3gm/kg) at 06:00, 10:00, 14:00, 18:00, 22:00, and at 02:00 hr for 2 consecutive days and the degree of analgesia was measured 30 minutes before and 30 minutes after drug administration. The repeated injection of ethanol resulted in tolerance to the analgesic response only if animals were treated during the light phase using the front and hind paw procedures. Results indicate that tolerance to the analgesic effects of morphine and ethanol is a circadian controlled phenomenon. The diurnal

fluctuation in tolerance development observed in the present investigations may be related to circadian variations in brain sensitivity to these drugs, or to diurnal fluctuation of some specific neurohormone in the brain.

1486 SEPTAL HIPPOCAMPAL NEURONAL NITRIC OXIDE SYNTHASE PLAYS A ROLE IN THE TRANSIENT COGNITIVE DECLINE INDUCED BY ETHANOL IN LONG EVANS RATS.

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The present study was conducted to evaluate the role of septal hippocampal neuronal nitric oxide synthase (nNOS) in the decline of spatial reference induced by ethanol. Male Long Evans Rats (200-250 g) were randomly divided in two groups (n=10 /group). Following a 3-day training period, the first group received a daily i.p. injection of ethanol in a dose of 3 g/kg (22.5% w/v solution in saline) for 10 consecutive days; the second group served as control and was injected i.p. with saline. Injections were performed daily at 13:00hr. One hour post injection the spatial reference memory was evaluated by measuring the latency to find the submerged platform on two separate trials, one minute apart. The same evaluation was conducted 24 hours later, immediately before the administration of the next ethanol dose. After the last evaluation, animals were sacrificed by decapitation, the septal hippocampus dissected and its nNOS levels measured by western blot. Results indicate that one hour post-injection, ethanol administration resulted in a significant increase in the time to find the platform ($P<0.0001$) as compared to controls. This impairment was consistent across testing days, indicating that no tolerance developed to acute intoxication. Spatial reference memory deficits as indicated by a significant increase in time to find the platform were also noted in ethanol-treated rats 24-hour post injection. This was mostly seen in day 3 ($p<0.0361$) and day 4 of treatment ($p<0.005$). Ethanol treatment resulted in significant increase (44%) in the levels of septal nNOS as compared to control. These findings clearly indicate that increase in septal nNOS may play an important role in the transient decline in spatial memory induced by ethanol. (Supported by VA Merit Project 0002 and NIH grant RR03020)

1487 ACQUISITION OF 8-ARM RADIAL MAZE (8ARM) TASKS DURING TREATMENT WITH SCOPOLAMINE (SCO), NICOTINE (NIC), ETHANOL (ETH), OR AMPHETAMINE (AMP).

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The 8ARM is used to identify potential learning and memory deficits in rodents after treatment with potentially neurotoxic therapeutics. We evaluated the utility of the 8ARM by treating adult male Sprague-Dawley rats with SCO (0.17 mpk), NIC (0.4 mpk), ETH (1500 mpk, Task 1 only) or AMP (0.125 mpk, Task 2 only) while being trained to learn one of two tasks. Both Task 1 and Task 2 are win-shift tasks, meaning the rats were rewarded for not revisiting arms. In Task 1, rats were trained to visit 6 baited of 8 arms within 5 min. In Task 2, rats were trained to visit 8 baited of 8 arms within 5 min. Evaluations included numbers and types of errors and time-to-complete (TTC). Rats treated with SCO, a muscarinic (M1) antagonist, required up to 2 min more TTC than controls and made significantly more errors. Although rats treated with NIC, a nicotinic agonist, required 20 sec less TTC, they consistently made more errors. TTC and numbers of errors made by rats treated with ETH or AMP were similar to controls throughout the study. Interestingly, rats in all groups continued to visit the unbaited arms in Task #1, thereby making the same number of reference memory errors that would be expected by chance (-1.75). To assess the effect of prior treatment on the ability to learn a new task, Task 2 rats were removed from all treatments after 5 weeks and trained to learn a new delayed non-match to position Task 3. Rats previously treated were not as proficient as controls in Week 1. By Week 3, there were no differences in performance between controls and any of the previously treated groups. This study was supported by NIAID Contract N01-AI-05417.

1488 PRELIMINARY NEUROLOGICAL AND 8 ARM RADIAL MAZE (8ARM) ASSESSMENTS AFTER TREATMENT WITH THE ANTIMALARIAL, β -ARTEETHER (β AE), TO NEONATAL AND ADULT RATS.

R. I. Erickson, K. L. Steinmetz, E. B. Defensor, D. R. Middaugh, J. Nimer and J. C. Mirsalis. *Mammalian Toxicology, SRI International, Menlo Park, CA.*

More than a million people die each year from malaria; 70% of these victims are children <5 years old. With increased drug resistance to current therapies, new treatments must be developed. Qinghaosu (artemisinin), the medicinal component

of the herb *Artemisia annua*, has been used for many centuries in Chinese traditional medicine as a treatment for fever and malaria. Since being isolated in 1971, artemisinin has shown to be potent and well-tolerated, prompting the World Health Organization to recommend that artemisinin derivatives be used in combination with other therapies to avert drug resistance. Although administered to humans (including children) without apparent toxicity, β AE given to adult rats causes severe brainstem toxicity including necrosis in the superior olive and trapezoid nucleus, regions which affect auditory, vestibular and motor functions. In our studies, adult rats treated intramuscular (i.m.) with β AE (12.5–50 mpk for 14d), did not have impaired spatial navigation in the 8ARM despite demonstrating severe gait disturbances and characteristic necrosis in brainstem nuclei. To date, nonclinical studies with artemisinins have been conducted almost exclusively in adult laboratory species which may not reflect β AE neurotoxicity potential on the developing brain. Beginning on postnatal day 7, neonatal rats (4 males and 4 females per group) were treated daily with β AE at 0, 5, 15, 30, and 90 mpk i.m. One pup (30 mpk) and 3 pups (90 mpk) died on Days 5, 5, 7, and 8, respectively. Neurobehavioral assessment on Days 1, 2, 5, 6 and 8 showed that 90 mpk impaired homing behavior. All pups receiving 90 mpk had significantly reduced weight gain and were found in moribund condition, requiring early termination of remaining pups. Surviving pups will be assessed as adults for neurological histopathology and functionality using motor activity, Functional Observational Battery, and learning and memory (8ARM). Work supported by NIAID contract N01-AI-05417.

1489 THE EVALUATION OF NEUROTOXICITY OF AN ARTIFICIAL DURA MATER CONTAINING DIBUTYLTIN IN RATS BY THE PREPULSE INHIBITION TEST AND AN OPEN FIELD TEST.

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A synthetic biodegradable artificial dura mater has recently been developed. The major components of dura mater are the co-polymers with poly-L-lactides. For the synthesis of dura mater, catalysts such as dibutyltin (DBT) are used. The effects of the artificial dura mater containing 100 ppm-DBT on the behaviors in rats after intracranial insertion were evaluated using both a prepulse inhibition (PPI) test and an open field test. Thirty rats were divided into three groups, the control, the artificial dura mater group, and the artificial dura mater containing DBT group. A circular bone of 5 mm in diameter was cut out from the cranium of each anesthetized rat. A piece of the artificial dura mater, or the artificial dura mater containing 100 ppm-DBT, was placed on the surface of the brain and covered with the bone. The control group rats underwent the same surgical operation without the insertion. After 4 weeks, PPI and open field tests were performed. In the PPI test session, four types of acoustic stimulation (a startle pulse of a burst of 120 dB, combined trials of prepulse [a burst of 70, 75, or 80 dB] followed with the pulse of 120 dB) were given to each rat in a chamber with a floor equipped with a scale. The percent of prepulse inhibition (% PPI) of a startle response at a prepulse of 80 dB (PP80), 75 dB, or 70 dB was calculated. The mean value of %PPI at PP80 in the artificial dura mater containing DBT group was significantly lower than that in the control. There were no significant differences among the groups in an open field test. The functions of cognition and learning may be impaired in the group for which the artificial dura containing 100 ppm-DBT was inserted.

1490 CIRCADIAN CONTROL OF THE RAPID DEVELOPMENT OF TOLERANCE TO THE ANALGESIC ACTION OF MORPHINE AND ETHANOL IN THE RAT.

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We have shown that the rapid development of tolerance to drugs is a phenomenon which is diurnally controlled, and we have coined the term "chronotolerance" to describe this phenomenon. The present investigations were designed to ascertain the presence of chronotolerance to morphine and ethanol in the rat. In the first experiment, four different groups of Sprague Dawley rats were injected with morphine sulfate 5 mg/kg, or saline at 06:00, 12:00, 18:00, and 24:00 hr for three consecutive days, and the degree of analgesia was assessed 30 minutes after drug administration in each animal. The results obtained indicate that animals exhibited circadian variation in their responsiveness to pain. The peak for pain sensitivity was found to occur during the light phase with a minimum sensation during the dark phase. Meanwhile, there was a circadian variation in the degree of analgesia produced

using each procedure. The results obtained also indicate that repeated injection after twenty-four hours was accompanied by a decrease in the degree of analgesia produced. It was interesting to note that the repeated administration of morphine resulted in tolerance development only if animals were treated during the light phase when assessment was made using the hot plate and tail flick methods. However, animals developed tolerance using the front paw procedure at each specific hour of treatment. In the second experiment, four different groups of male Sprague-Dawley rats were injected with ethanol (3gm/kg) at 06:00, 10:00, 14:00, 18:00, 22:00, and at 02:00 hr for 2 consecutive days, and the degree of analgesia was measured after drug administration. The repeated injection of ethanol resulted in tolerance to the analgesic response only if animals were treated during the light phase using the front and hind paw procedures. The present results indicate the presence of chronotolerance to the analgesic effects of morphine and ethanol. (Supported by NIH Grant # RR 03020)

1491 NICOTINIC-RECEPTOR BLOCKADE AND THE EFFECTS OF ANATOXIN-A ON THE MOTOR ACTIVITY OF RATS: COMPARISON WITH NICOTINE.

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Anatoxin-a is produced by several species of freshwater cyanobacteria and has caused numerous poisoning episodes in terrestrial and aquatic wildlife, livestock and domestic animals. Anatoxin-a is also a potent nicotinic agonist in the nervous system and at the neuromuscular junction. There has been little research on the behavioral effects of anatoxin-a, and virtually no characterization of the role of nicotinic receptors. These experiments determined the effects and interactions of anatoxin-a and selective nicotinic-receptor antagonists on the motor activity of adult male Long Evans rats. Parallel studies included nicotine. Motor activity was evaluated in a commercial photocell chamber during 20-min sessions. Each experiment included groups of 9 rats that received a dose of anatoxin-a or nicotine, a dose of a nicotinic antagonist, and their combination. Each experiment also included a vehicle-control group. Doses of anatoxin-a and nicotine were 0.6 mg/kg and 2.5 mg/kg, respectively, and were administered s.c. 5-min prior to a test session. Nicotinic antagonists included dihydro-beta-ethroindine (DHBE, 10 mg/kg) and methyllycaconitine (MLA, 10 mg/kg). MLA was given s.c. 15-min prior to testing and DHBE was given immediately before anatoxin-a or nicotine. Pretreatment with DHBE produced a transient blockade of the effect of nicotine, but did not block the effect of anatoxin-a. Pretreatment with MLA blocked the effect of anatoxin-a, but not that of nicotine. These results indicate anatoxin-a differs from nicotine in its site of action, most likely involving stimulation of alpha-7 nicotinic receptors in the nervous system.

This is an abstract of a proposed presentation; the information does not necessarily reflect Agency policy.

1492 BRAIN PGE2 CONCENTRATION IS ASSOCIATED WITH SUSCEPTIBILITY TO KAINATE-INDUCED SEIZURES.

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We have previously demonstrated that genetic deletion of cyclooxygenase (COX)-2, but not COX-1, the enzymes that metabolize arachidonic acid (AA) into prostanooids, can increase susceptibility to kainic acid (KA)-induced excitotoxicity. To further understand the mechanism of this increased susceptibility, we investigated the effect of chronic pharmacological inhibition of COX-2 on KA-induced excitotoxicity. Wild type mice were placed on a diet containing 0, 1000, or 3000 ppm celecoxib, a COX-2 selective inhibitor, for six weeks. At 12 weeks of age, mice were either euthanized by focused microwave irradiation of the brain or injected intraperitoneally with 10 mg/kg KA and euthanized 24 hours after injection. Celecoxib exposure did not alter mean group body weights over the course of treatment. A dose-dependent decrease in PGE2, a major COX-2 end product, was observed in the brains of celecoxib-exposed mice and COX-2 $-/-$ mice (0 ppm = 9.2 ng/g; 1000 ppm = 7.2 ng/g (21%); 3000 ppm = 5.3 ng/g (41%); COX-2 $-/-$ = 4.4 ng/g brain weight (52%)) with no effect of celecoxib treatment or genotype on brain levels of thromboxane B2 (TXB2), a COX-1 preferential end product. Kainate-induced seizure intensity, quantified by median Racine Seizure Scale (RSS), was increased in mice with decreased brain PGE2 concentration (0 ppm RSS = 1; 1000 ppm RSS = 1; 3000 ppm RSS = 2; COX-2 $-/-$ RSS = 3; Kruskal Wallis ANOVA $p=0.008$). This inverse relationship between PGE2 concentration and seizure intensity suggests that chronic inhibition of COX-2 may decrease the production of COX end products that regulate neuronal excitability. This data confirms our previous finding that genetic deletion of COX-2 increases susceptibility to KA-induced excitotoxicity and suggests that chronic exposure to COX-2 selective inhibitors may increase susceptibility to seizures and excitotoxicity. [Supported by the NIA IRP and NIH IRTA programs].

1493 NEUROPROTECTIVE ACTIONS OF PACAP38 IN MODELS OF DOPAMINERGIC TOXICITY.

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In this study we explore the possible neuroprotective actions of PACAP38 on dopamine toxicity. PACAP38 (pituitary adenyl cyclase activating polypeptide), a relative of VIP (vasoactive intestinal peptide), has been demonstrated to release growth factors and increase neurogenesis in the subventricular zone of the lateral ventricles (Mercer et al. 2004). Furthermore, endogenous expression of PAC1, a PACAP-specific receptor, is found in the substantia nigra pars compacta and acute direct injection of PACAP38 can protect nigral neurons against 6-OHDA toxicity (Reglodi et al. 2004). In our studies, a total dose of 1 mg/kg PACAP38 was administered to C57BL/6J retired breeder male mice via subcutaneous osmotic pumps over one week. Four weeks post-treatment, PACAP38 treated mice showed a 35% increase vesicular monoamine transporter-2 (VMAT2) in the striatum, but no change in tyrosine hydroxylase (TH) or dopamine transporter (DAT). Also, at two weeks post-PACAP38, a 56% elevation in vesicular uptake velocity of dopamine was observed along with a 19% increase in VMAT2 protein. Since we have shown that mice with genetic reduction of VMAT2 are more susceptible to methamphetamine- and MPTP-induced damage (Fumagalli et al. 1997), we decided to test if PACAP38 is protective in these models. PACAP38 treatment 48 hours after MPTP attenuated some of the behavioral deficits of these mice and was able to reduce VMAT2 loss by 50%; results from methamphetamine studies are pending. While administering PACAP38 after a toxic challenge is effective, PACAP38 pretreatment may provide enhanced resistance to dopamine neurotoxins and other oxidative challenges by elevating VMAT2 levels, thus reducing intracellular dopamine quinones. Since dopamine neurons are already vulnerable due to high basal levels of oxidative stress, reducing this load by increasing VMAT2 may also prolong the survival of these neurons in an unchallenged state as well.

1494 EXERCISE PROVIDES NEUROPROTECTION AGAINST KAINIC ACID TOXICITY THROUGH INDUCTION OF THE CHEMOKINE MCP-1 IN THE HIPPOCAMPUS OF C57BL/6J MICE.

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Physical exercise affords protection to neurons exposed to the excitotoxicant kainic acid (KA). We previously observed attenuation in KA-induced argyrophilia and blood-brain barrier disruption following a 14-day regimen of forced walking in male C57BL/6J mice. Here, we investigated the role of microglial activation in the hippocampus, and the production of microglial-derived factors in the protective mechanism. Mice were assigned randomly to one of four experimental groups: saline, kainic acid, exercise + saline, exercise + KA. Forced walking was achieved in a motorized exercise wheel (6 rpm, 60 min duration, 4:00 PM daily). Mice were acclimated to the wheels for three days, exercised for 14 days, and given an intraperitoneal injection of KA (25 mg/kg). Control animals were not exercised, and received a comparable injection of saline. Following a 12-hour survival, animals were sacrificed and their brains were dissected into regions for RNA extraction and analysis of microglial markers and secretory products by RT-PCR. KA treatment caused a four-fold induction in hippocampal levels of the neurotrophic factor Gdnf, and a two-fold induction in Igf-1; bdnf levels were slightly but non-significantly elevated. Microglial markers, F4/80, Iba1, Mac-1, and p67Phox were unchanged between experimental groups. KA treatment caused a 3-fold induction in IL-1 α and IL-6 levels, a 15-fold induction in Tnf- α levels, and an 8-fold induction in Mcp-1 that was increased to 23-fold by exercise pretreatment. Our data suggest the protective effects of exercise against excitotoxicity may occur through modulation of the neuroinflammatory response, and may be mediated through the chemokine Mcp-1. Subsequent experimentation will evaluate the protective effects of exercise in Mcp-1 knockout mice.

"The findings and conclusions in this report (abstract/presentation) have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy."

1495 SUSCEPTIBILITY TO 1-BROMOPROPANE EXPOSURE AND ALTERATION OF mRNA EXPRESSION IN RATS.

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The present study aimed to investigate the possible genes related with difference in susceptibility to 1-bromopropane (1-BP) exposure between two inbred strains of rat, F344 and Wistar Nagoya (WNA). Eighteen of 8wk old F344 or WNA male

rats were randomly divided into 2 groups of 9 each. Two groups of F344 or WNA were exposed to 1000ppm 1-BP and fresh air 8 hrs/d, 7 d/wk for 4 wks, respectively. Distal latency (DL) and motor nerve conduction velocity was measured in the rat tail. The liver was dissected out and frozen at the end of the experiment for oligo DNA microarray analysis. Another set of eighteen WNA or F344 were divided into two groups of nine and exposed to 1-BP and fresh air for 8 hrs and the liver was dissected out for the microarray analysis on acute effects of 1-BP exposure. Electrophysiological study showed a significant prolongation in the DL of tail nerve of F344 rats after 4 wk exposure compared to the control. Histopathological examination showed many scanty areas in the cytoplasm of liver cells around the central vein in the exposed F344 rats, but no change in WNA rats. Oligo DNA microarray and real time PCR showed exposure to 1-BP for 8 hrs and 4 wks upregulates mRNA expression of glutathione S-transferase, aflatoxin aldehyde reductase and epoxide hydrolase.

Although our analysis on the microarray has not shown interpretable difference related with the susceptibility between the two rat strains, the result showed consistent increase in mRNA expression of glutathione S-transferase, aldo-keto reductase and epoxide hydrolase after exposure to 1-BP at 1,000 ppm for 8 hrs and 4 wks in F344 and WNA. These genes might be related with the mechanism of 1-BP toxicity and the investigation on the polymorphism of these genes might be useful to understand the susceptibility to 1-BP exposure.

1496 NEUROPROTECTIVE EFFECTS OF SHORT REGULATORY PEPTIDES AND THEIR CYCLIC FRAGMENTS.

A. N. Petrov. *The Institute of Toxicology, ST-Petersburg, Russian Federation.* Sponsor: K. Khamidulina.

Experimental studies of short regulatory peptides and their cyclic fragments for treatment of experimental poisonings of neurotropic drugs has been conducted. One of the final stages in the chain of biological modification of some regulatory peptides involves cyclic peptides formation producing diketopiperazine ring from the residues of two C-terminal acids.

We have characterized neuroprotective effects of the following cyclic dipeptides: cAsp-Phe — cholecystokinin derivative, cLeu-Gly — oxytocin derivative, cHis-Pro — thyroliberin derivative. It was demonstrated that cLeu-Gly eliminated almost completely the short-term memory disorders in rats caused by the M-anticholinergic agent glypiline; cAsp-Phe prolonged the latent period significantly to convulsion onset and survival period in rats poisoned with corasole; cHis-Pro prevented haloperidol catalepsy in rats.

Thus, the studies showed that cHis-Pro, cLeu-Gly and cAsp-Phe have anti-cataleptic, anti-amnesic and anticonvulsant effects respectively. We suggest that some cyclic fragments of the short regulatory peptides can be used for selective impact of poisoning CNS disorders.

1497 DOPAMINERGIC AND GLIAL CELL MARKERS IN A CHRONIC MPTP NON-HUMAN PRIMATE MODEL OF PARKINSON'S DISEASE.

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The early detection of Parkinson's disease (PD) could be advantageous for the treatment and testing of drug therapies to arrest disease progression. We used positron emission tomography (PET) with radiotracers for dopaminergic neuronal and glial markers in a chronic 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) non-human primate model of PD. PET studies using [¹¹C]WIN35,428 for dopamine transporter (DAT), [¹¹C]DTBZ for vesicular monoamine transporter type 2 (VMAT2), and [¹¹C](R)PK11195 for peripheral benzodiazepine receptor (PBR) in glial cells were performed in baboons (n=5). MPTP was injected i.v. once/week at 0.1-0.2mg/kg for 6-7 months (m) and increased to 0.4-0.6mg/kg until the appearance of clinical signs. PET, movement and behavior were assessed at baseline, at 2, 6-7 & 9-10m after MPTP treatment. At 2m, there were significant (p<0.05) reductions of VMAT2 in caudate (46±5%) and putamen (35±5%) relative to baseline in the absence of an effect on behavior or DAT levels. Increased PBR levels were also measured in caudate (43±11%) and putamen (40±10%). After 6-7m of MPTP treatment, small but significant reductions of DAT were measured in caudate (22±8%) and putamen (17±6%) with persistent reductions of VMAT2 in caudate (46±11%) and putamen (32±8%). PBR levels were increased but did not reach statistical significance. After 9-10m of treatment, animals exhibited impairments in movement behavior and VMAT2 and DAT levels were both dramatically decreased in caudate (78±4% & 58±13%) and putamen (69±5% & 61±13%). However, PBR levels decreased to baseline values. Postmortem analysis of DAT, VMAT2 and PBR using quantitative autoradiography confirmed the PET findings. Our study

demonstrates that PBR could be a sensitive marker for early PD but lacks correlation with PD progression. Importantly, VMAT2 appears to be a more sensitive marker in the early stages of PD compared to DAT [M.J.Fox Found. and ES07062 grant to TRG]

1498 BUPRENORPHINE POTENTIATES METHAMPHETAMINE-INDUCED EXTRACELLULAR DOPAMINE RELEASE IN RAT CAUDATE-PUTAMEN.

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Methamphetamine (METH) abuse and addiction presents a major problem in the United States. Efficacy of the adjunctive medication with buprenorphine (BUP) has been demonstrated in the treatment of opiate and cocaine dependence. Using microdialysis, the effects of dopaminergic response to a combined BUP/METH treatment in the rat caudate-putamen was examined. In vivo microdialysis and high performance liquid chromatography with electrochemical detection (HPLC-EC) were used to measure baseline and METH-stimulated striatal dopamine (DA) and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) levels in adult male Sprague-Dawley rats weighing between 300 and 500 g. The animals received BUP either at 1 or 10 mg/kg s.c. 10 min before METH administration at 2 mg/kg i.p. Dialysates were assayed every 20 min for 320 min following METH administration. Compared with baseline (100%), a marked 312% increase in extracellular DA within 60 min was observed in animals pretreated with BUP at 1 mg/kg and a 566% increase in extracellular DA was observed in animals pretreated with BUP at 10 mg/kg. However, DOPAC efflux did not change. This BUP potentiation in DA response indicates that BUP may be effective in the treatment of METH addiction.

1499 A TOXICITY STUDY OF QUANTUM DOTS IN MICE.

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Quantum dots are nanomaterials with numerous possible applications in medicine. Studies specially designed for toxicological assessment are few. The aim of our study was to investigate their in vivo effect and body distribution.

Type I EviTags, carboxyl or amine modified, were intravenously injected in mice. In a first experiment high doses of 100, 20 and 4 µg were used and the animals were sacrificed after one hour. In a second experiment lower doses of 4, 0.4 and 0.04 µg were administered and the mice were examined 1, 4 or 24 hours afterwards. Organs were examined with fluorescence microscopy and the cadmium content was measured for assessing the body distribution. Also blood and BAL fluid were analyzed. Ex vivo, platelet aggregation was examined in presence or absence of ADP.

After injecting 100 µg carboxyl EviTags, 3 of 4 animals died immediately. Fluorescence microscopy revealed that their lungs were filled with EviTags containing thrombi; pulmonary thrombosis was also apparent with a dose of 20 and 4 µg of EviTags. In blood, the number of platelets was decreased one hour after injecting 20 µg carboxyl EviTags; in addition, 4 hours after administering 4 µg, the percentage of neutrophils was increased, along with a relative decrease in lymphocytes. Assessing the body distribution, the quantum dots were mainly found in lung and liver. In the BAL fluid, no influx of neutrophils or lymphocytes was observed. Ex vivo, carboxyl and amine EviTags enhanced the ADP induced platelet aggregation down to 0.4 µg/ml and 15 µg/ml, respectively.

In conclusion, our study indicates that the effects of quantum dots depend on the dose and surface charge. Carboxyl EviTags are more potent than amine EviTags. This highlights the need for further investigation of the toxicity of quantum dots before they can be adopted for in vivo or clinical applications.

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1500 BIOCOMPATIBILITY OF CARBON-BASED NANOMATERIALS.

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In evaluating biocompatibility of nanomaterials, defined as possessing one dimension equal to or less than 100 nm, it is important to understand how individual physicochemical parameters influence their toxicity. In this in vitro study, the cellular effect of four well-characterized carbon-based materials possessing diameters from micro- to nano- dimension was investigated using mouse keratinocytes (HEL-30) as a representative model for skin toxicity. The materials tested included conventional fibers (CF; 10 µm diameter), nanofibers (NF; 100 nm diameter), multi-

walled nanotubes (MWNT; 10 nm diameter), and single-walled nanotubes (SWNT; 1 nm diameter). CF and NF did not significantly impact cell viability. MWNT and SWNT reduced cell viability for the first 48 hours of exposure, but the cells recovered to mitochondrial function equal to control by the 72 hour time point. The mitochondrial membrane potential was unaffected by exposure to NF, MWNT, and SWNT after 24 hours. The results of this study suggest that decreasing dimension increases toxicological affects to cells. When decreasing the dimension of a material, however, the surface area and surface energy also increase. Therefore, further study is recommended to determine whether MWNT and SWNT exhibit toxicity due to dimension or the increase in one of these properties. Further studies were used to determine the toxicological effect of surface area and surface energy with constant dimension.

1501 THE EFFECT OF TITANIUM DIOXIDE NANOPARTICLES MOUSE KERATINOCYTES (HEL-30 CELLS).

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Titanium dioxide (TiO₂) nanoparticles are used in a wide range of products from paints to food to cosmetics and sunscreens. In order to assess the possible correlation between nanosize TiO₂ particles and toxicity, we examined the cytotoxic effect of varying sizes of TiO₂ nanoparticles on a mouse keratinocyte cell line. HEL-30 cells were continuously exposed to various sizes of TiO₂ nanoparticles (5, 10, 16, 40, 50, 72, 172, 194, or 223nm diameter particles) at concentrations ranging from 5 to 100 µg/ml. Cytoviva microscope analysis was used to visualize, and confirm, the uptake of the TiO₂ nanoparticles into the keratinocytes. Following 24, 48, or 96 hours of exposure, MTS and LDH assays were performed to assess for disruption in cell proliferation and membrane leakage, respectively. No significant effects of TiO₂ on cell proliferation were observed following a 24 h exposure at all concentrations and sizes. However, following 48 or 96 h exposures to either 50 or 100 µg/ml TiO₂, a significant decrease in cell proliferation for all sizes of TiO₂ was measured. The LDH data obtained following either 24 h or 48 h exposure found no significant increase in membrane leakage for all size particles and concentrations evaluated. Reactive oxygen species concentrations were quantified using a DCFH-DA. At 24 h post-exposure, at a concentration of 100 µg/ml, the particles that were ≥ 40 nm, showed a significant increase in the levels of oxidative stress. In contrast, there was no evidence of oxidative stress for all size particles when tested at a concentration of 10 µg/ml. Future studies are will correlate the crystallinity and structure of these particles with cytotoxic effects.

1502 THE USE OF STRUCTURE ACTIVITY RELATIONSHIPS (SAR) IN CHARACTERIZING THE TOXICOLOGICAL HAZARDS OF PLANT METABOLITES.

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Plant metabolites of the fungicide etridiazole, 5-ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole, were analyzed for their potential human toxicity utilizing DEREK for Windows from Lhasa Limited, Leeds UK. The assessment of the SAR modeling results in conjunction with available toxicity data provided the basis for a useful and favorable risk assessment on the initial metabolic pathway of etridiazole in plants. This assessment involved a step wise analysis of testing and SAR data on the parent and metabolites. The parent material, etridiazole, was modeled as possibly mutagenic in vitro by DEREK; however, a substantial battery of genotoxicity testing demonstrated that etridiazole is a promoter and not a mutagenic or genotoxic compound. One of the major plant metabolites, etridiazole acid was negative for mutagenicity in a battery of in vitro and in vivo assays and also in DEREK. This metabolite was also identified in animal metabolism studies. Other metabolites (3-hydroxymethyl etridiazole, 5-hydroxyethoxy etridiazole acid and dichloro etridiazole) are unique to plant metabolism and were not characterized in in vitro or in vivo studies. The dichloro etridiazole and 5-hydroxyethoxy etridiazole acid contained structural alerts for genotoxicity and further SAR modeling was conducted using Multicase on the 5-hydroxyethoxy etridiazole acid. The overall toxicological evaluation of the plant metabolites supported a favorable safety profile for use in human risk assessment.

1503 RISK AND SYMPTOMOLOGY FROM INHALATION EXPOSURE TO SOLVENT-BASED PAINT VAPOR.

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Seven women aged 24 to 58 claimed that vapor in their indoor office following the application of acrylic sealer and fire retardant coating to an external staircase caused skin, ear, eye, gastrointestinal, joint, neurological, respiratory, and sleep problems

fatigue; headache; heart palpitations; sore throat; and cancer. The women occupied the office for an 8-hour work day before being relocated due to odor complaints. No sampling was done, but the presence of vapor was supported by odor complaints. MSDS's indicate that the volatile constituents are toluene and methyl isobutyl ketone in the sealer, and toluene, xylene, and 1,3,5 triazine, 2,4,6 triamine in the fire retardant. In order to determine what vapors were emitted, weighed paint samples were dried for 3 hours at room temperature in sealed glass vials. The resulting atmosphere was analyzed by gas chromatography/mass spectrometry. In addition to the listed constituents, methyl methacrylate (MMA), 2 hydroxy 3-methyl butanoic acid, 2-propionic acid methyl ester, and tetramethyl cyclohexane were identified from the sealer, and MMA and ethyl benzene were identified from the fire retardant. Although no significant exposure pathway could be identified, the volatile compounds detected have low odor thresholds. MMA has the lowest odor threshold (0.3 mg/m³) and has a distinctive repulsive, acrid odor. For MMA, the OSHA permissible exposure limit (PEL) is 410 mg/m³, and the proposed EPA acceptable exposure guideline level 1 (AEG-L-1) for an 8-hour exposure is 70 mg/m³. MMA exposure at the odor threshold for 8 hours/day would be approximately 0.07% of the OSHA PEL and approximately 0.4% of the EPA 8-hour AEG-L-1. The analytical results and records available for the affected individuals supported a diagnosis of mass psychogenic illness (MPI). Symptoms associated with MPI include headache, dizziness, nausea, abdominal cramps, cough, fatigue, sore throat, difficulty breathing, irritated eyes, chest pain, difficulty concentrating, nervousness, rash, and itching, and correlate well with those related by the affected individuals.

1504 COMPARISON OF ORAL ABSORPTION AND SYSTEMIC AVAILABILITY IN THE RAT.

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In the safety assessment of pesticides, EU member states now tend not to include biliary excretion in the estimation of systemic exposure via the oral route, except when liver is the main target organ. This policy may result in a more conservative risk assessment for operator exposure. This paper compares estimates of systemic availability (F), calculated from plasma AUC values derived from total radioactivity measurements after oral and intravenous dosing, with values for oral absorption obtained in biliary excretion experiments for thirteen compounds. Out of a total of 25 determinations, nine values for systemic availability were lower than those for absorption, eleven values for systemic availability were higher than those for absorption and five values for systemic availability were similar to those for absorption. The limitations of using either biliary excretion or systemic availability to assess systemic exposure are discussed.

1505 RARE ASBESTOS-INDUCED HUMAN DISEASES.

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Inhalation of Asbestos fibers can cause different adverse effects especially according the lung; where the size and diameter of the fibers is critical for their toxicity. Whilst the knowledge of the most frequent asbestos-induced diseases like lung fibrosis, pleural thickening and mesothelioma is quite common, we report on mostly unknown diseases in humans caused by asbestos. In severe cases asbestos can cause mesotheliomas, i.e. malignant tumours of the pleura or peritoneum, with a long latency period of 20-40 years.

We report on a case of a veterinary doctor who died from peritoneal mesothelioma most probably induced by Asbestos from powdered medical gloves. Talcum used in sports and medicinal gloves can contain up to 10% asbestosis fibers. It is likely that other tumors following talcum exposure are not considered as those.

Other cases of two 74 and 76-years aged men were examined after diagnosis of pericarditis constrictiva. In both cases there were longstanding occupational asbestos exposure and no concurring causes for this special disease (e.g. Tuberculosis, infectious diseases, trauma, neoplasma). Cardiac examination results were relatively normal except the diastolic filling limitation due to constriction. Both had typical findings like fibrosis, pleural plaques and round atelectasis.

Conclusions:

When pleural or cardiac changes are etiologically unclear, former asbestos exposures should be taken into account. Possibly asbestos plays a more important role in the pathogenesis of pericarditis than actually known. Even if asbestos use is banned

in many industrialized countries, ongoing use of asbestos, e. g. in automotive industries, has been reported. If there was a relevant exposition to asbestos, an occupational disease should be supposed.

1506 EVALUATION OF THE POTENTIAL EFFECTS OF VARIOUS CIGARETTE INGREDIENTS ON SELECTED BIOLOGICAL ENDPOINTS.

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A series of in vitro and in vivo studies was designed to evaluate the potential effects of 253 ingredients on selected biological endpoints. The studies performed included Ames assay, a neutral red cytotoxicity assay, a 30-week dermal tumor-promotion evaluation of cigarette smoke condensate with SENCAR mice, and a 13-week subchronic inhalation study of mainstream smoke with Sprague-Dawley rats. These studies utilized Reference cigarettes, manufactured with a typical commercial tobacco blend without flavoring ingredients and Test cigarettes, identical to the reference cigarette except for containing a mixture of ingredients added as they are normally used in the manufacturing process but exceeding typical application levels. 253 ingredients were divided 4 subsets of 62, 67, 61 and 63 ingredients. The overall ingredient application rates were 1.97, 2.54, 2.43 and 2.94%, respectively, of the tobacco cut filler weight. Two subsets of studies were conducted, and each subset compared 2 Test cigarettes with the Reference cigarette. Genotoxicity, cytotoxicity, dermal tumor-promotion, and inhalation toxicity studies showed no differences between cigarettes with or without ingredients. It was concluded that the addition of ingredients to cigarette does not alter biological activity of mainstream smoke or mainstream smoke condensate with regard to the parameters investigated.

1507 EVALUATION OF POTENTIAL BIOLOGICAL EFFECTS OF SODIUM ALGINATE ADDED TO CIGARETTE PAPERS.

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Sodium alginate is an ingredient-added to cigarette paper. A series of biological assessments, including Ames and Neutral Red assays of cigarette smoke condensate and an inhalation study of cigarette smoke, was conducted with Test cigarettes with 2 different ingredient levels on cigarette paper (T-1, T-2) compared with Reference cigarettes using conventional cigarette papers (R). In Vitro studies showed no differences between cigarettes with or without the ingredients. For inhalation study, groups of 30 rats/sex were exposed by nose-only inhalation to filtered air (Sham) or 0.06, 0.2, 0.8 mg/L WTPM of smoke for 1h/d, 5d/wk for 13wk of exposure, and 13wk of recovery. Average concentrations of WTPM and CO measured in the highest dose group were 0.806 mg/L and 615 ppm for R group, 0.799 mg/L and 693 ppm for the T-1 group, and 0.800 mg/L and 788 ppm for the T-2 group. Blood COHb concentrations were increased in a dose-related manner in all smoke-exposed groups. Both T groups had significantly greater COHb concentrations at 0.8 mg/L than those of the respective R group. Male rats exposed to all cigarette types had depressed body weight/gains compared to Sham during the exposure phase. Hyperplasia of nasal respiratory and goblet cell epithelium were present in 0.8 mg/L groups from all three cigarette types. Squamous metaplasia and hyperkeratosis were observed in the laryngeal epithelium in almost all rats exposed at 0.8 or 0.2 mg/L from all cigarette types. Exposure to 0.8 mg/L of smoke from all cigarette types induced a minimal hyperplasia in the tracheal epithelium. Statistical comparison of the incidence and severity of microscopic lesions in recovery rats indicated no differences in the severity of lesions between T-1, -2 and R groups. Complete regression from those histological lesions and body weight gain were observed after the recovery period. Inhalation studies showed no differences between cigarettes with or without the test ingredients.

1508 A TWO-GENERATION REPRODUCTIVE TOXICITY AND LACTATIONAL INHALATION-PHASE EXPOSURE STUDY OF DIMETHYL DISULFIDE IN RATS.

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Dimethyl disulphide (DMDS) is a promising methyl bromide replacement candidate for soil fumigation applications with zero ozone depletion potential (ODP). Methyl bromide has an ODP of 0.38 and has been classified as a Class 1 stratos-

pheric ozone depletor. Daily exposure (6 h) of 4 groups of 30 SD rats/sex to vapor atmospheres of DMDS (0, 5, 20 or 80 ppm) did not cause any adverse effects on reproduction (reproductive performance, parturition, mean numbers of implantation sites and male spermatogenic parameters) across two generations (F0/F1). No reproductive target organs were identified. At 20 and 80 ppm, systemic toxicity was apparent in deficits in body weight, body weight gain and food intake. A NOAEL for parental systemic toxicity was established at 5ppm and a NOAEL for reproductive toxicity was established at 80 ppm. However, F1 pup body weight gains were reduced across all DMDS-exposed groups without any specific correlation to exposure concentrations. In order to determine if an exposure-response relationship exists between lactational inhalational exposure and pup body weight, and to determine the critical period of lactational inhalational exposure, 4 groups of 36 female SD rats were exposed in a comparable regimen during LD 5-12, 13-20 or 5-20. While the systemic parental toxicity was replicated, no effects on pup body weights were observed. In a parallel study, 4 groups of 10 female SD rats and their pups were simultaneously exposed to DMDS (0, 2, 20 or 80 ppm) in cylindrical maternity chambers during LD 5-20. Here, an exposure concentration of 80 ppm caused maternal toxicity with an accompanying reduction in pup body weight gain, but without any indication of selective juvenile toxicity. No effects were observed at 2 and 20 ppm. Collectively, these three studies established the threshold of neonatal inhalational toxicity of DMDS, in the two-generation study, as greater than 80 ppm.

1509 N-ACETYLATION OF AROMATIC AMINE HAIR DYES ANTAGONIZES HAPTENIZATION.

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A key element in the safety assessment of cosmetic chemicals is the prediction of their sensitizing properties when applied to human skin. For aromatic amine hair dye precursors of the phenylenediamine and aminophenol type, skin metabolism may be involved in the induction of skin sensitization, since they contain amino groups capable of undergoing enzymatic metabolism in the skin.

Since N-acetylation is known as a major enzyme reaction for aromatic amines in the skin, we tested N-acetylated metabolites of p-phenylenediamine (PPD) and 4-amino-2-hydroxytoluene (AHT) for their sensitizing properties *in vitro* using our dendritic cell (DC) activation assay (Aeby et al, 2004) and *in vivo* using the Local Lymph Node Assay (LLNA).

PPD itself did not induce activation of DC cells, but induced a positive LLNA response, as expected for a known human sensitizer. Activation of DC was obtained, when PPD was pre-incubated with the strong oxidizing agent K3[Fe(CN)6] or by exposing dissolved PPD to air oxygen for 24h. Under both conditions, the potent sensitizing PPD metabolite Bandrowski's base (BB) was identified among other oxidation products, indicating that formation of BB is a possible haptenization pathway. Mono- or diacetylation of PPD did neither induce DC activation nor a positive LLNA response.

For AHT, DC activation *in vitro* was induced without pre-treatment, reflecting the positive LLNA response. N-acetylation of AHT significantly reduced DC activation. Using human skin *ex vivo* and/or a human keratinocyte cell line, we found that a substantial amount of AHT and PPD, respectively, is N-acetylated under exposure conditions mimicking the use of hair dyes. We conclude that dermal N-acetylation of aromatic amine hair dyes antagonizes their haptenization.

1510 SAFETY AND THERAPEUTIC EFFICACY OF UNDENATURED TYPE II COLLAGEN (UC-II) IN HORSES.

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Currently, millions of horses suffer from arthritis, a chronic, crippling disease that affects their daily activities, especially running. This investigation was undertaken to evaluate the effectiveness of pain reduction and safety of UC-II in arthritic horses. Arthritic horses divided into four groups (n=5-6) were treated as: Group-I (placebo), Group-II (20 mg UC-II/day), Group-III (40 mg UC-II/day), and Group-IV (80 mg UC-II/day) for a period of 150 days. Group-I horses showed no changes in arthritic condition, while those in Groups II and III showed moderate improvement. Group-IV horses receiving 80 mg UC-II/day exhibited significant and marked improvement. Reduction in overall pain was from 4.7±0.36 to 1.0±0.0 (79%); in pain upon limb manipulation from 3.4±0.20 to 1.0±0.0 (71%); and in pain after physical exertion from 3.10±0.20 to 1.0±0.0 (68%). Overall, horses were running and more playful. No significant change was noted in body weight, pulse

rate, respiration rate, and body temperature. Horses receiving placebo or UC-II revealed no change in liver (ALP, GGT, or bilirubin) or kidney (BUN or creatinine) function. These findings suggest that UC-II at 80 mg/day ameliorates signs and symptoms of arthritis and is well tolerated in arthritic horses.

1511 A 90-DAY ORAL (GAVAGE) TOXICITY STUDY OF S-111-S-WB WITH A 60-DAY RECOVERY PERIOD IN SPRAGUE DAWLEY RATS.

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A mixture of perfluoro fatty acid ammonium salts of different carbon chain lengths (C7-C13) was administered by gavage for 90 days to CrI:CD®(SD)IGS BR rats at dosages of 0, 0.025, 0.125, and 0.6 mg/kg/day to evaluate its possible toxic effects. Males were more sensitive than females. Higher hepatic β -oxidation and liver weights indicative of mild peroxisome proliferation were present in the 0.125 mg/kg/day males and 0.6 mg/kg/day males and females. Lesions consisted of hepatocellular hypertrophy and eosinophilic foci in the 0.125 and 0.6 mg/kg/day males and hepatocellular degeneration and necrosis in the 0.6 mg/kg/day males. Other lesions consisted of ulceration, epithelial hyperplasia and acute inflammation in the duodenum and ulceration, erosion, squamous hyperplasia and chronic active inflammation in the stomach. Lower serum protein and higher bilirubin, urea nitrogen, and chloride concentrations were seen in the 0.6 mg/kg/day males and lower globulin and higher alkaline phosphatase concentrations in the 0.125 mg/kg/day males and 0.6 mg/kg/day males and females. After 2 weeks serum concentrations of pentadecafluorooctanoic acid (C8), heptadecafluoronanoic acid (C9), pentafluoroundecanoic acid (C11) and perfluorotridecanoic acid (C13) were similar at the sampling times with tmax ranging from 1-4 hrs post-dosing. After 90 days of dosing only C9 reached a steady state in the females dosed with 0.025 mg/kg/day. Serum C8 and C9 concentrations in the males were 10-fold higher than in the females, whereas C11 and C13 were similar for both genders. The main elimination for C8 was via the urine in males, C9 via the urine in females, and C11 and C13 via the feces. Elimination of C9 via the urine in males and via the feces in both genders was relatively low. Based on the results of this study the no-observed-effect-level was 0.025 mg/kg/day for the males and 0.125 mg/kg/day for the females.

1512 THE NONCLINICAL TOXICITY PROFILE OF ALEXIDINE, A DISINFECTANT FOR OPHTHALMIC USE.

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Alexidine is a biguanide disinfecting agent. A comprehensive toxicological investigation of the agent has not been published. Therefore, in these studies an evaluation of the safety profile of alexidine has been conducted. Endpoints in these evaluations include ocular safety, dermal sensitization, systemic toxicity, cardiovascular safety pharmacology, and genotoxicity potential. To evaluate the ocular effects of alexidine, an array of tests were conducted including an in vitro sodium fluorescein permeability assay, a single dose eye irritation study and a 14-day ocular safety study. A guinea pig maximization test was used to evaluate the dermal sensitization potential of alexidine. Systemic effects were evaluated in several rat and dog oral toxicity studies up to 28 days in duration while cardiovascular safety was evaluated in an in vivo conscious dog model. A battery of four assays (Ames, mouse lymphoma, chromosomal aberration, and in vivo micronucleus) was conducted to evaluate for genotoxicity potential. Alexidine demonstrated no significant effects in the ocular assays and skin sensitization model, and was negative in the genotoxicity assays. QT prolongation was not observed at very high oral doses (500 mg/kg) in the dog. Diastolic and mean arterial pressures were transiently increased in the dog at 500 mg/kg with no effects observed at the next lowest dose (100 mg/kg). The no observable adverse effect levels in the repeat dose studies were 15 mg/kg or higher with significant effects observed at 30 mg/kg or higher. In summary, these data support a favorable toxicity profile of alexidine.

1513 EVALUATION OF 4-(4-HYDROXY-4-METHYLPENTYL)-3-CYCLOHEXEN-1-CARBOXALDEHYDE (HMPCC) IN A 28-DAY ORAL SUBCHRONIC TOXICITY STUDY.

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HMPCC (4-(4-hydroxy-4-methylpentyl)-3-cyclohexen-1-carboxaldehyde) is a widely used fragrance material. A 28-day repeated dose oral subchronic toxicity study on HMPCC was conducted according to OECD Guideline 407 to evaluate

its systemic toxicity. Five rats/sex/dose were gavaged once per day for 28 days with 0, 15, 150, or 1000 mg/kg/day HMPCC in arachis (peanut) oil. Clinical signs, sensory reactivity to different stimuli, grip strength, motor activity, body weights, and food and water intake were monitored. Blood samples were drawn on day 28 for hematological and blood chemistry investigations. On day 29, all animals were euthanized and necropsied.

No effects were observed in rats treated with 15 mg/kg/day. In males treated with 150 mg/kg/day, increased albumin, decreased cholesterol, and increased liver weights were observed. Additionally, hepatocyte enlargement was observed in three males treated with 150 mg/kg/day. In females treated with 150 mg/kg/day, alkaline phosphatase was increased. Also, an increase in plasma enzymes, albumin and albumin/globulin ratio, and an increase in liver weights accompanied by centrilobular or generalized hepatocyte enlargement were observed in both sexes of the 1000 mg/kg/day group. Further, males treated with 1000 mg/kg/day showed a reduction in bodyweight gain and food consumption (Week 1 only), a reduction in plasma cholesterol, total protein and glucose, an increase in kidney weights, and centrilobular hepatocyte necrosis.

The no-observed-effect-level (NOEL) was determined to be 15 mg/kg/day. The histopathological changes observed in three males treated with 150 mg/kg/day were confined to adaptive liver changes, and as a result the no-observed-adverse-effect-level (NOAEL) was determined to be 150 mg/kg/day.

1514 EFFECTS OF PERFLUOROBUTYRATE EXPOSURE IN MICE DURING PREGNANCY.

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Perfluorobutyric acid (PFBA) is a fluorinated organic chemical found in the environment, perhaps as a contaminant of perfluoroalkyl acids (PFAA) production. Previous rodent studies from our laboratory have indicated developmental toxicity of PFAAs such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). The current study examines whether similar toxicity can be extended to PFAAs with shorter carbon chains such as PFBA. PFBA/NH₄⁺ was given to timed-pregnant CD-1 mice by oral gavage daily from GD 1-17 at doses of 35, 175 and 350 mg/kg; controls received water vehicle. These doses were chosen to approximate the effective doses of PFOA, after taking into consideration the shorter half-life of PFBA (estimated at ~3 h in non-pregnant mice). At GD 17, 24 h after the previous treatment, the serum PFBA levels have declined to 3.78, 4.08 and 3.12 μ g/ml, respectively in the three treated groups. PFBA did not significantly affect maternal weight gain, number of implantations, incidence of resorptions, fetal viability, or fetus weight. Gross examination of the fetuses did not reveal any anatomical malformations. However, maternal liver weights were significantly increased in the two highest dose groups. In contrast to PFOA and PFOS, PFBA exposure during pregnancy did not adversely affect neonatal survival or postnatal growth. These data therefore suggest that, unlike PFOA and PFOS, exposure to PFBA during pregnancy in the mouse is not associated with overt developmental toxicity, perhaps in part due to the relatively short half-life of the chemical. This abstract does not necessarily reflect US EPA policy.

1515 METHYL TERTIARY BUTYL ETHER (MTBE) 14-DAY DRINKING WATER STUDY IN WISTAR RATS.

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MTBE has been widely used as a gasoline oxygenate and identified in a variety of environmental media including groundwater. A series of studies designed to assess the potential toxicity of MTBE, administered in the drinking water, have been undertaken. In the current study, male and female Wistar rats were administered MTBE via the drinking water at concentrations of 0, 3, 7, and 15 mg/ml for 14 consecutive days. Body weights, clinical signs, and food and water consumption were monitored daily. Kidneys and testes weights were measured and blood was collected for the determination of concentrations of MTBE and its major metabolite, tertiary butyl alcohol (TBA). Additional blood analyses included hematocrit, BUN, and serum creatinine levels. Airborne concentrations of MTBE in animal room air and control cages averaged $\leq$ 0.2 ppm and in high dose animal cages $\leq$ 33 ppm throughout the study. Consumption of water was significantly reduced in treated versus control rats by approximately 20-30% and 35-39%, in males and females, respectively. There were no significant changes in body weights and food consumption. MTBE daily doses were: 371, 799, and 1624 mg/kg for males and 363, 843, and 1839 mg/kg for females. MTBE blood levels averaged $\leq$ 2.1 μ M, while TBA blood concentrations ranged from 38-116 μ M. Kidney weights were increased in male rats of the 15 mg/ml group. Testes weights in MTBE-treated rats were similar to control values. In summary, Wistar rats were found to tolerate MTBE in the

drinking water. Consumption of MTBE in water did not result in clear toxic effects as there were no adverse clinical observations, body weight gains and food consumption were unaffected, and there were no indications of dehydration. However, MTBE exposure resulted in increased blood concentrations of TBA, increased kidney weights in males, and BUN and hematocrit levels were depressed in females. [This study was sponsored by Shell Oil Company, Chevron USA, Inc., and ExxonMobil Corporation].

1516 A 28-DAY INHALATION
REPRODUCTIVE/DEVELOPMENTAL SCREENING
TOXICITY STUDY OF (3-
CHLOROPROPYL)TRIMETHOXSILANE IN SPRAGUE-
DAWLEY RATS.

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The potential toxicity of (3-chloropropyl)trimethoxysilane, a high production volume chemical, was evaluated in a combined repeated-exposure toxicity study with a reproductive/developmental toxicity screening test. The study design utilized an enhanced version of a protocol in accordance with the OECD and EPA test guidelines numbers 422 and 870.3650, respectively. Neurotoxicity endpoints, including Functional Observational Battery (FOB) and Motor Activity, were performed on male and female rats. The test substance was administered six hours per day by whole-body vapor inhalation to 10 rats/sex/group at 0, 5, 25 and 100 ppm. These exposure concentrations were based on previously conducted studies in which effects were seen in the renal system, liver and adrenal glands primarily at concentrations of greater than or equal to 100 ppm. In the current study no test article-related mortalities or clinical signs were noted throughout the study. There were no effects on body weight or food consumption among the groups. No treatment-related changes were observed in any FOB or motor activity parameters. There were no macroscopic differences upon examination of organs/tissues, nor alterations in organ weights, organ-to-body weight ratios, and organ-to-brain weight ratios among the groups. Histopathologic examination of tissues and organs from control and high exposure animals demonstrated no significant microscopic findings. No effects were observed in any of the reproductive parameters evaluated. Based on the results of this study, the NOEL for (3-chloropropyl)trimethoxysilane in rats via the whole-body vapor inhalation route was considered to be 100 ppm for all evaluations: toxicological, reproductive, developmental, and neurological. (Supported by the Silicones Environmental, Health and Safety Council of North America).

1517 PERFLUORO-N-BUTYL IODIDE (PFBI) 13-WEEK NOSE-
ONLY INHALATION TOXICITY STUDY IN RATS WITH
A 4-WEEK RECOVERY PERIOD.

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Perfluoro-n-butyl iodide (PFBI) is an environmentally acceptable (i.e., zero ozone depletion potential) replacement solvent for cleaning oxygen systems. A 90-day study was conducted in order to develop a health risk assessment. Fischer 344 rats (15 males & 10 females per group) were exposed for 6 h/day to 0 (Air Control), 500, 1500 or 5000 ppm of PFBI for 5 days/week for 13 consecutive weeks (at least 65 exposures) followed by a 4-week recovery period. Clinical observations, body weights, clinical pathology, thyroid function, organ weights and histopathology were conducted at the end of the treatment period for up to 10 animals/sex/group. Remaining animals (5 males/group) were held for a 4-week recovery period. Analytical results indicated that the PFBI test atmospheres were within 2% of target concentrations and were essentially vapor only. The target tissue following 13 weeks of daily inhalation exposure of rats to PFBI was the thyroid. The findings consisted of a minimal thyroid follicular cell hypertrophy occasionally accompanied by hyperplasia but without an increase in thyroid weight in the 500, 1500 and 5000 ppm males; only one 5000 ppm female had similar histopathological thyroid changes. At ≥500 ppm, there was also increased TSH in females and increased T3 and T4 in animals of both sexes. These effects resolved following a 4-week recovery period. Other changes were several minor clinical pathology variations at all exposure levels but with no biological significance, and a 9.4% reduction in absolute body weight in the 5000 ppm males. None of the organ weight changes were associated with histopathological correlates; these included increased kidney weights in the ≥1500 ppm females and decreased spleen weight in the 5000 ppm males. Increased adrenal weights at ≥1500 ppm (both sexes) with decreased thymic weight in the 5000 ppm males were indicative of stress while the effect on male accessory sex glands at 5000 ppm were considered secondary to decreased body weight.

1518 SAFETY AND THERAPEUTIC EFFICACY OF UC-II IN
COMBINATION WITH GLUCOSAMINE +
CHONDROITIN OR HYDROXYCITRIC ACID (HCA) +
CHROMEMATE (CM) IN ARTHRITIC DOGS.

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This investigation evaluated safety and therapeutic efficacy of nutraceuticals in arthritic dogs. Dogs in six groups (n=5-6) received daily treatment as follows: Group-I (placebo), Group-II (10 mg UC-II), Group-III (2,000 mg Glucosamine HCl + 1,600 mg Chondroitin Sulfate), Group-IV (UC-II + Glucosamine + Chondroitin), Group-V (1,800 mg HCA + 100 µg CM), and Group-VI (UC-II + HCA + CM). The treatment was given daily for 120 days, followed by a 30 day withdrawal. The dogs were evaluated for overall pain, pain upon limb manipulation, and exercise-associated lameness, on a monthly basis. Group-I dogs (placebo) exhibited no significant change in arthritic conditions. Following 120 day treatment, dogs in Group II, IV, and VI showed significant reductions in overall pain (62%, 57%, and 70%, respectively), pain upon limb manipulation (91%, 53%, and 67%, respectively), and exercise-associated lameness (78%, 53%, and 69%, respectively). Following a withdrawal of 30 days, all dogs (Group II-VI) experienced a relapse in pain. None of the dogs in any group showed any adverse effects or change in liver (ALT and bilirubin) or kidney (BUN and creatinine) function markers, or body weight and these supplements were well tolerated.

1519 THE RELATIONSHIP BETWEEN LOW DOSE PEANUT
EXPOSURES AND THE PERCENT OF PEANUT-
SENSITIZED AND PEANUT-ALLERGIC INDIVIDUALS
THAT REACT.

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Determination of the percent of peanut-sensitized and peanut-allergic individuals that react to the lowest exposures of peanut is important to characterize the risk of an allergic reaction in a population. Information on the number of peanut-sensitized (determined by skin prick test and/or IgE measures) and peanut-allergic (determined by positive reactions to food challenge tests) that reacted to different levels of peanut exposure can be derived from some food challenges studies. All available published literature studies were surveyed and the percentages of these subjects that reacted at the lowest levels of peanut protein exposure, or the lowest observed adverse effect level (LOAEL), for objective symptoms within each study were estimated when possible. The nature of the relationship between the dosage level of each study LOAEL and the respective percent of peanut allergic reactors was evaluated. The resulting association exhibited a linear relationship between the dosage level of the LOAEL value expressed as peanut protein and the percent of peanut-sensitized (r= 0.75) and peanut-allergic (r=0.8) individuals that reacted to these values. The lower study LOAEL values are associated with a lower percentage of subjects reacting at these dose values. However, when the relationship between the percent of the total number of subjects within a study that reacted to a challenge at any dose level during a peanut test and the respective study LOAELs across different studies were examined, a similar relationship was not found (r=0.3). Taken together, these findings indicate that these sources of data provide reasonable estimates of the percentage of individuals that would react at the "lowest" LOAEL value identified. The percent of peanut-sensitized and peanut-allergic individuals that reacted to the objective LOAEL (2 mg peanut protein) in the study identified as "critical" from which a reference value such as a tolerable acute intake would be based were, respectively, 7.1 and 12.5 percent.

1520 NANOPARTICLE (NP) TOXICITY BY TRADITIONAL
CYTOTOXICITY AND AFFYMETRIX MICROARRAY
ASSAYS.

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Nanotechnology presents many opportunities for improving human life, but it also poses potential dangers of adverse effects upon exposure. Strategies for testing biologic/toxicologic activities of NPs include the concept of ranking unknown NPs against well characterized benchmark NPs of high and low toxicity. We evaluated the cytotoxicity of TiO₂ (-20 nm, low toxicity) and of Cu (40 nm, high toxicity) as benchmarks in order to rank Ag (35 nm) and other NPs. To compare cytotoxicity of the three NPs (TiO₂, Cu, and Ag) and to decide on appropriate doses for a microarray study, lung epithelial cells (Type I like cell line R3/1) were incubated in 12-well or 6-well plates for 24 hrs with the NPs (0.5 to 40 µg/ml) for lactate dehydrogenase (LDH) and trypan blue exclusion assays. Cells were then incubated in 6-well

plates for 4 hrs with the NPs (TiO₂, 10 µg/ml; Cu, 4 µg/ml; and Ag, 0.3 µg/ml) for the array study. Results: 1. The LDH assay was not suitable for quantitative assessment of cytotoxicity of NPs due to the inactivation of LDH by the NPs. The limitation may apply to other ROS-producing toxicants. 2. Ag NPs were much more potent in causing cell death than Cu NPs, though the Cu NPs were more potent than the Ag NPs in producing ROS in other studies. So Ag NPs may exert toxic effects through other mechanisms in addition to posing oxidative stress. 3. The array study showed that more genes were differentially expressed after exposure to the Ag than Cu NPs. Many of the genes can be assigned to one or more of the following: cell cycle and apoptosis, MAPK signaling pathway, TGF-β signaling pathway, glutathione metabolism, and other oxidative stress related signaling pathways. TiO₂ NP exposure, on the other hand, led to changed expression levels of only a few genes. We suggest that Cu and TiO₂ are suitable candidates for benchmark particles. (Supported by the US DoD MURI grant FA9550-04-1-430)

1521 MAXIMUM RECOMMENDED GUIDANCE LEVELS (MRGLS) FOR ORGANOHALOGENATED IMPURITIES.

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Occupational Exposure Levels exist for many materials. Unless present in a blend, OELs rarely consider the presence of other substances. For blends of two or more materials that may present additive toxicity, the ACGIH's TLV Committee has published a method for estimating an OEL for the blend this is based on the following relationship:
 $Xa/OELa + Xb/OELb \dots = 1/OELblend$, where Xa is the mole fraction of "a", OELa is the OEL for "a", etc. The methodology presented in this paper has been derived for estimation of acceptable levels of impurities or contaminants in chemical products. That is levels of impurities which would not significantly impact on the OEL for the product. It is most applicable for cases where the product has a low toxicity and correspondingly high OEL while the impurity has a high toxicity and correspondingly low OEL. In this case the presence of a significant amount of the impurity could mandate a lower OEL to afford the same level of protection to those using this substance. The procedure involves first determining the OELs for the product and identified impurities either by using established OELs for these substances or when not available estimating them from OELs for similar substances. Safety factors are applied to account for additive toxicity and the potential for the impurity to fractionate. Treatment for multiple impurities, again assuming additive toxicity, is also considered. For a product with a single impurity:
The MRGL = [(Impurity OEL/safety factor x Impurity Mol. Wt)/ (product OEL x product mol. Wt.)] x 100. The calculation for multiple impurities will also be discussed.

1522 INHIBITION OF THE ADENINE NUCLEOTIDE TRANSLOCATOR BY N-ACETYL PERFLUOROOCTANE SULFONAMIDES *IN VITRO*.

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N-Alkyl perfluorooctane sulfonamides have been widely used as surfactants on fabrics and papers, fire retardants, and anti-corrosion agents, among many other commercial applications. The global distribution and environmental persistence of these compounds has generated considerable interest regarding potentially toxic effects. We have previously reported that perfluorooctanesulfonamidoacetate (FOSAA) and N-ethylperfluorooctanesulfonamidoacetate (N-EtFOSAA) induced the mitochondrial permeability transition (MPT) *in vitro*. In this study we tested the hypothesis that FOSAA and N-EtFOSAA interact with the adenine nucleotide translocator (ANT) resulting in a functional inhibition of the translocator and induction of the MPT. Respiration and membrane potential of freshly isolated liver mitochondria from Sprague-Dawley rats were measured using an oxygen electrode and a tetraphenylphosphonium-selective (TPP⁺) electrode, respectively. Mitochondrial swelling was measured spectrophotometrically. The ANT ligands bongkrekic acid (BKA) and carboxyatractylolide (cATR) inhibited uncoupling of mitochondrial respiration by 5 µM N-EtFOSAA, 30 µM FOSAA, and the positive control 5 µM oleic acid, but not FCCP induced uncoupling. ADP initiated respiration and depolarization of mitochondrial membrane potential were inhibited by cATR, FOSAA, N-EtFOSAA, and oleic acid, but not by FCCP. BKA inhibited calcium dependent mitochondrial swelling induced by FOSAA, N-EtFOSAA and oleic acid. 100 µM ADP also inhibited swelling induced by the test compounds, but cATR induced swelling was not inhibited by ADP. Results of this investigation

demonstrate that a direct interaction with the ANT by N-acetyl perfluorooctane sulfonamides results in an inhibition of ADP translocation and induction of the MPT; one or both of which may account for the metabolic dysfunction observed *in vivo*. (Supported by the 3M Co.)

1523 COMPARISON OF PHOTOTOXICITY TESTS: 3T3 NRU PT VS. MATTEK EPIDERM A 3D HUMAN EPIDERMAL SKIN MODEL.

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Currently, only the 3T3 Neutral Red Uptake Phototoxicity Test (3T3-NRU PT) has been approved as a non-animal phototoxicity test by governmental regulatory agencies. However, the 3T3-NRU PT has serious drawbacks as a model for human skin, specifically: it uses a monolayer cell culture consisting of mouse tumor cells, it is overly sensitive resulting in many false-positives, and test substances must be soluble in tissue culture media. Thus, we have developed a more relevant *in vitro* system based on a three-dimensional, differentiated human keratinocyte cultures, which can accommodate a wide range of vehicles and allow direct topical application of test substances, termed "Phototoxicity Assay in Reconstructed Skin" (PARS). Here we present a side-by-side comparison between the 3T3-NRU PT and the PARS test systems using the same Solar Simulated Light (SSL) source of UV and visible light and the 8 reference standard chemicals used for validation of the 3T3-NRU PT. The PARS test correctly predicts with 100% accuracy the phototoxic potential of all reference test substances. The concentrations of test agents needed to obtain the EC50s in the PARS MTT viability test, when compared to the Neutral Red assay in 3T3 fibroblasts, is one to two orders of magnitude higher, reflecting the thickness and complexity of a three-dimensional tissue structure. This better approximates the exposure levels of chemicals needed to induce phototoxic effect in animal tests and actual human skin. In addition, the most important practical advantage gained over the 3T3 NRU PT is that test substances can be applied topically, overcoming both the pH and solubility problems encountered when dosing via the culture media.

1524 REGULATORY REQUIREMENTS FOR *IN VITRO* SYSTEMS TO MEET PERFORMANCE STANDARDS OVER TIME AS WELL AS DURING VALIDATION.

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In vitro models are being proposed as alternatives to animal testing for safety evaluation of cosmetics, pharmaceuticals and consumer products. Currently available organotypic human models include dermal (EpiDerm, EpiDerm-FT), ocular (EpiOcular), ectocervical (EpiVaginal) and airway (EpiAirway). Some of these models have been, or are in the process of being, formally validated for testing of corrosivity, irritation or toxicity. However, regulatory agencies and other users also need to be reassured that the models will provide consistent, good quality data over time, not just during the validation process (K. Gupta et al., Regul Toxicol Pharmacol. 2005 Dec;43(3):219-24). Recommended guidelines include "full characterization of cells or tissues, sampling of each lot ... for performance, and regular use of controls and benchmark chemicals to provide assurance of consistency of assay performance" (A. Rispin et al. Regul Toxicol Pharmacol. 2006 Jul;45(2):97-103). The current poster summarizes the long-term reproducibility and performance of *in vitro* epidermal (EpiDerm) and ocular (EpiOcular) models against benchmark chemical treatment. Quality control of weekly batches of EpiDerm and EpiOcular was performed using the MTT assay. The exposure time needed to reduce the viability to 50% (ET-50) for Triton X-100 was determined. For EpiOcular, the yearly average ET-50 values have ranged from 20.6 minutes to 25.0 minutes. The coefficients of variation (CV) for the negative control tissue (exposed to ultrapure H₂O) have averaged under 6% for every year since 1997. In addition, the yearly average CV for all tissues has never exceeded 6.5%. For EpiDerm, the yearly average ET-50 values have ranged from 6.0 hrs to 7.5 hrs. The coefficients of variation (CV) for the negative control tissue (exposed to ultrapure H₂O) have averaged under 7.5% for every year since 1996. These results over the past 10 years of commercial production are designed to address regulatory concerns regarding performance standards over time.

1525 EVALUATION OF A NOVEL RELATIVE POTENCY APPROACH FOR AN HERBAL DIETARY SUPPLEMENT.

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Relative potency approaches for predicting the pharmacologic and toxicologic effects of similar chemicals have been applied in diverse areas of regulatory science, including environmental toxicology, risk assessment, pesticide regulation, and in

drug efficacy and safety evaluations. Relative potency approaches assume that the chemicals to be assessed behave as if they were dilutions of a reference chemical in producing a specific biologic response, and that any differences between the assessed chemicals and the reference chemical are quantitative rather than qualitative. The validity of these assumptions directly determines whether applying a relative potency approach is valid in any particular situation. Traditionally, several criteria are evaluated to determine how well a group of chemicals conforms to the assumptions of the relative potency approach. These include that the chemicals are molecular congeners, exhibit similar patterns of metabolism and detoxification, exhibit parallel dose response curves for the specific effect of interest in the same biological assay, share the same molecular target of action, have similar mechanisms of action for the effect of interest, and exhibit dose addition for the effect of interest when combined in mixture. We used these criteria to evaluate a novel relative potency approach adopted by FDA for predicting adverse cardiac effects of herbal ephedra. We found the approach unusual in several key aspects; it employs an indirect comparison of ephedra alkaloids to the reference compound epinephrine via a surrogate reference chemical, ephedrine; it assumes quantitative similarity based on potency for qualitatively dissimilar therapeutic indications, and; it employs recommended dose ranges for the quantitative comparison rather than dose response data. We discuss the validity of this novel approach for safety evaluation and its implications for future regulatory actions involving drugs and supplements.

1526 ASSESSMENT OF DIOXIN-LIKE COMPOUNDS IN MAINSTREAM SMOKE FROM SELECTED U.S. CIGARETTE BRANDS.

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Mainstream cigarette smoke (MS) from twelve U.S. cigarette brands was evaluated to determine concentrations of dioxins (i.e., polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and dioxin-like polychlorinated biphenyls). The study included three 'tar' ranges based on Federal Trade Commission (FTC) determination: Low Yield (LY) <5.5, Medium Yield (MY) 9.6 to 12.2, and High Yield (HY) >14.5 mg/cig. Cigarettes were produced by a single manufacturer and represent the principal design features found in the U.S. market. MS was generated by a machine smoking regime (60 cc puff volume of 2 sec duration, puffs taken twice per minute) more intense than FTC or ISO standards, collected on Cambridge filters and analyzed using EPA test methods. Of the brands studied, the HY cigarettes yielded the greatest mean concentrations of World Health Organization Toxic Equivalents (WHO-TEQs) on a per cigarette basis. WHO-TEQ levels in LY cigarettes were significantly lower than for HY cigarettes ($p=0.0497$) on a yield per cigarette basis and WHO-TEQ concentrations correlated with 'tar' yield ($r=0.69$, $p=0.013$), as did concentration on a WHO-TEQ per body mass per day basis ($r=0.69$, $p=0.013$). However, on a per mg Total Particulate Matter (TPM) basis, no statistically significant relationship between 'tar' range and WHO-TEQ concentrations was observed. Concentrations for all brands tested ranged from 0.48 to 4.82 fg WHO-TEQ/mg TPM. Maximum daily exposure estimates calculated from this range (0.003 to 0.109 pg WHO-TEQ/kg bw/d) are below the current WHO Tolerable Daily Intake range of 1 to 5 pg/kg bw/d. These results do not support findings from a 2005 study in which U.S. and international cigarette brands were smoked using a less intense ISO smoking regime. In contrast, we report lower maximum TEQ concentrations, found no relationship between 'tar' ranges and TEQ concentration on a per mg TPM basis, and a positive relationship between 'tar' yield and MS dioxin concentration on a per cigarette basis.

1527 CARCINOGENS IN TOBACCO SMOKE AND QUANTITATIVE CANCER RISK.

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Cigarette smoke contains more than 50 substances classified by the International Agency on Cancer Research (IARC) as known, probably or possible human carcinogen. It has been estimated that the excess cancer lifetime death risk from smoking in USA is 7.9×10^{-2} (1) The purpose of the present communication was to assess to what extent carcinogens quantified in mainstream cigarette smoke and for which quantitative data suitable for cancer risk assessment were available, could account for the cigarette smoke induced cancers. Thirteen carcinogens have been measured in mainstream cigarette smoke by the Canadian intense machine smoking regimen for which data for quantitative cancer risk exists. The data have been obtained from three different sources and the mean exposure based on a daily dose of 24 mg nicotine, which is representative for cigarette smokers in USA (2), has been calculated for the 13 substances. The lifetime cancer risk for the 13 substances was subsequently calculated by the T25 method (3) for quantitative risk characterisation of genotoxic carcinogens assuming 50-year exposure to cigarette smoke. In the calculations, no attempts were made to account for possible interactions between differ-

ent chemicals in the tobacco smoke. The 13 carcinogens could account for about 23% of the carcinogenic effects associated with smoking. PAH (with benzo(a)pyrene as indicator) represented the most important group of carcinogens accounting for nearly 75% of the carcinogenic potential identified. The four carcinogens acetaldehyde, N-nitrosomnicotine, acrylonitrile, and 1,3-butadiene accounted for about 20% of the carcinogen potential identified. The finding that the calculations could only account for 23% of the carcinogenic effect suggests synergistic interactions between the substances in the tobacco smoke and/or contributions from other carcinogens than the ones considered in the present calculations.

- (1) Fowles and Dybing. *Tob Control* 12: 424, 2003
- (2) National Cancer Institute Monograph No 13, 2001
- (3) Sanner et al. *Pharmacol Toxicol* 88: 331, 2001.

1528 USING DOUBLY-LABELED WATER MEASUREMENTS OF HUMAN ENERGY EXPENDITURE TO ESTIMATE INHALATION RATES.

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Doubly-labeled water (DLW) data is recognized as an improvement over alternative methods to quantify human energy expenditure (e.g., calorimetry, heart-rate monitoring, and accelerometer). More accurate and representative energy expenditure data can be converted into estimates of breathing rates using equations developed by Layton. DLW methods are advantageous for several reasons: the database is robust, they are direct measures, subjects are free-living, and the observation period is longer than what is possible from staged activity measures. DLW estimates of energy expenditure can resolve differences in breathing rate estimates used for risk assessment. These estimates are currently based on short duration heart rate measures of volunteers performing staged activities or energy expenditure estimates inferred from dietary recall surveys. The range of human energy expenditure is narrow with the maximum energy expenditure not likely greater than twice the minimum. Based on DLW estimates of energy expended by physically active adults expending, 2,850 and 2,250 kcal/day, average long-term breathing rates are approximately 16 m³/day and 13 m³/day, for males and females, respectively.

1529 PHYSIOLOGICAL PARAMETERS IN HEALTHY AND DISEASED ELDERLY.

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The availability of a central repository of human physiological data would be useful for developing PBPK models. The objectives of this study were to collect physiological data for healthy and diseased elderly, and to populate an ACCESS database. Following an extensive literature search, 528 peer-reviewed publications were retained for evaluation. Each publication was read to identify the specific parameter evaluated as well as the experimental design/protocol, sampling method, number of samples, statistical analysis, precision/variability, and any bias. However, no re-evaluations of the reported disease state (diabetes, COPD, obesity, angina pectoris and renal disease) were attempted. Following evaluation, 155 publications were retained for inclusion in the database. From these publications, a total of 1166 data records – relating to tissue volumes, blood flow rates, metabolic parameters, tissue composition, renal clearance parameters and pulmonary clearance parameters – were retrieved and entered into the database. The data availability for the various physiological parameters was greater for the older adults (50-64 years) and young elderly (65 – 74 years) groups than for the old-old (75-84 years) and oldest-old (> 85 years) groups. The physiological parameter measurements in diseased elderly are also limited and represent a major datagap, with the exception of a mixed group containing older adults and young elderly (60 – 65 years). Another datagap across the age groups relates to tissue composition data (neutral lipid, phospholipids and protein levels). Overall, the present study has assembled available data on physiological parameters in healthy and diseased elderly along with attendant identification of the data poor parameters and age groups.

*The views expressed in this manuscript are solely those of the authors and do not represent the policies and/or endorsement of the U.S. Environmental Protection Agency.

1530 HALOQUINONES, HALOGENATED FURAN DERIVATIVES, AND NITROSAMINES IDENTIFIED AS DISINFECTION BY-PRODUCTS (DBPS) OF POTENTIAL TOXICOLOGICAL CONCERN.

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Chlorinated drinking water has been epidemiologically associated with increased risk of cancer, especially in the bladder. A large number of chemicals have been identified as by-products of the disinfection of drinking water. Several by-product

classes such as the trihalomethanes and haloacetic acids have received substantial toxicological study. Although many of these have been found carcinogenic in animals, the combination of low potency as carcinogens and/or low concentrations in drinking water make them unlikely causes of the cancer risk of the magnitude suggested by epidemiology studies. To provide new direction for the resolution of this discrepancy, a strategy was developed to predict formation of novel by-products through reactions of chlorine with substructures found in natural organic matter (NOM). These potential disinfection by-products (DBPs) were examined using the QSAR program TOPKAT® to identify those chemicals of sufficient carcinogenic potency to be possible causes for the epidemiologic findings, if present. A "weight of evidence" strategy was developed to utilize the predictions of the multiple cancer models within TOPKAT and their respective training sets to identify those compounds that were most probably potent carcinogens. The likely potency of each compound was judged by predictions of the chronic LOAEL. Predictions were refined by literature review to determine whether probable modes of action would be consistent with bladder carcinogenesis. Several groups of chemicals appear to be of interest. Most prominent of these were several haloquinones, a series of halogenated furan derivatives, and nitrosamines arising from chloramine reactions with alkaloids. Most interesting, where halogen substitutions on 1,4-dibenzoquinone, compounds whose concentrations would be favored with chloramine rather than chlorine disinfection. (Supported by Awwa Research Foundation contract 2867).

1531 THE EPA MCLG FOR FLUORIDE IN DRINKING WATER: NEW RECOMMENDATIONS.

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The U.S. EPA has set the Maximum Contaminant Level Goal (MCLG) for fluoride (F-) in drinking water at 4.0 mg/L (4000 ppb). The Mar.06 report from the National Research Council (NRC) concluded that this is not protective of human health and should be lowered. No new MCLG was recommended by the committee, nor were any benefits of F- in drinking water assessed. The MCLGs for some essential elements (e.g. Ni, Cu) have to balance the risks from adverse health effects versus the benefits. F- has been shown to be a useful agent to combat dental decay, but its mechanism is now known to be topical, not systemic (F- is not an essential element). Water fluoridation, a 60-year old policy, generally exposes entire populations to 1 mg F-/L in the drinking water. However, there are people who retain greater than normal amounts of F-, ingest more than average amounts of F- or who have a particular intolerance to chronic exposures to small amounts of F-. These people have been shown to demonstrate adverse health effects such as dental and skeletal fluorosis. The purpose of this study was to conduct a risk analysis comparison between F- and other non-essential elements (e.g. Sb, As, Be, Cd, Hg and Tl). Most MCLGs of the toxic elements are in the 0-10 ppb range. We used two different approaches: a) first calculating a reference dose (RfD) from no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-health-effect levels (LOAELs), and deriving the MCLG from the RfD; b) the benchmark dose approach. Our results indicated that in all calculations, the new MCLG for fluoride in drinking water should be at most one tenth (0.4 mg/L) of the current MCLG of 4 mg/L, suggesting that the practice of fluoridation should be re-evaluated. Sponsored by SOT member James Murphy. Disclaimer: sponsorship of this abstract does not imply endorsement of its content.

1532 TOXICITY ASSESSMENT AND DEVELOPMENT OF REGULATORY STANDARDS FOR PERCHLORATE.

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In 2003, MassDEP decided to derive a drinking water standard (MCL) for perchlorate (ClO₄⁻) due to the need to protect public health from contaminated drinking water supplies and sites. As a primary mechanism of action, ClO₄⁻ interferes with iodide transport into the thyroid gland, which is essential for the synthesis of the thyroid hormones. Perturbation of thyroid function by perchlorate, especially in fetuses, neonates and developing children could result in irreversible neurotoxicity. In our evaluation, we used a weight of the evidence approach in which perchlorate toxicity data from animal and human studies were both evaluated to determine an appropriate reference dose (RfD) value and corresponding MCL. We put more weight on a human iodide uptake inhibition study performed in 2002 and identified the lowest dose in the study (0.007 mg/kg/day) as a LOAEL and calculated a chronic RfD of 7 x 10⁻⁵ mg/kg/day by applying a total uncertainty factor of a 100. This RfD equates to a health-based drinking water limit of 0.49 ppb using conventional adult exposure extrapolation, and a relative source contribution factor to account for other sources of exposure beyond that from drinking water (e.g., food, milk). In selecting a final MCL, MassDEP considers the RfD-derived limit and additional factors, including the frequency of contamination at

public water supplies in Massachusetts, analytical detection limits, treatment options, costs, and data that demonstrates that perchlorate can be introduced into drinking water from certain disinfection chemicals. To ensure the disinfection benefit is not compromised, MassDEP chose to set the MCL at a level that does not create any disincentive on the part of public water systems to introduce disinfection processes used to counter microbial contamination and associated risks to public health. The MCL of 2.0 ppb was determined to provide the best overall protection of public health, considering the benefits of disinfection, while retaining a margin of safety to account for uncertainties in the available data.

1533 EXTRAPOLATING ACROSS EXPOSURE DURATIONS: STATISTICS FROM MINIMAL RISK LEVELS (MRLS).

M. Fay and S. Chou. *CDC/ATSDR, Atlanta, GA.* Sponsor: M. Mumtaz.

ATSDR derives MRLs for hazardous substances based on available toxicological and epidemiologic information. These guidance values are estimates of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects. They are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effects(s) for oral or inhalation exposures, and are categorized into acute (1-14 days), intermediate (int.; >14-364 days), and chronic (365 days and longer) exposure durations. These substance-specific health guidance values serve as screening levels for ATSDR health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites or other exposure events. Due to a lack of sufficient information, MRLs are not always available for all durations and routes. Thus extrapolation across duration for a given route of exposure might be desired in emergency situations. They also serve as one of the few types of guidance values derived by an expert panel which address wide variations in exposure duration. We analyzed matched-pair ratios of MRLs across duration, for chemicals with more than one MRL for a given route. At this time, there are 347 MRLs offering 166 matched pairs for analysis. The median ratios for oral MRLs are: acute/chronic (acute MRL/chronic MRL) 5.0 (N=25), acute/int. 3.33 (45), and int./chronic 1.83 (32). For inhalation MRLs, median ratios are: acute/chronic 10.0 (17), acute/int. 2.86 (25), and int./chronic 2.75 (22). Additional statistics and ideas concerning extrapolation will be presented.

1534 ASSESSING THE HEALTH RISK FOLLOWING ENVIRONMENTAL EXPOSURE TO ENDOSULFAN.

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Endosulfan, an organochlorine (OC) insecticide that belongs to the cyclodiene group, is one of the most commonly used pesticides to control pests in vegetables, cotton and fruits. The present study was aimed to develop the PBPK model for endosulfan in rats and extrapolate to humans, to test the ability of the model to predict the pharmacokinetic behavior of endosulfan in humans. The neurotoxic risk, which utilizes the principles of PBPK modeling as well as in vitro experiments of cytotoxicity will be estimated. The PBPK model was constructed based on the pharmacokinetic data of our experiment following single oral administration of ¹⁴C-Endosulfan to male Sprague-Dawley rats. The model was verified by simulating the disposition of ¹⁴C-Endosulfan in vivo after single and multiple oral dosages and comparing simulated results with experimental results. Cytotoxic experiments on the glial and neuronal cell cultures from rat and human were used to estimate the neurotoxic risk. α -Endosulfan, β -endosulfan and endosulfan sulfate had cytotoxic effects on rat glial (C6) and neuronal (PC12) cell cultures as well as on human glial (CCF-STTG1) and neuronal (NT2) cell cultures. The effects of α -endosulfan were highly selective, with a wide range of LC50 values found in the different cultures, ranging from 11.2 μ M for CCF-STTG1 cells to 48.0 μ M for PC12 cells. In contrast, selective neurotoxicity was not so manifested in glial and neuronal cell cultures after exposure to endosulfan sulfate, as LC50 values were in the range of 10.4–21.6 μ M. CCF-STTG1 cells were more sensitive to α -endosulfan and endosulfan sulfate, whereas NT2 cells were more sensitive to β -endosulfan. Based on these experiments, there was no specific scientific evidence to prove the quantitative neurotoxic risks. However, we deduced that humans may likely develop some neurological diseases induced by endosulfan. It was known that nerve cells could not be regenerated when these cells were damaged. It is also hoped that the newly-developed PBPK model could be utilized in the human health risk assessment in the near future.

1535 A MODEL FOR ESTIMATING THE CUMULATIVE PESTICIDE EXPOSURE OF SUBJECTS IN A SECONDARY HEALTH DATABASE.

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A challenge in designing effective epidemiological studies assessing the impact of pesticides on human health is to obtain reliable pesticide exposure and health information at an individual level. The use of secondary health databases provides a

means to access information from a large population. However, obtaining pesticide exposure history on the subjects of secondary data sources is difficult. The objective of this project was to develop a model for estimating cumulative pesticide exposure (CPE) of subjects in a secondary health database. A proxy of CPE for cases in a dataset derived from the Mississippi Cancer Registry was calculated by using the age and year at diagnosis, county of residence, and surface area and annual amounts of pesticides (based on harvested crop records) used in the county of residence. Data mining techniques including factor, correlation, and logistic regression analysis were used to measure the strength of association between CPE and the occurrence of specific types of cancer while accounting for gender, race, and age. Because pesticides are tested for lack of carcinogenicity prior to registration, no associations were expected. Among the possible 6,790 potential univariate models of association between CPE of individual pesticides and cancer type, statistically significant associations were found between 13 pesticides and the occurrence of three types of cancer. Odds ratios were generally close to 1.00 suggesting that the associations were weak. The relatively few significant associations that were determined, only 0.002%, suggest that the model is quite specific and is not likely to report many false positives. The model will be applied to other secondary health databases to determine its utility in measuring associations between pesticides and other health outcomes. (Supported by NIH P20 RR17661)

1536 ALKYL BENZENE SULFONATES RISK ASSESSMENT.

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The USEPA OPP conducted a human health risk assessment for the linear alkylbenzene sulfonates as part of pesticide tolerance reassessment required by the 1996 Food Quality Protection Act (FQPA). These antimicrobial pesticides are disinfectants and food-contact sanitizers in agricultural, food handling and commercial settings. They are also inert ingredients in pesticide formulations. The EPA selected an oral no-observable adverse effect level (NOAEL) of 50 mg/kg/day, and an inhalation NOAEL of 1 mg/m³ for use in risk assessment. The Agency applied an uncertainty factor of 100 (10X for inter- and intra-species variability) to the NOAELs, and the FQPA safety factor was removed (1X). Chronic dietary exposure was evaluated for food-contact sanitizing solutions, fruit and vegetable washes, and for inert pesticide formulations applied to agricultural crops and animals. For the inert assessment, the Agency utilized the Dietary Exposure Evaluation Model (DEEMTM) that assumed 100% of all commodities and crops were treated. Residential exposures from the inert uses for turf treatment, hard surface cleaners, and pet flea products were evaluated using the EPA's Pesticide Inert Risk Assessment Tool (PiRat), and EPA's Residential Standard Operating Procedures (SOPs). Aggregate assessments were conducted following the USEPA OPP guidance. For children, the aggregate assessment includes average dietary exposure (food and drinking water) from both the active food contact sanitizer uses and the inert uses on agricultural commodities, in addition to estimated incidental oral exposures from hard surface cleaning products as an inert ingredient. For adults, the aggregate assessment includes food and drinking water exposure from both active and inert uses and residential inhalation exposures from wiping hard surface cleaning products. The aggregate oral and inhalation risks are not of concern for adults and children, and the EPA was able to make a safety finding for the existing pesticide tolerances.

1537 PROVISIONAL ADVISORY LEVELS (PALs) DEVELOPMENT FOR DICROTOPHOS.

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Provisional Advisory Levels (PALs) that are being developed for hazardous materials by the U.S. EPA represent general public emergency exposure limits. Initial PALs correspond to different effect severity levels (PALs 1, 2, and 3) and exposure durations (24 hours, 30 days, and 2 years). Application of PAL protocols has been performed for the organophosphate pesticide dicrotophos to estimate oral (in drinking water) and inhalation exposure limits, as experimental exposure data permit. The experimental data set for dicrotophos was marginally adequate, including only animal studies. Data gaps are identified. These estimates were evaluated and approved by an Expert Consultation Panel for Provisional Advisory Levels in June 2006. PAL estimates for dicrotophos will be presented. Oral PAL values for a 24-hour exposure duration to dicrotophos are 0.58 mg/L (PAL 1), 5.8 mg/L (PAL 2), and 9.3 mg/L (PAL 3); for 30-day oral exposure duration, the value is 0.11 mg/L (PAL 1); and for 2-year oral exposure duration, the values are 0.12 mg/L (PAL 1) and 1.2 mg/L (PAL 2). Data were insufficient to derive oral PAL 2 or PAL 3 estimates for the 30-day exposure duration or a PAL 3 estimate for the 2-year exposure duration. Data were insufficient to derive inhalation PAL values for all exposure durations. Route-to-route extrapolation was suggested. The Panel identified a need to compare oral vs. inhalation data for a range of organophosphates, and then use relative

potency and available data to assess the feasibility of using route-to-route extrapolation for estimating inhalation PAL values. In the future, route-to-route extrapolation may be accepted as an approach for deriving PAL values if adequate pharmacokinetic or other data are available. (This abstract presents PAL values that are subject to change pending further review.)

1538 OPTIMAL EXPERIMENTAL DESIGNS FOR TESTING COMPLEX NONLINEAR HYPOTHESES: COMPARING THE DOSE THRESHOLDS AND ED30'S IN ADULT VS. JUVENILE RATS.

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The 1996 Food Quality Protection Act set stringent standards to protect children from pesticide risks. To enforce these standards, the United States Environmental Protection Agency requires studies on pesticides to investigate the neurotoxicity of these chemicals. An important design strategy is to compare the dose responsiveness of adult versus juvenile rats. Of concern is that juvenile rats may be more sensitive than adult rats; here, we characterize sensitivity through the dose-threshold and ED30's. Our objective is to describe use of optimal experimental design strategies to test complex nonlinear hypotheses. Here we find the optimal design that minimizes the variance of the test statistic over dose (x) and proportion (q) of the total sample size for simultaneously testing the coincidence of the dose-thresholds and ED30's for adult versus juvenile rats. For illustration purposes we considered a single pyrethroid, Lambda-cyhalothrin. Preliminary estimates of the ED30 and dose threshold for adult Long Evans rats varying in ages from 60-66 days were taken from Wolansky et al. (ToxSci, 2005). We conjecture a 50% decrease in the dose-threshold and/or approximately a 30% decrease in the ED30 for the juvenile rats. Constrained optimal designs were found such that at least 5% of the sample is allocated to each control group. Based on the experimental range used by Wolansky, another constraint was implemented such that the maximum dose does not exceed 15mg/kg. We conclude that the optimal design for testing the hypothesis of coincidence between adult versus juvenile rats depends on (1) the preliminary assumptions about the dose-threshold and the shape of the dose response curves and (2) that the sample proportions are not equal across the dose groups.

1539 HUMAN RISK ASSESSMENT FOR CHLORPYRIFOS UTILIZING A CYP-SPECIFIC PBPK/PD MODEL.

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Protecting humans from the toxicological effects of organophosphorous pesticides (OPs) is dependent in part upon accurate physiologically based pharmacokinetic / pharmacodynamic (PBPK/PD) models. OP toxicity is dependent upon the balance between the formation of oxons by cytochrome P450 (CYP) catalyzed desulfuration (activation), dearylation (detoxification) by CYPs and paraoxonase (PON1), and inhibition of B-esterases by oxons. Current PBPK/PD models, which utilize single kinetic constants (Km, Vmax) for a given metabolic pathway, using rat liver microsomes, do not accurately reflect the variability in content and activity of key enzymes (CYPs, PON1) which regulate OP metabolism in humans. Simulations with chlorpyrifos have demonstrated that CYP-specific PBPK/PD models can better estimate exposure, target tissue dose and effects of chlorpyrifos in human populations by incorporating 1) kinetic parameters (Km and Vmax) for OP metabolism by specific human CYPs, 2) hepatic content of specific CYPs, 3) PON1 activity and content, and 4) serum protein binding data for OPs and metabolites into the PBPK/PD model of Timchalk et al., (2002). CYP2B6 and CYP2C19 exhibit low Km and high Vmax values for the desulfuration and dearylation of chlorpyrifos, respectively, supporting their role as the primary enzymes that regulate metabolism at low-level human exposures to chlorpyrifos. Since the hepatic levels of specific CYPs exhibit marked variability across both population and age groups, the CYP-specific PBPK/PD models should prove to be more accurate and more easily modified to address factors such as age, CYP content and genetic polymorphisms in CYP2B6, CYP2C19 and PON1 (biomarkers of susceptibility). CYP-Specific PBPK/PK models for OP pesticides, that better estimate variability in exposure, target tissue dose and effect, will be valuable tools for risk assessment efforts for chlorpyrifos and other OPs in the general population, special exposure groups, and susceptible individuals, exhibiting polymorphisms in CYPs and/or PON1. Supported by US EPA STAR grant R-83068301

1540 ASSESSMENT OF HEALTH EFFECTS RESULTING FROM POSSIBLE EMISSIONS FROM A PESTICIDE FORMULATION FACILITY.

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The cyclodiene insecticides, aldrin, chlordane, and heptachlor, were widely used in the 1950s and 1960s for insect control due to their persistence in soil, resistance to degradation, retention of biological activity, and selective toxicity. Cyclodiene pesti-

cides were often prepared for sale at "formulation facilities," wherein bulk quantities of pesticides were received and repackaged into bags of various sizes. In the late 1980s, near one such facility in a rural Illinois town, it was suggested that historical cyclodiene emissions may be posing a health risk to local residents. We designed and implemented a "risk-based" sampling methodology to focus data collection on the needs of a health risk assessment. Samples of residential soils, garden vegetables, well water, and suspended particulates were collected and analyzed. A stratified sampling plan was used to permit distinction between cyclodienes associated with facility emissions vs. those associated with other sources (e.g., homeowner application and/or drift from nearby treated crops). Incremental health risks were then calculated for the "facility-related" cyclodiene concentrations in these media. Three exposure scenarios were developed ("typical", "maximum", and "minimum"), and the following exposure pathways were considered: garden vegetable and incidental soil ingestion, dermal contact with soil, and inhalation of suspended surface soil particles. The estimated incremental carcinogenic risks were 4.72×10^{-7} , 9.73×10^{-6} , and 3.75×10^{-7} for typical, maximum, and minimum exposures, respectively. The greatest contributor to total carcinogenic risk from all sources combined was found to be the result of homeowner application of cyclodienes to lawns and gardens.

1541 PROVISIONAL ADVISORY LEVELS (PALs) DEVELOPMENT FOR G-SERIES NERVE AGENTS.

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Provisional Advisory Levels (PALs) that are being developed for hazardous materials by the U.S. EPA represent general public emergency exposure limits for 24-hour, 30-day and 2-year durations. Application of PAL protocols has been performed for chemical warfare nerve agents GA, GB, GD, GE and GF to estimate oral and inhalation/ocular exposure limits, as experimental exposure data permit. The experimental data set for agent GB was robust and included controlled human and animal studies. The remaining G-series agents were less well characterized, necessitating PAL estimation on the basis of relative potency. These estimates were evaluated and approved by the Expert Consultation Panel for Provisional Advisory Levels in August 2006.

PAL estimates for all 5 G-agents will be presented. Oral PAL values for 24-hour exposure are 0.037 mg GB/L (PAL1), 0.11 mg GB/L (PAL2), and 0.57 mg GB/L (PAL3); for 30-day oral exposure duration, values are 0.0081 mg GB/L (PAL1), 0.012 mg GB/L (PAL2), and 0.25 mg GB/L (PAL3); for 2-year oral exposure duration, the PAL1 value is 0.0020 mg GB/L. Data were insufficient to derive oral PAL 2 or PAL 3 estimates for the 2-year exposure duration.

Inhalation/Ocular PAL values for 24 hour exposure are 0.00020 mg GB/m³ (PAL1), 0.0010 mg GB/m³ (PAL2), and 0.015 mg GB/ m³ (PAL3); for 30 day inhalation/ocular exposure duration, values are 0.000018 mg GB/ m³ (PAL1) and 0.00073 mg GB/ m³ (PAL 2). Insufficient data are available to estimate a 30 day PAL3 value. For 2-year inhalation/ocular exposure duration, the PAL1 value is 0.000018 mg GB/ m³ (equivalent to PAL1 for 30 days) and the PAL2 value is 0.00016 mg GB/m³. Data were insufficient to derive an inhalation/ocular PAL 3 estimate for the 2-year exposure duration. (This abstract presents PAL values that are subject to change pending further review.)

1542 ALTERNATIVE APPROACHES FOR NONCANCER RISK ASSESSMENT OF PHOSGENE.

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Phosgene is primarily used in the polyurethane industry for production of polymeric isocyanates. The majority of phosgene for industrial applications is made on site by the reaction of carbon monoxide and chlorine gas using an activated carbon analyst. Inhalation is the primary route of exposure. The noncancer risk to humans from exposure via inhalation is assessed by deriving a chronic inhalation RfC. The RfC is an estimate of daily exposure to the human population that is likely to be without appreciable risk of deleterious effect during a lifetime. The RfC was based on two inhalation subchronic studies which measured immune response and pulmonary damage. These studies used the same strain of rats with similar exposure protocols. Support for the derivation of the RfC for phosgene came from the use of three different approaches: 1) the traditional NOAEL/LOAEL approach; 2) the benchmark dose (BMD) approach; and 3) the categorical regression (cat Reg) approach for the analysis of severity-graded pulmonary damage data using the recently developed USEPA cat Reg software. The BMD approach was used to determine the RfC for phosgene because it has several advantages compared to the NOAEL/LOAEL approach: 1) it is not restricted to be set of doses used in the experiments; 2) the result is not dependent on sample size; 3) it incorporates information on statistical uncertainty. The cat Reg approach allowed the incorporation

of data on the severity of the pathological lesions, and therefore it complemented the other approaches. Conclusions from the three approaches were consistent. The BMD approach could not be used for the immune response data because it was not possible to define an adverse effect level for bacterial resistance. Use of the NOAEL/LOAEL approach for that data was consistent with the RfC derived from the lung pathology data. This paper will summarize the three approaches used in derivation of the phosgene RfC. The derivation of the RfC uses uncertainty factors to account for variation between humans, extrapolation from animals to humans, and subchronic to chronic exposure duration.

1543 PERCEIVED RISKS AND HAZARDS OF NANOTECHNOLOGY.

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Nanotechnology is the study or application of any matter at the 1-100 nanometer scale. The study objectives were to measure and evaluate differences between nanotechnologists/nanoscientists and environmental health scientists in behavior, knowledge, beliefs and attitudes relating to nano-development. This study surveyed University of Washington faculty associated with the Center for Nanotechnology and the Department of Environmental and Occupational Health Sciences. The hypotheses were that 1) environmental health scientists will perceive greater risk and greater need for nano-development regulation and public awareness than will nanotechnologists and that 2) nanotechnologists will perceive greater benefit to nano-development than will environmental health scientists. Variances in knowledge, communication, and attitudes including trust, regulation and perceived benefits and risks were examined to better understand cross-disciplinary differences. Results indicated that nanotechnologists and environmental health scientists 1) did not trust regulatory agencies to minimize risks of scientific development; 2) tended to not trust business leaders within the nanotechnology industry to minimize risks associated with nano-development; and 3) tended to think the precautionary principle is an appropriate strategy for reducing risks. The two groups tended to differ in that 4) environmental health scientists were statistically significantly more likely to believe that current regulations were insufficient, while nanotechnologists general agreed but the finding was not significant; and 5) nanotechnologists were significantly more likely to think the benefits of nano-development would outweigh the risks, while the majority of environmental health scientists only concurred. These results can be used to improve cross-disciplinary communication about nanotechnology's benefits and potential hazards, and identify appropriate levels of nano-regulation, and spark discussion about methods for nano-regulation.

1544 DEPOSITION OF INHALED NANOPARTICLES IN THE HUMAN LUNG.

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Increased production of industrial devices based on nanostructured materials raises the possibility of environmental and occupational human exposure with consequent adverse health effects. Nanoparticles are suspected of having increased toxicity due to their internal structure and size characteristics that elicit unique carrier transport properties. For this reason, it is important to assess the dose and distribution of inhaled nanoparticles in the human lung. A model of nanoparticle transport and deposition in the human lung was developed based on the convective and diffusive properties of nanoparticles. Local, regional, and total lung deposition was calculated by performing a mass balance on particles moving through each airway of the lung during a breathing cycle. Particle losses due to diffusive transport were found to be insignificant in the upper airways of the lung because of high airflow convection. In the deep lung, particle transport by axial diffusion was significant and deposition tended to occur more distally compared to when axial diffusion was not included in the model. However, total deposition of nanoparticles in the lung was independent of axial diffusion because of high deposition efficiency (near 100%). The nanoparticle deposition model will yield more accurate predictions of site-specific and local deposition of particles and better correlations for dose-response relationships.

1545 CARDIOVASCULAR MORTALITY AND LUNG CANCER RISK FROM DIESEL EXHAUST PARTICULATE IN CALIFORNIA.

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Diesel exhaust particulate (DEP) was identified as a Toxic Air Contaminant by the Air Resources Board in California in 1998 following a lengthy process that included a quantitative risk assessment based on lung cancer in occupational settings.

DEP is one component of ambient particulate matter (PM) primarily in urban settings or near major roadways. Many studies demonstrate increased risk of morbidity and mortality from cardiovascular and respiratory illness, including lung cancer, associated with ambient PM. The risk management of diesel engine exhaust has been based primarily on the estimate of cancer potency developed by the California Office of Environmental Health Hazard Assessment (OEHHA). This paper describes estimates of both cancer and noncancer risks associated with ambient diesel PM exposure utilizing measured or estimated concentrations of diesel PM statewide and near-source, and compares estimated lung cancer risk derived from relative risk estimates in epidemiological studies of chronic exposure to ambient PM with estimates based on the lung cancer potency factor developed by OEHHA. Statewide average exposures to DEP based on emissions inventories have been estimated by the California Air Resources Board to be approximately $1.5 \mu\text{g}/\text{m}^3$ considering both indoor and outdoor exposures. Outdoor average concentrations estimates range between 0.2 and $3.6 \mu\text{g}/\text{m}^3$ in California. Measured near-source and in-vehicle exposures can be much higher (up to $50 \mu\text{g}/\text{m}^3$). Statewide average exposures in California are associated with excess cancer risk of 4.5×10^{-4} and a total estimated number of lung cancer cases of about 200 per year; cardiovascular mortality risks are on the order of 6×10^{-2} for a total number of about 3800 deaths per year. Near-source exposures are associated with higher risks. Estimates of cardiovascular mortality (up to several thousand deaths per year) exceed those for lung cancer (hundred of deaths/year) by a wide margin.

1546 THE EFFECTS OF DESERT SAND (PM10) ON THE RAT AIRWAY.

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Sand storm composition varies by geographical location and may contain contaminants such as metals or microorganisms on their surfaces or within their matrix. Military personnel deployed to areas where blowing sand is a more frequent occurrence are concerned about potential health consequences from inhaling the airborne sand particles. Airborne particulate levels (PM10; PM₁₀ < 10 μm) in such regions can exceed $10,000 \mu\text{g}/\text{m}^3$, significantly higher than the recommended military exposure guideline (MEG) of $150 \mu\text{g}/\text{m}^3$. Consequently, a single exposure to desert sand (PM10) particles from a known deployed region in the Middle East was investigated for airway inflammation or injury. Male Sprague-Dawley rats (n=80/time point) were intratracheally instilled with PM10 particles suspended in sterile saline at doses of 0.84 mg/ml, 4.2 mg/ml or 12.6 mg/ml (corresponding to 1, 5 and 15 mg/Kg body weight). Positive, negative and vehicle control animals were treated with silica, titanium dioxide and sterile saline, respectively. At select times (24 hours, 3 days, 7 days and 6 months) post single exposure, the lungs were lavaged with cold phosphate buffered saline and the bronchoalveolar lavage fluid (BALF) was analyzed for markers of inflammation and injury such as lactate dehydrogenase (LDH), total protein (TP), N-acetylglucosaminidase (NAG), cytokines, cell number, type, and viability. Positive trends were observed in the concentrations of TP and LDH in the BALF of sand exposed rats (15mg) from 24 hours to 3 days post-exposure. However, at 7 days and 6 months the results were comparable to the controls. Histopathology report at 7 days showed inflammation in the lung tissues from 15 mg of sand (PM10). The inflammation was characterized by alveolitis, alveolar hyperplasia, pneumonitis, silicosis and eosinophilic infiltration in the lungs. The 6 month study is on-going.

1547 PHYSICOCHEMICAL AND CELL-BASED APPROACH FOR PREDICTING OF PHOSPHOLIPIDOSIS-INDUCING POTENTIAL IN EARLY DRUG DISCOVERY.

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Sponsor: M. Kurata.

Some of the principal requisites of toxicity screening methods in drug discovery are their ease to perform and high throughput, as well as the possibility to predict the occurrence of clinical events. Drug-induced phospholipidosis is defined as the excessive accumulation of phospholipids in the cytoplasm as a result of medical treatment. The accumulation of phospholipids is observed as a distinct formation of lamellar inclusion bodies by transmission electron microscopy (EM). Various physicochemical approaches to predict the phospholipidosis-inducing potential (PLIP) of drugs have been reported. The purpose of the present study was to develop a new simple physicochemical prediction method also based on lipophilicity and a charge parameter. We employed a test set of 33 compounds including 11 in-house compounds. The PLIP of the test set compounds was determined by the fluorescence-labeled lipid accumulation assay using isolated rat hepatocytes. In addition, the formation of lamellar bodies in the cytoplasm was confirmed by EM. The

usefulness of the ClogP - most basic pKa (pKa - MB) plot to the PLIP of compounds was first examined. This plot was unable to predict the PLIP of zwitterions. In order to improve its predictability, the net charge of a given molecule (NC) was introduced instead of pKa - MB, since the NC corresponds directly to the ionization state of compounds in the organelles. Compounds with high ClogP (> 1) and high NC ($1 \leq \text{NC} \leq 2$) tended to be positive. This finding was also confirmed using 30 additional validation set compounds obtained from the literature. The ClogP - NC plot differentiated positive and negative compounds with more than 98% accuracy (62/63). The method based on ClogP and NC contributes to an adequate assessment of the structure-toxicity relationship and drug design. In the assay cascade, physicochemical and cell-based approach facilitates the selection of compounds prior to the implementation of further *in vivo* studies.

1548 ACETALDEHYDE INHALATION TOXICITY APPERARS SEVER IN ALDEHYDE DEHYDROGENASE 2 KNOCKOUT MICE; : 0, 125 AND 500 PPM 24HR 2WEEKS STUDY.

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Acetaldehyde is metabolized by aldehyde dehydrogenase 2 (ALDH2). Half of Japanese are inactive aldehyde dehydrogenase genotype (ALDH2*2/*2 and ALDH2*1/*2). ALDH2-inactive individuals were estimated to be at a higher risk of toxicity due to a higher internal exposure to acetaldehyde. However, inhalation toxicity of acetaldehyde has not been evaluated in these populations. We compare Aldh2 inactive gene targeting mice (Aldh2^{-/-}) and that of wild type mice (Aldh2^{+/+}) by 500, 125 and 0 ppm 24 hr/day 2 weeks acetaldehyde inhalation test. The average blood acetaldehyde concentration of Aldh2^{-/-} mice was higher than that of Aldh2^{+/+} mice in the acetaldehyde exposure group, observable effects by the acetaldehyde exposure on the lung and liver were not different between Aldh2^{+/+} and Aldh2^{-/-} mice. In Aldh2^{-/-} mice, the levels of (1) erosion of respiratory epithelium and the subepithelial hemorrhage in nose, (2) hemorrhage in nasal cavity, (3) degeneration of respiratory epithelium in larynx, pharynx and trachea, and (4) degeneration of dorsal skin were higher compared with Aldh2^{+/+} mice. The Aldh2^{-/-} mice expose higher level of internal acetaldehyde exposure and are more acetaldehyde-sensitive than Aldh2^{+/+} mice. In the case of the both mice have same absorption rate, same respiration and circulation status, and the blood acetaldehyde reach plateau status, one-compartment multiple dosing model figures out the risk factor of internal exposure for Aldh2^{-/-} mice is approximately 6 times higher than Aldh2^{+/+} mice. Based on this study, acetaldehyde inhalations were inferred to pose a higher risk to ALDH2-inactive human individuals.

1549 DEVELOPMENT OF TEFS FOR PCB CONGENERS BY USING AN ALTERNATE ENDPOINT - THYROID HORMONE.

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PCBs are ubiquitous global toxic contaminants. Health risk assessment for these chemicals is complex; the current TEF method only covers coplanar PCBs, dibenzofurans and dioxins, without involving non-coplanar PCBs. To address this deficiency, we correlated published data for several PCB congeners on toxicity related endpoints *in vivo*, with the aim of identifying one or more readily accessible parameters which correlate with toxic responses for both dioxin-like and non dioxin-like PCB congeners. In rats fed PCBs for ninety days, the order of EROD induction was PCB 126 > PCB 77 > PCB 118 > PCB 153 > PCB 28. This is consistent with the order of thyroid toxicity, i.e., PCB 77 > PCB 118 > PCB 153 > PCB 28. These data suggest a possibility by using the thyroid toxicity endpoint as an alternative to EROD induction in developing TEFS for non-coplanar PCBs. While some PCB congeners (PCB 77 and PCB 126) increase dopamine levels in the brain, most congeners decrease the dopamine level. The toxicity order for dopamine content is inconsistent with that for EROD or thyroid hormone effects, i.e., the dopamine levels decreased in the order PCB 28 > PCB 118 > PCB 153. The estrogenic endpoint for PCB congeners is also divergent: in general, lower chlorine PCBs have estrogenic effects, whereas higher chlorine PCBs are anti-estrogenic. Crofton et al. (2005) studied thyroid hormone decreases (ED30) caused by TCDD, PCDD, TCDF, and 12 PCBs, including both dioxin-like and non dioxin-like congeners. Based on their data, the calculation of TEF-THs, referred to TCDD by using the ED30, are identical to both TEF-WHO97 and TEF-WHO05 for dioxins and dioxin-like PCBs and their correlation coefficient are $r=0.73$, $P<0.05$; and $r=0.72$, $P<0.05$, respectively. A different 90 day rat experiment *in vivo* also found significant correlations for TCDD, PCB 126 and PCB 156 between reductions in plasma total thyroxine levels and liver enzyme induction. Therefore, we

propose to use thyroid hormone reductions and thyroid histological changes, as an endpoint to generate new TEFs which address the thyrotoxic and neurotoxic effects of PCBs.

1550 MECHANISM-BASED CANCER RISK ASSESSMENT FOR 1,4-DICHLOROBENZENE.

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The weight of scientific evidence indicates that cancer risk assessments for 1,4-dichlorobenzene (1,4-DCB) should be based on a nongenotoxic-mitogenic mode of action, rather than the linear default assumptions used in the proposed 2006 EPA IRIS assessments. The primary driving force exerted by mitogenic carcinogens is to stimulate the growth of spontaneous precancerous lesions and predicts a threshold dose level for carcinogenic activity corresponding to doses that are insufficient to exhibit mitogenic or promotional activity. Accordingly, the appropriate risk assessment approach for 1,4-DCB is to establish a no observed adverse effect level (NOEL) for tumor induction divided by a rational uncertainty factor to estimate a human daily oral exposure below which there is unlikely to be an increased lifetime risk of cancer. Two chronic studies showing increased incidences of liver adenomas and carcinomas in mice are available for 1,4-DCB, an oral gavage bioassay with 2 dose groups and an inhalation bioassay with 3 airborne concentrations. A coherent dose-response curve exhibiting a clear threshold is obtained when the two bioassays are assessed jointly using an absorbed-dose metric. Benchmark dose modeling of the combined dose-response curve provides the basis for assessing cancer risks under both a threshold and non-threshold set of assumptions. Using the threshold approach, derived safe exposure levels via oral and inhalation routes of exposure are somewhat higher than but consistent with the RfD and RfC values proposed by IRIS. Estimated cancer slope factor and unit risk based on the combined data sets under a non-threshold set of assumptions using the combined oral and inhalation data sets are approximately 10-fold lower than the values proposed by IRIS. The default IRIS calculation estimates a 1 in 100,000 increased lifetime risk of cancer at an airborne concentration of 0.0004 ppm, 250 fold lower than a representative mechanism-based threshold model and 187,500 fold lower than the NOEL of 75 ppm.

1551 RISK ASSESSMENT FOR SITES WITH VOLATILE CONTAMINANTS IN SHALLOW GROUNDWATER.

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In California, a number of hazardous waste sites are present in low-lying coastal areas with shallow groundwater (2 to 15 feet below ground surface) contaminated by volatile organic chemicals (VOCs). Shallow groundwater poses complex issues of site characterization and potential exposure pathways. In addition to potential ingestion and dermal contact through domestic water use, exposure can occur through indirect pathways. Inhalation of VOCs can occur via showering and other household uses of groundwater, following volatilization into outdoor air, and through vapor intrusion from soil or groundwater into indoor air. The vapor intrusion to indoor air pathway currently receives considerable attention in risk assessments. Exposure to VOCs from shallow groundwater can also occur via dermal contact or inhalation by construction workers and uptake of contaminated groundwater into home-grown produce consumed by residents. This paper illustrates how these issues were dealt with at specific sites in California. Risk assessment was performed at Site A for groundwater contaminated with benzene and methyl tertiary-butyl ether. A US Department of Energy model for home-grown produce predicted cancer risk as high as 3E-3 and a noncancer hazard index (HI) of 7. However, cancer risk and HI from the CalTOX model were much lower (2E-6 and 0.02, respectively), illustrating the uncertainty associated with this pathway. Excluding the food pathway, the highest cancer risk and HI were from the vapor intrusion pathway (2E-5 and 0.08, respectively). Our experience is that if significant concentrations of VOCs are present in soil or groundwater, the vapor intrusion pathway usually generates the highest cancer risk and HI. The risk assessment for Site A included an evaluation for construction workers. Although the estimated cancer risk and HI were de minimus for VOCs from groundwater in a trench scenario (3E-7 and 0.03, respectively), inhalation of VOCs from soil generated surprisingly high estimates for the outdoor air pathway (3E-6 and 52, respectively).

1552 DERIVATION OF A DRINKING WATER ACTION LEVEL FOR TRIS(MONOCHLOROPROPYL) PHOSPHATE ISOMERS.

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Tris(monochloropropyl) phosphate isomers (TMCPs) are used in thermoset and thermoplastic materials, and for textile finishes contacting potable drinking water. TMCPs are a mixture of two isomers: tris(2-chloroisopropyl) phosphate and

tris(2-chloropropyl) phosphate. When products are evaluated to national health effects standards for use in drinking water systems, chemicals extracting from these products must be below appropriate action levels. As defined by ANSI/NSF Standard 61, a Total Allowable Concentration (TAC) is the maximum concentration of a nonregulated contaminant allowed in a public drinking water supply; a default Single Product Allowable Concentration (SPAC) is 10% of the TAC. To determine an action level for TMCPs, a health effects evaluation was performed. TMCPs are not genotoxic based on mutagenicity, cytogenicity, and cell transformation assays. The systemic toxicity of TMCPs has been investigated in subacute and subchronic studies, and in a reproduction/developmental study. Effects were reported in repeat-dose toxicity studies at doses of 481 mg/kg/day and above, and included reductions in body weight and body weight gain, increased relative and absolute liver weights, mild hepatocellular swelling and cortical tubular degeneration, and mild hyperplasia of thyroid follicular and sternal bone marrow cells. Systemic toxicity was not reported at doses up to 160 mg/kg/day. No adverse maternal or developmental effects were reported at doses up to 1000 mg/kg/day following exposure during gestation days 0-20. Collectively, available toxicity data indicate that TMCPs are without adverse health effects at concentrations up to 160 mg/kg/day. A 0.2 mg/kg/day RfD was derived using a 160 mg/kg/day NOEL from a 13-week dietary study in rats and uncertainty factors totaling 1000. A TAC of 1.1 mg/L was derived for TMCPs based on the RfD, a drinking water source contribution factor of 0.20, and a drinking water intake of 2 L/day. The SPAC is 0.11 mg/L, or 10% of the TAC.

1553 TESTING FOR ADDITIVITY IN THE LOW DOSE REGION OF AN ENVIRONMENTALLY RELEVANT MIXTURE OF 18 POLYHALOGENATED AROMATIC HYDROCARBONS.

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A common default assumption in risk assessment of chemical mixtures is that the chemicals combine additively in the low dose region. Under additivity, with information from single chemical dose-response data, the risk associated with the mixture can be estimated. Our objective is to evaluate if there is sufficient evidence to claim additivity in a low dose region for the effects of an environmentally relevant mixture of 18 PHAHs on serum total thyroxine (T4). Young female rats were dosed via gavage with 18 different PHAHs [2 dioxins, 4 dibenzofurans, and 12 PCBs], for 4 consecutive days (Crofton et al, EnvHlthPersp, 2005). Serum total T4 was measured via radioimmunoassay. A mixture was custom synthesized with the ratio of chemicals based on the ratios of PHAHs found in breast milk, fish and other sources of human exposure. Following the work of Stork et al (Risk Analysis, 2006) we utilized equivalence testing methodology to test for additivity at three environmentally relevant low dose mixture groups. The ratio of predicted mean responses under additivity based on single chemical data to observed sample means at these mixture points was calculated with the corresponding 95% simultaneous confidence intervals. Two of the three confidence intervals were completely contained within predetermined additivity bounds of [0.80, 1.25]. In these two cases we conclude that there is significant evidence to claim that the T4 mean response is equivalent to that predicted from the single chemical data under additivity. In the lowest mixture dose group there is not enough evidence to claim additivity as the lower limit of the Bonferroni-corrected 95% confidence interval fell below 0.80. (This abstract does not necessarily reflect U.S. EPA policy. These data and research are not associated with Monsanto.)

1554 HEALTH RISK ASSESSMENT OF METHYL ISOBUTYL KETONE IN DRINKING WATER.

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Acceptable levels of methyl isobutyl ketone in drinking water were determined based on Annex A of NSF/ANSI 61 (2005). Limited human but numerous animal toxicity data were identified. Subchronic gavage or inhalation exposure caused transient narcosis, liver and kidney weight increases, and renal nephropathy in rats. Two subchronic oral studies were available, but a drinking water study evaluated an insufficient number of rats, used only one dose level, and included only females. Increased liver and kidney weights and nephropathy were observed without hepatic histopathology in a standardized subchronic gavage study in rats. Increased liver weights were likely an adaptive response to metabolizing high bolus doses of methyl isobutyl ketone, while renal effects were attributed to spontaneous and/or alpha-2μ-globulin nephropathy. Since narcosis was inconsistently or transiently observed in all studies, the transient narcosis observed after gavage but not after drinking water exposure at the same dose was attributed to bolus dosing. Renal lesions in rats

and mice chronically inhaling methyl isobutyl ketone were attributed to spontaneous or alpha-2u-globulin nephropathy or were within historical ranges, thus either not considered treatment-related or relevant to humans. However, interpretation of the effects in male rats was confounded by reduced survival. The weight of genotoxicity evidence suggests that methyl isobutyl ketone is not genotoxic. According to U.S. EPA (2005) guidelines, since no epidemiological studies in humans or chronic oral data in animals were identified, the data are inadequate for an assessment of human carcinogenic potential of methyl isobutyl ketone after oral exposure. The NOAEL of 1,000 mg/kg-day, based on the lack of adverse effects in the subchronic gavage study, was used to derive the Reference Dose (RfD), since it was a standardized study and oral data are preferred, due to the lack of kinetic or metabolism data in humans to reduce route-to-route or interspecies extrapolation. The RfD of 1 mg/kg-day was used to determine the Total Allowable Concentration in drinking water of 7 mg/L.

1555 SUPPORT FOR SELECTION OF A METHAMPHETAMINE CLEANUP STANDARD IN COLORADO.

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Methamphetamine production for illicit use occurs in makeshift labs and is associated with the release of numerous chemicals, including methamphetamine residues. The presence of these chemicals may pose a health risk to residents who reoccupy these structures after property seizures. Several states have established technology-based cleanup standards for methamphetamine, but none have examined the health-protectiveness of these standards. In response to Colorado House Bill 04-1182, exposure intakes correlated with three technology-based standards were calculated for various groups of individuals. Exposures were assessed for a one-year old and six-year old child, as well as for a female of child-bearing age. Exposure intakes were compared to toxicity reference values developed from developmental endpoints following methamphetamine exposure from the available literature. Uncertainty factors were applied to the lowest adverse effect levels observed in these studies to arrive at the toxicity reference values. These reference values were greater than the calculated intakes from each proposed technology standard, suggesting that all of the proposed standards would be protective of human health exposure. The cost and practicality of attaining each of the proposed standards was also factored into the decision making process. In their final regulation (6 CCR 1014-3), the CDPHE selected 0.5 ug/100 cm² as their final cleanup standard for methamphetamine residues.

1556 HEALTH RISK ASSESSMENT OF 4-CHLOROBENZOTRIFLUORIDE IN DRINKING WATER.

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Acceptable levels of 4-chlorobenzotrifluoride in drinking water were determined based on Annex A of NSF/ANSI 61 (2005). 4-Chlorobenzotrifluoride showed minimal potential for skin or eye irritation. Results of repeated-dose systemic studies from 14 days to three months duration, whether by the oral or inhalation route, showed liver effects manifested as increased liver weight and hepatocyte hypertrophy, which were not considered adverse in the absence of changes in hematology or clinical chemistry parameters, or adverse histopathology. In some shorter-term studies, increased cholesterol and triglyceride levels were observed and considered adverse. Kidney effects related to α -2u-globulin accumulation were observed in male rats, but were not considered relevant to human health based on U.S. EPA science policy. No effects on reproduction parameters were observed in a one-generation study, and neurotoxic effects were only observed in short-term studies at very high doses. Several distribution studies indicate this chemical has a small potential to accumulate in adipose tissue. 4-Chlorobenzotrifluoride produced negative results in standard and host-mediated *Salmonella typhimurium* reverse mutation assays, and in the mouse lymphoma forward mutation assay. No evidence of damage was observed in chromosomal aberration assays in vitro or in vivo. 4-Chlorobenzotrifluoride did induce unscheduled DNA synthesis and sister chromatid exchanges. Based on mixed results in genetic toxicity assays, and on the absence of human epidemiological data or a lifetime bioassay in laboratory animals, the data are inadequate for an assessment of human carcinogenic potential. A NOAEL of 45 mg/kg-day, based on lack of adverse effects in a subchronic gavage study in weanling rats, was used to derive the Reference Dose (RfD). The RfD incorporated a total uncertainty factor of 1,000, which accounted for inter- (10x) and intraspecies (10x) extrapolation, subchronic to chronic extrapolation (3x), and database deficiencies (3x). The RfD of 0.05 mg/kg-day was used to determine the Total Allowable Concentration in drinking water of 0.3 mg/L.

1557 AN EVALUATION OF THE MODE OF ACTION INFORMATION FOR HEXACHLOROETHANE USING U.S. EPA'S 2005 GUIDELINES FOR CARCINOGEN RISK ASSESSMENT.

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The Integrated Risk Information System (IRIS) Program is conducting a health assessment of hexachloroethane that will apply the mode of action framework of the U.S. EPA 2005 Guidelines for Carcinogen Risk Assessment. The guidelines highlight the importance of critically evaluating toxicological and epidemiological data to characterize the mode of action information for a chemical substance in order to interpret potential cancer risk to humans. Understanding a chemical's mode of action provides insight to determine the likelihood of toxicity in humans exposed to a chemical substance. A recent evaluation of the available information for hexachloroethane identified a two-year bioassay in which renal tubule tumors were identified in F344/N male rats. The production of these tumors is frequently associated with a group of chemicals that induce alpha 2u-globulin accumulation leading to early nephrotoxicity and progressing to renal tubule tumor formation. The male rats also exhibited a range of nephrotoxic effects that are characteristic of the renal lesions observed with the protein alpha 2u-globulin-associated nephropathy. Numerous studies for hexachloroethane have demonstrated toxic effects in male rats that are commonly associated with the alpha 2u-globulin protein mechanism. However, hexachloroethane also induced similar nephrotoxic effects in animal species that are not considered characteristic of the alpha 2u-globulin-associated mode of action. Based on the framework provided in the U.S. EPA 2005 Guidelines for Carcinogen Risk Assessment, the mode of action for hexachloroethane as it relates to the alpha 2u-globulin protein mechanism has been evaluated.

1558 MUCOSAL IRRITATION OF VAPORS OF HEXANAL, ACETIC ACID AND DIOXANE IN HUMANS.

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Chemosensory irritation of the mucous membranes is an important endpoint in the risk assessment of volatile chemicals. Information on health hazards associated with the handling of hexanal, acetic acid and 1,4-dioxane is sparse. We have performed controlled short-term chamber exposures with the intention to evaluate acute health effects of these chemicals, with emphasis on mucosal irritation. In pilot studies, the subjects where exposed to stepwise increasing levels (10 min per level) of each chemical. A questionnaire with visual analogue scales (VAS) was used for graded ratings of irritation and central nervous system symptoms. Based on these ratings, the subjects where exposed for 2 h at rest to vapors of hexanal (0, 2, and 10 ppm), acetic acid (0, 5, and 10 ppm) and 1,4-dioxane (0, and 20 ppm), respectively. In addition to the VAS ratings, effects in the airways were assessed by spirometry, nasal swelling was studied by acoustic rhinometry and nasal blocking index, blink frequency was measured by electromyography and inflammatory markers in plasma (C-reactive protein and interleukin-6) were analyzed. Exposure to 20 ppm 1,4-dioxane did not significantly affect any of the measured parameters. For hexanal, ratings of discomfort in the eyes and nose, solvent smell and headache increased significantly with exposure level. Blinking frequency was significantly increased at 10 ppm. Acetic acid caused significantly increased ratings of nasal irritation and smell at 5 and 10 ppm. No effects on pulmonary function, nasal swelling or on plasma inflammatory markers were detected in any study. In conclusion, minor effects were detected after exposure to hexanal (10 ppm) and acetic acid (10 ppm). No effects were detected for 1,4-dioxane at 20 ppm, the highest level tested.

1559 NASAL IRRITATION AND TUMORS IN RATS EXPOSED TO CHEMICALS IN DRINKING WATER MAY RESULT FROM DIRECT CONTACT WITH NASAL TISSUES.

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Exposure to 1,4-dioxane in drinking water has lead to nasal irritation and tumors in rats in several studies at concentrations of 0.5-1.8% (5,000 to 1,800 mg/L), but not following inhalation exposures to concentrations as high as 111 ppm (400 mg/m³). It has been hypothesized that the nasal lesions likely resulted from repeated, direct contact between inspired drinking water and nasal tissues producing localized high doses and chronic irritation. Magnetic resonance imaging was initially used to determine the amount of water inspired; however, this method was useful only when

air passages were completely occluded. Therefore, a series of water soluble dyes were evaluated for their palatability and ability to stain nasal tissues with a mixture of red fluorescent dyes selected for the definitive study. Three groups of five rats each were provided with either: 1) fluorescent dye mixture plus 0.5% dioxane; 2) fluorescent dye mixture alone; or 3) control drinking water for a single night. Nasal tissues were evaluated by fluorescence microscopy the following morning. Dye-specific fluorescence, indicating a direct contact with drinking water, was observed in nasal airways of all rats exposed to the dye mixture (with and without dioxane) in the anterior third of the nose where previous studies have identified irritation and tumors following drinking water exposures. These results provide a critical linkage between nasal tissue responses and localized high doses of dioxane inspiration of drinking water and a foundation for the hypothesized cytotoxic mode of action for nasal tumors. The nasal tumors observed only in rats exposed to dioxane in drinking water is therefore of questionable relevance for humans. Furthermore, these results have implications for the assessment of any chemical that causes nasal lesions in rats when exposed via drinking water. *Sponsored by the 1,4-Dioxane Risk Management Consortium.*

1560 DEVELOPMENT OF WEIGHTED DISTRIBUTIONS OF REPS FOR DIOXIN-LIKE COMPOUNDS (DLCs).

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Potential health risks associated with exposure to mixtures of DLCs are currently assessed using a toxic equivalency factor (TEF) approach. TEFs are single point estimates of the relative potency (REP) of DLCs recommended by WHO based on underlying REP values that represent a heterogeneous data set ranging across several orders of magnitude. Recently, the WHO and NAS reviewed the TEF methodology and acknowledged the importance of better characterizing variability and uncertainty inherent in the TEFs and indicated that the use of distributions of REPs would provide a means of characterizing the variability and uncertainty inherent in the TEFs. Development of a framework to quantitatively weight studies based on quality and relevance to human health risk assessment is a central component of developing distributions of REPs for each congener. The aim of this investigation was to develop a transparent and reproducible weighting scheme, to apply it to the REP₂₀₀₄ database, and to evaluate the impact on the distributions. The consensus-based weighting scheme incorporates several different measures of study quality and relevance including REP derivation method and quality as well as endpoint. Multiple iterations and numerical scales were assessed to determine the impact on the REP distributions. This weighting scheme provides an approach for placing greater emphasis on those REP values that are believed to be more well suited for health risk assessment purposes. Application of the weighting scheme to PCB 126 and 2,3,4,7,8-PeCDF, the two most data-rich congeners, had very little impact on the overall distribution. Other congeners were also assessed. While the weighting scheme did not have a significant impact on the overall distributions, the use of such a scheme yields a more transparent, reproducible, and consistent method for deriving TEFs from the underlying REP data. This abstract does not represent USEPA policy.

1561 CANCER POTENCY ESTIMATE FOR 2, 3, 4, 7, 8-PENTACHLORODIOBENZO(P)DIOXIN (4-PECDF).

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A cancer slope factor was derived for 4-PeCDF using data collected from the recently published NTP bioassay in female rats. The dose-response relationship for liver tumors in rats was assessed in terms of lifetime average daily liver burden (LADLB) and body burden (LADBB), using data collected from the lifetime and stop exposure components of the bioassay. Classical pharmacokinetic (PK) and physiologically-based pharmacokinetic (PBPK) models were compared and contrasted for the purposes of characterizing tissue burdens in rats and predicting tissue burdens in humans. Variations of the multistage model, both permitting and excluding a possible threshold term (dt), were used to characterize the dose-response relationship in terms of extra risk. Probability density functions were developed for the linear term of the multistage model (q1), dt, and the dose producing a 10% increase in extra risk (ED10) to permit characterization of central tendency as well as upper and lower bounds. Information regarding the mode of action for liver tumors support a nonlinear dose-response relationship, such that the potential risks at low doses are negligible. Linear estimates of cancer potency are presented for comparison purposes. The impact of alternative decisions for each decision point in the as-

essment will be discussed. The results are compared to potency estimates based upon the WHO toxicity equivalency factor (TEF) approach for the risk assessment of dioxin-like compounds.

1562 DIVERGENT TRANSCRIPTIONAL PROFILES INDUCED BY THE AHR AGONISTS 2, 3, 7, 8-TCDD AND 2, 3, 7, 8-TCDF IN PRIMARY RAT HEPATOCYTES.

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Environmental mixtures of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and structural related polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) are regulated using the toxic equivalency factor (TEF) or relative potency (RP) approach for risk assessment. The TEF approach is based on a number of assumptions such as the effects of individual compounds are essentially additive with parallel dose response curves, which is due to identical biochemical mechanisms of action following AHR activation. In order to test this fundamental TEF assumption, primary female Sprague-Dawley rat hepatocytes were treated for 24 hours with TCDD, 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) or 2,3,7,8-tetrachlorodibenzofuran (TCDF) and the induced gene array patterns were compared. TCDD altered the maximum number of genes (190) and the expression of 68 of these genes were also altered by PeCDF and TCDF. Induced genes in common between the three chemicals included Cyp1a1, Cyp1a2, and Cyp1b1 that are core members of the so called "AHR gene battery". Agglomerative hierarchical clustering (AHC) revealed two gene superclusters with one that is largely down-regulated and one that is up-regulated. In addition, AHC analysis identified that the furans (TCDF and PeCDF) clustered independent of TCDD. Similarly, Principal Components Analysis (PCA) revealed distinct clusters for the furans versus TCDD. Functional analysis identified several pathways specifically affected by TCDD and not by the furans that included glycolysis, arginine synthesis, cell survival and apoptosis, and bile acid synthesis. These results suggest that even among "TCDD-like" compounds there are significant differences in modulation of gene expression and their activation of the AHR. These results also suggest that the some of the fundamental assumptions of the TEF model for risk assessment of PCDDs and PCDFs may not be valid.

1563 TOWARDS AN INTEGRATED PHOTO SAFETY ASSESSMENT DURING DRUG DEVELOPMENT: EVALUATION OF MECHANISM-RELATED TEST APPROACHES BROADENING THE REGULATORY TEST BATTERY.

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Photosensitization – sunlight-induced toxicity – is a common phenomenon mediated by many natural and synthetic compounds including drug substances. For drug development there are already a few well-established test methods available (e.g. UV-VIS light absorption spectra, the in vitro 3T3 Neutral Red Uptake Phototoxicity Test and the UV Local Lymph Node Assay), which are routinely used to identify potentially photosensitizing compounds. Despite the fact that the individual test systems usually provide well-defined results, there often remain gaps in the understanding of the underlying mechanisms of phototoxicity and strategies to address those. We therefore addressed some of these shortcomings in order to identify methods providing additional insight and, in consequence, allowing for a more systematic and relevant risk assessment. Using several model substances such as lomefloxacin or chlorpromazine we generated photo safety data sets and suggest the impact and relevance of individual test results for a final phototoxicity evaluation. Along with the development of a modified alkaline Photo-Comet Assay, we first investigated in different in vitro and in vivo systems the relevance of short ultraviolet sunlight radiation (UV-B), which is cytotoxic itself and therefore often avoided in test systems. In addition, using custom-made filter sets which allow a selective UV-A and/or UV-B (only) exposure, we revealed pure UV-B effects. So far, no photogenotoxic compound was identified requiring exclusively UV-B light exposure for activation. In order to narrow the gap between absorption spectrum assessment and in vitro test results, we evaluated a general photo degradation test (using spectrophotometry and LC/MS) thereby imitating a cellular environment (PBS, prototypic reaction partners). First results indicate that fragmentation reactions could be monitored, but that specific trapping reagents might be necessary in order to cover the photo reaction mechanisms in total.

1564 PERFLUORO-N-BUTYL IODIDE (PFBI): OCCUPATIONAL HEALTH HAZARD EVALUATION.

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Perfluoro-n-butyl iodide (PFBI) is a promising alternative to CFC solvents used in aircraft maintenance operations, because it has zero ozone depletion potential and low global warming properties. To assess its health hazard potential, toxicity tests were conducted including acute (4-h), 4-week and 13-week (6 h/d, 5 d/wk) inhalation (nose-only) toxicity studies in rats, acute (10-min) inhalation cardiac sensitization study in dogs, in vitro chromosomal aberrations in human lymphocytes, and in vitro mutagenic experiments in *S. typhimurium* and *E. coli*. In the 13-week study, a separate group of male rats was assigned to a 4-week recovery. PFBI showed no mutagenic or clastogenic activity. As expected, the most sensitive tissue following repeated PFBI exposure was the thyroid. LOAELs and NOAELs were selected for the inhalation studies. Mild effects, attributed to the thyroid gland only and reversible with recovery, were considered adaptive responses, while effects external to the thyroid were considered potentially adverse. Also, the toxicity profile of trifluoriodomethane (CF₃I) was reviewed to assist with the health hazard evaluation of PFBI. A concentration of 3900 ppm PFBI was a NOAEL in the cardiac sensitization study, and this value was considered appropriate as an acute or ceiling occupational exposure level, since uncertainty factors are not generally applied to the NOAEL that results from this test. For repeated exposure, a NOAEL of 1500 ppm PFBI was selected. Following a dosimetric adjustment for daily exposure time (6 h to 8 h), uncertainty factors were applied to derive an 8-hr TWA of 40 ppm PFBI. The factors of uncertainty included animal to human extrapolation (3), interhuman variability (3), and extent of database (3). In comparison, recommended acceptable exposure limits (AELs) for CF₃I were 2000 ppm (ceiling) and 150 ppm (8-hr TWA). Uncertainty factors were less in magnitude when calculating AELs for CF₃I, because of availability of toxicokinetic and reproductive toxicity data.

1565 A RETROSPECTIVE ANALYSIS OF 12 HUMAN STUDIES WITH PESTICIDES (1992 TO 2001) TO ASSESS RBC ACHE ACTIVITY AND CLINICAL SIGNS.

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Inhibition of the red blood cell (RBC) acetylcholinesterase (AChE) in humans is considered an appropriate biomarker for exposure to N-methylcarbamate (NMCs) and organophosphate (OPs) pesticides and is used in EPA's hazard assessments. Clinical signs characteristic of NMCs and OPs are expected to occur in humans only after higher levels of inhibition are attained. In this retrospective assessment, RBC AChE and clinical signs data were evaluated from 12 human studies considered to have rigorous protocols from four laboratories that assessed NMCs (3 studies) and OPs (9 studies) (some chemicals having both acute and repeat dose protocols). All laboratories used modifications of Ellman's method for AChE analysis. Clinical signs were self-reported to trained medical staff. Data from a total of 356 males and 96 females ranging in age from 18-54 years were included and nearly all were Caucasian. Mean RBC AChE control values (IU/L) ranged from 8427 ± 1039 to 12493 ± 1176 for males and from 8576 ± 556 to 12475 ± 1808 for females. Data were evaluated based on: 1) controls (111 males and 36 females); 2) dosed subjects judged to be not showing RBC AChE inhibition (204 males and 46 females); and 3) dosed subjects judged to be showing RBC AChE inhibition; i.e., consistent depression (>10%) at critical times. For each group, the variability in AChE activity over time (predosing through postdosing) was identified. RBC AChE inhibition did not exceed 50% in any study. Evidence of RBC AChE inhibition corresponding with onset of clinical signs were judged to be occurring in only two males in two studies (both with NMCs). Clinical signs from these individuals consisted of one incident each of sweating and headache. This abstract does not necessarily reflect the views or policy of the USEPA.

1566 ACUTE EXPOSURE GUIDELINE LEVELS (AEGLS) FOR CROTONALDEHYDE.

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Crotonaldehyde (>95% trans and ≤ 5% cis isomer) is a potent eye, skin, and respiratory irritant. AEGls are airborne concentrations above which toxic effects of specified severity could occur in humans from one-time exposure for 10 min to 8 hrs: AEGL-1 (discomfort), AEGL-2 (disability), and AEGL-3 (death). AEGL-1 values were based on a study where workers exposed to 0.56 ppm for < 8 hrs had occasional minor eye irritation. An intraspecies uncertainty factor (UF) of 3 was used because eye irritation is a direct surface-contact effect not subject to pharmacokinetic variability. The resulting AEGL-1 value of 0.19 ppm was adopted for 10 min to 8 hrs because minor eye irritation is not expected to vary greatly over time. AEGL-2 values were based on a rat study where exposure to >8000 ppm-min

caused bronchiole proliferative lesions. AEGL-2 values were calculated by dividing 8000 ppm-min by 10, 30, 60, 240, or 480 min (concentration and time appeared equally important for toxicity). An interspecies UF of 10 was applied (actual exposure concentration and time were unknown and there was little supporting data), and an intraspecies UF of 3 (a greater UF yields 4 and 8-hr concentrations causing only mild irritation), yielding AEGL-2 of 27 to 0.56 ppm for 10 min to 8 hrs. AEGL-3s were derived from a rat LC₅₀ study, using calculated LC₀₁ values for 10, 30, 60, and 240 min, and scaling the 8-hr value from 240 minutes using $C^{1.2} \times t = k$. AEGL-3s of 44 to 1.5 ppm for 10 min to 8 hrs were obtained after use of an interspecies UF of 3 (LC₅₀ values for rats, mice, and guinea pigs were within a factor of 2.5), and an intraspecies UF of 3 (great human variability is unlikely given the homogeneity of the animal data, and a larger UF yields 8-hr concentrations causing only mild irritation). A preliminary cancer assessment indicated that a 10⁻⁴ excess cancer risk was associated with crotonaldehyde concentrations 25-fold greater than the toxicity-based AEGL-2 values. [This abstract presents Interim AEGL values that are subject to change pending further review.] ORNL is managed by UT-Battelle LLC for the US Department of Energy under contract DEAC0500OR22725.

1567 RISK ASSESSMENT FOR EXPOSURES TO PCBs FROM INTERIOR BUILDING SURFACES.

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As a consequence of the widespread historical use of polychlorinated biphenyls (PCBs) in industry, many older industrial sites have low level PCB contamination. We recently conducted a baseline risk assessment for an industrial property which considered health risks of low level PCB contamination on interior building surfaces. Testing revealed that wood and concrete floors in the building had low levels (<0.03 to 4 µg/sq cm) of PCBs. In many locations, these floors were covered with carpeting and padding, providing a potential barrier to exposure. In other locations, the concrete and wood floors were exposed. Air and surface dust sampling of work surfaces indicated nearly all samples were below analytical detection limits. Roller sampling of carpets also indicated no detectable PCBs. Potential worker exposure to exposed subfloors therefore constituted the only complete exposure pathway. In order to estimate baseline risks prior to any remedial activity, we evaluated potential on-site worker exposures to interior flooring using site-specific worker activity data as well as highly conservative and uncertain estimates of adult hand-to-floor and hand-to-mouth contact. We found that estimated cancer risks for a few job categories exceeded the 1E-4 cancer risk level, the typical upper bound acceptable risk for occupational scenarios. These elevated risks were due to potential worker exposures in a few high level locations. Estimated cancer risks for other job categories were in the 1E-5 and 1E-6 range. Interestingly, risk estimates developed with the OSHA PEL for PCBs as the exposure concentration were in the 1E-2 cancer risk range, clearly indicating a discrepancy between the OSHA and EPA approaches. Based on our initial work, spot remediation is being conducted to reduce average PCB concentrations to acceptable risk-based levels. Our experience in this case highlights the need for additional data to support realistic models for assessing risks of direct contact with chemicals on floors or other surfaces.

1568 CANDIDATE BENCHMARK BLOOD-LEAD AND BONE- LEAD CONCENTRATIONS.

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The purpose of this work was to identify candidate points of departure for development of a reference blood-lead or bone-lead concentration that can be used as a measure of acceptable lead exposure. A literature review was conducted to identify the most sensitive endpoints, sub-populations and life stages of inorganic lead exposure. Because a threshold of effects was not clearly identified for key endpoints, points of departure were based on a benchmark dose approach. Exposure was quantified as a biomarker of exposure and, therefore, the potential benchmarks are expressed as blood-lead or bone-lead concentrations. Decreased intelligence in children and increased blood pressure in adults were selected as the key endpoints. Canadian population data were used to define baseline distributions of blood pressure and Coronary Heart Disease (CHD) related mortality. Gender specific linear regression coefficients relating blood-lead and bone-lead concentrations to systolic blood-pressure (SBP) were used to estimate increased rates of hypertension (SBP > 140 mm Hg) associated with lead exposure. Relationships reported in the Framingham Heart Study were used to predict the incidence of CHD related mortality associated with increased rates of hypertension. A benchmark response of a 10⁻⁵ incremental cumulative CHD related mortality risk over the age span of 35-75 years was found to be associated with an incremental increase in blood-lead of 2 µg/dL and 9 µg/dL for males and females, respectively, and an incremental increase in bone-lead of 6 µg/g for males. Adjustments to these benchmark doses to account for sensitive sub-populations may be appropriate. Increased incidence of mild men-

tal retardation (MMR) was used as the benchmark response for neurocognitive effects in children. A benchmark dose low (BMDL₁₀) of 1 µg/dL corresponding to a 10% increase in cases of MMR was estimated from a recently published pooled analysis of longitudinal studies relating children's blood-lead concentrations to IQ. This work has identified age-specific benchmark concentrations that may be used to set acceptable exposure limits for lead.

1569 CARCINOGENICITY EVALUATION OF METHYL TERT-BUTYL ETHER (MTBE).

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MTBE is a gasoline additive, which has been found in ambient air and drinking water. In 1997, US EPA set levels of MTBE in drinking water to protect against taste and odor, and health effects, including cancer. In 2005 US EPA published new cancer assessment guidelines, which we used in conducting an in-depth analysis of tumors reported in rodents exposed to MTBE (i.e., leukemia/lymphoma, Leydig cell tumors (LCTs), kidney tumors, and liver tumors). We evaluated their relevance to humans, by comparing background rates to historical control rates (leukemia/lymphoma), conducting statistical analyses to take longevity into account (LCTs), using mechanistic information (kidney), and evaluating the results of genotoxicity testing.

At one laboratory, MTBE exposure was associated with excess lymphoma/leukemia in female Sprague-Dawley (S-D) rats. We identified several factors that weaken the relevance of these results: rates of lymphoma/leukemia were consistent with the historical background rate at that laboratory; there is no plausible mode of action given that MTBE is not genotoxic; the lymphoma/leukemia was found primarily in lungs (not in lymphatic tissue), and finally the excess could be a result of severe chronic respiratory disease found in the rat colony.

This laboratory also reported an increased prevalence of LCTs in treated vs. control S-D rats, but our statistical analysis (Poly-K) demonstrated that this increase was not significant when survival of control vs. treated rats was taken into account. In addition, the kidney cancer observed in rats exposed to MTBE is likely related to α 2u-globulin, a mechanism widely acknowledged to be irrelevant to humans, while liver tumors in female mice (in Chun et al., 1992) were not considered by the authors to support a carcinogenic effect.

Although positive findings were reported in animal bioassays with MTBE, they are either not reliable (i.e., leukemia/lymphoma, LCTs, liver cancer), or likely irrelevant to humans (i.e., kidney cancer). Further, MTBE is not recognized to be genotoxic. Hence, we conclude that MTBE is unlikely to be carcinogenic to humans.

1570 NOVEL PROTEOMIC APPROACH TO MAPPING SIGNALING PATHWAYS INDUCED BY SPECIFIC ISOMERS OF METHYLATED ANTHRACENES.

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Inhibition of gap junctional intercellular communication (GJIC) and activation of mitogen activated protein kinase (MAPK) are involved in tumor promotion. We previously determined that the 1-methyl but not the 2-methyl isomer of anthracene (1-MeA, 2-MeA) induced the release of arachidonic acid, inhibited GJIC and activated MAPKs using an in vitro rat liver epithelial cell model system. Inhibition of GJIC by 1-MeA is dependent on phosphatidylcholine specific phospholipase C (PC-PLC). Our current results directly measured PC-PLC and sphingomyelinase resulting in 1-MeA activating only PC-PLC, while 2-MeA had no effect on either enzyme. To provide further insight into the early upstream biochemical signaling events of 1-MeA induced inhibition of GJIC and MAPK, we demonstrate the suitability of advanced proteomics techniques. Cells were exposed to 1- or 2-MeA for 5 min and the cytoplasmic proteins were separated from those associated with membranes and then fractionated using Invitrogen ZOOM® isoelectric fractionator with pI values ranging within 1 pH unit affording greater resolution than traditional 2-D gels. Proteins affiliated with metabolism-, transcription, translation, cytoskeleton-trafficking and numerous signaling proteins were among those activated by 1-MeA as identified by mass spectrometry. Quantitative changes were further revealed by SILAC (a novel mass spectroscopy technique of Stable Isotope Labeling with Amino acids in Cell culture). Our study demonstrates advantages of recent proteomics techniques for detailed investigations of rapid and early signaling events that play an important role in tumor promotion processes such as inhibition of GJIC and activation of MAPKs. Support: NIEHS grants #R56 ES013268-01A2 and #R01 ES013268-01A2 to BLU.

1571 INVOLVEMENT OF I-KAPPA B KINASE IN ANILINE-INDUCED ACTIVATION OF TRANSCRIPTION FACTOR NF-KAPPA B IN RAT SPLEEN.

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Aniline exposure is associated with selective toxicity to the spleen leading to a variety of sarcomas, and fibrosis appears to be an important preneoplastic lesion. Earlier we have shown that aniline exposure in rats leads to oxidative stress and overexpression of profibrogenic cytokines in the spleen. We hypothesize that aniline-induced oxidative stress leads to transcriptional upregulation of fibrogenic cytokines via activation of redox-sensitive transcription factor NF-kappaB. To determine the activation of NF-kappaB, male SD rats were treated with aniline (1 mmol/kg/day via gavage) for 7 days, an experimental condition that precedes the induction of fibrosis. NF-kappaB activation was determined in the nuclear extracts (NE) of splenocytes using an ELISA specific for NF-kappaB p65, which showed a significant increase in NF-kappaB p65 binding activity in the NE from aniline-treated rats. Also, Western blot analysis showed increased phosphorylation of p65 subunit of NF-kappaB in the aniline-treated rats. To determine if NF-kappaB activation could be mediated through I-kappaB kinase (IKK) activation, phosphorylation of IKK was studied. Splenocytes from aniline-exposed rats showed increased phosphorylation of IKK. These results show that aniline exposure leads to an activation of NF-kappaB, which could be mediated through IKK pathway. Supported by NIH ES06476.

1572 THIOREDOXIN REDUCTASE IS REQUIRED FOR MAXIMAL NF- κ B-MEDIATED GENE EXPRESSION.

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The transcription factor NFkB mediates the expression of genes involved in inflammation and cell survival. NFkB is sensitive to redox regulation at multiple levels, and thioredoxin (Trx1) plays a major role in this regulation. Although thioredoxin reductase (TR1) is responsible for maintaining Trx1 in the active form, it is not clear how inactivation of TR1 by reactive electrophiles would affect NFkB activity. In the present study, we examined NFkB activation in HeLa cells stimulated with tumor necrosis factor- α (TNF) and co-treated with either curcumin or 1-chloro-2,4-dinitrobenzene (CDNB), electrophiles known to inhibit TR1 through covalent modification of its active site. At concentrations that inhibited TR1 activity by 95%, curcumin and CDBN completely blocked TNF-induced expression of an NFkB-dependent luciferase reporter construct. To determine the mechanism by which curcumin and CDBN inhibited the NFkB pathway, we measured degradation of the inhibitory protein I κ B- α by western blot, nuclear translocation of the NFkB subunits p50 and p65 by western blot of cytosolic and nuclear fractions, DNA binding activity of nuclear NFkB by gel shift analysis and subunit composition of the DNA-binding heterodimer by supershift analysis. The results showed that neither curcumin nor CDBN had any effect on these upstream processes, demonstrating that these electrophiles were not binding to known nucleophilic sites within IKK (which would have inhibited I κ B- α degradation) or p50 (which would have inhibited DNA binding activity). The role of TR1 inhibition was confirmed by repeating these analyses in cells in which 95% of the TR1 protein was depleted by siRNA. TNF-induced I κ B- α degradation and p50/p65 nuclear translocation were unaffected by TR1 siRNA, yet the NFkB reporter construct was inhibited by 35%. We conclude that TR1 is necessary for maximal transcriptional activity of NFkB in response to TNF, and that the toxicity of electrophiles that target TR1 may be influenced by inactivation of NFkB.

1573 SUPPRESSION OF TNF- α -INDUCED EXPRESSION OF CELL ADHESION MOLECULES BY THE COFFEE DITERPENE KAHWEOL VIA INHIBITION OF JAK2-PI3K/AKT-NF- κ B ACTIVATION PATHWAY.

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Endothelial cells produce adhesion molecules after being stimulated with various inflammatory cytokines. These adhesion molecules play an important role in the development of atherosclerosis. Recent studies have highlighted the chemoprotective and anti-inflammatory effects of kahweol, a coffee-specific diterpene. This study examined the effects of kahweol on the cytokine-induced monocyte/human endothelial cell interaction, which is a crucial early event in atherosclerosis. Kahweol inhibited the adhesion of TNF α -induced monocytes to endothelial cells and suppressed the TNF α -induced protein and mRNA expression of the cell adhesion molecules, VCAM-1 and ICAM-1. Furthermore, kahweol inhibited the TNF α -induced JAK2-PI3K/Akt-NF- κ B activation pathway in these cells. Overall, kahweol

has anti-inflammatory and anti-atherosclerotic activities, which occurs partly by down-regulating the pathway that affects the expression and interaction of the cell adhesion molecules on endothelial cells.

1574 PPAR γ -INDEPENDENT MECHANISMS REGULATING THE ACTIVATION OF SRC KINASE BY THIAZOLIDINEDIONES.

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Thiazolidinediones (TZDs) are used clinically in the treatment of type II diabetes and are synthetic ligands for the PPAR γ , but also elicit PPAR γ -independent effects, most notably activation of MAPKs. Following ciglitazone treatment Src kinase mediates EGFR transactivation, leading to activation of the MAPK, Erk; however, mechanisms involved in Src activation by TZDs are not fully understood. In this study both ciglitazone and troglitazone, induced rapid activation of Src kinase in the rat liver epithelial cell line, GN4. We observed an increase in Src Tyr416 phosphorylation that was accompanied by dephosphorylation of Tyr527. Changes in Src phosphorylation were observed with TZD derivative compounds which lack the ability to activate the PPAR γ . Inhibition of EGFR kinase had no effect on Src activation following TZD treatment. Interestingly, inhibition of Pyk2, a calcium-dependent tyrosine kinase that has been shown to mediate Src activation and is activated in by TZD treatment, was not required for TZD-induced Src activation. However, the Src kinase inhibitor PP2, but not the inactive analogue PP3, prevented Pyk2 phosphorylation following TZD exposure. Alterations in intracellular calcium concentrations have been shown to activate both Pyk2 and Src. Intracellular calcium chelation using BAPTA-AM prevented Src Tyr416 phosphorylation but had little effect on TZD-induced Src Tyr527 phosphorylation loss. Pretreatment of cells with pervanadate, a general protein tyrosine phosphatase inhibitor completely blocked TZD-induced Src activation and Tyr527 dephosphorylation. Our data demonstrate that ciglitazone and troglitazone activate Src kinase independent of PPAR γ and EGFR leading to subsequent Pyk2 activation. Furthermore, our data suggest that TZD-induced Src autophosphorylation, an event sensitive to intracellular calcium chelation is modulated by protein tyrosine phosphatase-dependent dephosphorylation of Src Tyr527.

1575 EARLY GROWTH RESPONSE GENE 1 (EGR-1) MEDIATES DEOXYNIVALENOL-INDUCED INTERLEUKIN-8 PRODUCTION IN HUMAN INTESTINAL EPITHELIAL CELLS.

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Deoxynivalenol (DON) and other trichothecene mycotoxins mediate a broad range of mucosal toxic and inflammatory effects on the human gastrointestinal tissues. The purpose of this study was to test the hypothesis that DON induces the pro-inflammatory cytokine interleukin-8 expression in human intestinal epithelial cells and that this is regulated at the level of immediate activated signaling molecule, early growth response gene 1 (EGR-1). Exposure of the human epithelial cells Intestine-407, HT-29 as well as HEK293 cells to DON markedly enhanced the production of interleukin-8 protein and mRNA. After acute exposure of human intestinal epithelial cells and mouse small intestinal tissues to DON, EGR-1 expression was markedly elevated along with extracellular, signal regulated protein kinases 1 and 2 (ERK1/2) mitogen-activated protein kinase (MAPK). ERK1/2 inhibition decreased both EGR-1 and interleukin-8 expression and promoter activity. Moreover, when EGR-1 was blocked with small interference RNA (siRNA), DON-induced interleukin-8 expression (protein and mRNA) and promoter activity were significantly suppressed in the human intestinal epithelial cells. Taken all, DON-induced EGR-1 via ERK1/2 MAPK mediated interleukin-8 production in the human intestinal epithelial cells, which provide insight into the possible general mechanism associated with mucosal toxic inflammation by trichothecene mycotoxin deoxynivalenol from food. (This work was supported by the Korean Research Foundation Grant funded by the Korean government (MOEHRD) (KRF-2006-312-E00163))

1576 CONSTITUTIVELY ACTIVE B-RAF COMPLEXES WITH 14-3-3 ISOFORMS AND RAF-1 IN TSC-2 NULL-CELLS.

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2,3,5-Tris-(glutathion-S-yl) hydroquinone (TGHQ), a hepatic metabolite of hydroquinone, is a renal carcinogen in Eker rats. Treatment of Eker rats with TGHQ results in loss of the wild-type allele of the tumor suppressor tuberous sclerosis-2 (TSC-2) in renal preneoplastic lesions and tumors. The development of these renal tumors is thought to be a consequence of disruption of the TSC-2/TSC-1 complex, which is a known inhibitor of TOR signaling. The tumor suppressor activity of TSC-2/TSC-1 may also negatively regulate the B-RAF/ERK/MAPK cascade.

TGHQ transformed cell lines from Eker rat proximal tubule cells (QTRRE cells) are null for TSC-2 and express constitutively active cytosolic B-Raf kinase, in conjugation with the constitutive phosphorylation of amino acid residues S496 and S603 (LC-MS/MS). In wild-type proximal tubule cells 14-3-3 isoforms are bound to TSC-2 at its AKT activation site. Coimmunoprecipitation revealed that within the cytosol of QTRRE cells, the active B-Raf forms a trimeric protein complex with Raf-1 and 14-3-3 isoforms β , γ , and τ . The formation of the 14-3-3/B-Raf/Raf-1 protein complex is consistent with increased ERK1/2 activity in QTRRE cells, as shown by Western blot analysis. Use of the Raf kinase inhibitor BAY 43-9006 reveals decreased activity of ERK1/2 and decreased expression of cyclin D1. Immunohistochemical analysis of tumors formed following 8-months of TGHQ treatment in Eker rats showed that cyclin D1 is overexpressed in proximal tubules surrounding tumors. Cyclin D1 is a key regulator of G1 to S phase progression in the mammalian cell cycle. Therefore, we suggest that the 14-3-3/B-Raf/Raf-1 protein complex is driving the increased expression of cyclin D1 during renal proximal tubule tumor formation. Further mechanistic studies of the 14-3-3/B-Raf/Raf-1 protein trimer may elucidate the mechanisms of proximal tubule proliferation leading to renal tumor formation. (GM39338, ES06694)

1577 REACTIVE OXYGEN SPECIES: INTEGRAL COMPONENTS OF B CELL ACTIVATION.

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The production of immunoglobulins (Ig) by activated B cells is a critical component of the humoral immune response. Ig production is mediated in part by a complex transcriptional regulation of the Ig heavy chain (IgH) gene. A component of this regulation involves binding of various transcription factors including the family of NF- κ B proteins to a regulatory region 3' of the IgH gene, termed the 3'IgH Regulatory Region (3'IgH RR). Activation of lymphocytes by lipopolysaccharide (LPS) has been shown to increase the production of reactive oxygen species (ROS) which are cysteine oxidizing agents known to induce tyrosine kinase activity and subsequent nuclear translocation of transcription factors. The NADPH Oxidase complex has been shown to be an intracellular source of ROS in response to LPS activation of lymphocytes. This study proposes that the production of reactive oxygen species plays a significant role in LPS-mediated Ig production in B cells perhaps by modulating the activity of the 3'IgH RR. Utilizing the CH12.LX B cell line, the increased production of ROS in response to LPS activation was detected by DCFDA fluorescence analysis. Pre-treatment with ROS scavenging molecules was found to decrease ROS levels in LPS activated cells. A transiently transfected transcriptional luciferase reporter driven by the 3'IgH RR and a variable heavy chain promoter, was utilized to show that LPS-activated B cells pre-treated with anti-oxidants decreased LPS-induced activation of the 3'IgH RR reporter construct. Pre-treatment of CH12.LX cells with NADPH Oxidase antagonists similarly decreased the activation of the 3'IgH RR reporter construct. These observations suggest that 1) the LPS-induced activation of B cells results in the production of ROS 2) the production of ROS is in part mediated by NADPH Oxidase, and 3) the production of ROS leads to the modulation of the 3'IgH RR. (Supported by the Wright State University Boonshoft School of Medicine).

1578 TRANSCRIPTIONAL RESPONSE TO OXIDATIVE STRESS IN EMBRYOS EXPOSED TO TBHQ OR TCDD.

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Oxidative stress resulting from environmental exposures has been implicated in a variety of human diseases ranging from chemical teratogenesis to cardiovascular and neurodegenerative diseases. Developing animals are especially sensitive to chemicals causing oxidative stress. The developmental expression and inducibility of anti-oxidant defenses through activation of NRF2 affects susceptibility to oxidants at these early life stages, but the embryonic response to oxidants is not well understood. The zebrafish (*Danio rerio*) is a valuable model in developmental toxicology. To assess the early response to chemical treatment occurring at different stages of development, zebrafish embryos at 1, 2, 3, 4, 5, and 6 days post fertilization (dpf) were exposed to DMSO (0.1%), TCDD (2 nM), or tBHQ (10 μ M) for 6 hr. Transcript abundance was assessed for 4 embryo pools per compound per time point by real-time qRT-PCR and microarray (Agilent 22k). Analysis by qRT-PCR showed strong (4- to 5-fold) induction of gstp1 by tBHQ as early as 1 dpf. tBHQ also induced gclc (2 dpf), but not sod1, nqo1, or cyp1a. TCDD induced cyp1a but none of the other genes. In 4-dpf embryos, microarray analysis revealed a large number of genes induced by tBHQ, including genes involved in glutathione synthesis and utilization (gstp1, gclc, gclm, ggt) as well as genes with roles in signal transduction (fos, jun, C/EBP β , cox-2) and DNA damage and stress response (hsp70, gadd45b, atf3). In contrast to results in mammals, there was no induction of sod1, nqo1, or heme oxygenases by tBHQ in these 4-dpf embryos. TCDD induced a more limited number of genes, with cyp1a and mpx being the most highly

induced. Few genes with detectable down-regulation were observed. These data show that zebrafish embryos are responsive to oxidative stress as early as 1 dpf, and that microarrays are capable of detecting altered expression of known and novel oxidant-responsive genes in whole embryos.
[R01ES006272 and Smith chair]

1579 HYPOCHLOROUS ACID ACTIVATES TRANSCRIPTION FACTOR NRF2-MEDIATED ANTIOXIDANT RESPONSE IN RAW 264.7 MACROPHAGES.

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Hypochlorous acid (HOCl) is a potent oxidant and important in the pathogenesis of many diseases. Transcription factor NF-E2-related factor 2 (Nrf2)-mediated antioxidant response represents a critical cellular defense mechanism that serves to maintain intracellular redox homeostasis and limit oxidative damage. In the present study, the effect of HOCl on Nrf2 activation was investigated in RAW 264.7 mouse macrophages. Exposure of RAW cells to NaOCl solution (initial concentrations up to 2 mM) resulted in a time- and dose-dependent increase in nuclear accumulation of Nrf2 and expression of Nrf2 target genes, which include NAD(P)H: quinone oxidoreductase 1 (NQO1), heme oxygenase-1, glutamate cysteine ligase (GCL) and GSH synthetase (GS), etc. Additionally, intracellular GSH, which is the prime scavenger for HOCl, decreased by 34% within the first hour of HOCl exposure. The decline was followed by a GSH rebound that surpassed the initial basal levels by up to 4 fold. This reversal in GSH levels closely correlated with the gene expression profile of GCL and GS: key enzymes in GSH synthesis. To study the mechanisms of Nrf2 activation in response to HOCl exposure, we examined the effects of several ROS scavengers and redox modifiers on the HOCl-induced Nrf2 activation. Pretreatment with cell permeable PEG-catalase (3300 U/ml), which reduces cellular H₂O₂ levels, markedly reduced expression of NQO-1 and GCL under basal and HOCl challenge conditions. A similar inhibitory effect was also observed by pretreating the cells with thiol compounds (N-acetyl-L-cysteine or GSH-monoethyl ester). These results indicate that H₂O₂ and/or depletion of intracellular reduced thiol are important for HOCl-induced Nrf2 activation. Taken together, we provide direct evidence that HOCl activates the Nrf2-mediated antioxidant response in mouse macrophages and likely underlies respiratory tract responses to chlorine which is hydrolyzed to HOCl upon absorption into tissues.

1580 KEAP1 CONFERS POST-INDUCTION REPRESSION OF AN ANTIOXIDANT RESPONSE BY ESCORTING NUCLEAR EXPORT OF NRF2.

Z. Sun, S. Zhang and D. D. Zhang. *University of Arizona, Tucson, AZ.*

The transcription factor Nrf2 has emerged as a critical factor in regulating cellular redox homeostasis. Through the antioxidant responsive element (ARE), Nrf2 regulates expression of an array of genes that encode antioxidants, detoxification enzymes, and cellular defensive proteins. Activity of Nrf2 is negatively modulated by Keap1. Recently, Keap1 has been identified as a substrate adaptor protein for a Cul3-containing E3 ubiquitin ligase. Under redox balanced conditions, Keap1 recruits Nrf2 into the multi-unit E3 ubiquitin ligase complex for ubiquitin conjugation and subsequent proteasomal degradation. Oxidative stress signal triggers activation of Nrf2 through inhibition of the Cul3-containing E3 ubiquitin ligase activity, which results in increased protein levels of Nrf2, facilitated nuclear import of Nrf2, and transcriptional activation of Nrf2-dependent genes. It is a well-accepted notion that Keap1 is a molecular switch that turns on and off the Nrf2 signaling pathway according to intracellular redox conditions. Although how the Nrf2 signaling pathway is turned on by oxidative stress is well studied, mechanisms of post-induction repression are still unclear. In this study, we have identified Keap1 as a post-induction repressor of Nrf2. When cellular redox homeostasis is reached again after oxidative stress, Keap1 translocates into the nucleus where it binds Nrf2 to dissociate Nrf2 from ARE. The Nrf2-Keap1 heterodimer is then transported out of the nucleus by the nuclear export sequence located in the Keap1 protein. Once in the cytoplasm, Keap1-Nrf2 dimer associates with the Cul3-containing E3 ubiquitin ligase, resulting in degradation of Nrf2 and inactivation of the Nrf2 signaling pathway.
(NIEHS 1R01ES015010-01)

1581 ARSENITE REGULATES TRANSCRIPTION FACTOR EXCHANGE AT DISTAL REGULATORY ELEMENTS OF HEME OXYGENASE-1: INTERCHANGE OF REPRESSIVE BACH1 FOR ACTIVATING NRF2.

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Arsenic is a major environmental contaminant that triggers widespread alterations in gene transcription, particularly of genes regarded as part of the antioxidant response battery, such as heme oxygenase-1 (HMOX1). Increased antioxidant gene

expression mitigates oxidative damage through induction of protective enzyme systems and prevents accumulation of oxidatively modified proteins through increased expression of heat shock proteins. Nrf2 (NF-E2 related-factor 2) is well established as the transcriptional activator of antioxidant response element (ARE)-regulated genes; however, little is known regarding the function and regulation of its repressive counterpart Bach1 (BTB and CNC homolog 1). Bach1 has been characterized as a heme-sensitive repressor involved in the regulation of β -globin genes during erythroid cell differentiation through its interaction with NF-E2/Maf recognition elements; similarly, Bach1 interacts with the closely related ARE motif in cells naïve to oxidative stress. It is thought that in order for Nrf2 to bind ARE motifs, Bach1 must first be released. Here it is demonstrated that while prooxidants, such as arsenite, trigger activation of the transcription factor Nrf2, they also rapidly inactivate Bach1. In a manner opposite that of Nrf2, inactivation of Bach1 is characterized by its rapid export from the nucleus to the cytosol. In chromatin immunoprecipitation experiments, Bach1 appears to be released from multiple distal HMOX1 AREs more rapidly and at lower arsenite concentrations than are associated with Nrf2 activation. In this respect, Bach1 inactivation is a more sensitive indicator of arsenic mediated oxidative stress than is Nrf2 activation. Since Bach1 interacts with at least two types of Maf recognition elements (NF-E2 and Nrf2), these data suggest the possibility that arsenite could trigger inappropriate expression of Maf-regulated genes through inactivation of the transcriptional repressor Bach1. (Supported by NIEHS P42 ES04908 and R01 ES010807)

1582 CYANIDE INDUCED HIF-1 α ACCUMULATION LEADS TO BNIP3 ACTIVATION VIA A REDOX-MEDIATED PATHWAY.

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Human exposure to cyanide continues to be a significant human toxicological problem and the response to cyanide also has served as a model to study the molecular basis of chemical-mediated neurodegeneration and selective vulnerability of the nervous system. In this study, an immortalized dopaminergic cell line, Mes23.5 was exposed to KCN to assess the extent and mode of cell death. Cyanide-treated cells exhibited a time and concentration dependent apoptotic cell death as demonstrated by the MTT assay and Hoechst 33258 staining. Cyanide induced a rapid burst of intracellular reactive oxygen species (ROS) generation, followed by p38 mitogen-activated protein kinase (MAPK) activation and accumulation of hypoxia-inducible factor-1 α (HIF-1 α). Pretreatment with antioxidants N-acetyl-L-cysteine (NAC), catalase or the p38MAPK inhibitor, SB203580 markedly attenuated p38MAPK activation and induction of HIF-1 α . This was followed by BNIP3 (Bcl-2/adonovirus E1B 19kDa interacting protein 3) upregulation at both the mRNA and protein levels. BNIP3 upregulation was reduced by HIF-1 α silencing with HIF-1 α siRNA. The role of enhanced BNIP3 levels in the cell death was determined by transiently transfecting cells with BNIP3 cDNA. Forced overexpression of BNIP3 produced mitochondrial dysfunction and a caspase-independent apoptosis. Moreover, overexpression of BNIP3 sensitized the cells to cyanide toxicity. Expression of a dominant negative BNIP3 mutant or BNIP3 siRNA knockdown significantly protected cells from cyanide. It was concluded that cyanide induced HIF-1 α accumulation via a redox-mediated pathway, followed by HIF-1 α -dependent BNIP3 transcription. The induction of BNIP3 expression then served as an initiator of mitochondrial-mediated apoptotic death. (Supported in part by NIH grant ES04140).

1583 THE HYPOXIA RESPONSE ELEMENT IN THE DEC1 PROMOTER SUPPORTS BOTH TRANSACTIVATION AND REPRESSION.

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DEC1 (differentially expressed in chondrocytes-1) belongs to a structurally distinct class of basic helix-loop-helix transcription factors. The expression of DEC1 is markedly increased in cells that undergo hypoxia. The proximal promoter of the DEC1 gene has two important response elements: an E-box and an HRE (hypoxia response element). The HRE supports the transactivation by hypoxia inducible factor-1 α (HIF1 α), whereas the E-box supports DEC1 autorepression. It was therefore, hypothesized that deletion of the E-box increases hypoxia response. In order to test this hypothesis, a DEC1 promoter reporter was subjected to site-directed mutagenesis to selectively disrupt the E-box. The resultant mutant reporter was tested for the responsiveness to DEC1, HIF1 α or in combination. Consistent with the hypothesis, the mutant reporter exhibited higher responsiveness to HIF1 α . Surprisingly, the mutant reporter retained the ability to respond to DEC1-autorepression. Similar repression was detected with an element reporter made of three copies of the HRE, and the repression was abolished by HIF1 α co-transfection. These findings suggest that the HRE supports both repression and activation depending on the cellular conditions (normoxic and hypoxic).

1584 EARLY GROWTH RESPONSE GENE 1 (EGR-1) VIA ERK SIGNALING PATHWAY ATTENUATES RIBOTOXIC STRESS-INDUCED CYTOTOXICITY.

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Sponsor: T. Jeong.

Endoplasmic Reticulum (ER) is the major signal transducing organelle that senses toxic insults and responds to changes of the homeostasis as a stress sentinel. Ribotoxic stress response can mediate cellular defense and repairing during the toxic insults to mucosal epithelium. The purpose of this study was to test the hypothesis that ribotoxic stresses by the translational inhibitors induce the early growth response gene product 1 (EGR-1) expression in human intestinal epithelial cells, which can play the defensive role in the gastrointestinal cytotoxicity. Exposure of the human epithelial cells Intestine-407, HT-29, HCT-116 to the translational inhibitors (cycloheximide, anisomycin and deoxynivalenol) markedly enhanced the production of EGR-1 production. Moreover, ribotoxic deoxynivalenol enhanced EGR-1 expression in the mouse small intestine. When signaling pathways were blocked with each mitogen-activated protein kinase (MAPK) inhibitors (SP600125, U0126, and SB203580), only the inhibition of extracellular, signal regulated protein kinases 1 and 2 (ERK1/2) suppressed toxin-induced EGR-1 expression. Generally, high levels of ER stresses by translational inhibitors are considered to cause the apoptotic cell death. However, suppression of EGR-1 expression by small interference RNA (siRNA) or ERK inhibition reduced the ribotoxin-induced intestinal epithelial cell death. Taken together, ribotoxic stresses enhance EGR-1 expression and ERK phosphorylation in the intestinal epithelial cells, which in turn resulted in the attenuation of the apoptotic cell death in the epithelial cells. These data provide insight into possible general mechanisms by which EGR-1 mediates the protective response to the ribotoxic stress in the gastrointestinal tract. (This work was supported by grant No. (R01-2006-000-10564-0) from the Basic Research Program of the Korea Science & Engineering Foundation.)

1585 CHRONIC ALCOHOL CONSUMPTION INDUCES TRB3 AND DISRUPTS INSULIN SIGNALING THROUGH INCREASED ER STRESS.

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Prospective cohort studies have shown that chronic and excessive alcohol consumption is an important and modifiable risk factor for type 2 diabetes. Alcohol consumption alters insulin signaling, but the molecular mechanisms underlying this effect are not well understood. We previously reported that chronic and excessive alcohol intake disrupts insulin signaling via alcohol-induced TRB3 (a negative effector of Akt) in rats. In this report, we provide evidence that alcohol-induced TRB3 effects are associated with increased ER stress. ER stress in FGC4 cells was blocked by 4-phenyl butyric acid and taurine-ursodeoxycholic acid ($p < 0.05$) and this was associated with a lower alcohol-induced increase in TRB3 ($p < 0.05$). In vivo treatment with diallyl sulfide abolished hepatic ER stress ($p < 0.05$), blocked alcohol's effect on TRB3 ($p < 0.05$) and restored insulin signaling. Alcohol induced CYP2E1 and this was blocked by diallyl sulfide. Our results suggest that enhancement of ER stress is a potential mechanism by which alcohol induces TRB3 and inhibits insulin signaling. CYP2E1 may mediate this alcohol effect. Furthermore, our results may have therapeutic implications for treatment of alcohol-associated diabetes. (Supported by NIAAA008645 TMB)

1586 EFFECT OF METALLOTHIONEIN ON MATRIX METALLOPROTEINASE 2 AND 9 GENE EXPRESSION.

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Recent studies show that metallothionein (MT), a cytosolic protein in resting cells, can be translocated transiently to the cell nucleus during cell proliferation and differentiation. These results indicate that MT may be associated with nuclear functions.

We previously reported that depressions of matrix metalloproteinase (MMP) 2 gene expression and activity were observed in the immortalized MT-null fibroblast cells, suggesting MT might regulate MMP2 gene expression. In present study, to further confirm the involvement of MT in the regulation of MMP2 gene expression we studied the changes in MMP2 gene expression by using wild-type and MT-null pri-

mary mouse fibroblast. Wild-type and MT-null mouse primary fibroblasts were obtained from infant mouse skin (1 day old). The primary fibroblasts were grown to 70-80% confluence and then cells were collected and isolated total RNA for RT-PCR of MMP2. MMP2 gene expression in both of cells was same level. The result showed that MT is not essential for MMP2 gene expression in primary fibroblast. These findings directly demonstrate a cooperative interaction of MT on MMP2 gene expression with aberrant gene expression induced by SV40 large T-antigen. In addition, the involvement of MT in the regulation of MMP2 gene expression and activities was examined in wild-type and MT-null mouse liver Itoh cells. Both of cells were immortalized by SV40 large T-antigen. The results showed again that MT is not essential for MMP2 gene expression. However, the wild-type Itoh cells secreted more a 94 kDa type IV collagenase encoded by MMP9 gene than the MT-null Itoh cells. These results suggest that MT may play a different role in different cell types. (Supported by a Grant-in-aid for General Research from the Ministry of Education, Sciences, Sports and Culture of Japan.)

1587 HOW WELL DOES GENOTYPE PREDICT THE NET ACTIVITY OF ANTIOXIDANT ENZYMES IN HUMANS?

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Genetic polymorphisms are commonly used as biomarkers of genetic susceptibility, to predict response to environmental challenges. However, limited evidence is available on the impact of genetic polymorphisms upon protein function in humans. Additionally, activity of inducible enzymes, such as antioxidant, is a function not only of genetic variation but also of the concurrent exposures.

We examined the effect of genetic polymorphisms on the activity of three antioxidant enzymes: mitochondrial superoxide dismutase (MnSOD), cytosolic glutathione peroxidase (GPX1), and peroxisomal catalase, in erythrocytes of 231 healthy student volunteers. DNA from blood clot was genotyped using Taqman PCR (C47T:MnSOD and C593T:GPX1) and standard PCR (C-262T:catalase). Genotype-activity relationships were analyzed with multiple linear regression adjusted for gender and ethnicity. Median activities for MnSOD, GPX1, and catalase were 2.8 U/g Hb, 13.2 U/g Hb, and 86.3 k/g Hb, respectively, and ranged 56-fold, 6-fold, and 8-fold, respectively, between lowest and highest levels. We found that a) GPX1 activity was reduced only in males, but not females, homozygotes for the variant T GPX1 allele; b) MnSOD activity was 16% higher in females than males, and it was 39% higher in CT or TT individuals versus CC individuals, despite in vitro evidence that the TT genotype is compromised in entering into the mitochondrial matrix; and c) catalase activity was on average 18.1 k/g Hb lower for TT subjects versus CC subjects, whereas the TT variant is reported to have higher transcription factor binding properties and therefore higher expression levels. In conclusion, genetic variation can only partially explain large inter-individual variability in enzyme activity in this cohort. The net oxidant balance determined by differences in diet, environmental exposures, and additional genetic factors may account for a substantial component of this variability.

1588 IMPACT OF GENETIC VARIATION IN ACRYLAMIDE METABOLISM ON HEMOGLOBIN ADDUCT LEVELS.

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Sponsor: K. Cheever.

This analysis explored contributions of genotype (genes encoding enzymes linked to acrylamide and glycidamide metabolism) and smoking on hemoglobin adduct levels of acrylamide (AA), its metabolite glycidamide (GA), and their ratio (GA/AA). Acrylamide has been shown to be genotoxic in animal studies. Human exposure occurs from diet, tobacco smoke, and occupational exposure. Blood samples from 39 consenting acrylamide industry workers with low potential risk for work contact with acrylamide were used. Variants of EPHX1 Y113H EX6+5C>T and GSTP1 I105V EX5-24A>G and gene deletions in GSTM1 and GSTT1 were identified by PCR. Hemoglobin adducts were measured by HPLC-MS/MS. Urinary cotinine was measured by immunoassay as a proxy for smoking (i.e., cotinine ≥ 200 ng/ml). Mean GA/AA ratios were calculated for dichotomized categories of EPHX1 (C/C + C/T=0.80, T/T=0.91), GSTP1 (A/G + G/G=0.88, A/A=0.87), GSTM1 (null=0.92, present=0.85), and GSTT1 (null=0.69, present=0.93). Separate linear regression models were run for log AA, GA, and GA/AA for each of these genotypes, with and without smoking adjustment. Smoking was highly significant when added to each model (p -values: 0.003 to <0.0001). EHPX1 T/T was significantly ($p=0.04$) associated with a higher GA/AA ratio after adjust-

ment for smoking. The GSTT1 null genotype was significantly associated with lowered GA/AA ratios both before ($p=0.0013$) and after ($p=0.0007$) adjustment for smoking. The GSTT1 null genotype was also significantly associated with higher AA levels, before ($P=0.02$) and after ($p=0.008$) adjustment for smoking. Genotype approached significance with AA or GA in several models. Conclusion: GSTT1 genotype appeared to affect AA levels and the GA/AA ratio, while EPHX1 Y113H affected the GA/AA ratio after adjustment for smoking. Low sample size may have limited detection of other effects. Disclaimer: The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy.

1589 ENVIRONMENTAL FACTORS AND POLYMORPHISM OF THE ENZYME GS-TRANSFERASE IN A CASE CONTROL STUDY OF ANENCEPHALY.

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Anencephaly, a type of NTD, occurs during the neurulation process as a result of failure of the neural folds in the cranial end of the neural plate to fuse and form the forebrain. This study examines the role of glutathione-S-transferase enzymes (GSTs) and environmental factors in a pilot case-control study of anencephaly at the US-Mexico border. GSTs catalyze some detoxifying reactions and may therefore affect penetration of pollutants to the fetus. Consenting obstetric patients were recruited from a hospital in Tijuana, B.C., Mex. (22 cases and 44 controls). Placentae were genotyped for GSTM1 (mu), T1 (theta) and P1 (pi) by PCR. Fathers of cases were more likely to work in a maquila (70.0% vs 42.9% of controls, $p=0.046$). Fewer mothers of cases reported having had prenatal care during the first trimester as compared to controls (52.2% of cases vs. 82.2% of controls, $p<0.01$). More mothers of cases reported being exposed to solvents during the first trimester than mothers of controls (23.8% vs. 4.9%, $p=0.026$). Cases had on average 26% lower serum folate than controls (not significant at $p=0.083$) and had resided fewer years in Tijuana (not significant at $p=0.068$). Cases and controls were not different for frequencies of GST mu and theta deletions (23.5% vs. 24.4%, GST mu, and 35.3% vs 36.6%, GST theta). GST pi genotype ile/ile was slightly higher in cases (36.8 vs. 25.6%), but this difference was not significant. In conclusion, this pilot study provides no evidence of increased frequencies of at risk GST mu, theta and pi genotypes in cases of anencephaly in a study at the US-Mexico border. However, the indications that solvent exposure and paternal occupation were associated with risk of anencephaly in this pilot study warrants further investigation.

1590 CHARACTERIZATION OF LIVER INJURY IN HUMAN HFE C282Y, Q283P, AND H63D HETEROZYGOTES USING α AND π GST QUANTITATION.

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Hemochromatosis (HH) occurs as an autosomal recessive disease of iron metabolism with the causative mutation occurring in a major histocompatibility complex class I-like gene (HFE). The Cys to Tyr change at position 282, occurs in about 10% of European descent, occurring in homozygous form in 90% of HH diagnosed patients. A HFE Q283P substitution has been shown to occur in 4-35% of HH patients, heterozygous with either C282Y, H63D, or S65C. Homozygous mutant genotypes demonstrate a disruption in regulation of iron uptake leading to iron loading in various organs and tissues, including the liver, whereas C282Y or Q283P heterozygotes show mild systemic iron deposition. New biomarkers have been developed for testing subclinical liver damage, α Glutathione-S-Transferase (α GST) and π Glutathione-S-Transferase (π GST). To examine the extent of proposed subclinical liver damage relative to HFE disease genotypes, α GST and π GST were quantified in serum and plasma (respectively) of 60 patients genotyped for C282Y, Q283P, and H63D. Samples (whole blood, serum, plasma, urine) were collected from random, anonymous individuals aged 20-45 years at Wright Patterson AFB clinical laboratory. Genotyping was accomplished with genomic DNA isolated from whole blood using standard PCR conditions and primer sets amplifying gene segments containing nt845 (C282Y), nt848 (Q283P) or nt187 (H63D). Clinical blood chemistries were analyzed on each sample (Serum alanine amino-

transferase, aspartate aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, total iron, and transferrin saturation). Additionally, π GST was quantified in urine of 14 individuals chosen at random. Analysis of samples heterozygous for C282Y (or Q283P) do not suggest a statistically significant increase in π GST levels in serum or, in tested samples, in urine. Interestingly, levels of α GST were found to decrease in C283Y heterozygotes.

1591 ASSOCIATION BETWEEN POLYMORPHISMS OF HUMAN LEUCOCYTE ANTIGEN GENES WITH SUSCEPTIBILITY TO TRICHLOROETHYLENE-INDUCED ALLERGIC DERMATITIS AMONG EXPOSED WORKERS.

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The aim of present study was to investigate the association between genetic polymorphisms of human leucocyte antigen, such as HLA-DQ and HLA-DRB1, with susceptibility to TCE-induced allergic dermatitis among the exposed workers. From June 1998 to March 2006, 112 cases with TCE-induced allergic dermatitis and 142 tolerant controls were recruited into the population based case-control study. The DNA sequence of HLA-DQA1 and HLA-DQB1 exon2, and HLA-DRB1 exon2 were performed by direct sequencing of polymerase chain reaction products. The frequencies distribution of allelic genotypes, SNPs and codon polymorphisms were compared, and odds ratio were calculated. There were significant differences in the frequencies of allelic genotypes of HLA-DQA1, HLA-DQB1 and HLA-DRB1 between cases and exposed controls. The frequencies of DQA1*0201,0601/0602 in cases were significantly higher than that in exposed controls (7.6% vs 3.5%, OR=2.25, 95% CI=1.01-5.02, 16.1% vs 7.0%, OR=2.53, 95% CI=1.42-4.50), while frequencies of DQA1*0103,0501/0101/0503/0505 and DQB1*0303 in cases were significantly lower than that in exposed controls (5.8% vs 10.9%, OR=0.50, 95% CI=0.26-0.98, 8.9% vs 17.3%, OR=0.47, 95% CI=0.27-0.82, 9.8% vs 19.8%, OR=0.58, 95% CI=0.34-0.99). The frequencies of 1501,1202 and 04 in cases were significantly higher than those in controls (18.8% vs 7.7%, OR=2.75, 95% CI=1.59-4.76, 12.5% vs 6.0%, OR=2.24, 95% CI=1.19-4.21, 17.4% vs 11.3%, OR=1.66, 95% CI=1.00-2.75), while frequencies of 0301,0901,13 and 1502 in cases were significantly lower than those in controls (0.4% vs 4.6%, OR=0.09, 95% CI=0.01-0.72, 7.6% vs 13.7%, OR=0.52, 95% CI=0.28-0.94, 1.3% vs 6.7%, OR=0.189, 95% CI=0.06-0.65, 2.7% vs 8.5%, OR=0.29, 95% CI=0.12-0.74). In conclusion, the genetic polymorphisms of HLA-DQ and HLA-DRB1 might be the factors influencing the individual susceptibility to TCE-induced allergic dermatitis among exposed workers.

1592 SEARCH FOR POLYMORPHISMS IN THE GCLC, GCLM AND ALDH2 GENES VERSUS RISK OF CORONARY HEART DISEASE IN A CHINESE POPULATION.

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Coronary heart disease (CHD) is a complex trait involving the interaction of many genes plus the environment over decades of life. Reactive oxygen species (ROS) are implicated in the development of atherosclerotic plaques in CHD. Glutamate-cysteine ligase (GCL) is pivotal in synthesis of the abundant antioxidant glutathione (GSH), which protects against ROS. Single nucleotide polymorphisms (SNPs) in the 5'-UTR of the human GCLC (catalytic) and GCLM (modifier) genes have previously been reported to be independently associated with increased risk for myocardial infarction in a Japanese population. We therefore investigated the association between variants in these two genes and susceptibility to CHD in 160 cases and 156 age- and gender-matched controls in a Chinese population; 43 SNPs in the GCLC gene (transcript 47,693 bp) and 13 SNPs in the GCLM gene (transcript 22,423 bp) were chosen to cover the region between 10 kb upstream and 10 kb downstream for each gene. We also examined the Glu504Lys mutation in the ALDH2 gene (transcript 43,437 bp). Genotyping was performed using the fluorogenic 5' nuclease assay, and association was analyzed by a linear-regression model. No association was observed with GCLM. Two GCLC SNPs, rs621571 in intron 6 and rs6922238 in the 3'-UTR, and the Glu504Lys mutation in the ALDH2 gene showed p values of 0.042, 0.041 and 0.047, respectively; however, none of the three

remained significant following Bonferroni correction for multiple-testing. In conclusion, we found no correlation between polymorphisms in either *GCL* gene or the *ALDH2* gene and increased risk of CHD in this Chinese population.—
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1593 THYMIDYLATE SYNTHASE POLYMORPHISM AND RISK OF NON SMALL CELL LUNG CANCER.

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Lung cancer is the most common cancer in the world and the leading cause of cancer death. The non-small cell lung cancer (NSCLC) and small cell lung cancer together account for over 90% of all forms of lung cancer. The chance of developing cancer depends on both genetic and non-genetic factors. Thymidylate Synthase (TS) catalyses the final step in the synthesis of deoxythymidine monophosphate using the substrate deoxyuridine monophosphate, limiting an irreversible step in *de novo* DNA synthesis. *TS* gene promoter enhancer region contains two different polymorphisms which can influence *TS* mRNA transcriptional and translation efficiency: a polymorphic tandem repeat sequence (2R and/or 3R). It was reported that *TS* genes with the 3R sequence have a greater expression activity than those with 2R sequence suggesting that *TS* repeat sequence plays an important role in the control of *TS* protein expression thus affecting the control mechanism. It has been reported that polymorphisms in *TS* modifies the risk and the survival of some types of cancer and no previous studies are published regarding *TS* polymorphisms in NSCLC. This study was designed to evaluate the role of *TS* polymorphisms in the susceptibility to NSCLC. Tandem repeat for *TS* polymorphisms were analyzed by PCR technique in DNA obtained from peripheral blood samples of 105 patients with NSCLC and a control group of 210 healthy volunteers. Results showed absence of risk associated with the 3R/3R genotype in recessive model analysis of NSCLC patients (OR=1.39; 95% CI=0.80-2.42). In non recessive model analysis a lack of significant risk associated with 3R tandem repeat was observed. Homozygous 3R and heterozygous 2R/3R carriers had no significant risk for NSCLC when compared with homozygous 2R (OR=1.73; 95% CI=0.83-3.61; OR=1.37; 95% CI=0.70-2.72, respectively). Therefore obtained data suggests that tandem repeat *TS* polymorphism may not predispose for an increased risk for NSCLC. Further studies will be helpful to understand the role of this polymorphism in disease progression, therapeutic outcome and survival.

1594 PCB, CYP1A1 POLYMORPHISM AND RISK OF BREAST CANCER IN POSTMENOPAUSAL WOMEN.

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BACKGROUND: Recent studies reported that postmenopausal women were at increased risk of breast cancer if they had a higher serum level of PCBs and the CYP1A1*2C gene. A meta-analysis was conducted to assess the association between the CYP1A1*2C gene, PCB exposure, and breast cancer and to quantify the relative risk of postmenopausal women with this genotype to develop breast cancer when exposed to PCBs.

METHODS: Meta-analyses were performed using odds ratio (OR) as the measure of effect size. Fixed effects method was used to combine data. Four studies were included in the analysis.

RESULTS: There was a statistically significant association between CYP 1A1*2C gene and the risk of breast cancer in postmenopausal women (OR = 1.46; 95% CI: 1.08, 1.96). No statistically significant association was found between high exposure to PCBs and risk of breast cancer (OR = 1.06; 95% CI: 0.88, 1.28) in postmenopausal women. Stratified analysis showed an increased risk of breast cancer (OR = 2.13; 95% CI: 1.38, 3.28) in postmenopausal women carrying the CYP1A1*2C allele and who had high PCB exposure, and no statistically significant increased risk (OR= 1.01; 95% CI: 0.66, 1.53) among women who had low PCB exposure. CONCLUSIONS: Overall, postmenopausal women carrying the CYP1A1*2C allele have 46% increased risk to have breast cancer. Moreover, carriers of CYP1A1*2C gene who had higher mean PCB serum levels had a 113 % increased odds to have breast cancer compared to women with low level of PCBs and wild type CYP1A1 gene.

Total character count: 1793

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Keywords, PCBs, Breast Cancer, CYP1A1, polymorphism, Categories: Genetic Polymorphism, Persistent Organic Chemicals (POPs), Epidemiology/Exposure Assessment

1595 DISTRIBUTION OF THE CYP1A1*2A ALLELE ON A RURAL GREEK POPULATION.

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CYP1A1 is a major extrahepatic component of the cytochrome P450. It contributes greatly to the toxicity of chemicals such as PAHs since it is responsible for the creation of the toxic DNA binding forms. The objective of our study was to investigate the possible role of the CYP1A1 detoxification enzyme in the incidence of various clinicopathological conditions in an isolated population group exposed to a variety of toxic factors. Our study focused on an isolated rural population in the south and east of Crete and central Peloponnesus. We collected an aliquot of peripheral blood as well as relevant personal and medical history data by interviewing 492 individuals. Genomic DNA was extracted and the genotype regarding the CYP1A1*2A allele was determined by PCR RFLP. We observed 1.02% CYP1A1*2A homozygotes, 19.31% heterozygotes and 76.67% wildtype individuals. The rare CYP1A1*2A allele frequency was 0.107 and the genotype frequencies appeared in Hardy-Weinberg equilibrium. Statistical analysis of the genotypes with the personal and medical history of the individuals revealed a significant positive correlation between the CYP1A1*2A allele and the myocardial infarction was observed ($p<0.001$), as well as a significant negative correlation between this allele and miscarriages ($p=0.003$) and stillbirths ($p=0.018$). Our results show the involvement of the specific mutant allele in relation to myocardial infarction and birth outcomes. This report is a part of our ongoing population and environmental study of exposure to pesticides and assessment of its effect on public health.

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1596 CLONING, EXPRESSION AND BIOCHEMICAL PROPERTIES OF CPOX4, A GENETIC VARIANT OF COPROPORPHYRINOGEN OXIDASE THAT MODIFIES THE EFFECT OF MERCURY EXPOSURE ON PORPHYRIN EXCRETION IN HUMANS.

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Coproporphyrinogen oxidase (CPOX) catalyzes the 2-step decarboxylation of coproporphyrinogen-III to protoporphyrinogen-IX in the heme biosynthetic pathway. Previously, we identified a specific polymorphism (N272H) in exon 4 of the human CPOX gene (CPOX4) and demonstrated that CPOX4 is significantly associated with a modified urinary porphyrin excretion pattern characterized by high concentrations of 4- and 5-carboxyl porphyrins as well as an atypical isocoproporphyrin in humans with low-level mercury (Hg) exposure. Here, we compared the biochemical properties of CPOX and CPOX4 to ascertain the role of CPOX4 in the etiology of this effect. Site-directed mutagenesis was performed on the wildtype CPOX cDNA (A814) to generate the CPOX4 mutant clone (C814). Wildtype and mutant CPOX cDNA-pGEX-2T constructs were expressed in *E. coli* BL21. CPOX-GST fusion proteins were purified from bacterial lysates by GSH affinity chromatography. Purified CPOX and CPOX4 were generated using thrombin to remove GST-tags. Copro'gen-III and pentacarboxy'gen-III (5dab) were incubated with purified human CPOX and CPOX4 enzymes at various concentrations, with or without Hg present. Both CPOX and CPOX4 formed proto'gen-IX from copro'gen-III or isocoproporphyrin from 5dab. However, the affinity of CPOX4 was 2-fold lower than that of CPOX using either substrate (CPOX $K_m=0.52\mu M$, $V_{max}=0.02$ pmol proto'rin-IX; CPOX4 $K_m=1.04\mu M$, $V_{max}=0.01$ pmol proto'rin-IX). Hg (0.1-1 μM) specifically inhibited the 2nd step of copro'gen-III decarboxylation (harder'gen to proto'gen-IX) in a dose dependent manner. These findings suggest that CPOX4 may be associated with impaired heme biosynthesis, which is limited further by Hg exposure. These observations are consistent with altered porphyrin excretion and potential neurotoxicity observed in humans with CPOX4 and Hg exposure (Neurotox Teratol 28:39-48,2006). Supported by ES04696, ES07033 and the Wallace Res. Fdn.

1597 GENETIC SUSCEPTIBILITY IN AUTISM.

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Autism is a common neurodevelopmental disorder with both genetic and environmental components. Genetic susceptibility to mercury (Hg) toxicity is recognized as a plausible explanation for autism in a subset of children. Even "safe" Hg levels

could be implicated in the etiology of autism due to genetic susceptibility that alters metabolism or intracellular compartmentalization. To identify genetic polymorphisms associated with autism that influence the extent of individual susceptibility to Hg neurotoxicity, we are conducting a thorough search for polymorphisms in four genes (MT1a, DMT1, LAT1 and MTF1) involved in Hg transport and clearance in the general population and in autistic individuals and assessing their frequency and association to the disorder. Using a sample pool of 48 unrelated individuals from both the general and autistic populations we employed Temperature Gradient Capillary Electrophoresis and Single Strand Conformation Polymorphism analysis to screen all of the exons, 5' and 3' UTR, 1kb upstream of the message start, and the exonic bordering regions for genetic variations. We have currently identified and characterized a number of polymorphisms in both MT1a and DMT1. Two of the MT1a polymorphisms are nonsynonymous, Thr to Asn at amino acid position 27, and Lys to Arg at amino acid position 51. Polymorphisms were evaluated for differences in allele frequencies using a standard chi-square analysis ($p < 0.05$). Preliminary findings failed to show association with autism. Further analysis with the TaqMan genotyping system in a larger dataset of 224 autistic samples and 224 controls is underway. Variations in MT1a, DMT1, LAT1 and MTF1 might increase susceptibility to Hg toxicity by altering the transport, binding, efflux, or tissue distribution of Hg in the body. Exposure to Hg might then act synergistically with these genetic susceptibility factors to manifest the behavioral and developmental deficits present in autism. This work was supported by grant #T32 ES007028.



1598 SOT PRESIDENTS – A PERSPECTIVE ON MIXTURES TOXICOLOGY.

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Several federal agencies such as the U.S. Environmental Agency, Agency for Toxic Substances and Disease registry, National Institute of Environmental Health Sciences, National Institute of Occupational Safety and Health and others have active programs of research and methods development for chemical mixtures. Recently, enormous resources have been committed by the international community including the American Chemistry Council, Dutch government, the European Union, the SETAC, and the HESI/ILSI steering committee on chemical mixtures. Also, the SOT established a Mixtures Task Force that organized a series of meetings that led to the inauguration of the Mixtures Specialty section at the 2006 annual meeting. These activities underscore the importance of the toxicity assessment of chemical mixtures that has gradually been introduced in federal laws viz., CERCLA (1980), SARA (1986), FQPA (1996), and SDWA (1996). Often decisions regarding the influence exerted by chemical exposures on the homeostasis and the complex mechanisms operative in biological or environmental systems are based on minimal information.

This session will review (1) the nature of mixtures we encounter and historically the means that have been used to minimize their risk, (2) the evolution from default methods, through innovative experimental design to the current use of in silico methods. Three SOT past Presidents Dr. John Doull, Dr. Glenn Sipes and Dr. Linda Birnbaum will share their insights.

1599 THE USE OF DOSIMETRIC ADJUSTMENT FACTORS AND PBPK MODELING TO PROTECT INFANTS AND CHILDREN FROM INHALED ACETALDEHYDE.

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Infants and children, especially those with asthma, are potentially more sensitive to irritating organic vapors, than adults. New risk assessment methodologies including benchmark dose analysis and PBPK modeling were used to derive draft 8-hour and chronic Reference Exposure Levels (RELs) for acetaldehyde. Previous reference exposure levels for acetaldehyde were determined using the LOAEL/NOAEL (400 and 150 ppm, respectively) approach. Using the same data from Appelman et al., 1982; 1986, a benchmark dose for nasal degeneration of the olfactory epithelium (99 ppm) was generated using a continuous modeling approach. In addition, a pharmacokinetic modeling (PBPK) was applied for dosimetric interspecies extrapolation of specific nasal olfactory tissue damage rather than the standard Human Equivalent Concentration (HEC) procedure. Based on a published computational flow dynamic model to estimate mass flux across the nasal passages, a dosimetric adjustment factor (DAF) was derived by OEHHHA (DAF = 0.85), which was the ratio of this value for the rat to that for humans. The model also took into account interspecies differences in the deposition of inhaled vapors and breathing rates. Using the BMC methodology and PBPK modeling with the DAF, a revised draft chronic REL of 25 ppb and a newly defined draft 8-hr REL of 105 ppb were deter-

mined. In conjunction with the DAF, an additional interspecies toxicokinetic factor of 2 was applied. A combined intraspecies uncertainty factor of 30 was applied, reflecting intra-individual toxicokinetic variation and greater toxicodynamic susceptibility of children and asthmatics. The 8-hour RELs, if adopted, will support assessment of the health impacts on children in schools, who can be exposed for up to 8 hours. Application of the benchmark dose and PBPK techniques lowers the uncertainty associated with reference exposure levels and serve as better risk assessment models for the protection of infants and children.

1600 LONG TERM (7-MONTH) CONTINUOUS INTRAVENOUS INFUSION IN THE ALBINO RAT.

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Continuous intravenous infusion via an indwelling catheter is frequently used in preclinical safety evaluation studies to mimic the proposed clinical route or to achieve higher blood drug levels than can be achieved by the defined clinical route. These studies have been conducted at our laboratory for over 20 years. Control data at our laboratory was evaluated to determine any effects of the presence of a femoral catheter and continuous intravenous infusion on the body weight gain, food intake and clinical pathology parameters over a 7 month period. A medical grade silicone-based catheter was surgically implanted under general anesthesia in the vena cava. Animals were continuously infused with saline at 0.4 mL/h for 24 hours/day over 7-months using a jacket and tether system. Body weight gains and food intake were unaffected when comparing data with historical control values for non-catheterized animals. Small differences in the clinical pathology data were observed between the catheterized and non-catheterized animals that could be associated with the presence of a foreign material.

It is concluded that in our laboratory the presence of an indwelling catheter and continuous intravenous infusion of saline at a rate of 0.4 mL/h in albino rats for up to 7 months had no adverse effects on growth compared with non-catheterized animals. Clinical pathology changes were minor and consistent with the presence of a foreign material.

1601 ESTIMATING HIGH DOSE HUMAN EQUIVALENT CONCENTRATIONS (HECS) CAN BE PROBLEMATIC EVEN WITH THE BEST PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS: METHANOL AS AN EXAMPLE.

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USEPA's procedure for establishing Reference Concentrations (RfCs) for inhaled substances typically involves determination of Human Equivalent Concentrations (HECs) corresponding to No Observed Adverse Effect Levels (NOAELs) or comparable Benchmark Doses (BMDs) developed with dose-response data for toxic endpoints evaluated in laboratory animal studies. Physiologically based pharmacokinetic (PBPK) models can facilitate this process by accounting explicitly for possible differences in 1) temporal exposure patterns, e.g., 7 hr/day vs. continuous exposure, 2) exposure route, and 3) absorption, distribution, metabolism, and elimination processes across species. However, when the most sensitive toxic endpoint (cervical rib malformations) is associated with saturation of metabolism and disproportionately high blood concentrations of the parent chemical (methanol), HEC estimation can require upward extrapolation to high airborne concentrations and/or exposure durations well beyond those for which in vivo human data are available. In such cases, the limited human data will exhibit essentially linear kinetics, and only the Vmax/Km ratio will be estimable with reasonable precision, making high dose HEC estimation problematic. To avoid this difficulty, we recommend that a Reference Increment be defined relative to the NOAEL or BMD estimate using the parent chemical's blood concentration as the dose metric, together with appropriate adjustment factors for residual pharmacokinetic uncertainty and interindividual variability. The resulting Reference Increment in blood can then be readily and accurately converted to an equivalent Reference Concentration in air using a well-calibrated and validated human PBPK model.

1602 IDENTIFICATION AND RISK ASSESSMENT OF HUMAN GENOTOXIC DRUG METABOLITES.

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Prior to clinical development, drug candidates are assessed in standard genotoxicity assays to identify potential genotoxic and carcinogenic hazards. Use of S9 for in vitro tests, as well as conduct of an in vivo study, allows detection of hazards related

to parent drug and metabolites. Due to the limitations of in vitro test systems, it is prudent to consider if the genetic safety of human metabolites has been adequately assessed as human ADME data becomes available during clinical development. We propose a process for identification and risk assessment of known or potential genotoxic metabolites. Human metabolites of concern are identified using a structure-based assessment, and human exposure to these concerning metabolites are estimated based on ADME excretory data. Similarly, rodent ADME data is used to estimate exposure to any concerning metabolites at the top dose intended for 2-year carcinogenicity studies. These data are submitted to a risk assessment model that determines the likelihood of not exceeding a 10⁻⁵ cancer risk from consuming a pharmaceutical with a known or suspect genotoxic metabolite. The risk assessment model is dependent on the probability that 1) a genotoxic metabolite is a human carcinogen, 2) human systemic exposure would result in a negligible cancer risk, and 3) in an oncogenicity study, animal systemic exposure to the metabolite would be high enough to cause a significant tumorigenic response in at least one animal species. A suggested process flow was developed based on results from a matrix of various clinical and nonclinical exposures. In general, if systemic exposure to the genotoxic metabolite in humans is $\leq 1.5 \mu\text{g/day}$, or in animals used in the oncogenicity study is $\geq 10 \text{ mg/kg/day}$, or there is a 1000-fold margin of safety (human exposure / animal exposure), it is unlikely that a 10⁻⁵ cancer risk will be exceeded. Based on case studies evaluated thus far, the approach seems to be a practical tool.

1603 ADDITIVITY ASSESSMENT OF TRIHALOMETHANE MIXTURES BY PROPORTIONAL RESPONSE ADDITION.

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If additivity is known or assumed, the toxicity of a chemical mixture may be predicted from the dose response curves of the individual chemicals comprising the mixture. As single chemical data are abundant and mixture data sparse, mixture risk methods that utilize single chemical data are useful risk assessment tools. The present study examined, by proportional response addition, the assumption of additivity for mixtures of the four regulated trihalomethanes (THMs) that are formed during chemical disinfection of water. Under proportional response addition, the expected (predicted) effect of the mixture is a proportionally weighted sum of the responses that result from exposure to the individual components at the concentrations at which they are present in the mixture. A series of 16 experiments were conducted, including evaluation of: dose response curves for the individual THMs (chloroform, chlorodibromomethane, dibromochloromethane, bromoform); the six binary combinations of the four THMs; and, mixtures containing all 4 THMs. Female CD-1 mice were exposed by daily aqueous gavage for 14 days. Hepatotoxicity was assessed by relative liver weight and serum levels of enzymes indicative of hepatic damage (SDH, ALT, AST). A statistical approach based on comparison of expected and experimentally observed means was developed and applied to the data. Generally, the experimentally observed and the predicted mixture responses were not statistically different. As the variance in the responses increased, the ability (power) to detect departures from additivity decreased. In summary, novel proportional response addition methodology was developed and applied to evaluate the assumption of additivity for mixtures of the regulated THMs. The mixture data were supportive of a conclusion of additive joint toxicity as few statistically significant deviations from additivity were detected. (This abstract may not reflect EPA policy.)

1604 TOXICITY TESTING SCHEMA FOR THE INITIAL RISK ASSESSMENT OF FOOD CONTACT PLASTICS BASED ON THE CONCEPT OF TTC AND USAGE PROBABILISTIC FACTORS.

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The producers of food-contact plastics are responsible to ensure that their products meet the requirements on migration specified in the national registration. The migration limit of each eluting chemical should be ideally derived from the acceptable daily intake of individual chemical. However, it is not realistic to assess fully the potential risks of all chemicals. FDA and EFSA had developed the safety assessment guideline of food-contact materials prior to the application. In Japan, there are particular migration limits for the specific or general plastic products as the national standards. As for the comprehensive guidance, several industry associations introduced independently the self-guidelines like that of FDA or EFSA. Overall, the required toxicity testing schemas depending on migration levels of all guidelines are similar, except the lowest threshold of 0.5 ppb (1.5 $\mu\text{g/person}$), which is used in

FDA's. This threshold had been greatly discussed so far, however, scientific basis of higher thresholds (e.g. 50 ppb, 1 or 5 ppm) are unclear. Recently, the concept of TTC (Toxicological Threshold of Concern) have been examined and expanded for more general toxicity endpoints (Kroes et al. 2000, 2004). While, it is also important for the concept of TTC to estimate the appropriate exposure levels. Therefore, we examined the possibility for the replacement of these higher thresholds to the similar level of the TTCs, and tried to develop the modified equation for estimating the exposure levels, using the usage probabilistic factor. These works suggested that the scientifically more transparent toxicity testing schema and/or the initial risk assessment guidance could be developed.

This study was supported by the grant from the Japanese Food Safety Commission.

1605 IN VITRO PREDICTIVE MYELOTXICITY ASSESSMENT FOR NOVEL ONCOLOGY DRUGS.

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It is commonly observed that at least 50% of oncology drugs, including those with a novel and target-specific mechanism of action, induce myelosuppression as the dose limiting toxicity in clinical settings. It is therefore of paramount importance to devise and validate predictive assays that would be useful to estimate the relevance of this liability in humans and derive an appropriate therapeutic index. In our laboratories, we have implemented and validated the HALO system (Hematopoietic Assay via Luminescence Output), which has recently been demonstrated to constitute a quantitative alternative to the classic clonogenic assay. This assay allows monitoring in a quantitative and precise manner and with medium throughput the effects of compounds on the hematopoietic precursor cells and therefore estimates the MTD due to neutropenia in early clinical trials. We will present the data we have obtained with a number of reference standard compounds and discuss how this assay can be used to support early candidate selection with respect to potency and animal species-specificity.

1606 CHARACTERIZATION OF SENSITIVITY OF RISK ESTIMATES TO UNCERTAINTIES ASSOCIATED WITH BIOLOGICALLY BASED MODELING OF FORMALDEHYDE CARCINOGENICITY.

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Regulatory acceptance of a biologically based dose-response model of formaldehyde-induced rat nasal tumors (*Tox Sci* 75, 432-47) has been hindered by concerns regarding the uncertainty of risk predictions obtained with the model. To address these concerns, we developed a novel, sequential Bayesian methodology for conducting a comprehensive, quantitative sensitivity analysis of the biologically based cancer model. A hierarchical Bayesian analysis was performed using the Markov Chain Monte Carlo (MCMC) algorithm to provide a multi-step evaluation of the propagation of uncertainty from the rodent experimental data and modeling to the human extrapolation and risk estimation. The analysis provided posterior parameter distribution estimates consistent with the values used in the original model. In addition, by taking advantage of the Bayesian technique, it was possible to develop an approach to resolve a key issue regarding the choice of historical control data. The analysis demonstrated that the most crucial prediction of the modeling – the existence of a dose-dependent threshold (DDT) for formaldehyde carcinogenicity at an inhaled concentration on the order of 1 ppm in the human – is robust to uncertainties in the model and the underlying data. Most of the uncertainties investigated affect the predictions of residual risk below this threshold, rather than the existence and location of the threshold itself. Even uncertainties amounting to several orders of magnitude did not necessarily change the conclusion that risks below the DDT are negligible.

1607 REFERENCE DOSE FOR 1, 1, 2, 2-TETRACHLOROETHANE.

J. Donohue. *HECD, U.S. EPA, Washington, DC.* Sponsor: E. Ohanian.

The National Toxicology Program (NTP) published a comprehensive subchronic study of 1,1,2,2-tetrachloroethane in F-344 rats and B6C3F1 mice in 2004. As part of the evaluation of this chemical as an unregulated drinking water contaminant, the U.S. EPA Office of Water selected the data from this study to develop a reference dose (RfD) for 1,1,2,2-tetrachloroethane after examining available data from studies of oral exposure in humans and laboratory animals. Rats were found to be more sensitive than mice to the noncancer effects of this chemical. Low dose effects included increased relative liver weights, cytoplasmic vacuolization of hepatocytes, increased serum levels of alanine transaminase (ALT) and sorbitol dehydrogenase (SDH), decreases in red blood cell hematological parameters, and decreases

in sperm motility. Male animals appeared to be more sensitive to the liver effects than females. Benchmark dose modeling for dose-response identified the increased relative liver weight as the critical effect supported by the cytoplasmic hepatocyte vacuolization (7/10 animals at 20 mg/kg/day), and increased serum levels of ALT (BMDL = 29 mg/kg/day) and SDH (BMDL = 32 mg/kg/day). The RfD of 10 ug/kg/day was derived from a BMDL of 10.71 mg/kg/day using a 1000-fold uncertainty factor. [The views expressed in this abstract are those of the author and do not necessarily reflect the views or policies of the U.S. EPA.]

1608 POTENTIAL AIR CRITERIA FOR TRICHLOROETHENE (TCE) BASED ON CARCINOGENIC EFFECTS IN HUMANS AND ANIMALS.

K. G. Bogdan, G. M. Recer and J. E. Storm. *Bureau Toxic Substance Assessment, NYS Department of Health, Troy, NY.*

We derived criteria for evaluating the carcinogenic risks from low-level chronic exposures using methods consistent with US EPA guidelines. Criteria based on chronic inhalation studies in mice (liver, lung, lymphoma) and rats (kidney, testes tumors) were derived using default and PBPK-based dose metrics and linear and non-linear low-dose extrapolations. Criteria were adjusted, when appropriate, for potentially increased sensitivity of children. Recommended criteria for each cancer endpoint were selected from a larger set of potential criteria using weight-of-evidence evaluations of dose metrics and modes-of-action. Recommended risk-specific (1×10^{-6}) criteria are 0.3 ug/m³ (lymphoma, default dose metric), 1.4 ug/m³ (liver, internal dose metric), 7.8 ug/m³ (kidney, default dose metric, adjusted for potentially increased sensitivity of children), and 3100 ug/m³ (kidney, internal dose metric). Criteria based on human data were used to check the plausibility of animal-based criteria because human studies were limited by several factors, including unavoidable uncertainties in exposure estimates. Criteria were derived from a study that showed increased risks of esophageal cancer and non-Hodgkin's lymphoma among TCE workers (Hansen et al., 2001). We used an average relative risk model, two relative risk estimates (MLE or 95% UCL) for each cancer, an estimate of the mean occupational air concentration, and three estimates of mean employment duration in our derivations. For criteria based on the 95% UCL on relative risk, the median of the six estimates of the risk-specific (1×10^{-6}) criteria is 0.4 ug/m³. These criteria, criteria based on non-carcinogenic effects, and other factors (e.g., background and analytical methods) were considered in the development of a TCE guideline of 5 ug/m³.

1609 POTENTIAL AIR CRITERIA FOR TRICHLOROETHENE (TCE) BASED ON NON-CARCINOGENIC EFFECTS IN HUMANS AND ANIMALS.

T. B. Johnson, K. G. Bogdan, G. M. Recer and J. E. Storm. *Bureau of Toxic Substance Assessment, New York State Department of Health, Troy, NY.*

Because of concern about potential exposures of New York State residents to TCE in air (e.g., in indoor air through soil vapor intrusion) the New York State Department of Health (NYS DOH) derived several potential air criteria for evaluating the non-carcinogenic risks from low level chronic TCE exposures using methods consistent with US EPA guidelines. Potential criteria were derived based on human (when available) and/or animal toxicity data on central nervous system, liver and kidney toxicity, and effects on development and reproduction. Critical studies for each type of non-carcinogenic toxicity were selected, and a physiologically-based pharmacokinetic model was used to estimate internal dose metrics (from experimental air concentrations) judged to be relevant to the mode of action for the toxic endpoint reported in each study. Points of departure for low-dose and cross-species extrapolation, expressed as an internal dose metric or air concentration, were obtained using the US EPA's Benchmark Dose Software or default methods, and associated human equivalent concentrations were estimated based on reverse PBPK or Category 3 gas dosimetry. Uncertainty factors were applied according to US EPA guidance, and for some endpoints were adjusted to account for lifespan sensitivity. Recommended criteria were selected from the larger set of potential criteria based on a weight of evidence analysis. Recommended criteria were 11 ug/m³ based on central nervous system toxicity, 160 ug/m³ for both liver and kidney toxicity, 20 ug/m³ to 32 ug/m³ for reproductive toxicity, and 19 ug/m³ to 38 ug/m³ based on developmental toxicity. These criteria, along with criteria derived for carcinogenic effects and other considerations (e.g., background and analytical methods) were used to support the NYS DOH air guideline of 5 ug/m³ for TCE.

1610 ESTIMATE TRICHLOROETHYLENE CANCER SLOPE FACTORS USING A HARMONIZED PBPK MODEL.

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Recently, a harmonized PBPK model for TCE was developed as a joint effort organized by TERA and supported by the US Environmental Protection Agency and the Department of Defense. This harmonized PBPK model was further calibrated

with Bayesian analysis, resulting in an updated TCE PBPK model. This updated PBPK model was used to compute updated cancer slope factors for liver tumor based on mouse bioassays, and for kidney tumor based on a rat bioassay. By using the corresponding animal PBPK models, we calculated the animal internal dose metric [i.e., the area under the TCA liver concentration curve (AUC) and liver AUC for DCA in mice, and kidney AUC for DCVC in rats] corresponding to the doses administered in the bioassays. Benchmark dose-response modeling was conducted to estimate lower bounds on the effective internal doses for 10% extra risk of liver or kidney carcinogenesis (LED10). The human PBPK model was then used to estimate the human inhaled concentration and orally ingested dose of TCE that will result in the estimated LED10s for causing liver and kidney tumors in animal bioassays. Finally, the human cancer slope factors for liver and kidney tumors were calculated using linear extrapolation from the risk at the human LED10 to 0. These slope factor estimates represent the most current estimate of the risk of liver or kidney carcinogenesis in humans from TCE exposure.

1611 SPACECRAFT WATER EXPOSURES GUIDELINES FOR C1-C4 MONO-, DI-, AND TRIALKYLAMINES.

J. M. Hampton. *Texas Southern University, Houston, TX.* Sponsor: C. Obot Akata.

Spacecraft Water Exposure Guidelines for C1-C4 Mono-, Di-, and Tri-Alkylamines

The Johnson Space Toxicology Group has developed spacecraft crew exposure guidelines for target and other alkylamines found as contaminants in humidity condensate and recycled water samples obtained from shuttle spacecraft. Target alkylamines were methylamine, ethylamine, propylamine, isopropylamine, n-butylamine, dimethylamine, diethylamine, di-n-butylamine, trimethylamine, triethylamine, tripropylamine and tributylamine. The National Research Council (NRC) provides guidance to the National Aeronautics and Space Administration JSC Space Toxicology Group on development of exposure guidelines for the protection of space crews from chemical contaminants of the internal atmospheres or water systems of crew spacecraft. Spacecraft water exposure guidelines (SWEGs) are established for exposures of 1, 10, 100, and 1,000 days (d). The 1-d SWEG is intended to prevent irreversible harm and degradation in crew performance during emergencies. Longer term SWEGs are intended to prevent adverse health effects and degradation in crew performance that could result from continued exposure, and to provide guidance for exposures under normal operating conditions. In developing the SWEGs, data on the physical and chemical characteristics of the contaminants are considered, as well as observed toxic effects from oral exposures for identification of the most sensitive target organ or body system affected. Risk assessments methods established by NRC SWEG guidelines, including consideration of uncertainty factors that adjust for weaknesses of uncertainties about available data and that are specific to spaceflight, were employed to establish Acceptable Concentrations (ACs) for toxic or adverse health end points for the alkylamines. Odor thresholds were selected as the most relevant adverse health end point, and were used to establish ACs of 2mg/L (water) for the mono-alkylamines, 0.3mg/L for the di-alkylamines and 0.4 mg/L for the tri-alkylamines, each respectively applied across all designated exposure periods.

1612 REDUCING THE DEFAULT UNCERTAINTY FOR SUBCHRONIC TO CHRONIC EXPOSURE FOR THE BROMOBENZENE REFERENCE DOSE (RFD) AND REFERENCE CONCENTRATION (RFC) USING DATA FROM CHLOROBENZENE.

C. L. Smallwood. *NCEA, ORD, U.S. Environmental Protection Agency, Cincinnati, OH.* Sponsor: H. Choudhury.

Due to the limited data available for risk assessment for some chemicals uncertainty can be quite high. Bromobenzene is an example of a chemical for which no chronic studies are available. EPA is in the process of developing a Toxicological Review for bromobenzene for the Integrated Risk Information System (IRIS). In the absence of chronic toxicity data for bromobenzene, this poster explores the possibility of using data from chlorobenzene to reduce the uncertainty for the bromobenzene RfD and RFC. Animal studies identify the liver as the most sensitive target of oral and inhalation exposure to bromobenzene. Numerous mechanistic studies in animals demonstrate that hepatotoxicity is associated with the metabolites of the parent compound. Repeated dose acute studies have shown that the liver appears to develop a tolerance to bromobenzene insult. Bromobenzene and chlorobenzene exhibit striking similarities in structure, with similar Pauling electronegativities, distribution, toxicokinetic properties, and toxicity to the liver in rats and mice. Metabolic schemes for bromobenzene and chlorobenzene include cytochrome catalyzed epoxidation to reactive epoxide intermediates and subsequent formation of corresponding metabolites. Dose-response relationships for liver effects from sub-chronic and chronic exposure to chlorobenzene appear to be similar, which suggest the development of some degree of tolerance to chlorobenzene during chronic exposure. It is reasonable to expect such similarities in dose-response relationships for

subchronic and chronic exposure to bromobenzene. Therefore, the data available for chlorobenzene may help to reduce the subchronic to chronic uncertainty and support development of a chronic assessment for bromobenzene.

1613 POPULATION SCREENING LEVEL RISK ASSESSMENT OF NASAL TUMORS DUE TO NAPHTHALENE EXPOSURE.

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The National Toxicology Program (NTP) has listed naphthalene as "reasonably anticipated to be a human carcinogen." EPA Region VIII and the State of California have calculated a unit risk level of -0.04 (mg/m³)-1 using the data on neuroblastomas of the olfactory epithelium and adenomas of the respiratory epithelium in male rat. However, there is a toxicological debate regarding the relevance of the rat nasal tumor data to human health risk assessment because of physiological and biochemical differences between rats and humans. To inform this debate, this paper investigates whether there is any evidence that nasal tumors occur in workers and the general public due to their long term exposures to naphthalene in the indoor and outdoor environment. First, an evaluation of the published health effects literature of workers who were exposed to several naphthalene-containing complex mixtures (creosote, coal tar and coal tar pitch) reveals no cases of nasal tumors causally linked to naphthalene. Second, a screening level risk assessment is performed on the general population to estimate the number of nasal tumors that would be expected in the U.S. population if the unit risk value for naphthalene is as noted above. The results are that the incremental excess lifetime cancer risk for nasal tumors would be predicted to range from 8×10^{-5} for the general population not exposed to wood smoke in the home to 2×10^{-2} for people who use naphthalene moth balls in their homes. A nasal tumor burden of 12,400 nasal tumors per year is predicted. However, the observed numbers of nasal tumors each year from all causes is approximately 2,000 per year, and this includes tumors of the nasal cavity, the middle ear, and accessory organs of the nasopharynx. The observed rate of nasal neuroblastomas, a very rare tumor for this site, is even less, at about 100 per year from all causes.

1614 PROVISIONAL ADVISORY LEVELS (PALs) DEVELOPMENT FOR DIMETHYL PHOSPHITE (DMP).

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Provisional Advisory Levels (PALs) that are being developed for hazardous materials by the U.S. EPA represent general public emergency exposure limits. PALs correspond to different effect severity levels (PALs 1, 2, and 3) and exposure durations (24 hours, 30 days, and 2 years). Application of PAL protocols has been performed for dimethyl phosphite (DMP), to estimate oral (in drinking water) and inhalation exposure limits, to the degree supported by the available data. In addition to being a chemical intermediate in the production of insecticides, herbicides and adhesives, DMP is used as a flame retardant, is a precursor for some chemical warfare nerve agents such as sarin, and is a degradation product of the chemically-related compound trimethyl phosphite (TMP) and some pesticides. The experimental data set for DMP was marginally adequate, including limited human data and a limited number of animal studies. Data gaps are identified. These estimates were evaluated and approved by an Expert Consultation Panel for PALs in June 2006. PAL estimates for DMP will be presented. Oral PAL values for a 24-hour exposure duration to DMP are 510 mg/L (PAL 1), 510 mg/L (PAL 2), and 750 mg/L (PAL 3); for 30-day oral exposure duration, the values are 25 mg/L (PAL 1), 25 mg/L (PAL 2), and 50 mg/L (PAL 3); and for 2-year oral exposure duration, the values are 13 mg/L (PAL 1), 25 mg/L (PAL 2), and 50 mg/L (PAL 3). Inhalation PAL values for a 24-hour exposure duration to DMP are 0.053 ppm (PAL 1) and 3.6 ppm (PAL 2); for 30-day inhalation exposure duration, the values are 0.021 ppm (PAL 1), 0.062 ppm (PAL 2), and 0.21 ppm (PAL 3). Data were insufficient to derive inhalation PAL 3 estimates for the 24-hour exposure duration or inhalation PALs 1, 2, and 3 estimates for the 2-year exposure duration. (This abstract presents PAL values that are subject to change pending further review.)

1615 ASSOCIATIONS BETWEEN ACUTE ASTHMA EXACERBATIONS AND AMBIENT SULFUR DIOXIDE EXPOSURE: POSSIBLE IMPLICATIONS FOR NATIONAL AMBIENT AIR QUALITY STANDARDS.

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Sulfur dioxide (SO₂) is a respiratory irritant regulated as a criteria air pollutant under the federal Clean Air Act. We sought to compare SO₂ exposure-response relationships for acute asthma exacerbations with current SO₂ national ambient air

quality standards (NAAQS). A time-series study of ambient SO₂ (and other pollutant) levels and asthma emergency department (ED) visits in New York City (NYC) and a systematic review and meta-analysis of other time-series studies relating SO₂ levels and acute asthma exacerbations were conducted. The time series study used Poisson regression to test for effects of daily variation in 14 air pollutants measured at a roof-top monitoring station in NYC on daily asthma ED visits among residents living near the air monitoring station. Core regression models controlled for seasonality, temperature and day of week. In single-pollutant models, SO₂ was one of four pollutants (in addition to ozone, nitrogen dioxide and fine particles) that were significantly associated with asthma ED visits, with significant relative risks (RR) for increments in 24-hour mean and 1-hour daily maximum air levels. SO₂ RR estimates remained statistically significant and roughly equal in magnitude in models controlling for effects of the other significant pollutants, suggesting an independent effect of SO₂ on acute asthma exacerbations. The meta-analysis included 26 time-series data sets that met inclusion criteria for relating acute asthma hospitalizations or ED visits to ambient SO₂ levels. RRs were normalized to a fixed SO₂ increment and 16 were statistically significant. Study mean SO₂ and maximum or 95% upper bound concentrations ranged from 5–53 and 21 to 293 ug/m³, respectively. These levels are well below the current 24-hour SO₂ NAAQS (365 ug/m³).

1616 ACRYLAMIDE CARCINOGENICITY AND A MECHANISM BASED SAFETY ESTIMATE.

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Acrylamide is a nongenotoxic carcinogen; It is considered a probable human carcinogen, therefore, no regulatory threshold value has been derived. Acrylamide (AA) and its metabolite glycidamide form DNA adducts, additionally AA has been shown to be clastogenic. However, animal experiments do not indicate a single target organ for acrylamide carcinogenicity and epidemiological data do not unanimously support human acrylamide carcinogenicity. Recent experiments have shown that acrylamide interferes with tubulin association. Since tubulin function is necessary for cell division, cell adhesion and growth regulation and neuronal transport, this mechanism may explain both acrylamide neurotoxicity as well as its non-specific carcinogenicity in a variety of organs. Additionally, tubulin interference explains the finding that acrylamide carcinogenicity depends on tumor promotion by e.g. TPA in animal bioassays. Assuming a toxic mechanism of action, risk assessment should be based on the derivation of a nontoxic threshold uptake rather than the current estimation of a cancer risk level of 1 / 105 (0.002 µg/kg/day) or 1 / 106 (0.0002 µg/kg/day uptake, EPA). In rat bioassays significant increases in peritesticular mesothelioma have been observed at 0.5 mg/kg/day, with possible increases in both mesothelioma and pheochromocytoma. This concentration was only marginally lower than the neurotoxic concentration of 1 mg/kg/day. For risk assessment a threshold value of 0.5 µg/kg/day is derived based on a LOAEL of 0.5 mg/kg/day and assuming safety factors of 10/10/10. There is evidence for low interspecies variability between mouse, rat and cat; additionally, the steep dose response curve for acrylamide toxicity in both in vivo and in vitro tests suggest lower safety factors. We therefore conclude that the average daily uptake of acrylamide from food estimated at 0.5–5 µg/kg/day explains the epidemiological data suggesting no or extremely low human carcinogenic potential.

1617 AN EVALUATION OF EARLY-LIFE SUSCEPTIBILITY AND EXPOSURES TO ETHYLENE OXIDE.

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Guidelines from the EPA state that, in the absence of chemical-specific data on childhood susceptibility, mode-of-action and other relevant studies should be used to determine age-related differences and possibly adjust potency. Relevant data exist for ethylene oxide and should be considered rather than automatically relying on defaults. Ethylene oxide is present endogenously in humans. Ethylene, a by-product of lipid peroxidation of membranes, metabolism of methionine, and metabolism by intestinal bacteria, is converted to ethylene oxide in the body. Higher levels of endogenous ethylene are not indicated in children. Currently, there are no animal toxicology or epidemiology studies that consider cancer risk of early-life exposure to exogenous ethylene oxide; however, there are data on the incidence of second cancers following exposure to other alkylating agents for the treatment of Hodgkin's disease that can be used as a surrogate. A review of these clinical epidemiological studies does not suggest greater leukemia or lymphoma risk among children, following exposure to alkylating agents. In addition, children generally tolerated doses of chemotherapeutic alkylating agents in Phase I clinical trials at least as well as adults. Furthermore, neonates and children have lower levels of

CYP2E1, the enzyme that oxidizes ethylene to ethylene oxide. Detoxification of ethylene oxide may be lower in children than adults, but the disparity is less than the assumed EPA default of 10-fold. Finally, fewer hemoglobin adducts indicate lower background ethylene oxide body burden in children.

1618 HUMAN HEALTH RISK ASSOCIATED WITH EXPOSURE TO PATHOGEN-CONTAMINATED SEDIMENTS.

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The presence of pathogens in environmental media can severely impact the public health, particularly when contamination involves human-derived pathogens such as *E. Coli* or *Giardia*. Previous environmental assessments have reported high levels of pathogenic microorganisms in the Lower Passaic River in Newark, NJ. Many combined sewer overflows (CSOs) are present in this portion of the river and have been identified as potential sources of pathogen release into the environment. Because river sediments are reservoirs for several types of waterborne pathogens that are associated with human fecal matter, the aim of this study was to characterize the pathogenic contamination of river sediments collected from mudflats near CSOs and to assess the human health risk associated with potential exposure to the pathogen-contaminated sediments. The sediments contained several types of pathogenic bacteria including fecal coliform, *E. Coli*, fecal *Streptococcus*, *Enterococcus* and *Pseudomonas aeruginosa*, as well as protozoa, specifically *Giardia* and *Cryptosporidium*. Antibiotic resistant strains of bacteria were also found in the sediments. Risk estimates were calculated for three adult exposure scenarios: recreators, visitors and transients. The risk of contracting gastrointestinal illness following incidental ingestion of sediments from fecal coliform was >1 for all scenarios, whereas the risk of illness from fecal *Streptococcus* and *Enterococcus* was as high as 0.058 and 0.049, respectively. Risk estimates for illness associated with transient exposure to *Giardia*-contaminated sediments exceeded 1 in 100. Results suggest that incidental ingestion and/or dermal contact with pathogen-contaminated sediments near areas of CSO discharge along the Lower Passaic River were associated with a high risk of illness.

1619 PROVISIONAL ADVISORY LEVELS (PALs) DEVELOPMENT FOR TRIMETHYL PHOSPHITE (TMP).

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Provisional Advisory Levels (PALs) are being developed by the U.S. EPA for contaminants that may be released to drinking water or air. PALs represent exposure limits for the general public that correspond to different effect severity levels (1, 2, and 3) and exposure durations (24 hours, 30 days, and 2 years). PAL protocols have been applied to estimate both oral (drinking water) and inhalation exposure limits for trimethyl phosphite (TMP) based on available data. TMP is used to make organophosphate pesticides and flame retardants and is also a precursor for certain nerve agents. This compound converts quickly to DMP in water and air, so continuous releases were assumed to support PAL development for durations beyond one day. Data gaps are evaluated as part of the PAL development process, and the experimental data set for TMP was found to be marginally adequate, with limited human data and a limited number of animal studies. These estimates were evaluated and approved by an Expert Consultation Panel for Provisional Advisory Levels in June 2006.

PAL estimates for TMP will be presented. Oral PALs for TMP in drinking water assume an adult ingestion rate of 2 L/d. For all three exposure durations, the PAL 1 is 17 mg/L and the PAL 2 is 19 mg/L, because these are based on developmental endpoints that could result from exposure within any of the periods. For the PAL 3, the 24-hour value is 350 mg/L, and the 30-day and 2-year values are 57 mg/L. TMP data were considered insufficient to derive any 2-year PALs or a 24-hour PAL 3 (per inconsistent reporting of saturation). The inhalation PAL 1 is 0.1 ppm for both 24 hours and 30 days. For the PAL 2, the 24-hour value is 15 ppm and that for 30 days is 0.5 ppm; the value for the 30 d PAL 3 is 2.9 ppm. (This abstract presents PAL values that are subject to change pending further review.)

1620 ASSESSMENT OF CHILDREN'S EXPOSURE TO TOLUENE.

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Toluene was included in EPA's Voluntary Children's Chemical Evaluation Program (VCCEP) Pilot program because it has been detected in the indoor air and in human blood and milk. The objective of this study was to quantify children's expo-

sure to toluene and aggregate exposures across scenarios and pathways. A child-centered approach was used to define exposure scenarios for children's interaction with toluene, including environmental background sources and source specific exposures such as tobacco smoke and consumer products. Chronic and short-term episodic exposures were quantified in terms of typical and high-end estimates, and the contributions of various sources to overall toluene exposures were assessed. The assessment of background exposures indicated that inhalation was the primary route of exposure; accounting for 95% of the aggregate dose for all age groups, except for nursing infants of occupationally exposed mothers. For these children, 68% of their total dose was via ingestion of human milk and 32% was via inhalation. The primary source of inhalation exposures was residential indoor air. Of the chronic source specific exposures, inhalation of mainstream smoke was the primary pathway of exposure where the estimated doses were 2-5 times higher than background. Exposures to toluene from ETS and refueling vehicles did not add significantly to the aggregate exposure. A human PBPK model for toluene was used to estimate blood concentrations that would be predicted in children of various ages given the inhalation exposure concentrations for background exposures used in this assessment. The PBPK results indicated that the average and peak predicted blood values were similar to those reported recently in the literature for children. In conclusion, robust exposure data sets exist for background exposure sources of toluene, and except for the nursing infant, no unique exposure scenarios were identified for children.

1621 QUANTITATIVE DOSE-RESPONSE ASSESSMENT OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES FROM MICE EXPOSED TO PRADOXIFLOXACIN.

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Genotoxicity endpoints have traditionally been evaluated only qualitatively (as either positive or negative), contributing primarily to the weight-of-evidence regarding an agent's mode of toxic action in risk assessments. However, many of these endpoints are remarkably sensitive, and they are amenable to quantitative dose-response evaluation on their own, provided data from a sufficient number and range of dose levels are available. Such analyses can provide information regarding the shape of the dose-response at very low doses as well as insights regarding an agent's mode of toxic action. An exemplary Benchmark Dose (BMD) analysis was undertaken of micronuclei data from two experiments in which young adult Hsd/Win:NMRI mice were exposed to a 40-fold range of doses of the veterinary drug candidate, pradofloxacin. An appropriate Benchmark Response (BMR) was first chosen to approximate a No Observable Effect Level (NOEL) for micronucleated cell (MNC) frequency among polychromatic erythrocytes (PCEs) relative to the baseline MNC frequency in unexposed animals. Statistical power calculations were employed to establish a BMR of only 1.27 additional MNC per 1,000 PCEs examined, nearly 100-fold lower than the 10% response increment typically used in BMD analyses. USEPA's Benchmark Dose software package (v. 1.3.2) was then utilized to estimate the parameters of a Weibull dose-response model as well as central (244 mg/kg) and lower 95% confidence bound (186 mg/kg) estimates of the BMD associated with the chosen BMR. The fitted dose-response model was markedly non-linear, suggesting that a threshold-based or non-linear approach to risk assessment is appropriate for pradofloxacin.

1622 COMPARISON OF TOOLS TO CALCULATE 95% UPPER CONFIDENCE LIMITS ON THE MEAN.

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Calculating a 95% upper confidence limit on the mean (95% UCL) for use as an exposure point concentration in risk assessment follows a two-step process. First, the data are subjected to goodness-of-fit tests to establish what type of distribution best fits the data (e.g., normal or lognormal distribution). A 95% UCL method is then selected according to the distributional characteristics of the data found in the goodness-of-fit test. A problem often encountered with environmental datasets is the existence of a number of non-detect values. The left censoring of data created by non-detect values can impose difficulties in correct assignment of distribution type and calculation of the 95% UCL. PROUCL 3, EPA software for calculation of 95% UCL values, had limited utility with datasets censored more than 15%. Due in part to this limitation, the Florida Department of Environmental Protection developed FLUCL, a software tool capable of calculating 95% UCL values from more heavily censored data. Recently, the EPA has developed PROUCL 4, which has improved capabilities with censored data. The performance of FLUCL and PROUCL 4 was compared using synthetic data sets with varying characteristics in terms of size, skewness, and censoring. Although FLUCL and PROUCL 4 can both handle

a variety of types of environmental data sets, there are differences in the way censored data are handled, distributional type is determined, and 95% UCL calculation method is selected. These differences can, in some cases, lead to substantial differences in the 95% UCL value derived. Skewness of the sample data was found to be an important factor in determining the conservatism of the 95% UCL estimates, and affected the two software tools differently. The 95% UCL software tools are a convenient means to calculate exposure point concentrations, but should not be treated as "black boxes." Existing tools have strengths and weaknesses that need to be understood in order to apply them properly in the calculation of exposure point concentrations for contaminated sites.

1623 A GLUTARALDEHYDE RISK ASSESSMENT USING BENCHMARK DOSES: DECISIONS, DECISIONS, DECISIONS!

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The objective of this study is to describe a quantitative risk assessment for inflammatory responses associated with glutaraldehyde exposure, using benchmark dose (BMD) methods, and to evaluate the impact of alternative assumptions on the estimated risk. These alternatives included the choice of which data set to use as a basis for BMD estimation, which toxicological endpoint(s) should be selected for extrapolation to humans, how the toxicologically-based BMDs should be scaled to humans, which statistical models in the BMD suite should be used, and how the different BMDs should be synthesized into a recommendation for human occupational exposures. Two NTP data sets were considered (13-week and 2-year), as well as RD₅₀ data. A total of 119 BMD models were fitted to various toxicological endpoints for male and female mice and rats in the two NTP studies. Within data set/endpoint combinations, different dose-response models were fit and a model-averaged BMD was obtained. BMDs based on squamous epithelial inflammation were chosen for extrapolation to humans; these ranged from 50-130 ppb in female rats and from 56-93 ppb in female mice suggesting that the BMD is not critically dependent on the choice of target species. Adjusting for 8-hr occupational exposures as opposed to 6-hr exposures in the NTP study reduces the rat BMD range to 38-98 ppb. The rat BMDs were then extrapolated to humans on the assumption of equal effects at equal exposure concentrations, regardless of species. A 32-fold uncertainty factor was applied, yielding an occupational exposure range of 1-3 ppb. This exposure range is intended to be protective against respiratory irritation due to chronic exposures to glutaraldehyde; however, it is not known whether it will also protect workers from respiratory sensitization.

Disclaimer: The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy.

1624 DEVELOPMENT OF SHORT-TERM, SUBCHRONIC AND CHRONIC ORAL REFERENCE DOSES (RfDs) FOR MICROCYSTIN-LR.

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Microcystins are monocyclic heptapeptide toxins produced by several species of cyanobacteria (blue-green algae), including members of *Microcystis*, *Anabaena*, *Nodularia*, *Nostoc* and *Oscillatoria*. Human exposure to microcystins may occur through the consumption of contaminated drinking water, ingestion of contaminated surface water during recreational activities or through the use of blue-green algae dietary supplements. Microcystins are potent hepatotoxins, primarily due to their preferential uptake into the liver via the bile acid transport system. The primary mechanism of action of microcystins is well known and involves the inhibition of serine/threonine protein phosphatases PP1 and PP2A leading to hyperphosphorylation of cytosolic and cytoskeletal proteins and subsequent hepatocyte disruption and hepatotoxicity. Additionally, microcystins have been shown to induce a number of other cellular alterations ranging from lipid peroxidation to apoptosis. At least 80 microcystin structural variants have been identified though the most commonly detected and best studied variant is microcystin-LR. In 1999, the World Health Organization (WHO) established a provisional 'Tolerable Daily Intake' (TDI) for microcystin-LR of 4×10^{-5} mg/kg-day. New repeated-dose experimental animal toxicity data have been generated by several laboratories since the WHO provisional TDI was published in 1999. Here, following a thorough literature review and the evaluation of all available toxicity information, oral Reference Doses (RfDs) for short-term (up to 30 days), subchronic (up to 10% of the average lifespan) and chronic (up to lifetime) exposure durations were derived for microcystin-LR using current US Environmental Protection Agency methodology.

1625 COMPARATIVE ECOLOGICAL RISK ASSESSMENT FOR A NEW ALUMINUM SMELTER IN ICELAND.

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Protection and maintenance of local populations and communities of plants and wildlife is gaining importance in corporate decision-making regarding industrial development. During the facility design phase for an aluminum smelter in East Iceland, it became necessary to determine if a wet (seawater) scrubber system should be installed to reduce overall sulfur dioxide emissions. In this case, predictive risk assessment was used to determine whether there would be a consequential difference in the level of risk to human and ecological receptors from constituents in air emissions from the aluminum smelter either with or without wet scrubbers. Benchmark exposure concentrations corresponding to negligible ecological risk were established for avian, mammalian, and plant receptor groups based on the toxicity literature. These benchmark exposure concentrations were then converted to concentrations in air and compared to air modeling predictions to develop risk estimates. Benchmark air concentrations were derived using plant uptake models for fluoride and PAH, and food-web modeling for birds and mammals. Spatially and temporally explicit exposure models were developed for all receptors based on predicted concentrations of constituents in air, and population-level effects were modeled for plant (lodgepole pine, heather, and mosses/lichen), bird (ptarmigan) and mammalian (wood mouse) receptors. The results of this work revealed that exposures under both scenarios are likely to be much lower than concentrations corresponding to risk thresholds and that overall risk was lower for a smelter operating without wet scrubbers. Thus, although mass loading of sulfur dioxide (and other constituents) would be reduced using wet scrubbers, corresponding risk to ecological receptors would actually be higher because of higher exposure-point concentrations in air.

1626 CANCER POTENCIES AND DAILY INTAKE LEVELS FOR SIX INDUSTRIAL CHEMICALS.

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Cancer potencies for six carcinogens (C.I. Direct Blue 218; 2,4-hexadienal; pyridine; bromoethane; propylene glycol mono-t-butyl ether; and N-methylolacrylamide) were estimated based on data from National Toxicology Program (NTP) rodent studies. Each of these chemicals was chosen for assessment of cancer potency because they are industrial chemicals (solvents, chemical intermediates, etc.) for which exposures of workers, consumers, and/or the general population are likely to occur. Based on a review of mechanistic data, the dose-response relationship was assumed to be consistent with linearity at low dose for all six chemicals, and a linearized multistage model was used to estimate cancer potency. The cancer potency estimate corresponds to the upper 95 percent confidence bound on the linear term of the multistage model fit to the tumor response data, adjusted to account for body size differences between humans and animals. One of the chemicals (N-methylolacrylamide) induced tumors at multiple sites (liver, lung and harderian gland) in mice. In this case, cancer potency was taken to be the sum of potencies associated with the three types of tumors in the male (most sensitive sex) mouse. Because of the statistical uncertainty in individual estimates of potency for each site, the linear terms were summed statistically, using Monte Carlo techniques. The upper 95 percent confidence bound on the summed linear terms was taken as the cancer potency estimate for N-methylolacrylamide. The cancer potency estimates range from greater than 1 (mg/kg-d)-1 for pyridine down to just over 0.01 (mg/kg-d)-1 in the case of C.I. Direct Blue 218. Daily intake levels corresponding to lifetime cancer risks of one in a million vary from several micrograms per day for the least potent carcinogens down to hundredths of a microgram per day for pyridine, the most potent of these six carcinogens.

1627 VALUE OF INFORMATION APPROACH FOR DEVELOPMENT OF MODELS OF THE DEVELOPING NEOCORTX AFTER EXPOSURE TO LOW DOSE RADIATION FROM INTERNALLY DEPOSITED RADIONUCLIDES.

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Low dose radiation exposures during particular gestational periods can result in permanent neuronal perturbations and can lead to abnormalities in behavior and mental activity. Multiple mechanisms underlie these effects including radiation-induced cell death among neuronal precursors in the proliferative regions of the neocortex. We developed a computational model based on diverse data sets to describe the extent and pattern of cell death in the neocortex and the altered patterns of mi-

gration. The model predicts the subsequent effects of cell death on neuron number at the end of neurogenesis and alterations in migration. Our results indicate that the induced death of a small fraction of neuronal precursors, dependent on the dose and time of exposure, leads to significant changes in migration and decrease in the final neuron count. The model provides a comparison of differences in absorbed dose, time of exposure, and duration of exposure. The model predicts a 1% decrease of neuronal precursor cells per cGy during the most sensitive time window of neurogenesis and about a 0.5% decrease per cGy during other times of neurogenesis. The data sets used to develop the models used single acute exposures from external sources of radiation. We have extended these models by linking to kinetic models for tritium, H-3, and Cs-137, internally deposited radionuclides commonly associated with environmental contamination. Based upon the extensions of the models we predict smaller effects from H-3 and Cs-137 than for external radiation when total doses during gestation are the same. We provide estimates for rodents and humans. Our value of information analysis indicate areas where future studies are needed to reduce variability, such as more detailed evaluation of postnatal neuron numbers. Support for this research was from Center for Child Health Risk Research, EPA RD-83170901 and NIEHS P01 ES09601, and Low Dose Program of the Department of Energy, DE-FG02-03ER63674.

1628 FUGACITY MODELING AND ESTIMATION OF SOURCE ALLOCATION FACTORS FOR DRINKING WATER CONTAMINANTS (DWCS).

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Source allocation factor (SAF) or relative source contribution (RSC) refers to an estimate of the proportion of the total exposure resulting from a specific exposure source (e.g., drinking water). A default SAF of 0.2 has been used in non-cancer risk assessments of DWCS with no obvious scientific basis. Since media-specific exposure data are often unavailable, uncertain or incomplete for most DWCS, the SAFs may be developed on the basis of model-derived values. The objective of the present study was to investigate the feasibility of developing SAFs for DWCS using the fugacity modeling approach. The approach involved using an equilibrium criterion (EQC) model based on the concept of fugacity (Level 1) to predict the concentrations of DWCS in various environmental compartments and subsequently using these results to compute human exposure doses. Using n-octanol:water and water:air partition coefficients as input parameters to the EQC model, the concentrations of several volatile organic chemicals (aromatic hydrocarbons, chloroethanes, alkanes and haloalkanes) in air, water, soil and fish were generated. These results were then used in other algorithms to compute chemical concentrations in vegetable, meat and milk. SAF for each chemical was then calculated as the proportion of total dose received via drinking water and it was well within 0.2 or 20% for most volatile organic chemicals, signifying that the current SAF is generous. The fugacity model-based SAF calculations are based on physicochemical characteristics of chemicals and it may not be appropriate for chemicals which are additionally generated by water treatment or water use processes. Therefore, the model-based or physicochemical parameter-based estimations of SAF should be considered along with other data (e.g., usage pattern, exposure related to consumer products), to derive the appropriate SAF value for use in DWCS risk assessment.

1629 DEVELOPMENT OF A SCREENING FRAMEWORK FOR DATA REVIEW AND ANALYSIS TO SUPPORT THE DERIVATION OF A NEURODEVELOPMENTAL TOXICITY VALUE FOR ETHANOL.

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U.S. EPA is performing a toxicity assessment for ethanol due to its increasing use as a gasoline additive. A screening framework was developed for review and analysis of data relevant to determining a toxicity value that incorporates the extensive knowledge of ethanol pharmacokinetics and neurodevelopmental toxicity. Epidemiology data were initially used to define relevant critical effects for low to moderate exposure to ethanol (i.e., problems with attention, learning, and memory). The review of animal data focused on neurobehavioral studies or mechanistic and neuroanatomical data that relate to learning and memory deficits. An iterative process of data review and targeted literature searching was applied, and recent secondary sources were relied upon to provide an overview of the current state of knowledge. A qualitative scheme describing possible modes of action (MOA) was developed to refine the determination of critical neurodevelopmental effects based on biological plausibility and human relevance, strength and specificity of association, and dose-response and temporal concordance. Possible key events identified were apoptosis

of post-mitotic neurons, oxidative stress, disruption of growth factor signaling, and altered function of cell adhesion molecules. Medline searches were used to provide a crude measure of the weight of evidence for each key event in the MOA scheme (expressed as # of hits). Multiple-dose studies characterizing the dose-response relationship for critical effects were chosen from the literature. Quantitative models were applied for analysis of ethanol pharmacokinetics and dose-response assessment. This framework combines mechanistic understanding with quantitative simulation to support the development of a neurodevelopmental toxicity value for ethanol. (The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA).

1630 RESPIRATORY EFFECTS OF PYROTECHNICALLY DISSEMINATED TITANIUM DIOXIDE SMOKE IN SPRAGUE DAWLEY RATS.

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Recently, the U.S. Army Chemical School and PM Obscuration performed an obscuration Mission Area Analysis (MAA) to identify the current critical uses of smokes/obscurants for the military. Operational capability gaps and limitations were identified which prevent accomplishing all aspects of the mission. In response to the small through medium area screening obscuration FNA, the Family of Tactical Obscuration Devices (FOTOD) has been developed as a solution to help reduce the risks associated with this functional need. Obscuration from the enemy is needed in restrictive terrain such as urban structures (i.e. 12' x 12' x 12' rooms) or smaller confined open terrain. Rapid, short duration effects are needed to satisfy this identified gap. Other requirements are for the item to be environmentally friendly and operator safe.

The Fast Obscurant Grenade (FOG) is a bursting type grenade that fulfills this small through medium area screening obscuration need. After an analysis was performed of the currently used military obscurant payloads, titanium dioxide (TiO₂) was chosen as the safest smoke, while maintaining the necessary performance characteristics. Many studies evaluated the toxicity of inhaled titanium dioxide; however most have evaluated long exposure times (i.e. 30 minutes) at low concentrations. For the current need, elevated concentrations for short exposure times would be the predominant operational scenario. Both acute and repeat exposures are therefore possible as maneuvers are performed in confined areas and in close proximity to the dissemination source. The current study evaluated clearance of the smoke material from the respiratory system as well as other biological effects. Groups of rats were exposed for 10 minutes to high concentrations of smoke generated from the FOG. Bronchoalveolar lavage, respiratory physiology, and pathology were performed to assess the toxicity of the inhaled smoke.

1631 GEOGRAPHICAL HETEROGENEITY IN BLADDER/LUNG CANCER RISK AND ARSENIC EXPOSURE IN SOUTHWEST TAIWAN.

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The bladder and lung cancer mortality data for a 42-village, six-township study in Southwest Taiwan (Wu et al., 1989) has served as the analytic base for cancer risk assessment from the ingestion of arsenic (NRC, 1999; 2001). A variety of analytic models have addressed this 42-village dataset, stressing issues of choice of comparison group, levels of stratification and aggregation, variation of arsenic levels within individual villages, population assumptions of body weight and water consumption, and additional sources of arsenic exposure. Although the earliest study of internal cancers from this area highlighted differences in risk based on water source [artesian wells] and on geography [township] (Chen et al., 1985), quantitative risk analyses have inherently assumed homogeneity in background risk for the 42-villages. Stratification by dependency has shown that increased bladder cancer mortality was limited to villages dependent upon artesian wells (Lamm et al., 2003). Further, stratification of the dataset by township has shown heterogeneity among the townships in background risk of bladder/lung cancer mortality (Lamm et al., 2006). The data from three townships show a significantly increased background risk of bladder/lung cancer mortality as calculated from regional rates. These villages show no apparent additional dose-response risk for arsenic exposure. The cause of this elevated background rate is not known and is being sought. In contrast, the other three townships show no increased background risk of bladder/lung cancer mortality. These second three townships do show a significant linear dose-response relationship with arsenic exposure that begins at 125-150 ug/L. Restriction of the analysis to villages not dependent upon artesian wells similarly shows now that the geographic heterogeneity is independent of artesian well-dependency. This analysis is consistent with modern theories of arsenic carcinogenicity.

1632 DETERMINING RISKS TO BACKGROUND ARSENIC USING A MARGIN OF EXPOSURE APPROACH.

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Inorganic arsenic (Asi) is a known human carcinogen. In certain areas of the world, where natural levels of Asi in the drinking water are extremely high (e.g., hundreds of ppbs), an association has been established between ingestion of Asi and cancer prevalence (skin, bladder and lung). Regulatory agencies use linear extrapolation to estimate Asi cancer risks; this results in risks from background exposure to Asi in food, water, and soil greater than 10⁻⁶, and, in some cases, 10⁻⁴, thus exceeding regulatory targets. However, epidemiological and mechanistic evidence strongly supports a non-linear dose response of inorganic Asi. We therefore propose a Margin of Exposure (MOE) analysis to characterize risks to background Asi exposures. For a point of departure, we use the no-effect level identified by Lamm et al. (2006). This value is based on a re-analysis of the Taiwanese data, demonstrating that the dose-response for Asi-related bladder and lung cancer has an apparent threshold, with bladder and lung cancer risk significantly increased (SMR ≥100) only at drinking water concentrations above 151 mg/L (CI: 42 to 229 mg/L). Using estimates of Taiwanese specific-weight and intake factors, we converted this drinking water level a no effect level dose of 0.013 mg/kg-day. We calculated MOEs for the US population by comparing the no effect level dose to estimates of background exposures to Asi in food, water and soil, considering distributions of intake as well as Asi concentration in the various media. We set our target MOE at 1 because the Taiwanese cohort was exposed to high Asi concentrations over a lifetime (i.e., included in utero, child and adult exposures) and, because of poor nutritional status, is likely more sensitive to Asi-related health effects than the US population. Our analysis demonstrated that typical and high end (95%ile) background exposures to Asi in food, water and soil for children and adults are well above the target MOE of 1 and do not present a toxicological concern in the US.

1633 RISK ASSESSMENT OF ESSENTIAL METALS: COPPER AS A CASE STUDY.

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Essential metals or trace elements (ETE) are inorganic micronutrients with specific biological functions that are indispensable for human health at all life stages. An element is identified as essential if deficiency or absence induces functional and/or structural abnormalities that are reversed by supplying the nutrient. Essential metals differ from other chemical compounds in that (1) appropriate intakes are required to maintain health and (2) complex homeostatic mechanisms have evolved to regulate absorption, excretion, distribution and storage in order to ensure a sufficient supply for performance of essential biological functions under conditions of varying intake levels. Both deficiency and excess can lead to adverse health outcomes, the dose-response curve is approximately U-shaped, and the dose-response slope is likely to be zero over a range of intake levels. Thus, approaches to risk assessment of essential metals differ fundamentally from those for conventional risk assessment. Severe or marginal ETE deficiencies are risk factors for several diseases of public health importance and can also impair reproduction, growth and development. The major challenge is to determine an "acceptable range of oral intake" (AROI) that meets nutritional requirements to prevent deficiency while simultaneously avoiding excessive intakes that can be toxic. Copper is a naturally-occurring metal that is found in all environmental media; it is also an ETE. In this case study, the hazards associated with copper excess and deficiency are summarized, a U-shaped dose-response curve is described and the AROI for copper determined. Margins of exposure are derived to determine the relative degree of human safety/risk of environmental exposures. This analysis is intended to serve as a model for characterizing the health risks of environmental metals that are also nutrients. From a public health perspective, it is important to resolve differences in methodologies and conceptual approaches between toxicologists and nutritionists in order to establish appropriate reference intake ranges for optimal health.

1634 HAZARD IDENTIFICATION AND PRELIMINARY DOSE-RESPONSE ASSESSMENT OF ORGANOTIN PLASTIC STABILIZERS FOUND IN DRINKING WATER.

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Organic derivatives of tin are used extensively as stabilizing agents in the manufacture of polyvinyl chloride piping for drinking water distribution systems. Leaching of these organotin compounds into drinking water may result in human exposure to mixtures of mono- and di-substituted methyl and butyl tins. Human exposure to these organotin species, irrespective of source, has been confirmed in blood and liver tissue samples. However, the toxicological significance of human exposure to

individual, or mixtures of, mono- and di-substituted methyl and butyl tins on human health remains unclear. Rodent models of oral exposure to these individual organotin species indicate multiple modes of toxic action. Specifically, organotin exposures in rats and mice suggest that the immune, central nervous, or female reproductive system and the developing fetus, may be the most sensitive targets of toxicity. Qualitative dose-response analysis suggests that toxicity in the developing fetus may be a common critical effect for the organotins of interest, and that dibutyltin may be the most potent. Candidate points of departure, using developmental toxicity as an endpoint, have been identified for future consideration in the derivation of single organotin oral reference values. However, due to the ubiquity of organotin mixtures in domestic waters, it will be imperative to develop human health risk assessments for mixtures of organotins. The most appropriate mixtures risk assessment approach will be highly dependent upon determination of mode of action. In this work, consideration of potential key events in a proposed toxic mode of action indicate that the organotins of interest induce adverse effects in fetuses independently of one another, suggesting that response addition would be an appropriate mixtures risk assessment approach. However, further mechanistic information is needed for a more informed evaluation of toxic mode of action for developmental endpoints and may lead to selection of an alternative mixtures risk assessment approach such as dose addition.

1635 RISK-RISK ASSESSMENT OF METHYLMERCURY (MEHG) EXPOSURE AND FISH CONSUMPTION.

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Assessment of public health risks of seafood consumption entails the quantization of the risks of exposure to environmental contaminants, like MeHg, and those associated with different levels of consumption of various fish nutrients. The estimation of risk of contaminant exposure as a matter of degree allows for the integration of other public health issues such as the nutritional benefits of fish consumption in an overall risk analysis. Estimates of seafood consumption were developed from several different sources: 1) CSFII survey (1989-91) 2) NHANES (2001-02) and 3) NMFS market share data (2002). An algorithm was constructed to account for the use of short-term consumption surveys to model long-term consumption. Variation in the types of species consumed as well as their market share and range of MeHg levels as well as the loss of water during cooking were accounted for. Dose-response relationships were developed for maternal exposure to MeHg and several different measures of neurobehavioral development that were compared by normalizing the outcomes relative to normal variation (i.e. Z-scores; $IQ = 100 + Z \cdot 15$). Dose-response relationships were also developed for potential effects of fish consumption on rates of coronary heart disease (CHD) and stroke. A variety of different consumption intervention scenarios were considered to assess the impact on these endpoints on four different U.S. subpopulations: women of childbearing age, older women, young men, and older men. The neurobehavioral models estimate that current levels of fish consumption are associated with a Z-score decrement of less than zero (i.e. there may be a benefit) to 0.01 (i.e. about 1/100th of normal variation). Fish consumption was also associated with reduction in the annual rate of both CHD and stroke by approximately 10-5 per person-year in men and women over the age of 45. Scenarios involving adjustments of seafood consumption via the selection of the species consumed or by increasing or decreasing impacted the resulting health outcomes.

1636 MARGIN OF EXPOSURE ANALYSIS FOR MTBE IN DRINKING WATER.

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MTBE is a gasoline additive used to reduce air emissions of carbon monoxide and nitrogen oxides, and can be found in drinking water at concentrations detectable by taste and odor. Although MTBE exposure is associated with development of tumors in rodents, there is a significant debate regarding the relevance to humans of the animal tumor findings. Even if the tumor findings are considered relevant in humans, the most plausible modes of action for the tumors observed in animals, as well as ample evidence that MTBE is not genotoxic, are consistent with a threshold dose response relationship. Hence, we evaluated potential cancer hazards associated with MTBE exposure using a Margin of Exposure (MOE) analysis.

We calculated the lower bound on the effective dose associated with a 10% increase in tumor incidence (LED10) for four tumor endpoints: Leydig Cell Tumors (LCTs) in male rats exposed via gavage; lymphoma/leukemia (L/L) in female rats exposed via gavage; kidney tumors in male rats exposed via inhalation; and liver tumors in female mice exposed via inhalation. We converted inhalation doses to oral doses, and converted oral doses to human equivalent doses using body weight to the ^{3/4} power.

We then converted the LED10s to drinking water equivalent levels (DWELs) assuming a time-weighted average body weight of 59 kg (6 years as a child, 24 years as an adult) and a time-weighted drinking water intake of 1.8 L/day. We calculated

MOEs from the DWELs assuming that individuals might consume drinking water with MTBE concentrations up to 100 µg/L, which is at the upper end of concentrations detected in drinking water, and also at the upper end of the taste threshold range. According to our analysis, MOEs ranged from 13,000 for L/L to 50,000 for liver tumors. Based on the magnitude of these MOEs, current concentrations of MTBE in drinking water would not present a carcinogenic hazard, regardless of whether or not MTBE is considered to be a human carcinogen.

1637 MTBE IS NOT ASSOCIATED WITH A STATISTICALLY SIGNIFICANT INCREASE IN LEYDIG CELL TUMORS IN SPRAGUE-DAWLEY RATS.

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The gasoline additive methyl tert-butyl ether (MTBE) has raised a potential human health concern because of reports of cancer in rodents. Belpoggi et al. (1995, 1997, 1998) reported an increase in Leydig cell tumors (LCTs) in Sprague-Dawley (SD) rats exposed to 1,000 (but not 250) mg/kg-day MTBE via gavage, but their statistical analyses took no account of the increased survival rate in the high-dose animals, which provides an increased opportunity for the occurrence of tumors. We re-analyzed the data using the Poly-3 method. Belpoggi et al. don't specify which rats had LCTs, so we conducted analyses for the three most extreme scenarios. In Case 1, tumors are assigned to occur in the youngest possible high-dose animals and in the oldest control and low-dose animals, resulting in the minimum dose response trend. In Case 2, tumors are assigned to occur in the youngest possible control and low-dose animals and in the oldest high-dose animals, resulting in the maximum possible dose response trend. Case 2 is not biologically plausible because any treatment-related tumors should occur in dosed animals at similar or earlier ages than in control animals. Thus, Case 3 is the maximum constrained dose-response trend that results when all tumors occur in the oldest animals in each dose group. P-values of 0.01 for pair-wise comparisons and 0.005 for trend tests of common tumors have been recommended in order to control for the overall false-positive error rate that can arise from multiple comparisons of tumor incidences in the large number of tissue/organ sites examined in chronic bioassays. Based on these significance levels, even for the biologically implausible scenario, MTBE does not cause a statistically significant increase in LCTs in SD rats. None of the recognized modes of action (MOA) for LCTs in rats is a likely MOA for MTBE, and the relevance of rat LCTs to humans is questionable. We conclude that the currently available data do not support a statistically significant association between MTBE and LCTs in rats.

1638 COMPARISON OF OBSERVED AND PREDICTED BLOOD LEAD LEVELS FOLLOWING LEAD PAINT ABATEMENT.

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Degradation of lead-based paint in U.S. homes built before 1978 continues to be a significant source of lead exposure for children and may result in subsequent neurological deficits. Various lead paint repair and maintenance (RM) techniques produce decreases in dust lead concentrations, but expectant reductions in children's blood lead levels (bLPb) are not similarly characterized. We compared the effects of lead paint abatement on actual and predicted bLPb in 56 children (39 M, 17 F; 2.4±1.1 yr on study admission) from urban Baltimore, MD, using a subset of publicly available data. As part of a previous EPA study on lead paint RM effectiveness, bLPb and environmental lead measurements were collected before and 6- and 12-months post-abatement. Two biokinetic models (EPA Integrated Exposure Uptake Biokinetic Model for Lead in Children, IEUBK; and O'Flaherty Model) were used to predict the effect of RM on bLPb. An average pre-RM bLPb of 13.7±7.1 µg/dL (geometric mean: 11.6±0.6 µg/dL) was observed and the dust lead concentration was 5973±10622 µg/g. For pretreatment, the IEUBK model predicted a slightly lower number of children with bLPb>10 µg/dL compared to those observed (36 vs. 41, respectively). At 6- and 12-months following RM activities, the IEUBK model predicted a greater percentage (29%) of children with bLPb <10 µg/dL. There was substantial disparity between the IEUBK and O'Flaherty model predictions (particularly when RM occurred after 2 yrs of age), which was partially attributable to differing age-specific default soil and dust ingestion rates used in the two models. In the context of using the models for predicting kinetics of bLPb changes following abrupt changes in exposure, potential contributors to the differences between model predictions and observations were evaluated. (Abstract does not represent EPA policy. This work was supported in part by an appointment to the Research Participation Program at NCEA administered by ORISE through an interagency agreement between DOE and EPA.)

1639 DEVELOPMENT OF A DRINKING WATER PUBLIC HEALTH GOAL FOR CADMIUM.

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A proposed health based drinking water criterion, or public health goal (PHG), was developed for cadmium. After an extensive literature review, renal toxicity was identified as the most sensitive toxicological endpoint. Increases in the levels of certain tubular proteins that are very sensitive indicators of the onset of renal toxicity were used to identify the onset of renal toxicity. Studies in humans showed the development of proteinuria when urinary cadmium levels exceed 2 to 5 µg/g creatinine. Proteinuria and other more severe signs of renal toxicity are not detected when cadmium levels remain at or below 1 µg/g creatinine. Thus a urinary cadmium level of 1 µg/g creatinine is considered to be equivalent to a no observed adverse effect level (NOAEL) for cadmium in humans. Toxicokinetic studies in humans were employed to estimate the long-term daily oral cadmium intake associated with urinary cadmium levels of 1 µg/g creatinine after 50 years of exposure. Humans exhibit first order elimination of cadmium, that is, the rate of urinary excretion is a function of body burden. A terminal half-life of 14 years was estimated in men based on the findings of Ellis and coworkers. With this half-life, an oral intake of cadmium of 19 µg/day would yield a urinary cadmium level of 1 µg/g creatinine after 50 years of exposure. Women are likely to be more sensitive to cadmium than men. Women have a higher body burden of cadmium, possibly related to increased oral absorption. Itai Itai disease, which is caused by cadmium, occurs principally in women. Also significant increases in mortality were observed in women but not men exposed to cadmium in rice in Japan. A PHG that is considered to be protective of women was developed based on a daily cadmium intake of 19 µg/day, an overall uncertainty factor of 50 and a relative source contribution from drinking water of 20 percent, yielding a proposed PHG of 0.04 µg/L. The current California and federal Maximum Contaminant Level is 5 µg/L.

1640 CREATING A DECISION CONTEXT FOR COMPARATIVE ANALYSIS AND CONSISTENT APPLICATION OF INHALATION DOSIMETRY MODELS IN CHILDREN'S RISK ASSESSMENT.

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Estimation of risks to children from air pollutants is complicated by the lack of reliable age-specific epidemiological data. Extrapolations from adults or animals present significant challenges: (1) differences in the mode of action (MOA) due to pharmacokinetic (PK) or pharmacodynamic (PD) differences among data sources; (2) integration of data ranging from in vitro biochemical to population studies; and (3) analysis of the quality and reliability of predictions. Numerous approaches address these issues, ranging from default uncertainty factors (UF) for interspecies and intrahuman variability to use of mechanistic models. PK and PD data are being developed for different life stages in animals and biomonitoring advances may provide measurements directly in humans. While these bring MOA data to bear on extrapolations, the problem of comparing disparate models remains; e.g., one model may empirically estimate age differences at the population level while another mechanistically predicts differences at the tissue level. We propose a decision analytic framework to systematically analyze and compare model structures; evaluate data interpretation, integration, and reliability; and rationally assess the quality of resulting model predictions. It uses a conceptual model to describe the process of interest in the target context (e.g., reactive gas uptake in nasal tissue), identify key parameters from exposure to clinical effect, and determine the relevance of data, parameter values, and extrapolation premises used to support a given model structure and predictions. The framework then assesses the rationality of predictions of the process in a target context (human children), including evaluation of the strengths and weaknesses of alternative parameters and model forms, the reliability of resulting risk estimates, and key sources of residual uncertainty - the latter to aid the design of research programs targeted at increasing the accuracy of risk predictions for children. (This abstract does not reflect Agency policy.)

1641 DEVELOPMENT OF DRINKING WATER HEALTH ADVISORY FOR BORON.

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The Health Advisory (HA) Program, sponsored by the Office of Water (OW), provides information on the environmental properties, health effects, analytical methods, and treatment technologies for regulated and unregulated drinking water contaminants. HAs establish nonregulatory concentrations of drinking water contaminants at which adverse health effects are not anticipated to occur over specific exposure durations (1-day, 10-days, several years, and a lifetime). This document is an update of the previous Office of Water draft HA for boron published in

1992 to reflect more recent health and environmental effects information for boron. Boron is a nonmetallic element present in compounds such as boric acid, and its sodium salt, borax. These compounds are mostly used in glass production and to a lesser extent, in pesticides, soaps/detergents and flame retardants. Boron is found naturally in many food items. The mean dietary intake in the US ranges from 0.85 to 1.47 mg/day. The results of a mid-1980's survey of 989 public ground water systems indicate that about 81.9% of these systems had detections of boron at concentrations ≥ 0.005 mg/L. A 2004 study funded by the American Water Works Association Research Foundation (AWWARF) found that boron tends to occur at higher concentrations in ground water, as compared to surface water sources. The lifetime and longer-term HAs for adults are derived from the chronic RfD of 0.2 mg/kg/day is based on fetal bodyweight from a developmental toxicity study in rats. The revised HA document is currently being prepared for peer-review. [The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S.EPA].

1642 THE NEWLY DEFINED ACCEPTANCE AND EVALUATION CRITERIA FOR THE MOUSE LYMPHOMA ASSAY AND THEIR IMPACT AND RELEVANCE.

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In the present study historical data from 313 mouse lymphoma studies (microwell method) performed in the years 2003-2005 at RCC were investigated in respect to the newly acceptance and evaluation criteria as defined in the workshop reports of the IWGT working group. First the defined acceptable background mutant frequencies for negative/vehicle (50-170 x 10⁻⁶) were investigated for these studies. In 23 studies either the vehicle control or the solvent control were out of the defined range where 19 studies were beyond and 4 studies below the range. Secondly our data were investigated in respect to the newly defined acceptance criteria for the positive controls (GEF, IMF300) and compared to our previously used criteria (mutation factor of 2.0). MMS, used at RCC as a positive control without S9 mix in the pulse and in the prolonged treatment, indicate that in 94 studies at least one of the newly defined criteria could not be fulfilled, even if the mutation factor of 2 was reached or exceeded.

A further investigation includes test items which were classified as genotoxic by the mutation factor approach compared to the newly defined evaluation criteria for the definition of a positive effect. In 37 out of 126 studies (29%) performed in 2005 at RCC, a positive result was obtained. 20 studies showed a positive response at pulse treatment, 17 at the prolonged treatment. In 4 studies the global evaluation factor 126 could not be reached even if the mutation factor of 2.0 was reached or exceeded (from 2.0 to 3.0).

The current presentation will discuss the impact and the relevance of the newly defined acceptance and evaluation criteria for the mouse lymphoma test based on RCC historical data and eventually a re-consideration of certain definitions.

1643 PHOTOMUTAGENICITY OF ANHYDRORETINOL AND 5, 6-EPOXYRETINYL PALMITATE IN MOUSE LYMPHOMA CELLS.

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Retinyl palmitate (RP) is frequently used as an ingredient in cosmetics and other retail products. We previously reported that under UVA light irradiation, RP is readily decomposed into multiple products, including anhydroretinol (AR) and 5,6-epoxyretinyl palmitate (5,6-epoxy-RP). We also determined that combined treatment of mouse lymphoma cells with RP and UVA irradiation produced a photomutagenic effect. In this study, we evaluated the photomutagenicity of AR and 5,6-epoxy-RP, two photodecomposition products of RP, in L5178Y/Tk⁺ mouse lymphoma cells. Treatment of cells with AR or 5,6-epoxy-RP alone at 10 and 25 $\mu\text{g/mL}$ for 4 h did not show a positive mutagenic response. However, because these doses did not induce the required amount of cytotoxicity, we are unable to determine whether these two compounds are mutagenic. Treatment of cells with 1-25 $\mu\text{g/mL}$ AR or 5,6-epoxy-RP under UVA light (315-400 nm) for 30 min (1.38 mW/cm²) produced a synergistic photomutagenic effect. At 10 $\mu\text{g/mL}$ (37.3 μM) AR with UVA exposure, the mutant frequency (MF) was about 3-fold higher than that for UVA exposure alone, whereas the MF for 25 $\mu\text{g/mL}$ (46.3 μM) of 5,6-epoxy-RP+UVA was approximately 2-fold higher than that for UVA exposure alone. Compared with previous results for RP+UVA treatment, the potency of the induced phototoxicity and photomutagenicity was: AR > RP > 5,6-epoxy-RP. To elucidate the underlying photomutagenic mechanism, we examined the loss of heterozygosity (LOH) at four microsatellite loci spanning the entire chromosome 11 for mutants induced by AR or 5,6-epoxy-RP. Most mutants lost the Tk⁺ allele, and

more than 70% of the chromosome damage extended to 38 cM in chromosome length. AR+UVA induced about twice more mutants that lost all 4 microsatellite markers from the chromosome 11 carrying the Tk⁺ allele than did RP+UVA or 5,6-epoxy-RP+UVA. These results suggest that the two of RP's photodecomposition products are photomutagenic in mouse lymphoma cells causing event that affect a large segment of the chromosome.

1644 GENOTOXICITY EVALUATION OF SODIUM TUNGSTATE DIHYDRATE AND TUNGSTEN POWDER.

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Tungsten (W) is a naturally occurring element which has several commercial and military applications. It is also under consideration to replace lead in bullets. Tungsten has been detected in the environment (air, soil and water) at certain production facilities and Army installations. The genotoxicity data on W are limited to only a few reports. We developed genotoxicity data under extramural contract. These tests were conducted in accordance with Environmental Protection Agency's Health Effects Testing Guidelines in compliance with Good Laboratory Practice. The summary of test results of completed work will be reported here. Sodium tungstate dihydrate (STD)(99.9%) did not produce mutagenic effects at any dose up to 5000 $\mu\text{g/plate}$ in *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* (WP2uvrA) tester strains with and without a metabolic activation system. Tungsten powder (TP) (APS 1-5 micron), when tested at concentration up to 5000 $\mu\text{g/plate}$, did not cause a positive increase in any these tester strains with or without metabolic activation. STD did not increase mutation frequency at levels up to 350 $\mu\text{g/mL}$ in mouse lymphoma L5178Ytk⁺ cells in the presence or absence of a metabolic activation system. TP at concentration 39.3 to 5000 $\mu\text{g/mL}$ was not cytotoxic and not mutagenic in mouse lymphoma cells with and without activation. The effect of STD or TP on in vitro chromosomal aberrations in Chinese Hamster Ovary (CHO) cells showed no effect for inducing structural chromosomal aberrations up to conc. 3500 $\mu\text{g/mL}$ and 500 $\mu\text{g/mL}$ respectively with and without activation. STD at 250, 500 and 750 mg/kg when tested in vivo in the mouse micronucleus assay was not mutagenic in mouse bone marrow. These studies reveal that STD or TP are not mutagenic in all above tests conducted. On the basis of these studies we believe that these compounds do not appear to pose genetic hazards. (Abstract does not reflect U.S. Army Policy).

1645 BIOLOGICAL SIGNIFICANCE OF DNA ADDUCTS: CORRELATION WITH OTHER ENDPOINTS OF GENOTOXICITY IN MOUSE LYMPHOMA CELLS TREATED WITH MMS AND COMPARISON OF INCREMENTS TO BACKGROUND.

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The biological significance of DNA adducts as a risk factor for mutation and cancer is under debate. In view of the background DNA damage this question could be addressed quantitatively by relating increments of DNA adducts from an exogenous source to the increments measured for other endpoints of genotoxicity. Background and induced levels of four adducts, O6-methyl-2'-deoxyguanosine (O6-mdGuo) and 7-methylguanine (7-mG), 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) and 1,N6-etheno-2'-deoxyadenosine (EdAdo) were measured by LC-MS/MS in DNA isolated from large batches of L5178Y mouse lymphoma cells treated with methyl methanesulfonate (MMS) at 0, 50, 100, 200 μM concentration. In parallel, background and increments were measured in three standard assays for genotoxicity in aliquots of the same cell suspension, (i) the alkaline version of the comet assay, (ii) the micronucleus test, and (iii) the thymidine kinase tk⁺ gene mutation assay. Means and standard deviations from at least four independent replicate studies were determined for all endpoints, and increments were expressed relative to controls. Fifty μM MMS resulted in a 1.4 to 2.2-fold increase above background for the biological endpoints, whereas O6-mdGuo and 7-mG increased about 8 and 10-fold. At 100 μM MMS, the biological endpoints showed 2.8 to 4.6-fold increases, while the factors for O6-mdGuo and 7-mG were 19 and 24-fold. At 200 μM , the respective factors ranged from 4.6 to 7.4 for general genotoxicity and were 27 and 252 for the guanine methylations. No dose-related increase was observed for either 8-oxodGuo or EdAdo. Except for the sublinear dose response observed for 7-mG at high dose, linearity could not be rejected for any endpoint. For a doubling of the background measure, the required MMS concentration was about ten-fold lower for DNA methylation than for the biological endpoints. A risk assessment based on adducts may therefore result in substantial overestimation of the biological consequences.

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1646 PREGNANE X RECEPTOR PROTECTS HEPG2 CELLS FROM BAP-INDUCED DNA DAMAGE.

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The pregnane X receptor (PXR) regulates the metabolism of a wide range of xenobiotics and endobiotics through coordinate regulation of gene expression of phase I and phase II metabolizing enzymes. Metabolism of benzo[a]pyrene (BaP), a common environmental contaminant, can result in either detoxification or bioactivation to its genotoxic forms. The genotoxicity of BaP in a particular organism, tissue, or cell type depends on the balance between these competing metabolic pathways. To examine the role of PXR in BaP genotoxicity, a cell culture model was developed based on the well-characterized human hepatoma cell line HepG2. Constitutive expression of PXR is undetectable in HepG2 and PXR-regulated responses are lost. PXR expression was restored by stable transfection of human PXR (hPXR) expression plasmid. DNA adduct formation was measured by 32P-postlabeling of parental HepG2 cells and hPXR-enhanced HepG2 cells treated with BaP at four doses (0, 0.5, 2, 5 μ M), with or without rifampicin (5 μ M) pretreatment. BaP treatment resulted in formation of DNA adducts in a dose-dependent manner in both cell lines. The presence of transfected hPXR significantly reduced the level of adducts ($p < 0.001$). Furthermore, the prototypic PXR agonist rifampicin markedly reduced adduct levels in the hPXR-enhanced cells ($p < 0.001$). These results suggest that PXR plays an important role in protecting cells from genotoxicity induced by exposure to BaP.

1647 LEVELS OF URINARY 8-HYDROXYDEOXYGUANOSINE IN FEMALE OFFICE WORKERS WHO ARE IN MENSTRUAL AND LUTEAL PHASES.

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The natural hormone 17 β -estradiol (E2) is considered as an endogenous cancer risk factor, and one possible mechanism of carcinogenesis by E2 is induction of oxidative stress. E2 is metabolized to two catecholestrogens, 2-OHE2 and 4-OHE2, by CYP1A1 and CYP1B1, respectively. Both metabolites can contribute to metabolic redox cycling, which induces ROS and causes the formation of 8-OHdG. The levels of E2 are changed according to menstrual cycle in women. In the present study, we investigated whether the levels of urinary 8-OHdG change according to phases of menstrual cycle of young female subjects.

The subjects were female office workers at a company. Information on demographic factors, phases of menstrual cycle and lifestyle factors were obtained from self-administered questionnaires. Subjects were then classified into menstrual phase (n=21), follicular phase (n=28), luteal phase (n=36), menopause (n=2), and pregnant (n=2) groups. Urine specimens were collected in the morning and were used to measure 8-OHdG and creatinine levels. The concentration of 8-OHdG was normalized by creatinine contents. Urinary 8-OHdG levels of the subjects who were non-smokers and did not take hormone-like medicines were analyzed in this study. The results showed that urinary 8-OHdG levels were $4.16 \pm 3.20 \mu\text{g/g creatinine}$ in the luteal phase group and $3.29 \pm 1.67 \mu\text{g/g creatinine}$ in the menstrual phase group, respectively. No statistically significant difference was found between both urinary 8-OHdG levels. No menstrual cycle dependence was observed for urinary 8-OHdG levels.

1648 OXIDATIVE DNA DAMAGE AND IN VIVO MUTAGENICITY IN THE LIVERS OF GPT DELTA MICE GIVEN DICYCLANIL.

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Dicyclanil (DC), a pyrimidine-derived insect growth regulator, is a female mouse liver carcinogen in spite of the negative results in various mutagenicity tests such as the Ames, chromosome aberration and micronucleus tests. In the present study, to explore DC-induced hepatocarcinogenesis, we examined oxidative stress and a re-

porter gene assay in the livers of male and female *gpt* delta mice given DC at a dose of 1500 ppm, a carcinogenic dose, in the diet for 13 weeks. In addition, early changes in 8-hydroxydeoxyguanosine (8-OHdG) levels were measured in the liver DNA of B6C3F1 mice, a back strain of *gpt* delta, given DC at the same dose as in the first experiment for 2 and 4 weeks. While thiobarbituric acid-reactive substances levels were not changed among the groups, 8-OHdG levels in the treated male and female *gpt* delta mice were significantly elevated as compared with the controls. In contrast to the negative outcome in the males, mutant frequencies of *gpt* in the treated females were increased with statistical significance, which were mainly due to GC:TA transversions. Four weeks exposure of DC to female mice, but not male mice was sufficient to induce 8-OHdG increment. The overall data suggested that the reporter gene assay could be a powerful tool to investigate mutagenicity of so-called non-genotoxic carcinogen. Additionally, it seemed likely that oxidative DNA damages might be implied in DC-hepatocarcinogenesis.

1649 THE SPORES OF CO-CULTIVATED ENVIRONMENTAL *STREPTOMYCES CALIFORNICUS* AND *STACHYBOTRYS CHARTARUM* INDUCE DNA DAMAGE IN RAW264.7 CELLS.

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We have recently shown that the actinobacterium *Streptomyces californicus* and the fungus *Stachybotrys chartarum* originating from moisture damaged buildings possess both immunotoxic and immunostimulatory characteristics, which are synergistically potentiated by microbial interaction. In the present study spore-induced genotoxic potential of these microbes was investigated. Dose related differences in genotoxic and cytotoxic effects in mouse RAW264.7 macrophages were studied after 24 hrs exposure to spores of *Str. californicus* or *S. chartarum* alone, spores of the co-cultivated microbes or mixture of spores of the separately cultivated microbes. The genotoxic effect of the exposures was determined by the Comet assay and cytotoxicity was assessed by using flow cytometric methods (DNA content analysis, live gate analysis and PI exclusion test) and also the MTT test. The present results revealed that the spores of *Str. californicus* alone induced a 1.5-fold DNA damage compared to control and also extensive cytotoxicity with G2/M cell cycle arrest. Interestingly, only the spores of the co-cultivated microbes caused DNA damage, cell cycle arrest and cytotoxicity even at the low dose, and at the highest dose a 2.5-fold DNA damage compared to control was reached. Instead, the mixture of separately cultivated spores or the spores of *S. chartarum* alone did not induce DNA damage or cell cycle arrest with any tested dose although they triggered significant cytotoxicity. Our results indicate that the spores of at least some environmental microbes have genetic activity. The genotoxic capacity of the spores seems to be potentiated when the microbes are grown in the same habitat. Thus, it is possible that the genotoxicity, at least in certain exposure circumstances, may contribute to the risk of cancer.

1650 EFFECT OF BREVETOXINS 2, 3, 6, AND 9 ON CELLULAR PROLIFERATION AND INDUCTION OF DNA DAMAGE IN JURKAT E6-1 CELLS.

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Brevetoxins (PbTx) are cyclic polyether compounds produced by several types of marine dinoflagellates (including *Karenia brevis* and *Chatonella antiqua*) during red tide events in the Gulf of Mexico, off the coasts of Florida and North Carolina. Necropsies of marine mammals killed by red tides in Florida have found inflammatory lesions in respiratory tissues with brevetoxin positive lymphocytes. Jurkat E6-1 cells, a T lymphocyte cell line, was used to examine four brevetoxins, PbTx2, 3, 6, and 9, specifically their effect on cellular proliferation and ability to induce DNA damage. Cellular Proliferation Assays determined the IC50 for PbTx2 to be 10–5.25 M, PbTx3= 10–4.5M, PbTx6=10–5M, and PbTx9= 10–4.25M. At Concentrations below 10–6M for PbTx 3, 6, and 9 and above 10–8M for PbTx2 showed increased proliferation above that of negative control. The Comet Assay was used to compare DNA damage following treatment with PbTx2, 3, and 6 at their respective IC50s. The data is given as the distributive moment, which is the percentage of DNA in the comet tail multiplied by the length between the center of the head and tail (arbitrary units). Comet Assays were performed in two groups,

PbTx2 and PbTx3 and 6. All PbTx treated samples had visible DNA damage. A t-test comparison revealed significant statistical difference for PbTx2, 3, and 6 compared with their respective negative controls. The negative control distributive moments were 15.97 (SE= 1.05) for the PbTx2 group and 26.30 (.54) for the PbTx3 and 6 group. The positive control (hydrogen peroxide, 1.75%) was 33.77 (2.62) for the PbTx2 group and 85.39 (2.15) for the PbTx3 and 6 group. PbTx2 was 27.09 (1.48), PbTx3 was 41.94 (1.91), and PbTx6 was 44.00 (2.28). Supported by the North Carolina Agromedicine Center and USDA/CSREES. PbTx was provided under NIEHS grant PO1 ES 10594.

1651 POTASSIUM DICHROMATE-INDUCED OXIDATIVE STRESS, GENOTOXICITY AND CYTOTOXICITY IN HEPG2 CELLS.

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Chromium is a widespread industrial waste. The soluble hexavalent chromium Cr (VI) is an environmental contaminant widely recognized to act as a carcinogen, mutagen and teratogen towards humans and animals. The reduction of Cr (VI) to Cr (III) results in the formation of reactive intermediates together with the oxidative tissue damage and a cascade of cellular events. However, the mechanisms of the toxic action of chromium are not completely understood. The main purpose of this study was to evaluate the occurrence of oxidative stress, genotoxicity and cytotoxicity in the human liver [HepG2] cells exposed to potassium dichromate. HepG2 cells were cultured following standard protocol and exposed to various concentrations [0 – 50 µM] of potassium dichromate. Following exposure to the toxic metal, we performed the MTT assay to assess the cytotoxicity, thiobarbituric acid test to evaluate the degree of lipid peroxidation as an indicator of oxidative stress and DNA damage evaluation using alkaline comet assay. Study results indicated that potassium dichromate is cytotoxic to HepG2 cells, showing LD50 values of 8.83 + 0.89 µg/ml, 6.76 + 0.99 µg/ml for cell mortality upon 24 and 48 hrs of exposure respectively; indicating a dose- and time-dependent response with regard to cytotoxic effect of potassium dichromate. Statistically significant increase in the concentration of malondialdehyde [MDA], an indicator of lipid peroxidation, was recorded in exposed cells [15.9 – 69.9 µM] compared to control [13 µM]. Similarly, a strong dose-response relationship (p<0.05) was also obtained with respect to potassium dichromate induced DNA fragmentation [comet assay] in HepG2 cells exposed [3.16 + 0.70 – 24.84 + 1.86 microns] to potassium dichromate than control [3.07 + 0.26]. Our results demonstrate that potassium dichromate is highly cytotoxic to HepG2 cells, and its cytotoxicity seems to be mediated by oxidative stress and DNA damage.

1652 THE CARCINOGENIC ASPECT OF POTASSIUM BROMATE GENOTOXICITY IN HUMAN LYMPHOBLASTOID TK6 CELLS.

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Potassium bromate (KBrO₃) is strongly carcinogenic in rodents and mutagenic in bacteria and mammalian cells in vitro. The proposed genotoxic mechanism for KBrO₃ is oxidative DNA damage. KBrO₃ can generate high yields of 8-hydroxydeoxyguanosine (8OHdG) DNA adducts, which cause GC > TA transversions in cell-free systems. In this study, we investigated the in vitro genotoxicity of KBrO₃ in human lymphoblastoid TK6 cells using the micronucleus (MN) test and the thymidine kinase (TK) gene mutation assay. In a 4-h treatment, KBrO₃ induced MN and TK mutations concentration-dependently. At the highest concentration (5mM), KBrO₃ induced MN and TK mutation frequencies that were over 30 times the background level. Molecular analysis revealed that 90% of the induced mutations were large deletions that involved loss of heterozygosity (LOH) at the TK locus. Ionizing-irradiation yielded similar mutational spectrum in our system. These results indicated that the carcinogenicity of KBrO₃ may be due to double strand breaks that lead to large deletions rather than to 8OHdG adducts that lead to GC > TA transversions, as is commonly believed. To better understand the genotoxic mechanism of KBrO₃, we analyzed gene expression profiles of TK6 cells using Affymetrix Genechip. Some genes involved in stress, apoptosis and DNA repair were up-regulated by the treatment of KBrO₃. However, we could not observe the similarity of gene expression profile in the treatment of KBrO₃ to ionizing-irradiation as well as oxidative damage inducers.

1653 AN AUTOMATED, SENSITIVE AND ROBUST VERSION OF THE FLUORESCENCE-DETECTED ALKALINE DNA UNWINDING (FADU) ASSAY FOR THE QUANTIFICATION OF DNA SINGLE-STRAND BREAKS AND DNA CROSS-LINKS IN CELLS.

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DNA single strand breaks are among the lesions directly introduced by ionizing radiation but also represent intermediates in several DNA repair pathways. Therefore quantification of DNA strand breaks and their rate of disappearance in living cells is an important endpoint in the assessment of genotoxicity of compounds and the cells' capacity to repair DNA damage. One of the available methods, the fluorescence-detected alkaline DNA unwinding (FADU) assay, is thought to be very sensitive, yet in its original, manually performed version (Birnbom HC, Jevcak JJ, Cancer Res. 1981;41:1889-92) is technically very demanding and requires large number of cells.

In order to render this assay more convenient and reproducible, to reduce the number of cells required and to increase throughput, we established an automated version of this assay based on the use of a commercial 8-channel liquid handling device and additional modifications of the assay protocol. The automated FADU is run in a 96-well plate format. It allows detection of DNA strand breaks induced with as little as 6 cGy of X-irradiation with high reproducibility. A dose of approximately 1 Gy allows robust determination of DNA strand break repair capacity. In addition, DNA interstrand cross-linking induced by mitomycin C can also be measured as the inhibition of DNA unwinding that would result from a fixed dose of ionizing radiation.

In conclusion, the automated FADU assay is a sensitive, robust and convenient method for the quantification of DNA single-strand breaks and DNA cross-links in cells and of DNA repair.

1654 A NON-ANIMAL-BASED, HIGH-THROUGHPUT UNSCHEDULED DNA SYNTHESIS (HT-UDS) ASSAY FOR SCREENING OF GENOTOXINS.

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For over 30 years, the Unscheduled DNA Synthesis assay has been used to detect genotoxic chemicals via measurement of the incorporation of 3H-Thymidine during the repair of damaged cellular DNA. This test is often employed to more fully characterize chemical and drugs when first-tier genotoxicity tests produce conflicting results. We have developed a rapid, non-radioactive "HT-UDS" assay based on flow cytometric methods to detect nucleotide analogs incorporated into human keratinocytes, hepatocytes, embryonic chick hepatocytes and cell lines, eliminating the need for test animals (rat liver or culture of primary hepatocytes). This assay was validated with 18 known genotoxins and non-genotoxins, attaining an accuracy of 94% without the over-prediction problem of many classical mutagenicity tests (very low false-positive rate). By employing cell types of both low- and high-metabolic potential, HT-UDS allows detection of compounds that are activation-dependent, activation-independent or detoxified by bio-transformation. Other advantages of the HT-UDS include: a rapid analysis time of less than 5 days (vs. 3-4 months), decreased cost, and an automated, quantitative procedure which replaces manual or subjective scoring of films or slides. HT-UDS can also be performed on a microplate-scale, where a 1000-fold greater number of nuclei can be rapidly analyzed and multiple chemicals can be simultaneously tested. The greatly increased throughput of HT-UDS enables allows a cost-effective and rapid genotoxicity pre-screening test and is the most significant advance in the UDS method in the last decade.

1655 CYTOTOXICITY AND RADIATION SENSITIZATION WITH OXALIPLATIN IN ESOPHAGEAL ADENOCARCINOMAS.

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The prevalence of esophageal adenocarcinoma in the U.S. is increasing and has a poor 5-year survival. A third generation platinum chemotherapy agent, oxaliplatin, is currently used in the treatment of colorectal cancer where it has described radiosensitizing effects. Oxaliplatin is known to form predominantly intrastrand guanine/guanine and guanine/adenine DNA adducts that block DNA replication and

transcription. We aim to determine the anti-tumor effects of oxaliplatin +/- radiation in esophageal adenocarcinomas. Clonogenic survival assays and MTT cell proliferation assays were performed in two esophageal adenocarcinoma cell lines (Seg-1 and Bic-1) treated with either 0-10 μ M oxaliplatin; 2, 4, or 6 Gy radiation; or oxaliplatin plus radiation. These assays demonstrated an increase in cell death as the oxaliplatin concentration increased and as the drug incubation time increased. The combination of oxaliplatin followed by radiation caused a further reduction in cell survival. Western blot analysis performed on the same esophageal cell lines after cells were treated with oxaliplatin and/or radiation allowed for the observation of double strand break (DSB) damage and repair through γ H2AX. Cells were harvested and lysed either immediately after treatment or after various repair times. The western results showed the combination of oxaliplatin and radiation caused more DSB than oxaliplatin alone. DSB repair appeared complete at 6 hours post-treatment. Oxaliplatin with radiation therapy is more cytotoxic to esophageal adenocarcinomas than radiation alone or oxaliplatin alone. The mechanism of this radiosensitization does not appear to be through inhibition of DSB repair, but it could possibly be through inhibition of base excision repair or nucleotide excision repair. Further experiments are needed looking at other DNA damage repair mechanisms and apoptosis.

1656 POLY ADP RIBOSE POLYMERASE ACTIVITY IN CULTURED HUMAN LUNG CELLS.

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Poly (ADP-ribose) polymerase-1 (PARP-1), is included in the cellular response to DNA injury and is decisive for the final response whether the targeted cell undergoes repair or apoptosis. It is widely unknown whether PARP-1 itself is a target for modulating factors. The scope of the study was to examine the time course PARP-1 activity in normal human bronchial epithelial cells (NHBE) during a follow up period of 10 weeks in culture. We have determined whether external factors such as cigarette smoke condensate or metals will modulate PARP-1 activity in vitro.

The expression PARP-1 protein was proved in normal human bronchial epithelial cells (NHBE) and tumor cell line A549 by Western Blot analysis. PARP-1 activity in normal human bronchial epithelial cells (NHBE) by H₂O₂ (100 μ M) for 5 min was measured by an immunofluorescence assay for ADP ribose polymers.

In the first explants of individual donors PARP-1 activity in (NHBE) varied by a factor of 1.5-2 (n=5). PARP-1 activity was analyzed in progressive generations and passages of bronchial and peripheral lung cells for 10 weeks. The activity was almost stable for about 4 weeks. After that the activity declined till the 5th generation to 50% of the start values (n=3).

Cigarette smoke condensate (CSC) 0.5 mg/L for 24 h induced PARP-1 activity in NHBE by factor 1.3 compared to controls (= basal cellular PARP activity). After H₂O₂ (100 μ M) PARP-1 activity was induced 1.4 fold and 1.8 fold by CSC (+ H₂O₂). Treatment of NHBE with copper sulfate (0.05 mM) alone did not trigger PARP-1 activity but decreased PARP-1 activity which was stimulated using H₂O₂ (0.1 mM) by 40%. Similarly mercury (II) chloride (0.03 mM) exerted no effect on PARP-1 activity and decreased the activity of PARP-1 upon co-incubation with H₂O₂ (0.1 mM) by 40%.

PARP-1 expression and activity can be monitored in primary human lung cells in vitro and it is likely that PARP-1 is modulated by adaptive responses in individuals.

1657 DNA DAMAGE RESPONSE TO ESTROGEN METABOLITES.

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DNA damage caused by estrogen metabolites is believed to be an essential process in the initiation of breast and endometrial cancers. Three pathways that are hypothesized to be the main cause of estrogen related DNA damage involve oxidative DNA lesions induced by redox cycling of estrogen metabolites, base damage directly caused by estrogen quinone metabolites, and depurination of adducted bases. In this study, we used the chicken B lymphocyte (DT40) cell line and its isogenic mutants deficient in DNA damage response genes to better understand the most biologically relevant types of DNA damage that may be responsible for the initiation of estrogen-related cancers. Using the estrogen metabolites 4-hydroxy-estradiol (4-OHE2) and 2-hydroxy-estradiol (2-OHE2), we exposed the isogenic mutants to varying metabolite concentrations by incubating the cells in the presence of the es-

trogen metabolites for ten cell replication cycles. After cultivation, XTT dye was used to measure the numbers of surviving cells. While DT 40 cells deficient in either xpa, parp-1, fen1, pol β , or pol θ were not hypersensitive to either 2-OHE2 or 4-OHE2, rev3- and msh3-deficient cells showed a marked increase in cell death when compared to wild type DT40 cells. These results provide insight about the nature of DNA damage caused by 4-OHE2 and 2-OHE2 and the subsequent cellular response. First, abasic sites induced by depurinating base adducts may not play a major role in the DNA damage response to major E2 metabolites. The mismatch repair protein Msh3 may possess a novel function related to the cellular tolerance to either bulky base damage or oxidized DNA lesions induced by E2 metabolites. Our data also suggest that translesion DNA synthesis polymerases such as Rev3 are essential for the survival of cells with stalled DNA replication forks due to lesions caused by estrogen metabolites.

1658 IN VITRO ASSESSMENT OF THE GENOTOXICITY OF BENZO-A-PYRENE USING SISTER CHROMATID EXCHANGES, HIGH FREQUENCY CELLS AND DNA SINGLE STRAND BREAKS ON HUMAN BLOOD LYMPHOCYTES.

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Benzo-a-pyrene (BaP) is a polycyclic aromatic hydrocarbon compound that can induce various genotoxic effects such as sister chromatid exchanges (SCE) and DNA single strand breaks (SSBs) in humans. *In vitro* studies provide a mechanistic comprehension of those effects and of the interindividual variation observed. Some studies with occupational exposure include the measure of high frequency cells (HFC) in their analysis of the SCE assay results, but this approach is not currently used in *in vitro* studies. In our report, human cultured lymphocytes were exposed *in vitro* to three concentrations of BaP dissolved in DMSO (0.1 – 1 – 5 μ g/ml) for 24 hours. Cells were obtained from 10 subjects (5 men / 5 women) for SCE test and 4 subjects (2 men / 2 women) for electron microscopy *in situ* end labelling (EM-ISEL) assay. Cells were harvested 24 hours after the end of the exposure and assessed for SCEs increase, the rate of HFC, and SSBs using the EM-ISEL assay. An important variation was present between individuals as some subjects presented a significant increase of SCEs or SSBs starting at [BaP] 0.1 μ g/ml, while other subjects showed such an increase only at [BaP] 1 or 5 μ g/ml. This observation was unrelated to gender for SCEs, while men presented twice more SSBs than women. Analysis of our results demonstrates a significant positive correlation between the concentration of BaP and the mean SCEs number ($r=0.666$, $p<0.001$) or HFC rate ($r=0.684$, $p<0.001$). Also, a significant positive correlation is present between the mean SCEs number and the HFC rate ($r=0.758$, $p<0.001$), supporting the hypothesis that HFC represents cells being more damaged following the *in vitro* exposure to BaP. Pursuing this study on a larger number of subjects will allow us to determine the usefulness and significance of HFC and SSBs in *in vitro* studies. This work was supported by a scholarship from the FRSQ.

1659 CLASTOGENICITY OF CIGARETTE SMOKE CONDENSATE AND DNA DAMAGING AGENTS IN TWO CELL LINES BY THE IN VITRO MICRONUCLEUS ASSAY.

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V79 and CHO cell lines are routinely used in the *in vitro* micronucleus (MN) assay aimed at testing genotoxicity of chemical and physical agents. The CHO cell line is the most predominantly used cell line in genetic toxicology. However, V79 is utilized by some laboratories since the *in vitro* MN assay was initially developed using this cell line. The aim of the current study was to compare the sensitivity of these two cell lines in testing the capability of cigarette smoke condensate and 3 known DNA damaging chemical agents to induce clastogenicity/augenicity using the micronucleus bioassay. The total particulate (TPM) phase of main stream reference cigarette smoke condensate (CSC) generated under laboratory conditions using International Organization for Standardization (ISO) and Canadian Intense (CINT) conditions from KY2R4F cigarettes and 3 DNA damaging chemical agents: colchicine, cyclophosphamide and mitomycin C were utilized in the current study. Clastogenicity was examined under three various exposure terms in both cell lines. Replicate cultures of CHO and V79 cell lines in 10% fetal bovine serum in F-12 Ham and DMEM medium, respectively, were established and treated with the 3 DNA damaging chemical agents and TPM of CSC at 0-500 μ g/ml. A total of 1000

cells were analyzed for MN presence. The CSC TPM phase and the 3 tested chemicals were clastogenic in both cell lines, with one cell line being about 2-3 times more sensitive than the other in both cases. Of the 2 tested cell lines, the V79 cells was the more sensitive cell line when testing the clastogenicity of TPM from CSC. However, an opposite trend in the cell lines sensitivity was observed following treatment with the three DNA damaging chemical agents.

1660 ASSESSMENT FOR THE USE OF SALMONELLA THYPHIMURIUM STRAIN TA100 IN THE AMES II ASSAY: A VALIDATION STUDY WITH 14 COMPOUNDS.

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Originally the Ames II was developed by Xenometrix to detect only the potential of compounds to induce mutagenicity using a modified protocol with Salmonella strains TA98 (frameshift mutations) and TAMix (TA7001 – 7006, base pair substitutions). The major advantages of this screening assay are the low amount of compound needed and the higher throughput that could be achieved compared to the standard Ames assay. The Ames II using TA98 and TAMix is a well validated test system to detect mutagens. The use of TA100 in a screening Ames II mutagenicity assay in microtiter plates is of interest, since a high number of compounds are only positive in this standard Ames test strain. In the past it was not possible to use the TA100 in the Ames II due to the high number of spontaneous revertants in the control group using the microtiter plate format. With a new study protocol, developed by Xenometrix, it should now be possible to use TA100 in the Ames II assay, as the number of spontaneous revertants was reduced. Fourteen chemicals described in literature to be positive in the standard Ames tester strain TA100 and partly in TA98 or TA97 were selected to evaluate a modified Ames II protocol from Xenometrix with the Salmonella strain TA100 in the absence and presence of S9-mix. The results of the 14 compounds tested lead to the conclusion, that there are no major differences between the use of TA100 and TAMix in the Ames II to detect base pair substitution mutations. The TAMix seems to be slightly more sensitive. This might be explained by the still higher spontaneous revertant frequency of the TA100 compared to TAMix. Therefore, for weak mutagens it could be difficult to detect a clear dose dependency of a weak mutagenic effect. Based on these results, the tester strain TA100 should not be included routinely in the Ames II assay. However, on a case by case basis, compounds originating out of a chemical class known to be positive in the standard Ames TA100 should be tested in TA100 in the Ames II.

1661 FORMATION OF GENOTOXIC BY-PRODUCTS DUE TO THE REACTION OF THE SOLVENT DMSO WITH COMPOUNDS FROM DIFFERENT CHEMICAL CLASSES.

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DMSO is a widely used standard solvent in in vitro studies. To investigate the influence of DMSO and other solvents on genotoxicity results, we tested several compounds from different chemical classes in the Ames II assay, a liquid microtiter modification of the standard Ames plate incorporation test, by using different solvents.

As a result of these studies, the chemical classes sulfonic acid halides and carboxylic acid halides could be identified so far, which showed different results in Ames II, depending on the solvent used. Most of the compounds from both chemical classes are described in the literature (NTP database...) as genotoxic compounds or they are alerted by the in silico tool DEREK (Deductive Estimation of Risk from Existing Knowledge) to give positive genotoxicity results. Compared to our results in the Ames II, we could confirm the positive results with and without metabolic activation in the Salmonella typhimurium tester strains TAMix and TA98, when using DMSO as solvent. In contrast to these results, the same compounds were tested negative in Ames II, when using other solvents than DMSO like water or ethanol.

These results may be explained by different reactions of the compounds in the solvents. Sulfonic/carboxylic acid halides in water should completely react to the corresponding and non-genotoxic sulfonic/carboxylic acids and hydro halide acids. But when using DMSO as solvent, besides the corresponding sulfonic/carboxylic acids, dimethylsulfide halides are also formed as reaction products of DMSO. These dimethylsulfide halides are alkylating agents, which are more stable in aqueous conditions and more likely to react with DNA, resulting in a positive Ames II.

As a consequence of these results, we recommend using other solvents than DMSO to test the genotoxic potential of sulfonic acid halides and carboxylic acid halides, to avoid false positive results from DMSO reaction products, not relevant for aqueous, physiological conditions.

1662 THE AMES MPF ASSAYS: NOVEL MUTAGENICITY TESTING IN LIQUID MICROPLATE FORMAT USING S. TYPHIMURIUM TA98, TA100, TA1535 AND TA1537.

S. Flueckiger-Isler and M. Kamber. *Xenometrix AG, Allschwil, BL, Switzerland*. Sponsor: E. Zeiger.

Genetic toxicity testing has moved towards the earlier stages of drug discovery in order to identify genotoxic liabilities of new compounds in the pipeline. Because in early development many compounds are available in very small quantities, a liquid microplate version of the original Ames plate incorporation test with different S. typhimurium tester strains was developed to decrease compound consumption and to increase the through-put of the assay.

The complete Salmonella plate incorporation test includes at least 2 frameshift strains, usually TA98 and TA1537 or TA97, and at least two base-pair strains. TA100 is the most sensitive of all tester strains, but some mutagens are positive in TA1535 only.

TA98 is already successfully used in the Ames II assay, in combination with TAMix, a mixture of strains to detect base-pair mutations. TA100 with its high spontaneous reversion rate, and TA1537 or TA1535 were as yet not available in the microplate format. We were able to decrease the spontaneous reversion rate of TA100 to a level low enough to be used in the microplate format without loss of sensitivity.

The mutagenic response to 24 reference compounds was examined in the microplate format with TA98, TA100, TA1537 and TA1535, and the results were compared with published plate incorporation data. Depending on the strain tested and on the reference citation, concordance between the two assay formats was 80 - 100%. Certain mutagens exclusively reverted TA1537 or TA1535, but not TA98 or TA100, irrespective of the assay format.

These new Ames MPF tests by Xenometrix using a liquid format and 384-well microplates offer a time and cost-effective pre-regulatory alternative to the plate incorporation method. As both formats use the same Salmonella strains, results can be compared with existing data sets. The new test kits include ready-to-use media and bacteria and allow rapid screening of a large number of compounds. They consume six times less test substances and consumables than the plate incorporation method, and reduce hands-on time.

1663 EVALUATION OF A MICROAMES SCREEN THAT IS FULLY PREDICTIVE FOR THE REGULATORY SALMONELLA/E. COLI MAMMALIAN MICROsome MUTAGENICITY TEST.

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In the pharmaceutical industry there is a need for rapid genotoxicity screens that mimic regulatory assays. Predictive screens are key for assessing genotoxic risk of low-yield isolated impurities, and are important to ensure positive genotoxic responses are not found during regulatory testing, after considerable time and resources have been allocated to a drug candidate. A unique miniaturized rapid throughput version of the Ames test has been developed that requires only 10 mg. The strength of this microAmes screen is the use of regulatory Ames tester strains and classical endpoints (colonies, cytotoxicity, precipitates). To assess the robustness of this screen, 20 compounds comprising diverse mechanisms and structures, many related to pharmaceuticals, were tested with 4 regulatory accepted tester strains of Salmonella (TA97a, TA98, TA100, TA1535) and one E. coli strain (WP2uvrApKM101). Positive and negative controls were used and included compounds previously shown to yield borderline results in the full Ames test. The microAmes screen correctly predicted Ames mutagenicity in all cases and correctly predicted Ames non-mutagens except for 2 controversial compounds, oxazepam and menadione which yielded positive responses in the microAmes. Menadione was positive in WP2 without S9, while oxazepam was positive in several strains with S9 but only at concentrations where precipitates were present. In the full Ames test, menadione displays small increases in WP2 colony formation (<2 fold), whereas strong elevations are seen in the WP2-OxyR deficient strain, a variant sensitive to oxidative mutagens. Although oxazepam is negative in the full Ames test, this compound is a known in vivo mutagen, in vitro clastogen, and carcinogen. These results indicate that the microAmes not only accurately predicts the full-plate Ames assay, but is sensitive enough to detect known mutagenic compounds. The microAmes screen is therefore an extremely valuable predictive tool that can be used with high confidence to evaluate drug candidates and impurities for genotoxicity.

1664 GENOTOXICITY ASSESSMENT USING A NEW, HIGH SPECIFICITY AND HIGH SENSITIVITY HUMAN CELL ASSAY.

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Recent analyses of published genotoxicity and carcinogenicity assessments make rather uncomfortable reading for genetic toxicologists: a positive result from an in vitro mammalian genotoxicity test is not a very reliable indication of potential carcinogenicity. The high sensitivity of the tests is compromised by poor specificity. This over-predictivity of carcinogenicity by the current in vitro tests results in many unnecessary in vivo tests, which waste time, money and most importantly, animals. We recently described a new in vitro reporter assay for the human GADD54a gene which provides high sensitivity along with high specificity. Assays are carried out in 96 well microplates and results are simply determined after 48 hours using spectrophotometric data. That initial work with 75 tested compounds has been published (Hastwell et al 2006).

We have now assayed over 150 compounds, comprising further carcinogens and non-carcinogens, and deliberately including many examples where other in vitro mammalian genotoxicity tests have given misleading indication of carcinogenicity hazard. Data from these more recent studies as well as data collected using imaging platforms and flow cytometry will be presented that confirm high sensitivity without loss of specificity. We shall also report on trials of robustness and reproducibility, and assays performed in the presence of S9.

1665 DEVELOPMENT OF A MIROTITER PLATE VERSION OF THE YEAST DEL ASSAY AMENABLE TO HIGH-THROUGHPUT TOXICITY SCREENING OF CHEMICAL LIBRARIES.

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Carcinogenesis can be the result of genetic instability and DNA deletions. The yeast DEL assay is a powerful molecular tool that can be used to assess the carcinogenic potential of many compounds. We have previously demonstrated that the yeast-DEL assay correctly differentiates between carcinogens and non-carcinogens. As a further development, the DEL assay has been modified into a colorimetric assay using the MTS tetrazolium compound, to allow for efficient quantification of chemical toxicity. It has been micro-scaled and can be performed in a 96 or 384-well format. Carcinogens previously characterized with the DEL plate-based assay were utilized to test the new well-based assay. Compounds included induced a range of genotoxicity from high to very low. The well-based DEL assay could readily determine the genotoxicity of the compounds; a lack of sensitivity was only discernible at extremely low genotoxic levels as determined with the plate-based DEL assay. We suggest that this new well-based version of the DEL assay can be used as an ergonomically favorable alternative to the plate-based assay to screen a large number of compounds, such as chemical libraries in a high-throughput screening (HTS) setting.

1666 THE YEAST DEL ASSAY.

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Genetic instability and deletions are involved in carcinogenesis. We have constructed and/or used assays (DEL assays) that select for DNA deletion events in yeast, human cells and in vivo in the mouse. DEL events in all three formats are inducible by a wide variety of carcinogens including ones, such as benzene and PCBs, that are negative in many other short-term tests. Furthermore, the yeast DEL assay correctly differentiates between carcinogen-non carcinogen structural analogs. Of 62 chemicals of known carcinogenicity status the yeast DEL assay detects 85% correctly compared to the Salmonella assay that detects only 33% correctly. More recently we have studied a variety of chemicals with the DEL assay including clastogens, alkylators, chromium(III) and Chromium(VI) and compounds that lead to oxidative damage. The DEL assays have been used to determine dose response relations and to investigate the biological activity of carcinogens. The yeast DEL assay is being further developed into a high throughput screening format and is currently used in industrial applications in attempts to identify the potential genotoxic activity of chemicals. Further validation is ongoing. We will present a review of our published

work with the DEL assay, compare other popular genotoxicity assays and discuss the mechanisms by which DNA deletions can occur. We will also present an updated correlation between the DEL assay and animal carcinogenicity data.

1667 KINETICS OF MICRONUCLEUS INDUCTION IN PERIPHERAL BLOOD OF RATS TREATED WITH DOXORUBICIN AND ETOPOSIDE FOR THE ANALYSIS OF BIOEQUIVALENCE.

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Evaluation of micronucleated reticulocytes (MN-RET) in the peripheral blood compartment of the rat is gaining acceptance as a valid alternative to conventional bone marrow micronucleus test for assessing cytogenetic damage. Recent validation studies have demonstrated that the role of rat spleen in selectively eliminating MN-RET was insignificant, especially when employing a flow cytometric methodology for enumerating the MN-RET. In this study, we evaluated the bioequivalence of two topoisomerase inhibitors with differing toxicological properties by comparing the induction of MN-RET frequencies using a flow cytometric method. Male Sprague-Dawley rats (5/dose) were given a single intravenous administration of etoposide (3, 6, or 9 mg/kg) or doxorubicin (1, 2, or 4 mg/kg) and the MN-RET in peripheral blood samples were evaluated at various intervals (24, 48, and 72 h). Cyclophosphamide (CP) served as a positive control at a single oral dose of 20 mg/kg. Peripheral blood samples were analyzed using the MicroFlow® protocol developed by Litron Laboratories. Methanol-fixed cells were treated with RNase, labeled with anti-CD71 and anti-platelet flow antibodies, and stained with propidium iodide to detect micronuclei. Samples were analyzed using a Coulter® EPICS-MCL™ flow cytometer. The MN-RET incidence peaked at 48 h for all dose levels. The highest dose of doxorubicin (4 mg/kg) and the lowest dose of etoposide (3 mg/kg) induced approximately the same magnitude of MN-RET at the 48 h sampling time (approximately 19 MN-RET per 1000 RET). The positive control chemical, CP, induced the expected increase in MN-RET (12 MN-RET per 1000 RET). These data further support the utility of rat peripheral blood for monitoring the cytogenetic damage in the bone marrow.

1668 FINDING IMPORTANT RELATIONSHIPS BETWEEN BIOLOGY AND CHEMICAL STRUCTURES BASED ON HIGH QUALITY GENETIC TOXICITY DATABASES.

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Data mining of toxicity databases is an important step in compound screening as well as in risk assessment. Many tasks involved in the use of databases are closely tied to data mining, hence database and data mining are essential technology pairs. Toxicity databases have a special role in predictive toxicology, providing ready access to historical information throughout the workflow of discovery, development, and product safety processes in novel product development as well as in review by regulatory agencies. To provide accurate information within a hypotheses-building environment, the content of the databases needs to be rigorously modeled using standards and controlled vocabulary. The utilitarian purposes of databases widely vary, ranging from a source for (Q)SAR datasets for modelers to a basis for "read-across" for regulators. In this poster, an example of comprehensive toxicity databases based on the combination of publicly available US FDA data with that from industry partners are used to find hidden relationships in biological endpoint profiles and their relationships with chemical structural domain. Combining data from FDA and industry partners, valuable profiles between genotoxicity and their relationships with chemical structural features are presented.

1669 THE IN VITRO HUMAN PERIPHERAL BLOOD LYMPHOCYTE MICRONUCLEUS SCREEN: A COMPARISON BETWEEN WHOLE BLOOD AND ISOLATED LYMPHOCYTES.

C. S. Farabaugh, M. A. Coleman, B. J. Harris, G. P. Stojhovic, H. Murli and L. F. Stankowski. Covance Laboratories, Inc., Vienna, VA. Sponsor: B. Fisher.

A comparative study was conducted to examine differences using whole blood (WB) and isolated lymphocytes (ILs) in the human peripheral blood lymphocyte (HPBL) micronucleus (MN) assay. Venous blood was collected and lymphocytes were purified from an aliquot of each WB sample by density gradient centrifugation. Thus, a direct comparison was made for each donor sample. Two clastogens, cyclophosphamide (CP) and mitomycin C (MMC), were evaluated with and without exogenous metabolic activation (Arochlor-induced rat liver S9), respectively. Two- or 10-mL cultures (containing 2 x 10⁶ ILs or 0.6 mL WB, respectively) were

initiated (at $t = 0$ hr) in 15-mL centrifuge tubes in RPMI 1640 containing 2% phytohemagglutinin M (PHA-M), and grown at 37°C in a humidified atmosphere of 5% CO₂ in air. Cultures were treated at 24 hr with CP (3 hr +S9) or MMC (3 and 20 hr –S9), and their concurrent solvent control. Treatment was ended by centrifugation and washing, and the cultures were re-incubated. Cytochalasin B (3 µg/mL for ILs, 6 µg/mL for WB) was added at 45 hr and the cultures were harvested at 72 hr. WB slides were stained with Giemsa and May-Grunwald; IL slides were stained with acridine orange. For each culture, 400 binucleated cells were scored for the frequency of MN (%MN-BN), and 200 total cells were scored for cytotoxicity (cytochalasin B blocked proliferation index, CBPI). Significant increases in %MN-BN were observed for each compound/treatment interval in both WB and ILs ($p < 0.05$ or 0.01). However, larger increases consistently were observed in ILs, and they reached significant levels at lower concentrations than in WB. In addition, ILs had a slower cell cycle time, indicating that the purification process may have a deleterious effect on the cells. Additional studies have been performed to compare the effect of recovery time (i.e., 96-hr harvest) on MN induction and recovery, and to select a suitable aneugenic positive control.

1670 IMPROVED CYTOTOXICITY ASSESSMENT ENHANCES THE UTILITY OF A HIGH CONTENT IN VITRO MICRONUCLEUS ASSAY.

J. C. Bemis, S. M. Bryce and S. D. Dertinger. *Litron Laboratories, Rochester, NY.*

This laboratory has described a flow cytometric method for scoring *in vitro* micronuclei [Environ. Molec. Mutagen. (2006) 47:56-66]. With this method, necrotic and mid-/late-stage apoptotic cells are labeled with ethidium monoazide (EMA). Cells are washed, stripped of their cytoplasmic membranes, and incubated with a pan-nucleic acid dye. This process provides a suspension of free nuclei and micronuclei (MN) that are differentially stained relative to chromatin associated with dead/dying cells. Experiments using a larger format *in vitro* design suggest that current methods of determining cytotoxicity, such as coulter counting, may underestimate cytotoxicity. This can lead to the inclusion of overly high concentrations of test article and an increase in the occurrence of recording false positive MN. Here, we report advances to the method aimed at acquiring concomitant cytotoxicity data in order to enhance interpretation of genotoxicity data in a miniaturized format. Experiments were performed with the genotoxins methyl methanesulfonate and mitomycin C, and the cytotoxic agents dexamethasone and staurosporine. After 24 hr continuous treatment, cells were processed for flow cytometry-based measurements of relative cell count (based on "counting beads"), cell cycle, and MN incidence based on the analysis of ≥ 5000 EMA-negative nuclei per culture. Dose-dependent MN induction was observed for each of the genotoxic agents. Induction of overt toxicity, which includes both apoptotic and necrotic processes, reveals the potential for recording spuriously high MN values. Preliminary data associated with the four disparate compounds suggests that relative to simple coulter counting, flow cytometric multiparameter cytotoxicity assessments facilitate better choices in selecting top drug concentration, thereby reducing the likelihood of false positive results. Collectively, these data suggest that high content *in vitro* MN scoring benefits from multiparameter assessments of test article-induced cytotoxicity, assessments that can be achieved concurrently with the MN scoring application.

1671 FURTHER DEVELOPMENT OF A RECONSTRUCTED SKIN MICRONUCLEUS ASSAY.

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We have previously reported initial investigations of a novel micronucleus assay which utilizes a 3-dimensional human skin model – EpiDermTM – supplied by MatTek Corporation (Ashland, MA). The advantage of this *in vitro* system is that it might potentially replace second-tier animal studies (or possibly first-tier non-specific traditional *in vitro* assays) in the genotoxicity evaluation of many materials, especially cosmetics ingredients. If so, it could then satisfy the requirement for non-animal testing of cosmetics ingredients after 2009 imposed by the European Seventh Amendment to the Cosmetics Directive. An important advantage of this assay system is that materials can be applied topically to the surface of the *in vitro* human skin model in a fashion similar to probable human exposure. In addition to previously reported positive responses with mitomycin C, vinblastine sulfate, MNNG and methyl methane sulfonate, four non-genotoxins (as shown by lack of micronucleus induction activity in an *in vivo* rodent skin model) have now been shown to induce no response in the reconstituted skin model. The non-genotoxins (4-nitrophenol, 1,2 epoxydodecane, trichloroethylene, and 2-ethyl-1,3-hexanediol) were tested up to levels of approximately 50% survival. The model has been further

characterized by measuring expression of xenobiotic metabolizing enzymes. Although many enzymes were constitutively expressed, 3-methylcholanthrene and β -naphthoflavone strongly increased expression of CYP1A1 and CYP1B1, and slightly enhanced expression of CYP2C19, CYP2D6, CYP3A4 and CYP3A5. Overall, the *in vitro* model showed an enzyme expression pattern very similar to normal human skin. It appears that this model may be very useful for detecting of micronuclei induction in a structurally complex tissue of human origin.

1672 NOVEL *IN VIVO* MUTATION ASSAY BASED ON THE ENDOGENOUS PIG-A LOCUS.

S. D. Dertinger and S. M. Bryce. *Litron Laboratories, Rochester, NY.*

Conventional approaches for measuring *in vivo* gene mutation have tended to rely on either endogenous genes such as *hprt* or else transgenes. Each method has its advantages and disadvantages. A significant advance in the field of *in vivo* mutagenesis would be the development of an assay that exhibited the best characteristics of these distinct systems: compatibility with any mammal, and no cell culture requirement. The experiments described herein were designed to test the utility of an assay that relies on glycosylphosphatidylinositol (GPI) anchored protein deficiency as a phenotypic marker of mutation at the *pig-a* locus. *Pig-a* resides on the X-chromosome, and the product is essential for anchoring GPI-linked proteins to the cell surface. The experiments described herein focused on Sprague Dawley rat erythrocytes and reticulocytes, as sufficient numbers of these cells are readily available via low volume venous draws. Fluorescent staining methods that permitted the enumeration of GPI-anchor deficient mature erythrocytes or reticulocytes via flow cytometry were developed and evaluated with reconstruction experiments. Following cell handling optimization, male Sprague Dawley rats were treated on 3 occasions (every other day) with 100 mg ENU/kg/day or 40 mg DMBA/kg/day. Peripheral blood specimens were harvested at weekly intervals for 5 weeks. Reticulocytes and total erythrocyte populations were analyzed for GPI anchored protein expression based on anti-CD59 labeling or else a pan-GPI ligand (i.e., FLAER, a fluorescent derivative of proaerolysin). All DMBA- and ENU-exposed animals exhibited elevated frequencies of GPI anchor deficient cells (ranging from 82 to 344 $\times 10^{-6}$, 310 to 647 $\times 10^{-6}$, and 0 to 70 $\times 10^{-6}$ for DMBA, ENU, and vehicle control rats, respectively). The elevated frequencies observed for the genotoxicant-exposed animals were highest in the reticulocyte subpopulation, tending to peak at 4 weeks post-exposure and plateau thereafter. These preliminary data support the hypothesis that a rapid *in vivo* mutation assay can be developed around flow cytometric analysis of GPI-anchored erythrocytes.

1673 PROTECTIVE ROLE OF METALLOTHIONEIN IN BENZO[A]PYRENE-INDUCED DNA DAMAGE AND CARCINOGENESIS.

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We investigated the role of metallothionein (MT) in benzo[a]pyrene (B[a]P)-induced DNA damage and forestomach carcinogenesis using MT-I/II null mice and wild-type mice. Ten-week-old male MT-I/II null mice and wild-type mice were orally treated with B[a]P 250 mg/kg and sacrificed at 6, 12, 24, 48 h after the administration. Micronucleus (MN) in the reticulocytes and 8-hydroxy-2'-deoxyguanosine (8-OHdG) in the liver were determined as indicator of DNA damage. At the 48 h after B[a]P administration, MN frequency in MT-I/II null mice was significantly increased compared with that in wild-type mice. Hepatic 8-OHdG level in MT-I/II null mice was significantly increased at 6 and 12 h after B[a]P administration, although wild-type mice were only slight changed. To study the suppressive effect of MT inducer on B[a]P-induced DNA damage, mice were subcutaneously administered ZnSO₄ once a day for 2 days and orally treated with B[a]P at 24 h after the last injection of ZnSO₄. B[a]P-induced MN frequency was reduced by ZnSO₄ pretreatment in the wild-type mice but not in the MT-I/II null mice. To study the sensitivity of MT-I/II null mice to B[a]P-induced carcinogenesis, MT-I/II null mice and wild-type mice were orally treated with B[a]P (100 mg/kg) twice a week for 4 weeks and sacrificed at 32 weeks after the last administration. Tumor incidence of MT-I/II null mice and wild-type mice was 100% and 78.6%, respectively. Tumor area of MT-I/II null mice was about 2.6 fold larger than wild-type mice. These results suggest that MT can protect B[a]P-induced DNA damage and forestomach carcinogenesis.

1674 INHIBITION OF DIHYDROOROTATE DEHYDROGENASE, AN ENZYME OF THE DE NOVO PYRIMIDINE SYNTHESIS PATHWAY, LEADS TO INDUCTION OF MICRONUCLEI BY NON-DNA REACTIVE MECHANISM VIA NUCLEOTIDE POOL IMBALANCE.

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Dihydroorotate dehydrogenase (DHODH) is the fourth enzyme of de novo pyrimidine synthesis, catalyzing the oxidation of dihydroorotate to orotate. The purpose of the study was to investigate the genotoxic potential of the DHODH inhibitor brequinar (IC₅₀ value ~ 100 ng/ml) and the possibility of a non-DNA reactive mechanism that could be antagonised by uridine supplementation of the test system.

In cell proliferation and DNA synthesis assays with L5178Y cells, it could be demonstrated that brequinar induced inhibition of cell proliferation, measured by BrdU incorporation. Uridine concentrations of 30 µg/mL were able to reverse cytotoxic potential of brequinar at concentrations up to 50 µg/mL. At 100 µg/mL the cytotoxicity of brequinar could not be reversed by Uridine supplementation.

The genotoxic potential of brequinar was investigated in a micronucleus assay conducted in L5178Y cells. Strong cytotoxic effects (population doubling ≤ 50 %) were observed at 49 ng/ml and above after 24 h treatment with brequinar in the absence of uridine. In parallel, the number of micronucleated cells was significantly increased at 49 and 98 ng/ml. In the presence of 30 and 100 µg/mL uridine the cytotoxicity was 1000 times less pronounced. Clear cytotoxic effects were observed only at 50 – 100 µg/mL brequinar. No induction of micronuclei was detected in the presence of Uridine even at highly cytotoxic concentrations (population doubling ~ 30%).

These results demonstrate that when the inhibition of pyrimidine synthesis is bypassed by the addition of uridine, neither cytotoxicity nor genotoxicity occurs. In conclusion, DHODH Inhibitors must be regarded as non-DNA reactive genotoxins, able to impact chromosomes via an exaggerated pharmacological activity.

1675 EVALUATION OF ALPHA-IONONE IN AN *IN VIVO* MOUSE MICRONUCLEUS TEST.

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alpha Ionone, a widely used fragrance ingredient was tested in an *in vivo* mouse micronucleus assay to evaluate the clastogenic potential as measured by its ability to induce micronucleated polychromatic erythrocytes in mouse bone marrow. This test is a short-term *in vivo* cytogenetic assay for detecting agents that induce chromosomal breakage and spindle malfunction. In the dose range-finding phase, a dose of 1200 mg/kg was estimated to be the maximum tolerated dose. The definitive micronucleus study consisted of several groups, each containing 5 male and 5 female ICR mice. The control groups were treated with the vehicle, corn oil, or cyclophosphamide monohydrate (CP) at 50 mg/kg as the positive control. α-Ionone was also tested at dosages of 300, 600, or 1200 mg/kg. Both the test and control articles were administered at a dose volume of 20mL/kg body weight by a single intraperitoneal injection and all animals were euthanized 24 hours after treatment. Bone marrow cells (polychromatic erythrocytes, PCEs) were collected 24 and 48 hours after the treatment and examined microscopically for the presence of micronuclei. Reductions (up to 21%) in the ratio of PCEs to total erythrocytes were observed and suggest bioavailability of the test article to the bone marrow target. There was no statistically significant increase in the incidence of micronucleated polychromatic erythrocytes observed in test article treated groups relative to respective vehicle control groups. Therefore, α-Ionone as a single intraperitoneal administration at doses up to and including 1200 mg/kg did not induce a significant increase in either male or female ICR mice and may be concluded as negative in the mouse micronucleus assay.

1676 THE GENETIC TOXICITY OF DIISOCYANATES IN HUMANS AND LABORATORY ANIMALS.

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Published *in vivo* genetic toxicity studies of methylene diisocyanate (MDI) and toluene diisocyanate (TDI) were reviewed to assess the potential for genotoxic effects from workplace exposures. These high production volume chemicals are used in manufacturing polyurethane foams, plastics, paints, adhesives and other products. Volunteers (previously exposed) demonstrated low levels of DNA damage in peripheral lymphocytes after MDI administration as measured by an electrophoresis method, but the Comet assay was negative. Positive results were confounded by

factors such as asthmatic responses, hypoxia, and concurrent medication in the participants. Authors speculated that immune responses and oxidative stress would have contributed to DNA damage rather than a direct interaction of isocyanate with DNA. Occupational exposure studies showed some weak cytogenetic effects, but suffered from some of the same confounders, and some effects could not be separated from other chemical exposures, including some known clastogens (e.g., benzene). Diisocyanates are reactive and there are a number of studies showing reaction with proteins at the site of exposure while producing no-to-minimal DNA binding. Studies with TDI in rodents elicited positive and negative responses for micronuclei and sister chromatid exchange, but not for aberrations, using peripheral lymphocytes. Positives were weak, sometimes non-dose related or associated with signs of toxicity, and often not reproducible. MDI yielded primarily negative micronucleus results except in one study, but this was not replicated by a different laboratory using the same protocol and rat strain. DNA adducts were at the level of detection and could not be clearly distinguished from background levels and incorporation of labeled metabolites. In summary, little confidence can be placed in the weak positive DNA fragmentation results as indication of direct DNA damage by diisocyanates. There remains insufficient evidence to conclude that MDI or TDI are genotoxic to humans *in vivo*. This review was sponsored by the International Isocyanate Institute, Inc.

1677 PELARGONIC ACID MORPHOLIDE – GENOTOXICITY AND INFLUENCE ON THE REPRODUCTIVE FUNCTION.

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The aim was to appreciate the possible genotoxic effect and influence on reproductive function of pelargonic acid morpholide (PAM). Studies were performed on un-bred white rats female. Investigated object was liquid mixture which containing PAM intended for filling out self-defense gas weapons. Genotoxic effect was evaluated after an intraperitoneal administration of PAM with dose 1/100 LD₅₀ according to DNA leucocytes indexes and hematological parameters. Experiments of PAM influence study on reproductive function were performed on pregnant female rats. The animals received intragastrically the PAM containing mixture with the dose 1/20 LD₅₀ every day during all pregnancy.

Evaluation of embryotoxic effect carried out according to death of fetuses, before-implantation and post-implantation, general embryonal mortality as well length and weight fetuses. The index of chemical factors influence was a coefficient of induced embryo death.

Results: The single intraperitoneal administration of the PAM mixture resulted in increase of lymphocyte amount in peripheral blood up to 60% in 24 hours and decrease of DNA index (in 24 hours and 30 days). General embryonal mortality was increased 2.3 times, including before-implantation – 2.2 times, and post-implantation – 3.2 times. High percent age of induced death was revealed.

Conclusion: The single intraperitoneal administration of the PAM mixture with dose 1/100 LD₅₀ resulted in early genotoxic effects in female blood detectable in the 30th day after exposure that allowed assuming the presence of a prolonged genotoxic sequels. The every day intragastric administration of the PAM mixture to pregnant rat females during the whole period of pregnancy led to true disturbances of reproductive function.

1678 MUTAGENIC ACTIVITY IN THE HPRT LOCUS OF LEUKEMIA HL-60 CELLS TREATED WITH SUCCINYL ACETONE.

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Succinyl acetone (SA) was initially identified in the urine of patients with tyrosinemia type 1, an autosomally recessive inherited disease. SA has been used to down-regulate the activity of myeloperoxidase (MPO) through its specific inhibition of heme biosynthesis and to investigate the biological properties of MPO in human myeloid leukemic (HL-60) cell line. The goal of this study is to evaluate the mutagenic potential of SA by determining the frequencies of somatic mutations in the hypoxanthine-guanine phosphoribosyl transferase (HPRT) reporter gene in HL-60 cells following treatment with the chemical. Treatments of HL-60 cells with 500 µM SA for 72 hours, a condition generally used to inhibit the MPO activity, resulted in a significantly increased HPRT mutant frequency (HPRT-Mf), compared with the control of untreated cells (47.25 x 10⁻⁶ versus 7.5 x 10⁻⁶, respectively, P < 0.01). Treatment of the cells with lower doses of SA also led to an increase in HPRT-Mf that is, however, significant only with 200 µM (28.67 x 10⁻⁶, p < 0.05) and not with doses lower than 100 µM (p > 0.05), compared with the control of untreated cells (7.5 x 10⁻⁶). These data show a dose-response increase in HPRT-Mf in HL-60 cells treated with SA, suggesting this chemical is mutagenic in these cells.

1679 DEVELOPMENT OF A HUMAN PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODEL FOR INORGANIC ARSENIC AND ITS MONO- AND DIMETHYLATED METABOLITES.

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A physiologically-based pharmacokinetic (PBPK) model was developed to estimate levels of arsenic and its metabolites in human tissues and urine after oral exposure to either arsenate (AsV) or arsenite (AsIII). The model consists of interconnected individual PBPK models for Asv, AsIII, monomethylarsenic acid (MMAv), and dimethylarsenic acid (DMAv). Reductions of MMAv and DMAv to their respective trivalent forms are also described in the overall model. Each submodel was constructed using flow limited compartments describing the mass balance of the chemicals in GI tract (lumen and tissue), lung, liver, kidney, muscle, skin, heart, and brain. The choice of tissues was based on physiochemical properties of the chemicals (solubility), exposure routes, and target and metabolic sites of inorganic arsenic and its metabolites. Metabolism of inorganic arsenic in liver was described as a series of reduction and oxidative methylation steps, in addition to the inhibitory influence of the metabolites on methylation. The inhibitory effects of AsIII on the methylation of MMAIII to DMA, and MMAIII on the methylation of AsIII to MMA were assumed to follow a noncompetitive mechanism. Most of the parameters used in the model were estimated from the literature. The remaining parameters were calibrated and evaluated using cumulative levels of inorganic arsenic and its metabolites in urine of exposed people. The calibrated and evaluated human PBPK model can be used, when combined with Monte-Carlo simulations, in identifying population-based distributions for some sensitive parameters (such as metabolic rate parameters differing due to genetic polymorphisms) and address the impact of these distributions on the human risks to arsenic exposure. (This abstract does not necessarily reflect EPA policy.)

1680 A PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR THREE DIHALOACETATES.

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Dihaloacetates (DHAs) occur as water disinfection byproducts commonly present in public water supplies leading to widespread exposure. Dichloroacetic acid (DCA), dibromoacetic acid (DBA), and bromochloroacetic acid (BCA) are all metabolized mainly by glutathione transferase zeta (GSTzeta). A physiologically based pharmacokinetic model was developed in support of the National Toxicology Program (NTP) bioassays for DBA and BCA. A single model was developed to enhance the understanding of the kinetics of these three structurally similar dihaloacetates. The PBPK model has flow-limited compartments representing kidney, liver, stomach, and other aggregated tissues. Elimination of parent and metabolite compounds occurs through urine or biotransformation. This model examines the biotransformation of BCA, DCA, and DBA to the metabolites glyoxalate (GXA) and oxalate (OXA) in detail. GSTzeta synthesis and degradation as well as GXA and OXA production external to the metabolism of the dihaloacetates are included. Molecular interactions of GSTzeta and the DHA derivative compounds are modeled including the covalent binding of an intermediate DHA metabolite with GSTzeta which effectively inactivates the enzyme. This suicide inhibitory behavior is characteristic of the dihaloacetates. Additionally for BCA, the model allows for stereospecific treatment of the BCA(+) and BCA(-) isomers due to published evidence of toxicokinetic behavioral differences between them. Model parameters not available from the literature were fit to the DHA, GXA, and OXA data available from IV, gavage, and drinking water studies. To evaluate the accuracy of this model, predictions are measured against data not used during parameter estimation. In particular, simulations of two-week drinking water exposures followed by a subsequent gavage challenge are compared to data.

1681 A PHYSIOLOGICAL MODEL DESCRIBING TISSUE DOSIMETRY OF INHALED SULFURYL FLUORIDE AND ITS METABOLITES FLUOROSULFATE AND FLUORIDE IN RAT.

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Sulfuryl fluoride (SF) is a structural and post-harvest fumigant used to control a wide variety insect pests. SF is a key alternative to methyl bromide, a stratospheric ozone depleting substance scheduled for phaseout by the Montreal Protocol. Repeated inhalation exposures to relatively high levels of SF cause inflammation of the nasal mucosal and lower respiratory tract and reversible neurological effects in

rodents. Although the mode of action of SF-mediated neurotoxicity is not clearly understood, the metabolic profile after inhalation exposure to SF suggests that the toxicity is likely due to metabolites of SF rather than parent compound. A preliminary physiologically based pharmacokinetic (PBPK) model describes the time-course concentrations of SF and its metabolites fluorosulfate (FS) and fluoride (F) in blood/plasma, tissues and urine during and after 4-hr inhalation exposures to 30 or 300ppm SF. Systemic uptake of SF is limited by extensive deposition and/or hydrolysis along the respiratory mucosa and/or rapid hydrolysis in the blood. Parent SF is not detectable in the blood immediately following inhalation exposure. Time-course concentrations of FS in blood and urine are described in the model as dose-dependent and primarily regulated by hydrolysis to F and elimination in urine. Appearance of F in plasma occurs by hydrolysis of SF and FS and removal from plasma occurs via urinary elimination and sequestration into bone. These model descriptions are consistent with data from our laboratory indicating that SF is rapidly hydrolyzed in blood ($t_{1/2} < 3$ min) while metabolism of FS to F is estimated to occur at a rate approximately one-half that of SF to FS. This current model provides an initial framework for examining the low-dose kinetic behavior of SF and its metabolites during and following exposure to SF and extending the model description to address possible pre-systemic clearance mechanisms of SF along the respiratory mucosa.

1682 APPLICATION OF A UNIFIED PBPK MODEL TO TWO KINETICALLY DISTINCT PHTHALATE ESTERS - DBP AND DEHP.

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Phthalate diesters are widely used plasticizers and environmental contaminants. Human exposure occurs primarily through drinking water and food consumption. Certain phthalates, such as di-n-butyl phthalate (DBP) and di-2,4-ethylhexyl phthalate (DEHP), disrupt testosterone dependent male rat sexual development. Recently, a PBPK model was developed in the non-pregnant and pregnant rat describing serum, urine, bile and fecal data for DBP, MBP and the glucuronide and oxidative metabolites across doses, routes and life-stages. With the exception of reduced maternal UGT activity, kinetic parameters did not differ in the naive and pregnant rat. For this model to be useful for risk assessment, however, it must account for simultaneous exposure to more than one phthalate. Despite similar toxicity, DEHP and DBP have very different metabolite kinetics. To use the DBP model for DEHP, it was necessary to reduce DEHP hydrolysis in the gut (10 v. 150 mg/kg-hr) and increase the capacity for oxidative metabolism of MEHP (30 v. 1 mg/kg-hr) based on in vitro data. UGT activity (maternal and fetal) was turned off based on in vivo excretion data. These changes allow the model to describe reduced bioavailability (30 vs. 0% of diester in feces) and enterohepatic recirculation (4% vs. 30% of dose in bile), and faster clearance of the monoester. Serum MEHP kinetics were well-described after a single iv or oral MEHP (20 - 400 mg/kg) or DEHP dose (30 - 2000 mg/kg). Using the same placenta/fetal transfer parameters as DBP, the model successfully predicted maternal serum MEHP time-course data after a DEHP dose on GD 20 and amniotic fluid MEHP levels on GD 18 after 10 daily doses of 300 mg/kg DEHP (3 mg/L). This suggests that the model may be successfully applied to other phthalates using in vitro metabolism data where in vivo data is not available, thereby facilitating a more integrated risk assessment for the phthalate family.

1683 A PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR DEVELOPMENTAL EXPOSURE TO PBDE-47 IN RODENTS.

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Polybrominated diphenyl ethers (PBDEs) are used commercially as additive flame retardants and have been shown to transfer into environmental compartments where they have the potential to bioaccumulate in wildlife and in people. Of the 209 PBDE congeners, approximately 5 dominate the profiles measured in humans, particularly 2,2',4,4'-tetrabromodiphenyl ether (BDE 47), demonstrating a need to understand the toxicokinetic properties associated with this class of chemicals. A small subset of the PBDES have been investigated in toxicokinetic studies; these studies have shown that binding proteins, elimination, adipose tissue mass, and placenta transfer may all play roles in the distribution of PBDEs. The aim of this work was to develop a developmental PBPK model for BDE-47 in mice and rats with the perspective of extrapolating the model to humans. The model consists of seven

compartment including liver, brain, fat, kidney, placenta/foetus, blood and rest of the body. Parameters such as tissue volumes, blood flows, and partition coefficients were extracted from previously reported studies. Optimization was performed using mouse exposure data for adult female and neonates determined in our laboratory. The optimized model was extrapolated to rats and validated using data from the literature. The model validation used experimental data from acute and subchronic exposures prior to and during gestation. The simulations predict that BDE-47 tissue concentrations in the maternal compartments are within the standard deviation of the experimental data. This PBPK model presented here is the first reported for any PBDE. This model may provide a framework for the development of a human PBPK model to predict both adult and developmental PBDE concentrations for use in risk assessments. (This abstract does not reflect Agency policy. Supported in part by EPA cooperative agreements CR830756 and DESE CT 826513.)

1684 PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELING OF INHALED MANGANESE IN RATS.

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Manganese (Mn) is an essential element found in food, water and air. Mn-containing fuel additives introduce small amounts of Mn particulates into the atmosphere after combustion. Symptoms of central nervous system toxicity from Mn are known to occur at high concentration inhalation exposures. However, Mn is an essential element, required for normal tissue growth and function. Tissue [Mn] is closely regulated to prevent accumulation to toxic levels. The purpose of this research is to develop a pharmacokinetic model to describe the regulation of tissue [Mn] in the face of increasing concentrations of inhaled manganese. We have developed a PBPK model to describe the tissue kinetics of Mn following inhalation of Mn by rats. Each tissue compartment had shallow and deep regions to describe discrete tissue Mn stores. The PBPK model parameters were calibrated using dietary data for tissue Mn in rats following 14 days of inhalation exposure to MnSO_4 at specific doses. The steady state levels of Mn predicted by the PBPK model were tested against rat tissue measurements at dietary intakes of 2, 10 and 100 ppm of Mn. Dose dependent hepatic biliary excretion, noted in other studies, was then necessary to regulate the tissue levels of Mn in rats measured following 14 days of inhaled exposure to 0.0, 0.03, 0.3 mg Mn/m³. The hepatic controller in the model consisted of an empirical adjustment of biliary elimination rate constants of Mn based on net daily uptakes from ingestion and inhalation. At the highest inhalation exposure, the hepatic Mn controller increased the bile excretion rate constant in the PBPK model by a factor of 10 times the steady state rate. This PBPK model with the control by enhanced biliary excretion control was able to predict tissue regulated Mn levels in rats following inhalation. Multi-dose route PBPK models for Mn will provide support for the evaluation of tissue-dose based risk assessment of this essential metal.

1685 EVALUATION OF ADULT-NEONATE DIFFERENCE IN METABOLIC INTERACTIONS AMONG ALKYL BENZENES (ABS) USING A MIXTURE PBPK MODEL.

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A physiologically-based pharmacokinetic (PBPK) model for mixtures of ABs (toluene (T), m-xylene (X) and ethylbenzene (E)) has previously been developed and validated in rats and humans. This PBPK model, accounting for physiological and metabolic differences between rats and humans, provided simulations of the extent of metabolic interactions among the ABs. The objective of the present study was to evaluate adult-neonate differences in metabolic interactions among ABs using this mixture PBPK model. The approach involved the substitution of physiological parameters (cardiac output, breathing rate, tissue volumes and blood flows) in the previously validated adult PBPK model while considering physicochemical parameters as age-invariant, based on available data. The maximal velocity of metabolism of ABs was computed on the basis of the hepatic CYP2E1 protein content in neonates relative to adults. For this purpose, the subject-specific data on CYP2E1 in neonates (≤ 1 month old) were obtained from published literature. The mixture PBPK model, with the physiological and metabolic data for neonates, was used to simulate the inhalation kinetics of ABs previously evaluated in adults (1-17 ppm T, 1-33 ppm X and 1-33 ppm E; 7 hr). The simulated peak venous blood concentrations (C_{max}) of ABs were greater in neonates compared to adults by a factor of <2 . However, the additional change in C_{max} during mixed exposures in neonates (upto a factor of 1.4 for a mixture of 17 ppm T + 33 ppm X + 33 ppm E; 7 hr) depended upon the subject-specific content of CYP. There was considerable interindividual variation in both neonates and adults such that the subject-specific

magnitude of metabolic interaction was primarily influenced by the CYP content as well as the relative importance of perfusion and enzyme content in the hepatic clearance of ABs. Overall, this study showed that the extent of metabolic interactions can be predicted using PBPK models as a function of hepatic enzyme content and age-dependent physiological data.

1686 PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING OF METABOLIC INHIBITION FOR INTERACTION BETWEEN TRICHLOROETHYLENE AND CHLOROFORM.

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Trichloroethylene (TCE) and chloroform (CHCl_3) are two of the most common environmental contaminants found in water. PBPK models have been increasingly used to predict target dose in internal tissues from available environmental exposure concentrations. A closed inhalation (or gas uptake) system was used to estimate metabolic parameters using male F344 rats at different initial concentrations. Individual chemicals were first tested separately using the following initial concentrations: 100, 500, 1000, and 3000 ppm. The decrease in chamber concentration reflecting metabolism was measured for up to 6 hours. A PBPK model was then used for each individual data set to obtain the metabolic parameters describing saturable metabolism, V_{max} (maximum velocity of reaction) and K_m (affinity constant). The next set of experiments described co-exposure of different binary combinations, including 500 ppm for both chemicals, 500 ppm TCE and 10 ppm of CHCl_3 , and 500 ppm TCE with 2000 ppm CHCl_3 . PBPK modeling was used to test three types of different metabolic inhibition: competitive, uncompetitive and noncompetitive. Results of the simulations suggest that competitive inhibition gives the closest agreement between the inhalation data and the simulations, resulting in an inhibition constant (K_i) of 0.33 mg/liter, which is similar to the K_m value of 0.36 mg/liter. This interaction model was used to identify sensitive parameters for the design of future experiments. In summary, PBPK modeling results were consistent with competitive inhibition for TCE and CHCl_3 co-exposure, leading to an overall decrease in metabolism when both chemicals are present simultaneously. (This abstract does not reflect EPA policy. Research support for Karen Yokley is provided by grants EPA T829472 and EPA CT833237.)

1687 ESTABLISHING CHANGES IN METABOLISM OF CARBON TETRACHLORIDE IN THE PRESENCE OF TRICHLOROETHYLENE IN THE RAT THROUGH THE USE OF PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING.

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Toxicological interactions of chemicals can affect metabolism, often decreasing overall associated metabolic rates; and changes in metabolism can be evaluated through the use of mathematical models. Trichloroethylene (TCE) and carbon tetrachloride (CCl_4) are common contaminants in water and at superfund sites. A closed inhalation (or gas uptake) system was used to collect metabolic data using F344 rats at various initial concentrations of TCE and CCl_4 . The initial concentrations used for the exposures were: 25 ppm CCl_4 and 1000 ppm TCE, 25 ppm CCl_4 and 100 ppm TCE, 100 ppm CCl_4 and 500 ppm TCE, 100 ppm CCl_4 and 1000 ppm TCE, and 1000 ppm CCl_4 and 500 ppm TCE. This particular binary mixture pair is an example of metabolic synergy as opposed to the more common metabolic inhibition expected during exposure to multiple chemicals. A previously developed physiologically based pharmacokinetic (PBPK) model of carbon tetrachloride in the rat was verified to fit with gas uptake data for CCl_4 alone (with initial concentrations of 25 ppm, 100 ppm, 250 ppm, and 1000 ppm) and then used to simulate chamber concentration predictions for the aforementioned mixtures. In order to fit chamber concentration data for CCl_4 when administered with TCE, the parameter of the maximum rate of metabolism (V_{max}) was increased. An increase of 2.8 times the V_{max} value from the original model of carbon tetrachloride alone produced reasonable predictions for mixture data with higher concentrations (500 ppm or 1000 ppm) of TCE, and an increase of 1.4 times the original V_{max} worked well at predicting CCl_4 chamber concentration for mixtures with 100 ppm TCE. The increase in V_{max} suggests that metabolism of carbon tetrachloride is amplified by the presence of trichloroethylene and that this amplification is dose-dependant. (This abstract does not reflect EPA policy. Research support for Karen Yokley is provided by grants EPA T829472 and EPA CT833237.)

1688 PBPK MODELING ASSESSMENT OF THE COMPETITIVE METABOLIC INTERACTIONS FOR M-XYLENE, ETHYLBENZENE, AND A LUMPED AROMATIC FRACTION OF JP-8 JET FUEL VAPOR.

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Jet Propellant 8 (JP-8), a kerosene-based jet fuel used in the military, is composed of hundreds of hydrocarbons. A PBPK model was developed to assess the metabolic interactions of JP-8 vapor on two prominent aromatic constituents of JP-8 vapor, m-xylene (XYL) and ethylbenzene (EBZ). A limited number of rats were exposed to JP-8 vapor in a Leach chamber for 4 hr to 390, 1100, or 2700 mg/m³. The chamber atmosphere was monitored during exposure with several individual hydrocarbons being quantified, including XYL, EBZ and the total hydrocarbon concentration. Blood and liver was harvested (end of exposure and 0.5 h post exposure) and analyzed by a novel headspace SPME/GC-MS method which allowed for identification and quantification of low levels of individual hydrocarbons. The PBPK model was able to describe both the single exposure data previously published for XYL and EBZ as well as the metabolic interactions between XYL, EBZ and a lumped aromatic fraction of JP-8 vapor that was estimated to be approximately 25% of the fuel vapor. Competitive inhibition of XYL and EBZ metabolism was observed for JP-8 vapor inhalation exposures of 1100 and 2700 mg/m³. Future inhalation studies with jet fuel include aerosol exposures and expansion of the PBPK models to include other hydrocarbons such as n-alkanes and upper respiratory tract dosimetry of aerosol droplets.

1689 PHYSIOLOGICALLY-BASED TOXICOKINETIC AND TOXICODYNAMIC (PBTK/TD) MODELING OF A TERNARY ORGANOPHOSPHORUS INSECTICIDE MIXTURE IN RATS: MODEL DEVELOPMENT AND VALIDATION.

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A PBTK/TD model was developed, from the open literature, to predict the toxicokinetic disposition and toxicodynamic response (acetylcholinesterase inhibition) of a ternary OP insecticide mixture: chlorpyrifos (CP), methyl parathion (MP) and parathion (P). In vivo studies were conducted in adult male Sprague-Dawley rats, orally administered a CP/ MP/ P mixture (2.5, 0.5, 0.5, or 5, 1, 1 mg/kg, respectively) with selected tissues (blood, brain, diaphragm, liver, lung and skeletal muscle) collected at 30min, 4, 12 and 24hr postdosing. Low dosages were studied so the mixture did not result in significant disruption of cardiovascular function nor invalidate the model's underlying general physiological assumptions. The data were used to validate the model. The metabolites of CP (CP-oxon, 3,5,6-trichloro-2-pyridinol (TCP)), and both MP and P (their respective oxons and 4-nitrophenol), were quantified in the tissues of interest. Peak concentrations of CP were attained by 4hr in all tissues with the exception of the liver, whose peak occurred at 30min; this was supported by the model simulations. MP, P, and their respective oxons were below limits of quantitation for the lower dosage, however, were quantifiable at the higher dosage. No toxicokinetic interactions were observed. Cholinesterase inhibition in the tissues ranged from 11- 37% for the lower dosage, and 29-93% for the higher dosage group; with few exceptions, inhibition was generally additive and was also supported by the model simulations. This study demonstrates the utility of using previously developed individual PBTK/TD models and in vitro/ in vivo data from the open literature to construct reliable mixture PBTK/TD models.

1690 BAYESIAN PHYSIOLOGICALLY BASED PHARMACOKINETIC/PHARMACODYNAMIC (PBPK/PD) MODELING OF MIXED ROUTE EXPOSURE OF CARBARYL.

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Bayesian techniques enable characterizing uncertainty and variability based on prior and experimental information applicable for risk assessment of pesticides. Amongst the N-methyl carbamate family of pesticides under re-evaluation is carbaryl, a common insecticide which reversibly inhibits neuronal cholinesterase activity where the extent of knowledge is indeterminate. The objective of this work was to estimate biologically relevant doses associated with cholinesterase activity through Bayesian modeling of the carbaryl pharmacokinetics following different exposure scenarios. A physiologically-based pharmacokinetic and pharmacodynamic (PBPK/PD) model for carbaryl was developed to estimate tissue doses of carbaryl and its metabolites (1-naphthol and other hydroxylated metabolites) and particularly the cholinesterase activity in blood and brain. A Markov Chain Monte Carlo (MCMC) technique was implemented to characterize distributions of model

physiological and biochemical parameters (mean, variability, and uncertainty). Calibration of model parameters was obtained using prior literature distributions, experimental tracer data of carbaryl and its metabolites, and inhibition of cholinesterase activity measurements in rats which were exposed by dermal, oral, and intravenous routes at low and high doses ranging from 0.8 to 103 mg/kg body weight. Based on the experimental data, the converged posterior estimates of the model parameters displayed less than 0.5 fold deviations from the mean values. Monte Carlo simulations with the resulting MCMC posterior estimates and PBPK/PD model adequately predicted the tissue doses of carbaryl and 1-naphthol as well as the cholinesterase inhibition by carbaryl with an interval of confidence less than 1 fold deviation from the mean. The Bayesian PBPK/PD modeling approach developed here will serve as a prototype for developing mechanism-based risk models for other N-methyl carbamates.

1691 BAYESIAN ANALYSIS OF HUMAN TOLUENE PBPK MODEL AT STEADY-STATE.

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Bayesian statistical approaches are relevant for characterizing the population distribution of pharmacokinetic determinants and tissue dose of chemicals. Chronic inhalation exposure to volatile organic chemicals (VOCs) leads to the onset of steady-state, at which the blood and tissue concentrations of the parent chemical are constant. The steady-state blood concentration (C_{ss}) might vary among individuals as a function of the population variability of critical determinants. The objective of this study was to conduct Bayesian analysis of the inhalation pharmacokinetics of toluene using a human PBPK model at steady-state. At steady-state, the blood concentrations are influenced by alveolar ventilation rate, liver blood flow, blood:air partition coefficient and intrinsic clearance. The prior information on these input parameters for the toluene PBPK model was obtained from the literature. The algebraic equation representing the steady-state PBPK model was written in Winbugs (version 1.4; MRC and Imperial College of Science, Technology and Medicine, Cambridge, U.K.) software for conducting Markov chain Monte Carlo (MCMC) simulations. The mean and variability of C_{ss} of toluene observed in a human volunteer study (n=4) was used as a basis to create a distribution (normal) from which 16 samples were drawn using Monte Carlo approach. Based on these individual C_{ss}, MCMC simulations were conducted and the convergence of the posterior estimates of the parameters was obtained after 10000 iterations. Monte Carlo simulations were then carried out using Crystal Ball 7 (Decisioneering, Denver, CO) with the MCMC-generated posterior density estimates of input parameters. The calculated C_{ss} values compared well with the individual and population values. Overall, this study has demonstrated the feasibility of conducting MCMC simulations of C_{ss} using WinBugs, particularly during data-poor situations.

1692 STATISTICAL APPROACHES FOR EVALUATION OF PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELS.

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PBPK model evaluation is a crucial step in its development since it ensures that the conceptual and functional representations of the real system are adequate to yield reasonable simulations in the range of its intended use. Unfortunately, the model evaluation step is often limited to a subjective analysis consisting of visually comparing simulations to experimental data. The objective of the present study was to investigate the applicability of different statistical methods for the evaluation of PBPK models. Amongst the various statistical methods, three were selected for the present study: i) the lack of fit F-test, ii) the bootstrap with bias-corrected (α) (BC α) confidence intervals, and iii) regression analysis. These tests were applied to a validated rat PBPK model for a ternary mixture of toluene, m-xylene and ethylbenzene. The lack of fit F-test doesn't seem to be an adequate statistical test because it relies on the variability of the simulations attributed to input parameters almost uniquely influenced by body weight. The regression of the error (i.e., difference between simulated and corresponding experimental data) over their sum (i.e., simulations plus corresponding experimental data) offers an adequate analysis but the assumption of residual normality on which it relies is seldom respected. The bootstrap re-sampling technique coupled with the generation of BC α confidence intervals for the error is an approach that is much more versatile than the others but necessitates a priori residual analysis in order to ensure homoscedasticity of simulated data. This study suggests that an objective evaluation of PBPK models is possible using a two step approach that combines a residual analysis with a bootstrap-BC α confidence intervals test. (SH is recipient of a research scholarship from FRSQ)

1693 MODELING THE TOXICOKINETICS OF INHALED TOLUENE IN RATS: THE IMPACT OF FEEDING STATUS, PHYSICAL ACTIVITY AND STRAIN.

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Toluene is found in petroleum-based fuels and used as a solvent in consumer products and industrial applications. The critical effects following inhalation exposure involve the brain and nervous system in both humans and experimental animals whether exposure duration is acute or chronic. The goals of this modeling effort were two-fold: (1) evaluate and explain the influence of feeding status and activity level on toluene pharmacokinetics, and (2) apply this model to the analysis of data in the published literature to explain differing toluene kinetics. Compartments in the model are lung, slowly and rapidly perfused tissue groups, fat, liver, GI tract and brain; tissue transport is blood-flow limited and metabolism occurs in the liver. Chemical-specific parameters and initial organ volumes and blood flow rates were obtained from the literature. Sensitivity analysis revealed that the most influential parameters for our experimental conditions were cardiac output, alveolar ventilation and fat volume; other parameters (e.g., partitions and metabolic rate parameters) were either well defined (multiple consistent experimental results with low variability) or relatively non-influential (e.g. organ volumes other than fat). Model-based analysis of our data and that of other investigators suggests that there is a toluene-specific effect on ventilation parameters that differs between active and sedentary rats and is dose-dependent as well. However, rats that were weight-maintained compared to free-fed rats in our studies and those of other investigators could be modeled with a single set of parameters provided that experimental data were obtained from rats exposed to toluene in whole body inhalation chambers. These results point to (1) the importance of experimental conditions and physiological status in explaining apparently conflicting kinetic data and (2) the need to match simulation conditions to experimental conditions when estimating internal doses for effects in studies that lack kinetic data. (This abstract does not necessarily reflect EPA policy.)

1694 CYP2E1 MEDIATED EXTRAHEPATIC METABOLISM IN PBPK MODELING OF LIPOPHILIC VOLATILE ORGANIC COMPOUNDS.

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Physiologically based pharmacokinetic (PBPK) models increasingly are available for environmental chemicals and applied in risk assessments. Often a simplified representation of a real biological system is used in order to reduce uncertainties in the PBPK predictions caused by uncertainties in input parameters. Volatile organic compounds (VOCs) are important pollutants in air, soil and water. CYP2E1 metabolically activates many VOCs in animals and humans. Despite of its presence in some extrahepatic tissues, the metabolism by CYP2E1 is often described as restricted to the liver in PBPK models, unless the risk assessment requires target tissue dose metrics in extrahepatic tissue. The impact of accounting for extrahepatic metabolism (EHM) by CYP2E1 on the estimation of in vivo metabolic parameters and the prediction of dose metrics was evaluated for the lipophilic VOCs - vinyl chloride, trichloroethylene, and carbon tetrachloride. Metabolic parameters estimated from fitting gas uptake data in rats with- and without- EHM were very similar. The impact of EHM on PBPK predictions was evaluated under relevant inhalation exposure scenarios for animal toxicity studies and human risk assessment. Although small, the relative role of EHM and the differences in the predictions of dose metrics were concentration dependent with greater impact at low exposure concentrations. The extent of the impact was varied by several factors such as Km for CYP2E1, age, and species. The current study suggests PBPK modeling for VOCs that are CYP2E1 substrates is not very sensitive to a lack of accounting for EHM, implying that using simplified liver-only metabolism for PBPK modeling of lipophilic VOCs is a reasonable approach. The PBPK predictions using this assumption can be applied confidently for risk assessment, but care should be taken when applying this conclusion to VOCs that are metabolized by other enzymes (Supported by EPA-NRC #CR82879001. This abstract may not represent official EPA policy.)

1695 PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING IN THE ELDERLY.

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PBPK models have been developed for a number of chemicals in test animal species and adult humans; however, few models have been developed to evaluate the kinetics and internal dose of chemicals in elderly. The objective of the present study was

to develop an inhalation PBPK model for a group of healthy elderly using toluene as the model substrate. The PBPK model consists of four tissues (liver, fat, slowly perfused tissues (SPT), and richly perfused tissues (RPT)) interconnected by systemic circulation. Values for cardiac output, ventilation rate, tissue volumes, and blood flows were obtained from the published literature. The partition coefficients (blood:air and tissue:blood) are the same as those used previously in an adult toluene PBPK model, since there is a lack of data suggesting significant change in tissue and blood solubility of chemicals in elderly compared to adults. The volume-normalized intrinsic clearance, obtained from the adult PBPK model, was multiplied by the liver volume in elderly, and was used along with the liver blood flow rate in elderly to compute toluene metabolism in the model. The PBPK model was used to simulate the kinetics of toluene following 7 hr exposure to 17 ppm. The simulated blood kinetic profile was compared with the experimental data collected in adult human volunteers following a similar exposure scenario. The predicted values in elderly were slightly lower than those observed in the adult male volunteers. Overall, the PBPK model developed in this study could be useful for evaluating the extent of adult-elderly differences in dose surrogates of relevance to mode of action of chemicals for risk assessment applications.

*The views expressed in this manuscript are solely those of the authors and do not represent the policies and/or endorsement of the U.S. Environmental Protection Agency.

1696 PHYSIOLOGY-BASED PHARMACOKINETIC MODELING: A TOOL FOR SIMULATING PLASMA AND TISSUE CONCENTRATION-TIME CURVES OF COMPOUNDS.

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Physiology-based pharmacokinetic (PBPK) modeling provides a useful tool for simulating plasma and tissue concentration-time curves for different routes of administration of compounds to humans and preclinical animal species. The present study was aimed at evaluating a PBPK model for simulating tissues and plasma concentration-time curves following different routes and schedules of administration of a compound to rodents. The first basic model, developed considering only IV administration, included a system of differential equations describing 13 tissue compartments. It required the inclusion of a minimal amount of information on the compound, obtained from in silico or in vitro investigations: octanol:water partition coefficient, ionization constant(s), fraction unbound in plasma, solubility, permeability and hepatic intrinsic clearance. Tissue partition was modeled using a set of tissue composition-based equations. Hepatic and renal clearances were obtained scaling the intrinsic clearance obtained in vitro from liver preparations, and assuming only the glomerular filtration, respectively. The model was tested comparing the predicted and the observed pharmacokinetics in plasma, after intravenous dosing, of 113 molecules synthesized as part of various discovery programs in different therapeutic areas.

Oral administration was also included by adding differential equations from an advanced compartmental absorption and transit (ACAT) model that was integrated into the PBPK model to determine the rate, extent and approximate gastrointestinal location of drug dissolution and passive absorption. Fraction absorbed, bioavailability and plasma concentration-time profiles were predicted and compared to literature data.

Multivariate analysis was used for identifying the characteristics leading to the possible risk of bias and the influence of different sources of variability using stochastic simulations.

1697 NON-INVASIVE, TELEMETRICALLY-RECORDED SURFACE ECG IN BEAGLE DOGS IN REGULATORY TOXICOLOGY STUDIES. VALIDATION WITH SUBCUTANEOUS ISOPROTERENOL.

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Recording of ECG in regulatory toxicology studies faces several constraints, among which predominate infrequent handling of the animals (generating stress) and recording of ECG over minutes instead of hours or days, which prevents the analysis of additional qualitative (shape of complexes, detection of arrhythmias) and quantitative data (measurement of the duration of several ECG parameters). Such constraints become critical in the evaluation of potential QT interval-prolonging properties of new chemical entities. We took advantage of our GLP validated telemetric environment to assess whether non-invasive and non-stressful investigation of ECG in freely-moving beagle dogs could be incorporated in regulatory toxicology studies. The telemetric environment consisted of an implant (DSI® Technologies, USA) and signal processing software (Notocord®, F). ECG was recorded with adhesive electrodes connected to an implant body (model

TL11M2D70-PCT), embedded in a jacket, to which the dogs had been previously accustomed. The primary objective was to assess whether this configuration could record high-quality ECG for at least 20 hours. Two dogs were subcutaneously injected with isoproterenol (0.25 mg/kg), while another 3 dogs were injected with water. The treated dogs developed marked tachycardia (up to 248 beats/min) starting 30 minutes after injection. The heart rate then stabilized about 35 beats/min above the pre-test values for the remaining duration of the recording period. No such changes were seen in control dogs. Ventricular extrasystoles developed in all isoproterenol-treated dogs and lasted for 20 hours. All ECG traces were of excellent quality and exploitable by the software. This telemetric method appears sufficiently robust to be used in regulatory toxicology studies.

1698 INVESTIGATION OF QT-INTERVAL PROLONGATION IN CONSCIOUS TELEMETERED BEAGLE DOG WITH CLASS III ANTIARRHYTHMIC DRUG.

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Drug-induced effects on the QT-interval with the possibility of inducing fatal arrhythmias have become a new challenge for drug development and regulatory approval. The conscious telemetered Beagle dog recently became a major in vivo model for the cardiovascular safety pharmacology studies, and the duration of QT-interval has been used as the determinant of the risk of drug-induced torsades de pointes or arrhythmias (ICH S7B). However, interpretation of the QT-interval in the light of heart rate changes is often complex. The purpose of this study was to define the sensitivity of the in vivo method of safety pharmacology assessment of cardiovascular functions, and investigated a method for quantifying beat-to-beat QT-interval in a conscious free-moving telemetric canine model using an antiarrhythmic class III reference drug, d,l-sotalol. A telemetry transmitter (TL11-M2-D70-PCT) from Data Sciences International (DSI) was surgically implanted into each dog following the standard procedures. The animals' blood pressure, heart rate, ECG, body temperature and locomotor activity were simultaneously and continuously acquired by DSI OpenArt system up to 24 hours prior to and following a single bolus intravenous administration of saline (control), or d,l-sotalol at 16 mg/kg. The heart rate-adjusted QT interval (QTcB, QTcF and QTcV) was determined using the rate-correction formula in Ponemah analysis program. In addition to standard parameters an advanced beat-to-beat analysis (Tpeak-Tend duration, QT variability and P-wave duration) was performed. The d,l-sotalol induced QT prolongation was observed with maximal effects at 2-hr post dose when compared to control. Beat-to-beat variability of QT-interval was assessed from Pioncare plots (QT vs QTn-1) of 60 consecutive beats at time of maximal QT effect. In conclusion, the conscious telemetered dog has been shown a reliable pre-clinical model for detection of drug-induced ECG abnormalities.

1699 HEMODYNAMIC AND ELECTROCARDIOGRAPHIC EFFECTS OF FLECAINIDE, QUINIDINE AND MOXIFLOXACIN IN CONSCIOUS, INSTRUMENTED BEAGLE DOGS MONITORED BY TELEMETRY.

S. R. Arthur, A. K. Gupta, B. D. Car and B. Gemzik. *Discovery Toxicology, Bristol-Myers Squibb, Princeton, NJ.*

Assessment of cardiovascular (CV) safety has become an integral part of nonclinical safety evaluation of all new drug candidates because several pharmaceutical products have been found to delay ventricular repolarization and increase the risk for life threatening ventricular arrhythmias. Inhibition of hERG ion channel activity is the most common mechanism of delayed ventricular repolarization by pharmaceuticals, but sodium and/or calcium ion channel effects can also result in CV liabilities. Considered with cellular electrophysiology evaluations of ventricular repolarization and ion channel activity, intact animal models allow an integrated CV assessment. We have profiled 3 cardiac ion channel inhibitors as part of a reference dataset in a canine telemetry model. Conscious, unrestrained instrumented beagle dogs (N=4) were given single oral doses of vehicle (control), or flecainide (sodium ion channel inhibitor), moxifloxacin (hERG ion channel inhibitor), or quinidine (mixed ion channel inhibitor). Parameters including heart rate, systemic blood pressure, myocardial contractility, and ECG waveform morphology and interval duration were evaluated simultaneously. Plasma drug concentrations were determined by a limited sample collection during study, and confirmed by a toxicokinetic profile in non-instrumented dogs. Flecainide at 10 mg/kg increased P wave width (≈ 10 msec), QRS (3-5 msec), and QTc (10-25 msec) intervals. Dose-related increases in heart rate, +dP/dt, and QTc (≈ 10 & 25 msec) were seen with quinidine at 10 and 25 mg/kg, with additional effects at 25 mg/kg. Moxifloxacin at 50 mg/kg increased +dP/dt (15-20%), QTc (≈ 30 msec) and heart rate. The CV changes in dogs were consistent with known ion channel profiles of these agents and reproduced their clinical effects at plasma concentrations that were within the range of their therapeutic levels. Our results demonstrate that beagle dogs can exhibit a similar sensitivity as humans and contribute to a comprehensive CV safety assessment of new drug candidates.

1700 TELEMETERED RESPIRATORY EMG/INTRAPLEURAL PRESSURE VS LIFESHIRT®: MONITORING OF BRONCHOCONSTRICTION AND VOMITING IN THE DOG.

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The objective of this study was as follows: a) to investigate the feasibility of non-invasive recording of respiratory muscle activity, in conscious freely-moving dogs; b) to compare the efficacy of a telemetric versus an external recording approach; c) to validate the efficacy of both approaches by triggering bronchoconstriction and emesis. Although bronchoconstriction can be measured in the dog, it is usually done in anaesthetized and instrumented animals. In addition, direct measurement of respiratory muscular activity during emesis could circumvent the unreliable and time-consuming assessment of emesis by observation of video recordings. To achieve this, inspiration was recorded by two leads of a radio transmitter implanted into the diaphragm (Dia) or into the external intercostal (EI) muscles. Expiration was recorded by two leads implanted into the rectus abdominis (RA). The intrapleural pressure (IP) was also measured, based on the method of Murphy et al. The LifeShirt® system, an ambulatory version of respiratory inductance plethysmography, consists of a shirt with embedded sensors to measure respiratory activity. Emesis was telemetrically evidenced by a series of synchronized bursts of the DIA/EI and RA, with large variations of IP. This pattern was also accurately detected by LifeShirt®. Bronchoconstriction was telemetrically evidenced by a large recruitment of the inspiratory and expiratory components, and an increase in IP. With LifeShirt®, this reflex was mainly evidenced by a decrease in the peak to mean expiratory flow relationship, and an increase in the relative tidal volume related to a change in thoracic volume. This was associated with an increase in the lung resistance invasively measured in the anesthetized dog. This study shows that both telemetry monitoring of respiratory EMG and LifeShirt® are suitable for evaluating bronchoconstriction and emesis in the dog.

1701 MET-KINASE INHIBITION DOES NOT IMPAIR RECOVERY FROM TOXICANT INDUCED ACUTE HEPATO-RENAL INJURY IN MICE.

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Dysregulation of MET (Hepatocyte Growth Factor receptor, a cell surface receptor protein tyrosine kinase) signaling has been shown to play an important role in tumorigenesis, particularly in tumor invasion and metastasis, and is an attractive oncology therapeutic target. In addition to its established role in neoplasia, MET signaling pathways contribute to hepato-renal development and function, and in adult livers, induce mitogenesis, are cytoprotective and antifibrogenic, as well as contribute to survival functions in liver physiology. MET conditional knock-out mice exhibit impaired recovery from acute hepatocellular injury, whereas, loss of MET function is not detrimental to hepatocyte function under normal physiologic conditions. These observations suggest that a small molecule inhibitor of MET kinase may attenuate recovery from acute hepato-renal injury. We evaluated a small molecule MET kinase inhibitor, BMS-698769, in a carbon tetrachloride (CCl₄)-induced acute hepato-renal injury model in mice for its effects on recovery from toxicant-induced effects. Male Balb/C mice were given either vehicle or BMS-698769 at 100 mg/kg/day orally for 7 days, beginning 24 hours after a 50 microliters/kg i.p. dose of CCl₄. Hepatic and renal effects were monitored by serum clinical pathology and tissue histology over the 7-day period. BMS-698769 did not affect the rate or extent of recovery from CCl₄-induced hepatocellular and renal tubular toxicity when administered at a dose and plasma drug exposure that provided maximum efficacy in tumor xenograft models, and suggest a low potential for hepatic mechanism-based liabilities with the use of targeted small molecule MET kinase inhibitors.

1702 RELATIONSHIP BETWEEN DOSES THAT PRODUCE THE REINFORCING EFFECT AND GROSS BEHAVIORAL EFFECTS IN RHESUS MONKEYS - 2ND REPORT.

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The dose-response relationships of drugs that possess the reinforcing effect generally show inverted U-shaped curves when administered to animals by intravenous self-administration. Therefore, it is important to select appropriate dose-ranges to

assess the reinforcing effect of drugs. In the 66th annual scientific meeting of the College on Problems of Drug Dependence, it was reported that the most frequently self-administered doses of cocaine, pentazocine, pentobarbital, nicotine and caffeine were 1/8 to 1/125 of the respective minimal effective doses in gross behavioral observations following single administrations (Wakasa et al., 2004). In this study, the dose-response relationships of 2 different types of anesthetics, ketamine hydrochloride and thiopental sodium, were evaluated by gross behavioral observations following cumulative dosing and by the self-administration method. In the cumulative dosing procedure, the drugs were administered approximately 15 minutes apart and gross behavioral changes in monkeys were observed immediately and 10 minutes after each administration. In the intravenous self-administration procedure, monkeys were allowed to self-administer the drug solutions under a FR5 with time-out 1 minute schedule for 2 hours per day. The minimal effective doses in gross behavioral observations were 0.5 mg/kg with ketamine and 1 mg/kg with thiopental. The most frequently self-administered doses ranged from 0.06 to 0.125 mg/kg/infusion with ketamine and from 0.25 to 0.5 mg/kg/infusion with thiopental. These doses were 1/2 to 1/8 of the respective minimal effective doses in gross behavioral observations. Therefore, it is suggested that the reinforcing effect of ketamine and thiopental may be detected at doses less than the minimal effective doses by gross behavioral observations following cumulative dosing, as with cocaine, pentazocine, pentobarbital, nicotine and caffeine.

1703 PANCREATIC TOXICANTS DETECTED USING RINm5F CELLS.

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In the course of examining high doses of several novel compounds in rats, blood glucose elevations and pancreatic islet beta cell vacuolizations were noted after several days. These diabetes-inducing effects were reminiscent of those observed with cyproheptadine and structurally similar compounds—cyclizine, pizotyline, and azacylonol—in rats (Fischer et al, *Tox Appl Pharm* 26:288-298, 1973). The rat pancreatic RINm5F cell line, which makes and secretes insulin but has lost response to glucose, had proven an effective in vitro model to study cyproheptadine-induced diabetes (Miller et al, *J Biochem Toxicol.* 8:127-34, 1993), and we modified this model to screen compounds for pancreatic toxicity. Insulin content was determined via ELISA in cells and media, and cytotoxicity was measured via resazurin reduction. Since many drugs share structural similarities with cyproheptadine (particularly those with structures similar to 4-diphenylmethylpiperidine; Hintze et al, *Toxicology* 7:133-40, 1977), it was not surprising that several—including terfenadine, desloratidine, chlorpromazine, doxepine, clopenthixol, thietilperazine, thio-properazine, thiothixene—produced decreases in insulin content and release in this model. Most of these drugs produced effects only at supra-therapeutic levels, or only at near cytotoxic concentrations. Possible exceptions included several antipsychotics, many linked to type II diabetes in the clinic. For example, olanzapine (3 to 30 uM) markedly decreased insulin cellular content and insulin release from RINm5F cells after 24 hours treatment with no concurrent cytotoxicity. RINm5F cells provide a simple model for screening compounds for effects on insulin content and release, and should be an early step for structural series containing diabetogenic compounds or those structurally related to 4-diphenylmethylpiperidine.

1704 APPLICATION OF THE MICROAMES SCREEN AND MULTICASE SAR FOR ASSESSING THE GENOTOXIC AND CARCINOGENIC POTENTIALS OF THE PHARMACEUTICAL IMPURITY, DIFLUOROANILINE.

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In the pharmaceutical industry, impurities from raw materials, intermediates, degradation products, synthetic reactants, and residual solvents should be assessed for genotoxic potential. Early in drug development, isolated impurities are often of insufficient quantity to fully test for genotoxicity and thus predictive screening tools become important for risk assessment. Difluoroaniline (DFA), a model impurity, was evaluated for genotoxicity by screening in the microAmes, an assay predictive for the full plate Ames test but requiring only 10 mg when used with 5 tester strains with or without an S9 metabolic activation system. DFA was also assessed for carcinogenicity using MultiCASE computer-assisted SAR coupled with ICSAS expert system. The National Toxicology Program in 1984 reported DFA to be negative in the Ames test with rat liver S9 but positive using 30% hamster S9. To understand the human relevance of this finding, DFA was evaluated in the microAmes screen using up to 30% human liver S9. The microAmes screen demonstrated DFA to be positive with hamster S9 at concentrations down to 10% and negative with rat S9 at concentrations up to 30%. DFA results using human S9 at concentrations up to 30% were also negative. These results indicate that activation of DFA by human liver S9 is more similar to rat and unlike the hamster. These results are supported by literature data indicating that hamster induced-liver microsomes have

higher rates of arylamine N-hydroxylation (activation step) and lower rates of arylamine ring-hydroxylation (detoxication step) than rat microsomes. Using MultiCASE, the overall carcinogenicity potential of DFA via ICSAS expert system was also negative. These assessments demonstrate the utility of 1) using the microAmes screen for exploring the potential genotoxicity properties of impurities, particularly when the amount of an isolated impurity is limited; and 2) employing well validated SAR databases to evaluate impurities for their carcinogenic potential.

1705 A PROPOSAL FOR REFINEMENT OF THE METHOD OF EUTHANASIA EMPLOYED IN THE MURINE LOCAL LYMPH NODE ASSAY.

A. Robertson and D. Dreher. *Covance Laboratories Ltd., Harrogate, United Kingdom.* Sponsor: D. Everett.

In the interests of animal welfare, and for ethical and legal reasons, methods of euthanasia employed at Covance Laboratories are intended to minimise the discomfort, pain and distress caused to animals. Whether euthanasia by carbon dioxide inhalation is humane has recently been considered. Although widely used, guidance on how it can be humanely administered is lacking.

A recent meeting to consider problems associated with CO₂ euthanasia concluded that both using chambers pre-filled with CO₂ and administering rising CO₂ concentrations posed animal welfare problems. Using chambers containing a >50% concentration of CO₂ causes pain for up to 15 seconds prior to loss of consciousness. Exposure to rising CO₂ concentrations causes stressful "air hunger".

The Murine Local Lymph Node Assay is the preferred method used in the UK to determine the potential of a chemical to cause delayed contact hypersensitivity ("skin sensitisation") by indirectly determining lymphocyte proliferation at the lymph nodes draining the sites of topical application. The assay is conducted in CBA/Ca-strain mice which are subjected to euthanasia by a rising concentration of CO₂ after six days. Incorporation of intravenously-injected 3H-methyl thymidine is then measured as disintegrations per minute (DPM) and the mean DPM values are used to calculate the Stimulation Index (SI) value for each test group.

It is considered that a refinement to this assay could be effected by the use of alternative methods of euthanasia.

Additional high dose groups which were subjected to euthanasia by exposure to Halothane(TM) have been added to studies performed with known mild-to-moderate sensitising chemicals in order to establish if the method of euthanasia affects the DPM and SI values. It was not possible to determine from these studies whether the method of euthanasia had any effect on DPM and SI values.

This poster presents the results of additional work, designed to examine whether it is possible to change the method of euthanasia and produce a refinement in the method without affecting the validity of the assay.

1706 COMPARABILITY OF *IN VITRO* HEMOCOMPATIBILITY ASSAYS WITH HUMAN AND NON-HUMAN SPECIES TO SUPPORT CANDIDATE DRUG DEVELOPMENT.

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In vitro hemocompatibility assays have been utilized by pharmaceutical companies as a means to assess the hemolytic potential of candidate intravenous drugs prior to their use in humans. However, several disadvantages exist. First, a criterion for the determination of a compound as being hemolytic has not been universally accepted. Secondly, many blood compatibility assays require the use of large amounts of compound and vehicle formulation. Finally, extrapolations to in vivo are unclear. Three blood compatibility assays were compared in our experimental design: (1) The Dacie method utilizes serial dilutions of each test article with 0.9% physiological saline (2) The Salauze method uses ratios of test article to blood that mimic an intravenous injection immediately upon dosing and after distribution and (3) The Reed and Yalkowsky which differs from standard in vitro methods, analyzes the amount of hemoglobin in intact erythrocytes.

Known hemolytic (10% Ativan and 0.5% SDS) and non-hemolytic (10% Cremophor El and 10% Tween 80) co-solvents were used to evaluate the accuracy of each of the three methods tested. The co-solvents were selected based on published information regarding their hemolytic capability. Heparinized human blood, pooled rat and rabbit blood, and dog blood were analyzed. Our experimentation included a comparison of between analysts and between day performances of each assay. In order to accomplish this, we compared the 10% and 50% ratio, which was common for all methods, co-solvents and species. Results for Dacie and Salauze methods did not always lead to conclusions that were consistent with known properties of the co-solvents. The results from this evaluation demonstrated that the Reed and Yalkowsky method was a more accurate indicator of erythrocyte hemolysis using co-solvents reported as hemolytic. In addition, the Reed and Yalkowsky method demonstrated a higher level of reproducibility in all co-solvents evaluated for all species tested.

1707 CHROMOSOMAL INTEGRATION, IMMUNOGENICITY, AND TOXICITY OF REPEATED INTRAVENOUS INJECTION OF GENE THERAPEUTICS FOR X-CGD IN MICE.

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As a prerequisite for a human clinical trial, integration, immunogenicity and toxicity of gene therapeutics for X-CGD (murine bone marrow-derived stem cells transduced with MT-gp91 retrovirus), administered intravenously twice at an interval of two weeks at dose level of 5×10^7 cells/kg, was evaluated in C57BL/6 mice. The integration of gene therapeutics for X-CGD into host genome was evaluated by a polymerase chain reaction (PCR) assay in the reproductive tissues (testis, epididymis, seminal vesicle and prostate) 20 days after the final administration. Gene therapeutics for X-CGD was not detected in gonads in the treated groups indicating no potential of genomic integration. Immunogenicity test was performed by FACS analysis in order to investigate whether anti-gp91 antibodies are produced in sera which were collected 20 days after the final injection. No significant change in anti-gp91 antibody production was noted in sera from mice treated with gene therapeutics for X-CGD compared with sera from the vehicle control group. In the toxicity study, no treatment-related abnormalities were observed in clinical signs, body weight, food and water consumption, ophthalmologic examination and urinalysis while an increase in white blood cells and a slight decrease in albumin/globulin ratio were observed in the test substance-treated groups. The increase in both absolute and relative spleen weights was consistent with red pulp hyperplasia in the spleen noted in microscopic examination and it was considered to result from the accumulation of bone marrow-derived stem cells in the spleen.

1708 SAFETY PHARMACOLOGY EVALUATIONS ASSOCIATED WITH mRNA TARGET INHIBITION BY 2'-MOE ANTISENSE OLIGONUCLEOTIDES.

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Antisense oligonucleotides (ASO) continue to make great progress as therapeutic agents and being used in clinical trials in diseases as diverse as cancer, hyperlipidemia, asthma, and diabetes. Independent of the molecular target, the toxicology profiles of ASOs tend to be qualitatively similar because of the consistency in pharmacokinetic properties from one sequence to the next. To further investigate effects on major organ systems related to chemical class and those related to the inhibition of mRNA targets on critical organ functions, the rat-specific and cross-species active ASOs were studied in rats. In the current study, four different ASOs were used to evaluate the effects of antisense inhibitors on the safety pharmacology core battery including the cardiovascular, respiratory and central nervous systems. Rats (8/group) received three doses of PBS, 50, 100 or 150 mg/kg ISIS 116847 (phosphatase and tensin homolog inhibitor, PTEN), ISIS 379804 (rat-specific apolipoprotein B inhibitor, apoB), ISIS 345198 (glucocorticoid receptor inhibitor, GCCR), or ISIS 141923 (control oligonucleotide). Treatment with the ASOs to PTEN, apoB and GCCR produced a dose-dependent and statistically significant reduction in liver PTEN (~60%), apoB (~75%) and GCCR (~50%) mRNA relative to control, respectively. However, there were no effects on the general behavior, body temperature, heart rate, blood pressure, respiratory rate, tidal volume and minute volume in the rats treated with up to total 450 mg/kg/rat antisense inhibitors. In hERG assays, there were no effects on ion current by concentrations of antisense drugs up to 150 M. There was also no evidence inhibition of the expression of PTEN, apoB and GCCR was associated with effects on critical organ functions. Collectively, these safety pharmacology data suggest that these ASOs as representatives of the chemical class do not alter the function of major organ systems.

1709 TARGET IDENTIFICATION OF DRUG INDUCED MITOCHONDRIAL TOXICITY USING MONOCLONAL ANTIBODY CAPTURE OXPHOS ACTIVITY ASSAYS.

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Mitochondrial dysfunction has been shown to be a pharmacotoxicological response to a wide variety of currently-marketed therapeutics, such as certain antiviral agents, anti-diabetic and anti-lipidemic drugs, antibiotics, analgesics and certain cancer chemotherapeutics. In order to reduce attrition due to mitochondrial toxicity, HTS applicable screens should be applied early in drug discovery and pre-clinical stages (Discovery Toxicology Paradigm). No true structure-activity relationship (SAR) has been established to date which would allow to predict the ability of a compound to induce mitochondrial toxicity. Consequently, in order to prevent new

chemical entities (NCEs) from having any possible liability, a screening paradigm needs to be in place. The goal is to establish a screen which identifies the target of inhibition, is suitable to high throughput screening (HTS) and amenable to structure-activity relationship (SAR) approaches. Here we describe five HTS applicable screens for compounds that directly inhibit complex I, II, III, IV, and V of the electron transport chain (ETC). We determined intra and assay variation and tested for specificity by using classical mitochondrial inhibitors. The HTS format of the OXPHOS Complex activity assays allowed for rapid in vitro screening of 23 drugs in parallel and for easy IC₅₀ determinations. Results show direct effects of some of these drugs on OXPHOS complexes, whereas others showed no effect. Compounds were rank-ordered according to their relative toxicities to one another and to well-known inhibitors of each complex. In summary, NCEs can easily be screened on a daily basis and the identification of mechanism can greatly assist in structure-activity-relationship (SAR) approaches.

1710 IN VITRO AND IN VIVO COMPARABILITY ASSESSMENT OF A POTENTIAL SURROGATE PRECLINICAL TOXICOLOGY MODEL.

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hCBE11 is an agonist anti-Lymphotoxin-beta receptor (LTBR) monoclonal antibody developed for the treatment of solid tumors. Due to restricted species cross-reactivity, IND-enabling preclinical toxicology studies were conducted in chimpanzees. A preclinical surrogate model was evaluated for potential use in GLP chronic toxicology studies. hBHA10, another agonist monoclonal antibody to LTBR, with similar activity to hCBE11 and broader species cross-reactivity, was assessed as a potential surrogate model in the cynomolgus monkey. hBHA10 recognizes cysteine-rich domain 2 (hCBE11 recognizes domain 1) of the LTBR, and has biological activity in macaques, chimpanzees, and humans. The criteria used to assess the surrogate model focused on the pharmacological and toxicological similarities between hBHA10 and hCBE11, and also considered the technical feasibility of developing a surrogate (e.g. immunogenicity, production process). Both in vitro and in vivo assessment studies were performed comparing hBHA10 to hCBE11, which included cytokine and transcript profiling, antibody dependent cellular cytotoxicity, complement dependent cytotoxicity, pharmacokinetic comparability, tissue cross-reactivity, and in vivo cytokine induction. Using a weight of evidence approach and considering the limitations of the chimpanzee, the in vitro and in vivo assessment studies supported the potential use of hBHA10 in the cynomolgus monkey in toxicology studies.

1711 RESULTS OF LONG-TERM TOPICAL TESTS IN GÖTTINGEN MINIPIGS AND WISTAR-HAN RATS WITH TINOSORB®M.

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TINOSORB®M (Bisocetrisole) is a broad spectrum sunscreen ingredient currently under review by the US FDA for addition to the OTC Sunscreen Monograph. Presently it is used as a sunscreen active in 39 countries and has over 6 years of safe global use in consumer topical sunscreen products. Previously we reported on the lack of photo(co)carcinogenic effects in hairless mice and no areas of concern based on low bio-availability by oral dosing in shorter non-clinical studies. New results from non-clinical long-term topical dosing studies in Göttingen minipigs and Wistar-Han rats are presented. Göttingen minipigs received daily topical doses applied in vehicle without occlusion for 39 weeks at 0 (vehicle), 500, 1000 & 2000 mg/kg/d. Plasma concentrations of test article were below analytic LOQ (5 ng/ml) in all groups at all time points. Systemic toxicity did not occur as evidenced by clinical signs, clinical biochemistry, and hematology. Macroscopic observations were unremarkable; microscopic examination indicated an absence of test article related changes in all organs and tissues at the systemic level; dermal changes related to the test article did not occur at the site of topical dosing. Wistar-Han rats received lifetime (104 wks) single daily topical doses of 0 (untreated), 0 (vehicle), 100, 400, & 800 mg/kg/d, in vehicle, without occlusion. Survival and growth rates were unaffected by treatment; there were no clinical signs; clinical biochemistry parameters were similar across groups; plasma concentrations of test article did not show time or dose-related increases during the study. Histopathological findings indicated the test article did not induce neoplastic changes at the dose site and systemic non-neoplastic and neoplastic changes were not rare findings or at a frequency that was related to the test article. For dermal carcinogenicity the NOEL is 800 mg/kg/d, the highest dose tested. In conclusion, TINOSORB M is a safe UV filter ingredient that can be used to make highly photostable, broad-spectrum sunscreens.

1712 RESULTS OF LONG-TERM TOPICAL TESTS IN GÖTTINGEN MINIPIGS AND WISTAR-HAN RATS WITH TINOSORB® S.

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TINOSORB® S (Bemotrizinol) is a broad spectrum sunscreen ingredient currently under review by the US FDA for addition to the OTC Sunscreen Monograph. Presently it is used as an active sunscreen ingredient in 31 countries and has over 6 years of safe global use in topical sunscreen products. Previously we reported on the lack of photo(co)carcinogenic effects in hairless mice and on shorter non-clinical studies indicating no areas of concern based on low bio-availability by oral dosing. Here we present new results from non-clinical long-term topical dosing studies in Göttingen minipigs and Wistar-Han rats. Göttingen minipigs received daily topical doses applied in vehicle without occlusion for 39 weeks at 0 (vehicle), 250, 500, & 1250 mg/kg/d. Plasma concentrations of test article were below analytic LOQ (2 ng/ml) at all time points in each group during the study. Clinical biochemistry, hematological and urinalysis results were similar between all groups. Macroscopic and microscopic changes were not different in the dosed vs. control groups; dermal changes related to the test article did not occur at the site of topical dosing. Wistar-Han rats received lifetime (105 wks) topical applications in vehicle at single daily doses of 0 (untreated), 0 (vehicle), 100, 500, & 1000 mg/kg/d without occlusion. All animals showed good survival to study termination, growth within expected ranges; clinical chemistry, hematology, and urinalysis changes were not related to the test material; plasma concentrations of test article did not show time or dose-related increases during the study. Histopathological findings indicated the test article did not induce neoplastic changes at the dose site and systemic non-neoplastic and neoplastic changes were not rare findings or at a frequency that was related to the test article. For dermal carcinogenicity the NOEL is 1000 mg/kg/d, the highest dose tested. In conclusion, TINOSORB S is a safe UV filter ingredient that can be used to make highly photostable, broad-spectrum sunscreens.

1713 ULTRASONOGRAPHY QUANTIFICATION OF PROSTATE SIZE IN THE CYNOMOLGUS MONKEY.

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Experimental techniques permitting the *in vivo* monitoring of the size of the prostate in large laboratory animal models can be of particular value in the development of novel therapeutic approaches to the treatment of benign prostatic hypertrophy (BPH) and prostatic cancer or in the investigation of changes in prostate size related to variations in androgen levels. Measurement of prostate size may also be a useful addition to the range of available parameters for evaluating the onset of male sexual maturity. We have used ultrasonography to obtain noninvasive measurements of the prostate size in male cynomolgus monkeys. As in man, the prostate of the cynomolgus monkey is not amenable to imaging by a ventral approach and the transrectal approach must be used. The monkeys were tranquilized with ketamine and the prostate was imaged by transrectal ultrasonography, using a IOE323 intraoperative probe coupled with an Esaote Megas CVX ultrasound system. Triplicate images were recorded digitally for each lobe of the prostate and calculated values for the volume of the right lobe, left lobe and combined volume were then estimated from measurements on these images. This work was performed in compliance with GLP (Good Laboratory Practice). Using this technique we obtained an estimate of $2.24 \pm 0.78 \text{ cm}^3$ for the (combined lobes) prostate volume of sexually mature cynomolgus monkeys with a mean body weight of 5.2 kg (n=6). We were able to perform repeated measurements on animals and to monitor and quantify prostate growth over a period of twelve months. This technique should prove to be a useful addition to current possibilities for *in vivo* monitoring of large laboratory animals.

1714 VALIDATION OF A HEAD-OUT PLETHYSMOGRAPHY MODEL FOR CONSCIOUS PULMONARY FUNCTION ASSESSMENT IN THE MOUSE: EFFECTS OF ORALLY ADMINISTERED MORPHINE.

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ICH Guidelines stipulate assessment of pulmonary function for all test agents before human use. Mouse is a small, inexpensive animal that requires smaller amount of test article for testing. Specific mechanism of action or pharmacokinetic properties may also require the use of mice in pulmonary studies. Quality of data obtained from freely moving mice using whole body plethysmography has been inadequate and many times questionable. In the present study we evaluated the effects of morphine in order to validate a head-out plethysmography model for conscious animal pulmonary function studies.

Tidal Volume, Minute Volume and Respiratory Rate were acquired in mice using DSI Dataquest Open ART & PONEMAH Physiology Platform. Morphine (40 mg/kg) and vehicle (5 mL/kg) doses were administered subcutaneously to mice. Each animal was placed in the plethysmographic chamber before and 15 min, 1, 2, 3 and 4 hours after the administration of morphine or vehicle for the measurement of pulmonary parameters. Morphine-induced changes in pulmonary parameters were statistically analyzed using Two-way repeated measures ANOVA followed by a Bonferroni Multiple Comparison Test (SigmaStat, v2.03).

Vehicle (5 mL/kg) did not induce any significant effects on respiratory rate, tidal volume or minute volume in conscious mice in this study. However, morphine (40 mg/kg) produced a significant decrease (124 ± 20.67 at 1 hour, 167 ± 30.29 at 4 hour), compared to the 285 ± 8.39 breaths per minute pre-dose value in this group. Similarly the minute volume values were significantly lower (51.5 ± 7.17 at 1 hour and 68.0 ± 13.51 at 4 hours) compared to the $109.0 \pm 7.02 \text{ mL/min}$ pre-dose value. The respiratory rate and minute volume values were significantly lower compared to the respective vehicle-group values. No significant change in tidal volume was caused by morphine in these animals. Consistency with the published data on morphine-effects on pulmonary function validates the use of this system for pulmonary function studies.

1715 UTILIZATION OF A HUMAN FACIAL SENSORY DISCOMFORT ASSAY TO EVALUATE COSMETIC FORMULATIONS.

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Cosmetic products are intentionally and repeatedly applied to consumers' skin and thus require a high degree of mildness. Objective clinical evaluations such as patch testing and safety in use studies are employed to identify formulations that elicit significant dermal irritation and dermal sensitization. They rely on observable damage to the skin including erythema, edema, and dryness. However, formulations that pass these stringent tests may still evoke disagreeable and unacceptable sensations. The facial sensory assay provides a measure of the sensory perception of stinging or burning caused by a topically applied product. In this study, we report the use of a modified Frosh-Kligman protocol to evaluate facial sting across classes of skin care products and to determine the influence of the preservative system in a selection of those classes. All panelists were lactic acid sensitive Asian females. The split-face study design was conducted double-blinded so that neither the panelists nor the technicians know the identity of the products. The test formulations were applied to one side of the nasolabial region and to the cheek area. Reference formulations, with extensive use experience, were applied to the contralateral side. At intervals of 0, 2.5, 5, and 10 minutes, the panelists rated the formulations for stinging or burning sensations. Moisturizers were very mild with only a small fraction of panelists responding at all. Cleansers and toners showed slightly higher values that peaked at 2.5 minutes. Masks showed the highest scores with the maximum values coming at 5 minutes. Furthermore, there was a concentration-dependent correlation between the amount of the preservative phenoxyethanol and the stinging sensation perceived by the panelists. In additional studies in the mask product, panelists rated other preservative systems to determine mildness. A formulation with diazolidinyl urea and mixed parabens received scores similar to the unpreserved formulation control. These data suggest the importance of facial sting as a tool in formulation development and overall product assessment.

1716 APPLICATION OF FLOW CYTOMETRY TO BONE MARROW EXAMINATION IN RATS: A SIMULTANEOUS MEASUREMENT OF TOTAL NUCLEATED CELL COUNTS AND CELL DIFFERENTIALS.

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(Purpose) The bone marrow examination of drug toxicity study consists of measuring total nucleated cell counts (TNC) and differentiation of bone marrow cells. There is no method available regarding simultaneous analysis of TNC and cellular differentiation using a flow cytometry in rat bone marrow. The purpose of this study was to establish the flow-cytometric method that allows us to simultaneously measure both TNC and main cellular differentials of rat bone marrow.

(Methods) The bone marrow cells were obtained from the femur of male rats (CrI:CD(SD)), and were suspended in phosphate-buffered saline containing bovine serum. The cells were stained with CD11b/c-FITC, CD71-PE, CD3-FITC, CD45RA-PE and the anthraquinone fluorescent DNA stain (DRAQ5), and were analyzed using a flow cytometry in the presence of internal standard beads (Flow count).

(Results) The population of nucleated cells was distinguishable using DRAQ5. The TNC was calculated using the internal standard, and was well correlated with that measured by a classical method (the electrical resistance method). The nucleated

cells were further classified into myeloids and erythroids using CD11b/c-FITC and CD71-PE, respectively. In another test tube, T- and B-cells were also classified using CD3-FITC and CD45RA-PE, respectively. The ratios of cells, together with myeloid / erythroid ratio (ME), were equivalent to that obtained by a manual microscopic method as well as another flow-cytometric method (differentiation by cellular size and 2'7'-dichlorofluorescein-positive peroxidase activity). (Conclusion) The present flow cytometric method allows us to simultaneously measure not only the total nucleated cell counts but also cellular differentials of the rat bone marrow.

1717 COMPARISON OF TWO COMMON TECHNIQUES USED IN MICROAUTORADIOGRAPHY EXPERIMENTS FOLLOWING INTRAVENOUS ADMINISTRATION OF [3H]RDP58.

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Microautoradiography offers the advantage of determining cellular distribution of drug/metabolites by studying the distribution of radioactive markers by light microscopy. Histological evaluation of cellular distribution can then establish a mechanistic basis for pharmacological/toxicological activity.

Two microautoradiography approaches have evolved, as described by Appleton and Stumpf. Each approach administers a radioactive test substance [¹⁴C or ³H] to test animals, necropsy at selected times and harvesting the required frozen tissues. Subsequent sections are placed in contact with a photographic nuclear emulsion coated slide. Following exposure the samples are photographically processed and then histologically stained. Microscopically, the stained tissue morphology will be overlaid with silver grains; the latter denoting the presence of radioactivity in the adjacent tissue.

The principal difference between the two approaches is that for the Appleton method, the frozen section is placed in contact with frozen nuclear emulsion to minimise thawing and/or subsequent 'relocation' of radioactive material. The Stumpf method indicates that sections may be thaw mounted, as the thawing/drying processes are transient as the sections are prepared at 4µm or less and any relocation is deemed insignificant.

Due to known retention of RDP58 related material in the liver, this test substance was considered an ideal candidate for studying and comparing tissue distribution at the microscopic level using both the above methods.

Each animal received a single intravenous administration of [3H]RDP58, by tail vein injection and duplicate animals were necropsied and the livers harvested at 0.5 & 24 hours and 7 & 28 days post dose.

The results indicated few apparent differences when comparing autoradiographic evidence prepared by the methods of Appleton and Stumpf. The results indicate that the Stumpf method is technically easier and is recommended for use for establishing a mechanistic basis for pharmacological or toxicological activity.

1718 EVALUATION OF A NEW QUICK-CONNECT INTRAVENOUS INFUSION CATHETER/TETHER SYSTEM IN RATS.

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Intravenous infusion is used in preclinical toxicology studies to mimic intravenous administration in humans. Historically, small animal catheter/tether systems were comprised of a continuous indwelling femoral catheter with an external catheter tethered to an external cage-side syringe pump. This type of infusion system requires that animals be leashed to individual cages and maintenance solutions be administered continuously between dosing cycles to maintain catheter patency. This study evaluated two new quick-connect intravenous delivery systems in rats; the Strategic Applications and the AVA Biomedical system. These infusion systems offer greater flexibility in study design, allow disconnection from the tether system between dosing cycles, are less expensive to purchase, and require less time and labor. Two groups of twelve male and female rats each received Lactated Ringer's Solution, at 30 mL/kg/day. Animals were continuously infused for four cycles (four days each). To evaluate intermittent infusion, a subset of 3 animals/sex/group were disconnected from the system after the fourth dosing cycle, catheters were locked with a heparin solution, and patency reestablished. System assessment was based on the incidence of infusion line breaks, leaks, occlusions, and disconnections. Tolerability was based on clinical signs, body weights, food consumption, and clinical and anatomical pathology findings. Both systems allowed continuous and intermittent infusion. No system-related alterations in clinical signs, body weight or food consumption, clinical or anatomical pathology were observed. Infusion-associated findings were similar for both groups and were consistent with findings observed in studies using continuous catheter/tether systems. However, the AVA Biomedical system had the fewest number of catheter breaks and dosing interrup-

tions of the systems evaluated. Based on the results of this study, we adopted the use of the AVA Biomedical quick-connect catheter/tether system for intermittent and continuous infusion studies in rats.

1719 CONTINUOUS INTRAVENOUS INFUSION IN THE WEANLING BEAGLE DOG OF 50 DAYS OF AGE.

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Continuous intravenous infusion via an indwelling catheter is frequently used in preclinical safety evaluation studies. Previously, our laboratory has shown that continuous intravenous infusion was both feasible and well tolerated in dogs of 70 days of age. The purpose of this study was to investigate the feasibility of 28 days of continuous intravenous infusion via an indwelling femoral catheter in weanling Beagle dogs commencing at approximately 50 days of age. This age group was investigated as it could be used to evaluate products intended for use in human pediatric populations. A medical grade silastic catheter was surgically implanted under general anesthesia in the vena cava. Animals were continuously infused with saline at 1.5 mL/kg/h for 24 hours/day for 28 days using a modified jacket and tether system. Body weight gains, food intake and neurological conditions were unaffected when comparing data with control values for non-catheterized weanling dogs of the same age. Minor changes in clinical pathology were associated with the presence of a catheter.

It is concluded that, in our laboratory, continuous intravenous infusion of saline at rates of 1.5 mL/kg/h in weanling dogs approximately 50 days post partum for 28 days had no adverse effects on growth compared with non-catheterized animals. Clinical pathology changes were minor and commonly seen in catheterized animals. The experimental procedures were well tolerated and no adverse effects were observed in clinical signs, body weight or body weight gains, behavioral or neurological conditions, clinical pathology, macroscopic observations, organ weights and histopathological information of infused animals.

1720 DEVELOPMENT AND VALIDATION OF IMMUNOPHENOTYPING PROTOCOLS IN SPRAGUE DAWLEY RATS TO EVALUATE PERIPHERAL BLOOD, THYMUS AND SPLEEN LYMPHOCYTES.

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The purpose of this study was to develop procedures for immunophenotyping rat lymphocytes, and to evaluate the assay's ability to precisely measure total T lymphocytes (CD3+), T helper/Inducer lymphocytes (CD3+4+), Cytotoxic/Suppressor T lymphocytes (CD3+8+), B lymphocytes (CD3-45RA+), NK T cells (CD3+161+), and Natural Killer cell (CD3-161+) populations in peripheral blood, thymus, and spleen. Red blood cell lysis procedures and antibodies were optimized for the study. Three antibody panels CD3PE-CD4FITC-CD8PerCP-CD45APC, CD3PE-CD45RAFITC-CD45APC, and CD161aFITC-CD3PE-CD45APC were developed to evaluate the lymphocyte subsets in naive 8-10 week old Sprague-Dawley rats by flow cytometry. Results obtained for blood (47% CD3+, 31% CD3+CD4+, 17% CD3+CD8+, 38% CD3-CD45RA+, 8% CD3- CD161a+, 0.5% CD3+CD161a+), spleen (34% CD3+, 19% CD3+CD4+, 15% CD3+CD8+, 3% CD4+CD8+, 54% CD4-CD8-, 46% CD3-CD45RA+, 4%CD3- CD161a+), and thymus (77% CD3+, 6% CD4+CD8-, 6% CD4-CD8+, 0.3% CD3- CD45RA+, 87% CD4+CD8+, 2% CD4-CD8-, 0.19% CD3- CD161a+, 1% CD3+CD161a+) were similar to those reported in the literature. Evaluation of assay precision for all parameters was acceptable with a coefficient of variation less than 10% for most parameters. Results from sample stability testing showed all stained samples to be stable for at least 24 hours. The results illustrate that new immunophenotyping protocols have been developed and validated and accurately measure lymphocyte subsets in rat tissues. Considering that new FDA and CPMP guidelines may require, under certain circumstances, immunophenotyping assays for rat peripheral blood (PB), spleen, and/or thymus, these assays have application to future regulatory studies.

1721 FEASIBILITY STUDY TO CONDUCT ANTIBODY PLAQUE-FORMING CELL (AFC) ASSAY AND SPLEEN HISTOPATHOLOGY SIMULTANEOUSLY USING FISCHER 344 RATS.

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The assessment of a rodent's antibody response towards a T-cell dependent antigen via the AFC assay has served as a standard for some 50 years. The AFC response requires a single cell suspension of the spleen for proper representation of B cells, T

cells and antigen presenting cells, limiting the flexibility in conducting the AFC assay on standard toxicology studies without including additional animals exclusively for the spleen. This study investigated the ability to measure the AFC response to sheep red blood cells (SRBC) and evaluate spleen histopathology from the same spleen. Rats were immunized with a single injection of 2×10^8 SRBC via the lateral tail vein. On four consecutive days, designated rats received an ip injection of cyclophosphamide (CP) at 20 mg/kg. Spleens were harvested and three transverse sections approximately 2-mm thick were collected in 10% neutral buffered formalin for histopathology. The remaining spleen was homogenized to a single cell suspension and the AFC response evaluated. Histological changes in the spleen following immunization with SRBC alone, or in combination with CP treatment, were effectively evaluated using spleen sections. Rats immunized with SRBC had more germinal centers in the spleen than controls, whereas CP eliminated almost all germinal center formation. The remainder of the sectioned spleen from rats immunized with SRBC showed a mean response of 537 AFC/ 10^6 spleen cells as compared to 329 AFC/ 10^6 using whole spleens. Rats immunized with SRBC and treated with CP had a mean of 4 AFC/ 10^6 from sectioned spleens. A second study conducted to verify the robustness of the AFC response following spleen sectioning showed 186 AFC/ 10^6 in sectioned spleens as compared to 259 AFC/ 10^6 in whole spleens. These preliminary data suggest spleen histopathology and AFC response can be successfully performed simultaneously. This approach would allow for assessment of immune function via the AFC assay on standard toxicology studies without the need for additional animals.

1722 THE OPTIMIZATION OF AN ACUTE RAT MODEL TO EVALUATE EFFECTS ON INNATE IMMUNITY INDUCED BY ANTI-INFLAMMATORY AGENTS.

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Evaluation of cellular and humoral immunosuppression by pharmaceutical agents is routinely performed in targeted immunotoxicity studies and/or standard toxicology studies. A more thorough evaluation of suppression of the innate immune response requires infection with a pathogen to clearly demonstrate outcomes on host resistance. We have optimized an acute disease model using intranasal administration of *Streptococcus pneumoniae* to Lewis rats and validated effects on the pulmonary innate immune response using two anti-inflammatory agents, dexamethasone (Dex) and methylprednisolone (MP). Female Lewis rats (n of 15/group) were treated orally 2 hours prior to infection with 30, 100, and 200 mg/kg MP or 5, 15, and 30 mg/kg Dex and monitored 1, 4, and 24 hours post-infection for clearance of the *S. pneumoniae* from the lungs. To measure bacterial clearance, lung homogenates were prepared and plated, and colony counts performed. Cyclophosphamide (CY, 100mg/kg) was used as a positive immunomodulatory control by intraperitoneal administration 4 and 1 days prior to infection. Cyclophosphamide, all doses of DEX, and the 15 and 30 mg/kg doses of MP showed suppression of bacterial clearance at 24 hours, defining a relative immunosuppressive potency of DEX>CY>MP. In addition to our model in the Lewis rat, a similar suppression of the innate immune response can also be demonstrated using both the Fischer 344 and Sprague Dawley rat strains.

The relevance of this acute model to predict potential drug suppression of the innate immune response to *S. pneumoniae* is especially important given similarities of this rodent infection model with human pneumococcal pneumonia. Moreover, the ability to rank order the severity of innate immune suppression with multiple test articles in the same study make this model effective in screening potential drug candidates.

1723 EVALUATION OF VARIOUS KLH DOSING REGIMENS FOR THE CYNOMOLGUS MONKEY TDAR ASSAY.

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The T-cell dependent antibody response (TDAR) assay is suggested to assess the immunomodulatory effects of new drugs by several regulatory agencies. The purpose of this study was to evaluate various keyhole limpet hemocyanin (KLH) dosing regimens for use in the cynomolgus monkey TDAR assay. Monkey were administered KLH by ID, IV, IM, and SC injections at various dose levels with and without FIA. Toxicology endpoints included clinical observations, clinical pathology, necropsy and histopathologic evaluations. The kinetics of the KLH antibody responses were compared using peak titers, time to peak titer, and area under the titer-time curve. No remarkable clinical observations, body weight changes, food consumption changes, clinical pathology findings, macroscopic findings, organ weight changes, or microscopic findings were associated with a particular dosing regimen. These data suggest that satellite cynomolgus monkeys are not necessary for the monkey TDAR assays during routine toxicology testing. Some animals failed to show any KLH titers, while others exhibited quantifiable predose KLH titers. No compelling differences were observed when comparing KLH responses

via different dose routes. Administration of two KLH injections produced increased antibody responses. Increasing dose levels of KLH did not produce a consistently proportional increase in kinetic parameters. Administration of KLH with FIA did not produce a remarkable change in IgM kinetics but did produce generally higher IgG, although more variable, kinetics. In general, mean maximal IgM and IgG titer values ranged from 6 to 13 days and 12 to 26 days (respectively) across all dose groups. The results of this study can be used as guidance for implementing a monkey TDAR assay using KLH. For a particular drug development program, TDAR variables should be kept constant whenever possible. Potential sources of variation include primate species, animal source, KLH dose levels and dosing regimen, serum sampling intervals, ELISA conditions, and test article interference.

1724 HUMORAL RESPONSE TO KLH AND LYMPHOCYTE SUBSET ANALYSIS IN DOGS TREATED WITH DEXAMETHASONE.

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Regulatory immunotoxicity studies are most often performed in the rat. However, the ICH S8 guideline states that the most pertinent species should be selected based on the effects observed in the standard toxicity studies. Therefore, the dog should also be considered as a relevant species for immunotoxicity investigations. In the present study, the effects of dexamethasone given by the oral route at 0.5 mg/kg/day for 28 days was tested on the humoral response against KLH and on lymphocyte subsets in 4 male and 4 female beagle dogs. The animals were immunized with KLH on days 14 and 21. Blood samples were collected before each injection and again on day 28 to determine the primary (IgM) and secondary (IgG) responses to KLH. Lymphocyte subsets (T cells, B cells, CD4+ and CD8+ T cells) were evaluated by flow cytometry before treatment, and on days 14 and 28. Dexamethasone treatment induced a 40-50% decrease in both B and T cell counts, whilst neutrophil counts were increased 30-35%. Anti-KLH IgM and IgG levels were also markedly decreased in the dexamethasone-treated dogs. These results show that the humoral response to KLH and lymphocyte subset analysis are suitable tools for the detection of immunosuppressive compounds in the dog.

1725 DELAYED-TYPE HYPERSENSITIVITY RESPONSE IN RATS CHALLENGED WITH TETANUS TOXOID (TTX) FOR IMMUNOTOXICITY EVALUATION.

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[Purpose] Delayed-type hypersensitivity response is one of a battery of functional immunotoxicity tests. The purpose of this study was to assess TTx, an antigen used for delayed-type hypersensitivity response in rats; moreover, Cyclosporin (CY) suppresses delayed-type hypersensitivity response in rats challenged with TTx. [Method] CY at 0 (physiological saline), 3, 9 and 15 mg/kg/day was administered orally to 6 male and 6 female Crl:CD (SD) rats per group once daily for 4 weeks (Day 0 to 27). Rats were challenged with TTx at 1 Lf/site (10 Lf/mL, 0.1 mL/site) intradermally in the dorsal scapular region and intramuscularly in the femoral region, a total of 3 points (3 Lf/body) per sensitization, 7 and 14 days after oral administration of CY. Eleven days after final sensitization (25 days after CY administration), intradermal challenge with TTx at 0, 0.3, 1, 3 and 10 Lf/mL (10 µL/site), at 5 points in the dorsal scapular region was performed. Reaction at challenge sites was observed at 24, 48 and 72 hours after challenge. Blood was drawn at 7, 14, 21 and 25 days, and anti-TTx-specific IgM and IgG antibodies in sera were measured by ELISA. [Result] In the physiological saline and 3 mg/kg CY groups, edema scored as 1 or 2 was observed in all animals at 24, 48 and 72 hours after challenge. At 9 and 15 mg/kg of CY, the number of animals in which reaction was observed decreased. At 15 mg/kg of CY, no reaction was observed in any animal at 48 or 72 hours after challenge. At 9 and 15 mg/kg of CY, anti-TTx-specific IgM and IgG antibodies in sera were decreased at 21 days, and 21 and 25 days respectively compared with the physiological saline group. But the level of specific-IgM antibody was low. [Conclusion] In rats were sensitized twice with TTx at 3 Lf/body, induction of delayed-type hypersensitivity response and suppression by CY (9 mg/kg) were confirmed.

1726 VALIDATION OF IMMUNE FUNCTION TESTING DURING A 4-WEEK ORAL TOXICITY STUDY WITH CYCLOSPORIN.

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[Purpose] T cell Dependent Antibody Response (TDAR) using Keyhole Limpet Haemocyanin (KLH)-specific antibodies is regarded as one of the most predictive assay for immunotoxicity testing. The purposes of this study were to validate immunization of rats with KLH as a means of investigating the influence of drugs on

TDAR. [Method] Cyclosporin (CY) at 0 (physiological saline), 3, 9, and 15 mg/kg/day was administered orally (gavage) to 8 male and 8 female Crl:CD (SD) rats per group once daily for 4 weeks (Day 0 to 27), and influence on TDAR was investigated using KLH. KLH was administered subcutaneously (without adjuvant) once (2 mg/kg/body) on Day 10. KLH-specific antibodies (serum IgM and IgG) were determined by ELISA on Days 14, 17, 21, 24, and 28. Hematology, blood chemistry, gross pathology, and histopathological examinations were performed, and organs were weighed. Immunophenotyping (FACS analysis) in the peripheral blood, thymus, and spleen was also conducted. [Result] In CY groups, when compared with the physiological saline group, no significant changes were noted in body weight or organ weights (thymus, spleen, or adrenals), and no abnormalities were observed in gross pathology. In females in the 15 mg/kg CY group, a decrease in leukocyte count was noted at the end of the dosing period. In the CY groups, KLH-specific antibody production was suppressed dose-dependently in both sexes. [Conclusion] KLH-specific antibody production was suppressed by CY (9 and/or 15 mg/kg). Accordingly, it is thought that TDAR test using KLH is suitable for evaluation of immune system by investigational new drugs.

1727 EVALUATION OF DRUG-INDUCED ALTERATIONS IN LYMPHOID TISSUES BY OBJECT ORIENTED IMAGE ANALYSIS USING VIRTUAL IMAGING.

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This work compares the quantitation of changes in B- and T-cell populations of rat lymphoid tissue obtained using Definiens eCognition software with Nanozoomer virtual images to results obtained using laser-scanning cytometry (LSC). Male Sprague-Dawley rats received intraperitoneal injections of sterile saline (vehicle) or cyclophosphamide (6 mg/kg) for 14 days to induce immunosuppression. On day 10, rats received keyhole limpet hemocyanin (KLH) (100 µg/animal) intravenously to induce a T-cell dependent antibody response. Animals were sacrificed on day 15, and spleen and thymus organ weights were recorded. Anti-KLH IgM endpoint titers were measured using an enzyme-linked immunoabsorbent assay (ELISA). Formalin-fixed, paraffin-embedded samples of spleen, thymus and mesenteric lymph node were routinely processed and stained with hematoxylin and eosin for histologic evaluation by a pathologist and fluorescent and chromogenic staining for T-cell (CD3) and B-cell (CD79a) populations for assessment with LSC and a custom Definiens ruleset respectively. Cyclophosphamide treated rats had a mean 98% decrease in anti-KLH IgM response, decreased splenic and thymus weights (30% and 25%, respectively), and histopathological evidence of lymphoid depletion in the spleen. Neither Definiens nor LSC analysis of T-cell populations revealed significant differences in the spleen or mesenteric lymph nodes in vehicle vs. treated animals. Examination of B-cell populations revealed significant decreases in the spleen (29.5 ± 3.1 vs. 10.3 ± 1.4 by LSC and 37.9 ± 3.5 vs. 16.0 ± 1.1 by Definiens) and mesenteric lymph nodes (12.9 ± 2.7 vs. 3.8 ± 0.9 by LSC and 19.8 ± 4.1 vs. 8.6 ± 1.8 by Definiens) of treated animals. In summary, image analysis of virtual images using Definiens eCognition software can be used to quantitate T- and B-cell populations in rat lymphoid tissues and these data correlate with LSC data and serologic evidence of decreased IgM.

1728 THE IMMUNOTOXICOLOGY INTER-LABORATORY PROJECT (IILP): VALIDATION OF A T CELL-DEPENDENT ANTIBODY RESPONSE (TDAR) USING KEYHOLE LIMPET HEMOCYANIN (KLH) IN RATS.

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Aims of the IILP project are to: (1) establish a common design for a TDAR using KLH, (2) validate and test design robustness and sensitivity by inter-lab testing, (3) compare with results obtained in Plaque Forming Cell assays. Main study design, based on 4 pilot studies, included 3 treatment groups with each test compound and 2 (vehicle and a positive) control groups. Rats were treated with test compounds, cyclophosphamide (cp), azathioprine, cyclosporin A and levamisole, for 33 days and were iv immunized with KLH twice, at days 14 and 28. Serum anti-KLH-IgM/IgG titers were determined at days 19, 28 (primary KLH-IgM and IgG response, respectively) and 33 (secondary KLH-IgM and IgG response) using a standardized ELISA. Each compound was tested in the KLH assay at 4 different labs in Wistar- and at 3 different labs in SD rats and in addition once in a PFC assay.

Pilot studies indicated 300 mg KLH iv/animal provided optimal responses to cp-induced suppression. SD versus Wistar rats showed quantitatively different responses. Inter-lab comparisons showed that developed KLH specific antibody ELISAs were easily transferable with a low variability (intra-assay: <10%, inter-lab: <20%) and a high reproducibility. Initial main study results (comparison within labs) showed in general a good concordance between KLH primary IgM responses and PFC assay results. Depending on dose level immunosuppressive treatment can result in enhanced or reduced primary or secondary IgM and IgG responses. In conclusion, the developed and optimized KLH model appears to be robust with respect to inter-lab variability and reproducibility of the applied methods. Moreover intra-lab comparison shows a good concordance between primary KLH-IgM responses and PFC results.

1729 APPLICATION OF VIRTUAL MICROSCOPY AND OBJECT BASED IMAGE ANALYSIS FOR AUTOMATED DETERMINATION OF AGE-RELATED CHANGES IN THE THYMUS OF CYNOMOLGUS MACAQUES.

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There has been considerable interest in the use of expanded histopathology as a primary screen for immunotoxicity assessment. To date, this assessment has primarily relied on semi-quantitative methods. In this study, we report the use of virtual microscopy and object-based image analysis to develop an automated method for quantitative assessment of thymic morphology in a non-human primate. Histologic sections of thymus from 94 male and female cynomolgus macaques were stained with hematoxylin and eosin (H&E) and trimmed at 3 micrometers. Sections were evaluated quantitatively and semi-quantitatively, related to organ, body and brain weights and analyzed based on age (young: <3 years; adult: 3-6 years; older adult: 7-15 years). For quantitative assessment, H&E stained sections were scanned on the Nanozoomer imaging platform. Definiens eCognition custom rulesets were written to 1) identify the thymus from adjacent non-thymic stromal and lymphoid tissues; 2) recognize and determine the area of the cortex; 3) recognize and determine the area of the medulla; 4) in older adult adults, compose a ruleset to quantify thymic tissue in animals with no appreciable medullary area. Semi-quantitative assessment used visual scoring by a pathologist. No appreciable differences were noted between the two methods of assessment. There was a trend for a decrease in thymic weights and ratios with increased age that was more evident in males than females. This correlated to smaller medullary and/or cortical areas. The data presented here indicate that automated phenotypic analysis of the thymus in cynomolgus macaques using conventional staining is practical, using virtual imaging and object oriented image analysis and that these results correspond to those from a pathologist.

1730 EXTENSION OF A T-DEPENDENT ANTIBODY RESPONSE MODEL IN THE RAT FOR ENHANCED PRECLINICAL IMMUNOTOXICITY ASSESSMENTS.

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The T-dependent antibody response (TDAR) model in the rat has recently been validated with potent immunosuppressive drugs and highly practical analytical methods. However, the utility of the model to detect mild immunosuppression and/or increased sensitivity over standard immune tests (i.e. hematology and histopathology of lymphoid organs) has not been convincingly demonstrated. Here we describe a TDAR model that combines IgM and IgG antibody measurements with *ex vivo* functional parameters (e.g. immunophenotyping, cytokine profiling, and lymphocyte proliferation) for enhanced hazard identification and characterization. Male Sprague Dawley rats were administered vehicle or cyclosporine (CsA) at a low dose of 5 mg/kg/day and a maximum tolerated dose of 20 mg/kg/day by oral gavage for 4 weeks. Immunization with keyhole limpet hemocyanin (KLH) by the intravenous route on Days 14 and 24 enabled assessment of a primary IgM and IgG (Day 19 and 24, respectively) and boosted IgG (Day 29) response. Immunophenotype analysis on peripheral blood was evaluated on Days 1, 19, 24 and 29. Splenocyte function post stimulation with KLH or Pokeweed mitogen was assessed on Day 29. Administration of CsA at 20 mg/kg/day caused significant decreases in the following: anti-KLH IgM and IgG antibodies (>90%; p<0.001); peripheral blood CD3+ T cells (40 to 50%; p<0.01); splenocyte proliferation (~70%; p<0.05); and IFN-γ, IL-4, and IL-10 production (40 to 80%; p<0.05). Rats administered 5 mg/kg/day CsA had statistically significant decreases in anti-KLH IgG antibodies (~80%; p<0.001) and CD3+ T cells (15 to 30%; p<0.05) but not anti-KLH IgM antibodies. Thus, the IgG parameter combined with immunophenotype analysis in the described TDAR model demonstrated greater sensitivity in identify-

ing mild immunosuppression caused by the low dose of CsA. *Ex vivo* functional parameters aided in the interpretation of the biological significance of immunotoxic effects observed in this model.

1731 HOST RESISTANCE STUDIES FOR COMPLIANCE WITH THE ICH-8 IMMUNOTOXICOLOGY GUIDELINE.

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As per the ICHS8 guideline "Immunotoxicity Studies for Human Pharmaceuticals", the evaluation of potential adverse effects of new drugs on the immune system should be incorporated into standard drug development. Host resistance assays that examine possible adverse effects due to immunosuppression in appropriate models may also be required for compliance with Regulatory Agency guidelines. Several pathogens may be used to demonstrate these effects, such as bacteria, viruses, fungi and tumor cell lines. In our laboratory, we have used several strains of mice to demonstrate deleterious effects of agents such as cyclophosphamide on the immune system. Intraperitoneal injection of 80 mg/kg cyclophosphamide for three days prior to infection in Balb/c mice with *Listeria monocytogenes* resulted in mortality, decreased body temperature and recovery of bacteria from the liver for surviving animals at necropsy. Similar results were seen in the same strain of animals following dosing with *Candida albicans* with the organism recovered from the spleen at termination. C57BL/6 mice were also treated with a B16 melanoma cell and examined for lung metastases 14 days later. Various immunostimulatory herb extracts were shown to reduce the lung colony counts by up to 40% compared with those seen in control animals. Overall, this work was considered to demonstrate the utility of host resistance assays in providing an overall assessment of immune status in relation to an animal's ability to combat an infection or tumor. These types of study are valuable as follow-on investigations to support mechanistic immunotoxicology, particularly if an effect on specific cell types has been shown for a test compound. In summary, although these studies demonstrated the versatility and utility of infectious animal models in assessment of immunological status, it should be remembered that translation to human risk assessment is more complicated, particularly due to difficulties in determining realistic infectious doses in humans.

1732 VALIDATION OF THE KLH-ASSAY AS ALTERNATIVE TO THE PFC-ASSAY: A COMPARATIVE STUDY WITH CYCLOPHOSPHAMIDE.

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For immunotoxicity testing the IgM-Plaques Forming Cell (PFC) assay using Sheep Red Blood Cells (SRBC) as antigen is regarded to be the most predictive functional assay. Because various drawbacks of this assay alternative tests like the KLH-assay, using Keyhole Limpet Hemocyanin (KLH) as antigen, are being developed. A common design for a KLH-assay was established in an Immunotoxicity Inter-Laboratory Project (IILP), using well known immunosuppressive compounds. Using cyclophosphamide (CY) the robustness and sensitivity of the KLH-assay was determined and compared with the PFC-assay. In both the KLH- and PFC study Wistar rats were exposed to 1, 3 and 10 mg cyclophosphamide/kg bw by gavage for respectively 33 and 28 days. Primary- and secondary immunization with KLH (300 µg i.v.) was performed on days 14 and 28. The KLH-specific IgM and IgG antibody responses were determined in blood at days 19, 28 and 33. In addition effects on body weight, hematology, lymphoid-organ weight and -pathology are determined. Additional control and top dose groups were included which were not immunized with KLH to assess if KLH immunization will affect routine immune histopathology. In the PFC-assay immunization with SRBC was performed on day 24 and the number of IgM-PFC in the spleen was assessed on day 28. The primary and secondary anti KLH-IgM titers were dose-dependently decreased at day 19 (significantly decreased at the 2 top dose levels) and day 33 (significantly decreased at all dose levels), respectively. At day 28 the primary anti KLH-IgG titers were still low and only significantly reduced at the top dose of CY. The secondary anti KLH-IgG titers were significantly decreased at the two top doses of CY. In the PFC-assay the number of IgM-PFC in the spleen was dose-dependently decreased which was significant at the two top doses of CY. In conclusion it was evident that the KLH and PFC-assay were equally sensitive, and that the histopathology of the lymphoid organs was not affected by KLH immunization.

1733 BONE MARROW BIOPSY IN NON-HUMAN PRIMATES: COLLECTION AND EVALUATION IN RHESUS MONKEYS.

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Examination and quantification of bone marrow cells is a valuable tool for assessment of toxicological or pharmacological effects of certain drugs, whose intended (or unintended) actions target the bone marrow. Non-terminal, serial bone marrow

collection can be used to establish a time-course of effect, and to thereby gain a better understanding of a compound's effect on progenitor cells. Our laboratory established methods to serially collect, process and evaluate bone marrow. Baseline from forty (40) untreated Rhesus monkeys is presented. Prior to collection, animals were anesthetized with an injectable. The right or left shoulder and the right or left hip were shaved and aseptically prepared. A spinal needle (Terumo, 20G, 3 1/2 inches) was inserted in the medulla of the right or left humerus and right or left femur, through the proximal epiphysis. Serial bone marrow collections were performed on each animal by alternating between left and right sides. On each biopsy occasion, a first sample of (circa 1 mL) was collected in EDTA for smear preparation while a second sample (circa 1 mL) was collected into a syringe containing circa 2 mL of culture media (RPMI1640, penicillin 50U/mL, streptomycin 50 µg/mL, 100 U/mL of DNase-1 and 90 IU/mL of preservative-free sodium heparin). Syringes with culture media were weighed pre (including the culture media) and post bone marrow collection. Samples were then transferred to sterile collection media container(s). One (1) tube was analyzed by flow cytometry after staining with CD3/CD71/CD34/CD41a antibodies. The second tube was used for automated counts of nucleated cells using a hematology analyzer. Anticoagulated bone marrow samples (unfiltered), protected against environmental temperature changes, were used for clonogenic assays (GM-CFC, CFU-E, BFU-E, MK-CFC, F-CFC). The bone marrow progenitor cells were characterized and normal values are presented. Significant inter-individual variability was noted. Serial evaluations is recommended to allow comparison of post treatment results with baseline values.

1734 EVALUATION OF THE IMMUNE SYSTEM IN RATS AND MICE ADMINISTERED AMMONIUM PERFLUOROOCTANOATE (APFO).

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Few studies have evaluated immune system function following repeated exposure to APFO. Male CD rats and CD-1 mice were given doses by oral gavage ranging from 0.3 to 30 mg/kg of linear APFO for 28 days. Anti-sheep red blood cell (sRBC) IgM levels, clinical signs, body weights, food consumption, selected hematology and lipid parameters, liver weights, spleen and thymus weights and cell number, selected histopathology and serum corticosterone concentrations were evaluated. An additional group of animals was dosed with 30 mg/kg APFO for 22 (rat) or 23 (mouse) days and, following sRBC immunization, with vehicle for the remaining days prior to sacrifice on day 29. In rats no suppression of the ability to make anti-sheep RBC antibody was observed at any dose. Ten and 30 mg/kg resulted in systemic toxicity as evidenced by decreases in body weight gain (26 and 63% of control, respectively) and increases in serum corticosterone levels to 135 and 196% of control, respectively. In mice exposed to 0.3 or 1 mg/kg, no significant suppression of the ability to make anti-sheep RBC antibody was observed. At 10 and 30 mg/kg APFO, marked general toxicity and stress was observed, as evidenced by a loss in body weight of 3.8 and 6.6 g, respectively (despite a tripling of liver weight), ~230% increase in serum corticosterone, and increases in absolute numbers of blood neutrophils and monocytes with an accompanying decrease in absolute lymphocyte numbers. A number of immune-related findings occurred at 10 and 30 mg/kg that likely represent secondary responses to the systemic toxicity and stress observed at these doses: decreased IgM antibody at 10 (20% suppression) and 30 mg/kg (28% suppression); decreased spleen and thymus weights and cell numbers; microscopic depletion/atrophy of lymphoid tissue starting at 10 mg/kg in the thymus and 30 mg/kg in the spleen. In summary, no immune-related changes occurred in rats, even at doses causing significant systemic toxicity. In mice, immune-related changes occurred only at doses causing significant systemic toxicity and stress.

1735 EVALUATION OF EFFECTS OF INTRAVENOUS AND SUBCUTANEOUS ADMINISTRATION OF A CYNOMOLGUS MONKEY ANTI-IL-15 ANTIBODY IN THE CONTEXT OF REPEATED DOSE SAFETY EVALUATION.

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Inhibition of interleukin-15 (IL-15) is currently being evaluated for treatment of inflammatory disease. Effects of intravenous and subcutaneous administration of a cynomolgus monkey IL-15 antibody (cyIL-15 Ab) were evaluated in the context of two safety studies of 1- and 3-months duration. The purpose of these studies was to determine potential toxicity, toxicokinetics (TK), and to explore potential biomarkers of a cyIL-15 Ab, when administered weekly for 5 weeks by intravenous (IV) or subcutaneous (SC) injection or for 3 months by SC injection. In addition, recovery from test article-related effects was evaluated following a treatment-free period. For the 1-month study, five groups of monkeys (5/sex/group) were treated once weekly for five weeks at 0, 30, 60, or 150 mg/kg IV or 150 mg/kg SC. For the 3-month study, five groups of monkeys (6/sex/group) were treated once weekly for 3 months

at 0, 5, 30, or 150 mg/kg SC. Parameters evaluated included mortality, clinical signs, body weights, ECGs, ophthalmic examinations, clinical pathology, immunophenotyping, natural killer cell (NKC) activity, organ weights, gross and microscopic pathology, TK, and antibody analysis. For both studies, all animals survived to scheduled necropsy with no test article-related effects on body weights, ECGs, ophthalmic examinations, organ weights, gross pathology, or histopathology. In the 3-month study, a few animals in the test article-treated groups manifested gastrointestinal findings associated with *Shigella* infection. Test article administration produced a marked reduction in individual absolute and relative NKC values as early as one week after the first dose; this change was associated with a concomitant decrease in NKC activity. Based on these results, weekly IV or SC treatment with cIL-15 antibody for up to 3 months in cynomolgus monkeys was fairly well tolerated, and pharmacodynamic activity of IL-15 inhibition (i.e., NKC depletion) was observed.

1736 ORAL EXPOSURE TO DISULFOTON FOR 28 DAYS BY GAVAGE PRODUCED MINIMAL IMMUNOMODULATION IN FEMALE B6C3F1 MICE.

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Disulfoton (DST), O,O-diethyl S-[2-(ethylthio)ethyl] phosphorodithioate, is a selective, systemic organophosphate insecticide and acaricide that is effective against multiple insects. It is used to control aphids, leafhoppers, thrips, beet flies, spider mites, and coffee leaf miners. Disulfoton products are used on a wide variety of food and nonfood crops with the highest use on cotton and wheat. DST has been found to bioaccumulate in fish and other organisms. DST inhibits cholinesterase, and cholinergic muscarinic receptors have been identified on lymphocytes. In the present study, DST was administered to female B6C3F1 mice daily for 28 days by gavage at five dose levels (0.01, 0.05, 0.1, 0.5 and 1 mg/kg) in order to determine the potential effects of DST on the immune system. There were no significant changes in the terminal body weight and weights of liver, lungs, kidneys, spleen and thymus. Blood was collected from the animals by the retro-orbital route under carbon dioxide anesthesia on day 29 for hematological evaluation. The erythrocyte count, hemoglobin, hematocrit, MCV, MCH, MCHC, %reticulocytes and platelet counts were unaffected after exposure to DST. Although an increase in total leukocyte count (primarily lymphocytes and neutrophils) was observed in the 0.05 and 0.5 mg/kg-dose groups, the increase was not dose-dependent. Flow cytometric analysis suggested that a decrease in the percentage of splenic B cells at the dose level of 0.1 mg/kg; however, the absolute value was not significantly changed. Five functional assays including the spleen IgM antibody-forming cell response, spleen cell proliferative response to anti-CD3 stimulation, natural killer cell activity assay, and spleen mixed leukocyte response and cytotoxic T cell activity assay were performed. There were no biologically meaningful changes associated with any of the assays. Taken together, oral exposure to disulfoton for 28 days by gavage produced minimal immunomodulation in female B6C3F1 mice (Supported by NO1-ES-05454).

1737 LOVASTATIN, THE CHOLESTEROL LOWERING DRUG, DOES NOT ALTER IMMUNE RESPONSES IN FEMALE B6C3F1 MICE.

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The statins are a class of compounds shown to be highly effective in lowering serum cholesterol levels, particularly when changes in diet and exercise prove ineffective. World wide, millions of people take one of the statin drugs, on a daily basis, in order to lower their cholesterol levels. In addition to their ability to decrease cholesterol levels, the statins have been reported to have anti-inflammatory activity which may contribute to their ability to help in reversing coronary artery disease as well as the possible prevention of heart attacks and strokes. Lovastatin (LOV) was selected, as a representative statin drug, for evaluation of its ability to modulate the immune response. Female B6C3F1 mice were administered LOV once daily for 28 days by oral gavage in a 0.5% methylcellulose vehicle. Dose levels consisted of 0.5, 2, 5, 20, and 50 mg/kg/day. On day 29, animals were evaluated for toxicological and immunological effects. In animals receiving LOV, no significant differences were observed in terminal body weights or selected organ weights, including liver, spleen, lung, thymus and kidneys at any dose level as compared to the vehicle controls. Similarly, hematological parameters and the splenic differential, as determined by flow cytometry, were unaffected. No effect was observed in functional assays used to measure humoral immunity including the antibody forming cell response to sRBC. Cell-mediated immune responses, including the MLR, CTL, and anti-CD3 mediated proliferation were also unaffected following LOV administration. In the parameters used to evaluate innate immunity, including NK cell activity and the functional activity of the mononuclear phagocyte system (MPS) there were no significant differences observed between vehicle control animals and the animals treated with any of the LOV doses. In summary, treatment with LOV for 28 days at dose levels up to 50 mg/kg/day did not result in modulation of the immune response in B6C3F1 mice. (Supported by NIEHS NO1-ES-05454)

1738 INHIBITION OF THE MURINE ALPHA 1 BETA 1 INTEGRIN DOES NOT AFFECT THE PRIMARY IMMUNE RESPONSE TO INFLUENZA IN BALB/C MICE.

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Preclinical development of a monoclonal antibody targeting inhibition of the alpha 1 beta 1 integrin, a member of the integrin family of cell surface molecules involved in cell-cell/ cell-matrix adhesion and communication, indicated the potential for the recrudescence of a plasmodium infection in non-human primates. Therefore, immunocompetence was tested in mice administered a hamster anti-mouse alpha 1 beta 1 integrin monoclonal antibody (HA31/8) using an influenza challenge, considered the 'gold standard' for identifying an immunotoxic effect. This model consisted of a main phase to measure viral clearance, influenza-specific IgG, cytokines, and spleen natural killer (NK) cell and cytotoxic T lymphocyte (CTL) activity, and satellite phases to measure lung NK, lung CTL, and macrophage function in the spleen and lung. Female Balb/c mice (n=50) were administered phosphate buffered saline (PBS), 0.3, 3 or 30 mg/kg mHA31/8, or 30 mg/kg P1.17 (a negative control antibody) twice weekly via intraperitoneal (IP) injection prior to viral influenza infection (Days -7 to -1) and up to 3 weeks after viral influenza infection (Days 1 to 21). Dexamethasone, a positive immunomodulatory control article, was administered (n=50) at 20 mg/kg/day orally, from Day -3 through Day 21. Animals were infected (on Day 0) with mouse-adapted influenza virus (MAIV). Necropsies for the main study groups were conducted on Days 0 (naïve animals only), 2, 6, 8, 10, and 21. Satellite phase mice were necropsied on Day 2 (lung NK and macrophage function) or Day 8 (lung CTL). Treatment with mHA31/8 did not affect mortality or spleen weight, caused only minor changes in terminal body weight, and increased lung weight. Although there were variable effects of the test article on certain cytokine levels, mHA31/8 at doses up to 30 mg/kg did not affect influenza-specific IgG levels or the clearance of influenza and was not considered to be immunotoxic.

1739 QUANTITATIVE EVALUATION OF THE TIME COURSE OF EFFECTS OF ACUTE ETHANOL ON POLY I:C INDUCED PRODUCTION OF CYTOKINES, CHEMOKINES, AND CORTICOSTERONE.

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Previous studies in our lab suggested a role for an ethanol-mediated stress response as a feedback mechanism in the suppressed production of one pro-inflammatory cytokine (IL-6) and the enhanced production of an anti-inflammatory cytokine (IL-10). However, these effects were only measured at one time point, so the overall effects of ethanol were not clear. The purpose of the present study was to measure both corticosterone and serum cytokine and chemokine concentrations over time, to allow calculation of the area under the concentration vs. time curves and quantitative comparisons of changes in corticosterone and changes in cytokine and chemokine production. Groups of mice (5 per group) were given no treatment (naïve), poly I:C (100 µg/mouse, I.V.), ethanol (6g/kg, oral gavage), or both, and blood samples were analyzed for corticosterone (by RIA) and cytokines and chemokines (by multiplexed bead array assay). Blood samples were taken 1, 2, 4, 6, 8, and 10 hours after administration of poly I:C and/or ethanol. Statistical analysis of the corticosterone AUCs indicate a significant increase in the poly I:C + ethanol groups versus the poly I:C or ethanol groups. Cytokine and chemokine concentrations and AUC values were calculated, and poly I:C induced readily measurable quantities of IL-1 beta, IL-12 (p40), IL-12 (p70), IL-6, IL-10, TNF-alpha, KC, CCL3, and CCL4. Concentrations of IFN-gamma and GM-CSF were near the lower limits of detection. In general, ethanol decreased the response of pro-inflammatory cytokines to stimulation by poly I:C, but increased the production of the anti-inflammatory cytokine IL-10. The time course of changes was unique for each cytokine/chemokine. Further studies will be needed to determine which of these alcohol induced effects are due to the changes in corticosterone induced by the stress response, but AUC values indicated that changes in some cytokines caused by ethanol were much greater than the slight increase in corticosterone AUC. This work was supported by NIH grant AA009505.

1740 IMMUNOTOXICITY EVALUATION OF METHOTREXATE IN RATS BY ORAL ADMINISTRATION.

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Methotrexate (MTX), a folate antagonist, is widely used in the treatment of rheumatoid arthritis. Although it is a well-known immunosuppressant, its immunotoxicological profile has not been much reported. In this study, we evaluated

several immunological parameters in rats treated with MTX. MTX was intermittently given orally to male and female Crl:CD(SD) rats for 2 weeks (3 times weekly at dose levels of 0, 1, 2 or 5 mg/kg/day). The following evaluations were conducted: T-cell dependent antibody response (TDAR), serum immunoglobulin levels, lymphocyte subset analysis (on the peripheral blood, thymus, spleen and mesenteric lymph nodes), mitogen (PHA, ConA, LPS)-induced proliferation and cytokine induction on splenocytes, hematology and pathology. In the TDAR, anti- Σ RBC IgM antibody production obviously decreased. In addition, slight decreases in IgA, IgE, IgG and IgM were observed. The lymphocyte subset analysis revealed no significant changes in lymphocyte phenotypes in the peripheral blood, thymus, spleen and mesenteric lymph nodes. In the T-cell- or B-cell- mitogen response in the splenocytes, no significant changes were observed in splenocyte proliferation or in humoral immune response related cytokines (IL-4 etc.), although inflammatory cytokine production (TNF- α etc.) decreased in the highest dose group. The hematological and histopathological changes associated with the immune system were not observed in the peripheral blood, thymus, spleen and mesenteric lymph nodes. This study demonstrated that MTX decreased the humoral immune function that is complicatedly associated with several kinds of immune system cells (antigen presenting cells, T cells, B cells etc.), but it did not change the population, proliferation and response related cytokine induction potency of T-cells or B-cells. It seems likely that the immunosuppressive effect of MTX is mainly involved in inhibition to the other immune cells rather than to T-cells or B-cells. As well, the results of the analysis including other immune cells will be presented in this study.

1741 INVESTIGATING IMMUNE RESPONSES IN FEMALE BROWN NORWAY RATS GAVAGED DAILY WITH NEVIRAPINE FOR A WEEK.

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Nevirapine, used for the treatment of HIV infections, is associated with a significant incidence of skin rash and liver toxicity. Formerly the discovery of an animal model of nevirapine-induced skin rash in female Brown Norway rats fed with 150 mg/kg/day drug mixed in their diet has been reported, with rats developing red ears on day 7 and skin rash on day 21 of the treatment. In the current study we aimed at establishing and utilizing a new model of nevirapine-induced skin rash by gavaging and not feeding rats with the drug. We gavaged rats with either a control (0.5% methylcellulose) or commercially available nevirapine solution (75 mg/kg/day) twice a day for a week. During the treatment we regularly checked nevirapine serum concentration levels. At the end of the treatment we performed ear and liver tissue pathologies, and we analyzed rat sera, lymph nodes and splenocytes for immunological changes. Our data showed that in the rats gavaged with nevirapine drug blood concentration reached 45 μ g/ml by day three of the treatment and it subsequently subsided to 11 μ g/ml by day 7. No red ears were observed at day 7 in the nevirapine-treated rats; however, increased serum IL1 α , IL2, IL6 and IL10 levels were detected at this time point. Organ analyses showed increased liver and auricular lymph node weights as well as nodal cellularity (predominantly in T, B and macrophage cell populations) in the nevirapine-treated rats. Pathology analysis showed diffuse moderate acute inflammation in nevirapine-treated rats, characterized by prominent vessels with intravascular inflammatory cells (eosinophils) and mixed dermal infiltrate composed of polymorphonuclear and mononuclear cells. Results from our study indicate that: a) rats gavaged or fed nevirapine-diet show comparable immune response to the drug, b) gavaging rats twice per day with 75 mg/kg nevirapine induces similar immunological responses as does feeding them with 150 mg/kg and c) rats from different breeders show similar immune responses to nevirapine treatment.

1742 IMMUNOTOXICOLOGICAL ACTIVITY OF INDOOR AIR PARTICLES AND BIOAEROSOLS BEFORE AND AFTER RENOVATION OF MOISTURE DAMAGED BUILDING.

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Many of the building-related health problems coincide with moisture damage and mould growth within the building. This has been shown both as increased incidence of adverse health effects in moisture damaged buildings, and as a decrease in prevalence of symptoms after a thorough renovation. The aim of this study was to follow the success of repairs in two cases by analyzing the immunotoxicological activity of filter samples collected from 1) indoor air of moisture damaged building before and after renovation, 2) reference buildings and 3) outdoor air. The effects of collected bioaerosols were tested by exposing mouse macrophages (RAW264.7) for 24 hours to particle suspension extracted from the filters. Viability of the exposed cells (MTT-test) and production of inflammatory mediators (nitric oxide, IL-6 and TNF α) in cell culture medium were measured by ELISA immunoassay.

The samples collected before renovation on Case 1 were more toxic and induced higher production of inflammatory mediators in mouse macrophages compared to samples collected after the renovation. Samples from outdoor air and reference building had only minor effect, both before- and after the renovation. Instead, in Case 2 the renovation measures did not cause any evident change in before and after-samples, whereas the samples collected from the reference building induced more intense responses after renovation. The samples collected from outdoor air in Case 2 caused strikingly similar responses compared to indoor air samples. In conclusion, in the Case 1 the decrease in immunotoxicological activity of the indoor air particles coincided with the repairs. In the Case 2, the inflammogenic and toxic properties of the indoor air particles seemed to reflect the situation in outdoor air rather than changes in the indoor air. This indicates, that the adverse health effects caused by exposure to moisture and mold damaged houses are not necessarily of the same origin.

1743 KIOM-79 INHIBITS LPS-INDUCED P65 NUCLEAR TRANSLOCATION.

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We demonstrate that KIOM-79, combined extracts obtained from *Magnolia officinalis*, *Pueraria lobata*, *Glycyrrhiza uralensis*, and *Euphorbia pekinensis*, inhibits LPS-induced expression of iNOS gene in RAW 264.7 cells. Treatment of RAW 264.7 cells with KIOM-79 inhibited LPS-stimulated nitric oxide production in a dose-related manner. Immunohisto-chemical staining of iNOS and RT-PCR analysis showed that the decrease of NO was due to the inhibition of iNOS gene expression. Immunostaining of p65, EMSA, and reporter gene assay showed that KIOM-79 inhibited NF- κ /Rel nuclear translocation, DNA binding, and transcriptional activation, respectively. Western immunoblot analysis of p38 kinase showed KIOM-79 significantly inhibited the phosphorylation of p38 kinase which is important in the regulation of iNOS gene expression. Collectively, this series of experiments indicates that KIOM inhibits iNOS gene expression by blocking NF- κ /Rel and p38 kinase signaling. Due to the critical role that NO release plays in mediating inflammatory responses, the inhibitory effects of KIOM-79 on iNOS suggest that KIOM-79 may represent a useful anti-inflammatory agent.

1744 INHIBITION OF INOS EXPRESSION BY RADICICOL IN CYTOKINE-STIMULATED PANCREATIC BETA CELLS.

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In the present study, we show that radicicol, a fungal antibiotic, produces a marked inhibition of inducible nitric oxide synthase (iNOS) transcription by the pancreatic beta cell line MIN6N-8a in response to cytokine mixture (CM: TNF- α , IFN- γ , IL-1 β). Treatment of MIN6N-8a with radicicol inhibited CM-stimulated nuclear factor- κ B/Rel (NF- κ B/Rel), which plays a critical role in the iNOS transcription, in a dose-related manner. Our data also show that the extracellular signal-regulated protein kinase-1 and 2 (ERK1/2) pathway is specifically involved in CM-induced NF- κ B/Rel activation and iNOS expression because NF- κ B/Rel DNA binding and iNOS mRNA production in the presence of a specific inhibitor of ERK1/2 pathway, PD98059, were dramatically diminished. In contrast, SB203580, a specific inhibitor of p38 had no effect on NF- κ B/Rel activation and iNOS expression. Collectively, this series of experiments indicates that radicicol inhibits iNOS gene expression by blocking ERK1/2 signaling. Due to the critical role that NO release plays in mediating destruction of pancreatic beta cells, the inhibitory effects of radicicol on iNOS suggest that this potent anti-fungal agent may represent a useful anti-diabetic agent.

1745 PANAX NOTOGINSENG ATTENUATES DENDRITIC CELL FUNCTION THROUGH INHIBITION OF NF- κ B ACTIVATION.

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The transcription factor NF- κ B is involved in the regulation of many genes in the immune system including inflammatory cytokines. Agents that modulate NF- κ B activation are actively sought after and have great potential as novel treatments of inflammatory diseases. In this study, the natural product *Panax notoginseng* was

evaluated for its immunomodulatory effects on murine bone marrow-derived dendritic cells (BMDC). BMDC were stimulated with 1 μ g/ml LPS and concurrently treated with notoginseng for 24 hours at varying concentrations ranging from 0-200 μ g/ml. Notoginseng inhibited the production of the inflammatory cytokines TNF- α , IL-6 and IL-12. The expression of cell surface molecules required for the productive interaction of dendritic cells (DC) and T cells were also modulated by notoginseng. CD40 expression was decreased, while MHC class II and CD86 were unperturbed unless BMDC were pretreated with notoginseng. DC had reduced ability to phagocytose FITC-conjugated ovalbumin antigen, with variable effects on antigen-specific activation of CD4+ T cells following notoginseng treatment. Since these processes can be influenced by the transcription factor NF- κ B, the effects of notoginseng on its activation were examined. Notoginseng reduced the LPS-induced activation of NF- κ B in BMDC. Taken together, these results demonstrate that notoginseng can reduce DC activation and function presumably by altering NF- κ B activity. Supported by NIH grant RR17670 and NSF grant EPS0091995.

1746 FLAVONOID GALANGIN, BUT NOT CATECHIN OR TAXFOLIN INDUCES MITOCHONDRIAL DYSFUNCTION IN HEPG2 CELL LINE AND LEADS TO CELL DEATH.

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Flavonoids are benzo- γ -pyrone derivatives widely distributed in the vegetable kingdom, possessing well-recognized antioxidant properties. Their fundamental structure comprises two benzene rings linked through a heterocyclic pyran or pyrone ring. The structural features favouring the antioxidant activity of these compounds are well known, however there are few studies on their toxicological potential. Objective: The study addressed aspects of mitochondrial energetics of HepG2 cells exposed to the flavonoids Galangin, Taxfolin and Catechin in context of the mitochondrial mechanisms potentially involved in the cell death. Methods: Cell viability was determined by the LIVE/DEAD assay by flow cytometry. Adenine nucleotides content was quantified by HPLC. Caspases -9 and -3 activities was quantified by measuring the cleavage of colorimetric peptides Ac-LEHD-pNA and Ac-DEVD-pNA. Apoptosis was quantified by determining the percentage of cells with nuclear morphologic changes of apoptosis detected by fluorescence microscopy of Hoechst 33342-stained fixed cells. Results: When Galangin was tested in HepG2 cells it showed a decrease in cell viability as a consequence of a decrease in the mitochondrial membrane potential and ATP levels. Catechin and Taxfolin however, did not show an effect on the mitochondria energetic process. An increase in the activity of caspases 3 and 9 exposed to Galangin was also observed, and again no effect was observed for the other two flavonoids. On the other hand, there was no statistical difference for cells with nuclear morphologic changes of apoptosis detected by fluorescence microscopy amongst all three flavonoids. Conclusions: These results suggest that galangin is the only one of the tested flavonoids that present a toxicological potential, and that changes in the mitochondrial energetics is involved in its effects, possibly leading to cell necrosis and/or apoptosis. (Financial support: Capes, FAPESP)

1747 INDUCTION OF HEME OXYGENASE-1 BY GINSENOSE RB1 INVOLVES THE ACTIVATION OF THE NRF2/ANTIOXIDANT RESPONSE ELEMENT PATHWAY.

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Ginsenosides are the most important component isolated from ginseng. Recently, most effects of Panax ginseng have been attributed to its antioxidant action. Heme oxygenase-1 (HO-1) is known as a stress-inducible protein and functions as an antioxidant enzyme. It has been shown that HO-1 is induced rapidly by a variety of chemical and physical stimuli. However, little is known about the HO-1 induction by ginsenoside and its cellular signaling events. In this work, we assessed the ability of ginsenoside Rb1 (Rb1) to up-regulate HO-1 gene expression via activation of NF-E2-related factor 2 (Nrf2) in cultured mouse Hepa-1c1c7 cells. HO-1 enzyme activity and protein expression in the cells were significantly increased by Rb1. Transfection studies using a human HO-1 ARE/EpRE reporter construct demonstrated that Rb1 activated the ARE/EpRE. Furthermore, Rb1 induced nuclear localization of the Nrf2 transcription factor. Taken together, these results demonstrate that Rb1 induced HO-1 expression through the activation of the Nrf2/ARE pathway.

1748 HEPATOPROTECTIVE POTENTIAL OF HEMIDESMUS INDICUS: ETHNOPHARMACOLOGICAL APPROACH.

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Since ancient times, plants have been an exemplary source of medicine. Various indigenous plants are known to play a vital role in the management of liver diseases but the perusal of literature reveals lack of scientific authenticity of the traditional medicine. Hemidesmus indicus, popularly known as "Anantamul" grows abundantly in most parts of the Asia. The ethanolic extract (50% v/v) of the roots of H. indicus was subjected against carbon tetrachloride (0.6 ml/kg i.p.) induced hepatic damage in Sprague dawley rats. The extract when administered to mice as a single i.p dose of 1000 mg/kg was found to be non toxic. The concentrations of AST (aspartate transferase), ALT (alanine transferase), ALP (alkaline phosphatase), total bilirubin in serum and malondialdehyde contents in liver were significantly elevated in carbon tetrachloride induced rats. Pre treatment with the extract of H. indicus roots (200 and 400 mg/kg x 7 days p.o) significantly inhibited (P<0.01) AST (aspartate transferase), ALT (alanine transferase), ALP (alkaline phosphatase) and total bilirubin levels. The liver malondialdehyde content in carbon tetrachloride rats treated with the extract was also significantly (P<0.05) inhibited. The effect of the reference drug (silymarin) on AST, ALT, ALP, total bilirubin and malondialdehyde levels were nearly similar to that of extract treated at 400 mg/kg dose. Histopathological examinations of livers challenged with carbon tetrachloride showed centrilobular necrosis, ballooning degeneration, inflammatory infiltration of lymphocytes and fatty changes. The liver sections of rats treated with the extract against CCl4 challenge showed well preserved architecture. In conclusion, the present study states that the roots of Hemidesmus indicus possess fairly good hepatoprotective activity.

1749 PROTECTIVE EFFECTS OF 3-CAFFEYOYL-4-DIHYDROCAFFEYOYL QUINIC ACID ON THE PROGRESS OF CARBON TETRACHLORIDE-INDUCED HEPATIC FIBROSIS.

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3-caffeoyl-4-dihydrocaffeoyl quinic acid (CDCQ) is a new chlorogenic acid derivative isolated from the Salicornia herbacea. The protective effects of CDCQ on the progress of acute carbon tetrachloride induced hepatic fibrosis were investigated in mice. CDCQ significantly inhibited carbon tetrachloride-induced hepatic necrosis and inflammation, as determined by serum enzymatic activities of alanine and aspartate aminotransferase and serum TNF- α levels in a dose-dependent manner. In addition, increased hepatic lipid peroxidation and fibrosis after acute carbon tetrachloride treatment were suppressed by the administration of CDCQ. CDCQ also significantly prevented the elevation of hepatic hydroxyproline and collagen content and α -smooth muscle actin expression in the liver of carbon tetrachloride intoxicated mice. These results suggest that the protective effects of CDCQ against the acute carbon tetrachloride-induced hepatic fibrosis possibly related to its ability to block both hepatic inflammation and the activation of hepatic stellate cells.

1750 PROTECTIVE EFFECTS OF PLATYCODON GRANDIFLORUM ON LIVER INJURY MEDIATED BY IMMUNE RESPONSE IN A CONCAVALIN A-INDUCED HEPATITIS MODEL.

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Administration of concanavalin A (Con A) induces T cell-dependent liver failure in mice and is considered to be a model for human viral hepatitis. Platycodon grandiflorum is a traditional oriental herbal medicine that is known for its immunomodulatory and anti-tumor effects. The present study showed the protective effects of the saponins isolated from the roots of Platycodon grandiflorum A. DC (Campanulaceae), Changkil saponins (CKS), on Con A-induced hepatotoxicities and the possible protective mechanisms involved were investigated in mice. Pretreatment with CKS prior to the administration of Con A significantly prevented the increase in serum alanine aminotransferase and aspartate aminotransferase activity in a dose-dependent manner. The protective effect of CKS prior to Con A injection was associated with reduction of serum inflammatory cytokine lev-

els (TNF- α , IFN- γ , IL-6) and blockade of mRNA expressions for these cytokines. Activation of NF- κ B by Con A, which regulates TNF- α production, was inhibited in the liver of mice treated with CKS. These results suggest that the protective effects of CKS against the Con A-induced hepatotoxicity through inhibition of inflammatory cytokines-mediated immune disorders.

1751 ECHINACEA PURPUREA EXTRACTS MODULATE MURINE DENDRITIC CELL FATE AND FUNCTION.

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Echinacea is a top-selling herbal remedy that purportedly acts as an immunostimulant. However, the specific effects of Echinacea on the immune system remain to be elucidated. We are focused on defining the effects of Echinacea purpurea extracts in dendritic cells (DCs)- which are centrally involved in the generation of both innate and adaptive immune responses. We hypothesize that E. purpurea extracts enhance murine bone marrow-derived DC (BMDC) activation, ultimately leading to increased immune responses. We evaluated the fate and function of DCs following exposure to E. purpurea extracts. BMDCs were exposed to extracts for 48 hours and assessed by flow cytometry. A polysaccharide-rich extract from the root of the plant increased the expression of MHC class II and CD86; whereas an alkylamide-rich leaf extract inhibited the expression of both cell surface molecules. Although both extracts promoted the production of IL-6 and TNF- α when compared to ethanol controls, a general trend towards a concentration-dependent increase (root extract) or decrease (leaf extract) in the production of both cytokines was observed following the exposure of BMDCs to E. purpurea. In a separate experiment, E. purpurea extracts from leaf, but not root, inhibited the enzymatic activity of cyclooxygenase-2, an inducible enzyme involved in inflammation. In addition, pre-treatment of BMDCs with the leaf extract of E. purpurea prior to antigen loading with OVA peptide resulted in a significant reduction in the clonal expansion of naïve, OVA peptide-specific CD4⁺ T cells when compared to the ethanol controls. Collectively, these results suggest that Echinacea has the capacity to be immunostimulatory, immunosuppressive, and/or anti-inflammatory depending on the portion of the plant utilized and how it is extracted. Supported by NIH grant RR17670 and NSF grant EPS0091995.

1752 MAST CELL MEDIATED HISTAMINE RELEASE AND PRO-INFLAMMATORY CYTOKINE PRODUCTION ARE ATTENUATED BY GALLIC ACID.

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The discovery of drugs for the treatment of inflammatory allergic diseases such as, asthma and allergic rhinitis is an important subject in human health. Gallic acid is a polyphenyl natural product from gallnut and green tea. The aim of the present study was to elucidate whether gallic acid modulates the inflammatory allergic reaction and to study its possible mechanisms of action. Gallic acid attenuated IgE-induced histamine release from mast cells by the modulation of cAMP and calcium. Gallic acid decreased the phorbol 12-myristate 13-acetate plus calcium ionophore A23187-stimulated pro-inflammatory cytokine gene expression in human mast cells. The inhibitory effect of gallic acid on the pro-inflammatory cytokine was nuclear factor- κ B and p38 mitogen-activated protein kinase dependent. In addition, gallic acid inhibited compound 48/80 or IgE-induced systemic allergic reaction. Our findings provide evidence that inhibitory effect of gallic acid on the mast cell-derived inflammatory allergic reactions, and suggest the mechanisms of action. Furthermore, in vivo and in vitro anti-allergic effect of gallic acid suggests a possible therapeutic application of this agent in inflammatory allergic diseases. This work was supported by Brain Korea 21.

1753 BREVETOXIN INDUCED-BRONCHOCONSTRICTION: CORRELATION OF STRUCTURAL DATA WITH PHARMACOLOGICAL RESPONSES.

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Brevetoxins (PbTx) are potent polyether neurotoxic compounds produced by the dinoflagellate *Karenia brevis*. Inhalation of PbTx aerosolized by surf and wind induces nasal and/or tracheobronchial irritation, and pM concentrations can produce asthma-like symptoms in humans. beta-naphthoyl-PbTx, a synthetic derivative of

the natural PbTx-3 binds with high affinity ($K_i=1.2$ nM) to the neuronal voltage-sensitive sodium channels (VSSC) without increasing sodium flux, and antagonizes PbTx-induced bronchoconstriction in sheep. Thus, beta-naphthoyl-PbTx has been defined as the lead compound for investigating the mechanism by which inhaled brevetoxins induce bronchoconstriction, and by which selected derivatives reverse those effects. A series of beta-naphthoyl-PbTx analogs have been synthesized. Affinities for VSSC were determined by competitive displacement of [³H] PbTx-3 from rat brain synaptosomes. Effects on intact airways were determined by lung resistance measurements in live sheep. Extensive conformational searches were also conducted on β -naphthoyl derivatives and other analogs, as well as on the parent PbTx-2 and PbTx-3 structures to attempt to correlate structural data with pharmacological and physiological responses. Preliminary binding assays and lung resistance measurements have revealed that an ester linkage between PbTx and the synthetic functional group is necessary for maximum binding to the VSSC, and that electron withdrawing groups on the naphthoyl group induce bronchoconstriction. Additionally, conformational searches revealed that PbTx-2 and PbTx-3 have two non-degenerate ground-states. One is the traditional "staircase" conformer, the other exhibits a curved form similar to a helix. In the case of the β -naphthoyl-PbTx, the curved conformers are favored by approximately 10 kcal/mol, while the "reverse ester" derivative favors the traditional backbone conformers by approximately 6 kcal/mol over the helical forms.

1754 NATURAL AND SYNTHETIC BREVENALS: NEW THERAPEUTICS FOR MUCOCILIARY DYSFUNCTION AND CF.

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Our collaborations have resulted in the complete synthesis of brevenal, a natural product from Florida red tide that stimulates mucociliary clearance and relieves many of the toxicological effects of brevetoxins produced by the red tide. Different epimer derivatives of synthetic brevenal were also produced as were several derivatives of the natural material. Experiments on all synthetic products and the natural materials were conducted in vitro in rat brain receptor bindings assays, and by observing the effects of the materials on bronchoconstriction, tracheal mucus velocity, and whole lung clearance in sheep. Synthetic compound ME-1 induced pharmacological events nearly identical with those induced by natural brevenal while the other synthetic products differed significantly. All chemicals produced significant effects following 20 breaths of a solution in the pg/mL concentration range. Further, the data suggest that the character of side chains, when reduced, confer a substantial (circa 20%) increase in bronchoconstriction. Receptor binding studies utilizing tritiated brevetoxin show a reduced displacing ability ranging from 10-100 fold when very conservative reductive or epimeric modifications are made to the molecule. All derivatives still displaced tritiated brevenal from its specific receptor site, a site allosterically-linked to the brevetoxin site. From the data collected, including spectroscopy, we conclude that brevenal stereochemistry at C-26 is *epi* to that originally published because that synthetic material most closely matches the pharmacology when compared to the natural product. We further conclude that reduction of chemical loci in either of the two side chains reduces the ability to antagonize the effects of natural brevetoxin or to confer therapeutic effects in TMV displayed by natural or synthetic brevenal.

1755 BREVETOXIN-INDUCED NEURAL INSULT IN THE RETROSPLENIAL CORTEX OF MOUSE BRAIN FOLLOWING INHALATION EXPOSURE.

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Brevetoxins (Polyether breve toxins; PbTx) are polyether neurotoxins produced by the marine dinoflagellate *Karenia brevis*, an organism associated with red tide blooms in the Gulf of Mexico. Brevetoxin-3 (PbTx-3) is a major component of the array of brevetoxins found in marine aerosols. Humans exposed to aerosolized brevetoxins for short periods of time often suffer respiratory irritation whereas ingestion of contaminated shell fish produces a neurotoxic syndrome. Inasmuch as toxicokinetic studies indicate that brevetoxins distribute to the brain, it was of interest to assess the potential for aerosolized brevetoxin to produce a neurotoxic response. Female Balb/c mice were exposed nose-only for two consecutive days to

PbTx-3 aerosol, with a two hour exposure on the first day and a four hour exposure on the second day. The average PbTx-3 exposure concentration on Days 1 and 2 were $312 \pm 113 \mu\text{g}$ brevetoxin $3/\text{m}^3$ and $278 \pm 24 \mu\text{g}$ brevetoxin $3/\text{m}^3$, respectively. Coronal sections of mouse brains were evaluated for neuronal damage using both silver- and Fluoro-Jade B-staining to identify degenerating neuronal elements. PbTx-3 inhalation exposure produced neuronal degeneration in the posterior cingulate/retrosplenial cortex of mice as evidenced by silver-positive degenerating neurons in this region. No staining was found in other regions of the PbTx-3 exposed mouse brains or in brains of control, sham-exposed mice. The existence of a neurotoxic insult in PbTx-3-exposed mice was confirmed using Fluoro-Jade B to label degenerating neurons. Fluoro-Jade-positive neurons were observed in the retrosplenial cortex of PbTx-3 exposed, but not control, mice. Inhalation exposure to PbTx-3 produced a similar pattern of neurotoxicity in rat brain. These results suggest that subacute exposure to PbTx-3 for two days is sufficient to induce neuronal degeneration in a discrete region of the mouse cerebral cortex.

1756 GAMBIEROL ACTS AS A FUNCTIONAL ANTAGONIST OF NEUROTOXIN SITE 5 ON VOLTAGE-GATED SODIUM CHANNELS IN CEREBELLAR GRANULE CELLS.

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The marine toxin gambierol, a polyether ladder toxin derived from the marine dinoflagellate *Gambierdiscus toxicus*, was evaluated for interaction with the voltage-gated sodium channel (VGSC) in cerebellar granule neuron cultures (CGN). At concentrations ranging from 10 nM to 10 μM gambierol alone had no effect on the intracellular Ca^{2+} concentration $[\text{Ca}^{2+}]_i$ of exposed CGN. Furthermore, there was no evidence of neurotoxicity in CGN exposed for 2h to gambierol (1 nM to 10 μM). Gambierol was however a potent inhibitor ($\text{EC}_{50} = 189 \text{ nM}$) of the elevation of $[\text{Ca}^{2+}]_i$ that accompanies exposure of CGN to brevetoxin-2 (PbTx-2). Conversely, the influx of Ca^{2+} resulting from KCl-induced membrane depolarization was not affected by gambierol. To further explore the potential interaction of gambierol with VGSC, we assessed the influence of gambierol on the neurotoxicity of PbTx-2. Gambierol reduced the PbTx-2-induced efflux of lactate dehydrogenase in exposed CGN in a concentration-dependent manner ($\text{EC}_{50} = 471 \text{ nM}$). It is noteworthy that the potencies of gambierol as an inhibitor of PbTx-2-induced Ca^{2+} influx and cytotoxicity were coincident. In agreement with a previous report, gambierol produced a concentration-dependent inhibition of the high-affinity binding of [^3H]PbTx-3 to intact rat synaptosomes. These results provide evidence for gambierol acting as a functional antagonist of neurotoxin site 5 on neuronal VGSCs.

1757 HIPPOCAMPAL C-FOS EXPRESSION AND MICROGLIA ACCUMULATION IN RATS EXPOSED TO THE MARINE TOXIN DOMOIC ACID.

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Introduction: Domoic acid (DA) is a marine neurotoxin that may cause Amnesic Shellfish Poisoning in humans by binding to ionotropic glutamate receptors in the hippocampus, an area responsible for learning and memory processing. We investigated neuronal c-FOS expression and macrophages/microglia (BMΦ) accumulation in the hippocampus of rats treated with DA. **Hypothesis:** We hypothesized that differential behavioral responses, FOS expression and BMΦ accumulation in the CA 3 region of the hippocampus would be observed in rats injected with either DA or saline. **Methods:** Twelve rats were injected with either 2.2, 3.3 mg/kg DA or saline (control) intraperitoneally and behavioral responses were observed over a 3 hour period. A DA specific ELISA was used to quantify DA in rat serum. Neuronal FOS protein was determined by immunohistochemistry and BMΦ CD11b by immunofluorescence. **Results:** Rats receiving either 2.2 or 3.3 mg/kg DA showed significantly different behavioral responses when compared to controls which correlated with serum levels of DA: 2.2 mg/kg DA and 3.3 mg/kg DA, $>10,000 \text{ pg/ml}$ ($n=5$). A statistically significant increase in the number of CA 3 hippocampal FOS positive neurons per coronal section was observed in DA-treated rats when compared to controls: Saline, 2.9 ± 1.3 ($n=4$); 2.2 mg/kg DA, 101.5 ± 50 ($n=4$, $p<0.05$); 3.3 mg/kg DA, 220.8 ± 88.6 ($n=4$, $p<0.05$). In contrast, no difference in BMΦ accumulation was observed: mean BMΦ per 1,000 DAPI-stained nuclei; Saline, 23.7 ± 4.2 BMΦ ($n=3$); 2.2 mg/kg DA, 23.2 ± 1.1 BMΦ ($n=3$); 3.3 mg/kg DA, 26.6 ± 7.1 BMΦ ($n=3$). **Conclusions:** Our data provides partial support to our working hypothesis by demonstrating that during a 3 hour observation period, DA significantly affected rat behavioral responses and hippocampal FOS protein in a

concentration- and time-dependent manner, but did not result in changes in BMΦ accumulation. Supported by NIH (AMSM) and the Biomedical Sciences Program, College of Health Sciences, Midwestern University.

1758 THE EFFECTS OF ALOE VERA WHOLE LEAF EXTRACT ON THE GROWTH AND SHORT CHAIN FATTY ACID PRODUCTION BY *BACTEROIDES FRAGILIS*, *BIFIDOBACTERIUM INFANTIS*, AND *EUBACTERIUM LIMOSUM*.

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The effects of changes in the environment of the gastrointestinal (G.I.) tract upon the growth and activity of gut microflora are poorly understood. Aloe vera, a popular herb and dietary supplement, is widely promoted as a prophylaxis in the treatment of intestinal disorders. Short chain fatty acids (SCFA), produced by anaerobic microflora, are the preferred energy source for the G.I. mucosa. The aim of this study was to investigate the fermentation properties of Aloe vera whole leaf extract in isolated and mixed human gut bacterial cultures. The Aloe vera extract at 0, 0.5, 1.0, 1.5, and 2.0% (wt/vol) was incubated in 24 and 48h batch culture fermentations, and bacterial growth and SCFA production were used to determine changes in populations of *B. fragilis*, *B. infantis*, and *E. limosum*. Experiments were conducted in triplicate, and SCFA production was measured by gas chromatography/mass spectrometry analyses. Results indicated that the Aloe vera extract enhanced the activity of bacteria *in vitro*. A significant linear growth response to Aloe vera supplementation was observed at 24h for each of the bacteria cultures, regardless of species or mixture. There were no significant differences in bacterial numbers among cultures at 48h with the exception of the mix, which showed a significant linear growth response. In isolated bacteria cultures, a significant positive dose response to Aloe vera supplementation was observed in the production of acetic acid by *B. infantis* and of butyric acid by *E. limosum* at 24 and 48h. Aloe vera significantly inhibited the production of propionic, iso-butyric, iso-valeric, and valeric acid in the mixed culture at 24 and 48h in a dose dependent fashion. In contrast, the production of butyric acid in the mixed culture was enhanced by Aloe vera supplementation at both time points, suggesting that Aloe vera may boost the production of butyrate in the gut. Research supported by the National Toxicology Program.

1759 POSSIBLE FALSE POSITIVE RESULTS IN THE SALMONELLA TYPHIMURIUM MUTATION ASSAY DUE TO THE PRESENCE OF HISTIDINE IN NATURAL PRODUCTS.

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High and low molecular weight semi-purified fractions extracted from the mushroom *Agaricus blazei* Murril were tested for their potential to cause mutations at the histidine operon of *S. typhimurium* strains TA98, TA100, TA1535 and TA1537, and at the tryptophan operon of *Escherichia coli* strain WP2 uvrA. The extract was diluted in water and tested for toxicity to strain TA100 in a Range Finding Test at concentrations ranging from 5.0-5000 $\mu\text{g}/\text{plate}$. The tester strains were exposed to the test article in the absence of exogenous activation and in the presence of Aroclor induced rat liver S-9 plus cofactors. Toxicity was evaluated based on: 1) reversion frequency, 2) viability, and 3) integrity of the background lawn. The results of the Range Finding Test indicated that the extracts were nontoxic. However, TA100 treated with the highest extract concentration without and with activation had significant increases in revertant colonies and relative cloning efficiency as compared to the solvent controls.

Treatment of strains TA98 and TA1535 yielded similar results; a dose dependent increase in the number of revertants although the maximum increase was not always two-fold or greater than that of the solvent control plates. The most sensitive strain was TA100.

It was suspected that the natural histidine content of the mushroom might be the cause for the increase in the number of revertant colonies. Therefore, the histidine content of the extract was determined and additional assays were conducted in which control cultures for each extract concentration were treated with equivalent concentrations of histidine. These assays produced similar dose-dependent responses in both the extract treated and histidine treated cultures. Correcting for the increase in revertants due to naturally occurring histidine in the extract by subtracting the number of revertants in the corresponding control plates produced negative responses.

1760 SUBCHRONIC ORAL TOXICITY STUDY OF RHAMSAN GUM, A NATURAL FOOD POLYSACCHARIDE PRODUCED FROM SPHINGOMONAS ATCC 31961, IN RATS.

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Rhamsan gum is a microbial polysaccharide belonging to the gellan family produced by *Sphingomonas* strain ATCC 31961. The material is of considerable importance, since it possesses unusually good stability under shearing pressure, superb compatibility with high salt concentrations, and excellent suspension characteristics. It has therefore found major employment as a food additive.

This study was designed to evaluate and characterize any subchronic toxicity of rhamsan gum when administered to both sexes of Sprague-Dawley rats at dietary levels of 0 (control), 0.5, 1.5, and 5.0% (10 rats/sex/group) for 90 days. During the study, the treatment had no adverse effects on clinical signs, survival, body weights and food and water consumption, or on findings of urinalysis, ophthalmology, hematology, or blood biochemistry. Examination of gross pathology and histopathology exhibited no differences of toxicological significance between control and treated rats. Increased relative cecum (filled) and cecum (empty) weights, evident in males of the 1.5% group and both sexes receiving 5.0%, were considered to be a physiological adaptation.

In conclusion, the results indicated any toxic level of rhamsan gum to be more than 5.0%, and the no-observed-adverse-effect level (NOAEL) was concluded to be 5.0% (3362 mg/kg body weights/day for males, and 4304 mg/kg body weights/day for females) from the present study.

1761 A 1-YEAR ORAL TOXICITY STUDY OF PURPLE CORN COLOR, IN CRL:CD(SD) RATS.

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Purple corn color (Maize morado color, PCC), extracted from the seeds of corn, *Zea mays* LINNE. is a natural food colorant used in beverages, jellies, and candies in Japan. A chronic oral toxicity study was here performed with groups of 20 male and 20 female Crl:CD(SD) rats fed the agent at dietary levels of 0, 0.5, 1.5 and 5.0 % for 1 year. Citric acid monohydrate (CA) was contained at the concentration of 2.64% in the PCC used in this study. Therefore, a CA group consisting of 10 male and 10 female rats received a dietary level of 0.14% CA (the value contained in 5.0% PCC diet) was included in this study. In the PCC treatment group, no treatment-related changes in the survival rates, body weight, food and water consumption, ophthalmology, hematology and organ weight data were observed. Regarding general conditions and gross pathology, staining of fur (5.0%, both sexes) and black feces (1.5% and 5.0%, both sexes) were noted. In addition, changes of urine coloration with dose-dependence were observed. These alterations were all considered due to the anthocyanin content. On blood chemistry determinations, phospholipid (PL) and triglyceride (TG) were significantly decreased in the 5.0% female group, but these were not considered to be toxicologically significant. Histopathological lesions observed in the dose groups were equivalent to that of the control group. Based on these results, the no-observed-adverse-effect-level (NOAEL) was judged to be 5.0% in the diet for both sexes (male; 2,689 mg/kg/day, female; 3,889 mg/kg/day) for purple corn color under the present experimental conditions.

1762 THIRTEEN WEEK TOXICITY STUDY OF INDOLE-3-CARBINOL IN B6C3F1 MICE AND FISCHER 344 RATS.

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There is substantial evidence that indole-3-carbinol (I3C), a natural component of cruciferous vegetables, can reduce the risk of certain cancers induced by several known carcinogens, but can also function as an initiator and tumor promoter. Similar to dioxin-like compounds, I3C induces hepatic cytochrome P450 1A enzymes through the Ah receptor, a process often associated with toxicity. The National Toxicology Program is currently investigating the toxicity and carcinogenicity of indole-3-carbinol in a 90-day toxicity study and forthcoming 2-year rodent bioassay. In the current study, Fischer 344 rats were exposed to up to 300 mg I3C/kg body weight and B6C3F1 mice were exposed to up to 250 mg I3C/kg body weight five days a week for 13 weeks by gavage. There were no significant treat-

ment-related effects on body weight, survival, clinical chemistry, hematology, or gross or microscopic pathology. Dose-dependent increases were observed in liver weights in male and female rats and mice. In mice, hepatic CYP 1A1 and 1A2 activities were maximally increased 2- to 4- fold. In rats, hepatic CYP 1A1 activity was dose-dependently increased with a maximal induction of 109- and 81-fold in males and females, respectively, at 300 mg/kg. Hepatic CYP 1A2 activity was also dose-dependently increased with a maximal induction of approximately 5-fold in both males and females. The magnitude of induction of CYP 1A2 activity by I3C in rats was comparable to that induced by ng/kg doses of 2,3,7,8-tetra-chlorodibenzo-p-dioxin (TCDD) in previous subchronic NTP studies in female Fischer and Sprague-Dawley rats. CYP 1A1 activity maximally induced by I3C in female rats exceeded previously observed activities induced by TCDD at doses that induced neoplastic and nonneoplastic lesions in multiple tissues at 2 years, including moderate to marked hepatotoxicity and liver tumors. Therefore, the 2-year I3C studies will provide further insight into the relationship between marked CYP 1A1 and 1A2 induction and liver tumor development in rats.

1763 MODULATION OF PROSTATE CANCER CELLS BY NATURAL "PUTATIVE" ANTI-ANDROGEN.

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Plant-derived compounds such as isoflavones, flavones, and lignans have been proposed as the cancer-protective agents in populations exhibiting low incidences of prostate disease. Plant products may contain isoflavones such as genistein with estrogenic and non-estrogenic properties that could influence both endocrine and growth-factor-signaling pathways within the prostate. Anecdotal reports suggest that Bizzy Nut may be useful for a number of medical purposes, including prevention of prostate cancer. Thus, the goal of this study is to evaluate the effect of an acetone extract of this common herbal medicine on prostate function. We examined the effect of an acetone extract of Bizzy Nut on prostate by assessing its effect on the growth of LNCaP cells, production and secretion of prostate secretory protein, androgen receptor status, and induction of androgen dependent genes. The Acetone extract of Bizzy Nut elicited a dose and time-dependent inhibition of LNCaP cells with GI50 of 50ppm. Secretion of PSA, a biomarker of prostate function, was inhibited by 2-fold within 24hr exposure to the Bizzy Nut extract. Furthermore, the acetone extract of Bizzy resulted in a down-regulation of 5 α -reductase type II in LNCaP cells. Taken together, these results suggest that Bizzy Nut may prevent prostate cancer by interfering with prostate cell growth.

1764 THE EFFECTS OF BORIC ACID ON THE ENDOPLASMIC RETICULUM IN LNCAP AND DU-145 PROSTATE CANCER CELL LINES.

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The incidence of prostate cancer has increased 3% annually worldwide, and represents 33% of new male cancers, ranking second for male cancer deaths in the United States. Dietary intake and exposure to certain elements strongly influences the risk for prostate cancer. Boron has been shown to decrease prostate cancer risk in a dose dependent manner in epidemiological studies. Boron is a naturally occurring, ubiquitous, trace element that circulates in blood as boric acid. It is essential for plant growth and occurs in high levels in fruits, nuts, legumes, vegetables, and wine. Treatment with boric acid has been shown to inhibit the growth of prostate tumors in mice and proliferation of DU-145 and LNCaP prostate cancer cell lines over an 8 day period. The purpose of this study was to determine how boric acid affects DU-145 and LNCaP prostate cancer cells over 24 hours. Using a broad proteomics approach, we found that low doses of boric acid cause endoplasmic reticulum (ER) stress in DU-145 and LNCaP prostate cancer cell lines, inducing the unfolded protein response (UPR). Through 2 dimensional (2D) proteomics and western blot analysis, we have shown that the hallmarks of the UPR such as induction of chaperones BiP/GRP78, and calreticulin, as well as phosphorylation of eif2 α , occur in LNCaP and DU-145 prostate cancer cell lines. In addition, marked ultra-structural changes occur in the ER of treated cells. My future research involves determining how boric acid initiates ER stress by looking at ER calcium regulation in the presence of boric acid by using confocal microscopy. Supported by DAMP 17-03-1-0067 and TSRTF.

1765 RESVERATROL AND PICEATANNOL CYTOTOXICITY.

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Resveratrol (3,4',5-trihydroxy-trans-stilbene) is a plant antioxidant with proven cancer-preventive, anti-proliferative and anti-inflammatory activities. The toxicity potential of resveratrol remains to be fully defined. The present study has evaluated

the *in vitro* toxicity of this compound on transformed mouse macrophages (RAW 264.7), tumor-derived human T-cells (CEM) and human epidermoid carcinoma cells (A-431). Resveratrol was found to inhibit the viability of all three cell types in a concentration-dependent manner (LC50 ranging from 12-50 μ M; 24-48hr), with T-cells being the most sensitive (~12 μ M; 48hr). Viability was assessed using an MTT-based assay. Paradoxically, while higher concentrations of resveratrol (25 and 50 μ M) inhibited the proliferation of all three cell types, a low concentration (5 μ M) stimulated the proliferation of macrophages and T-cells. Scanning electron microscopy (SEM) revealed that resveratrol induced morphological changes consistent with toxicity in both immune and skin cells (0-100 μ M; 24-48 hr). The effect of resveratrol on the cellular levels of glutathione (GSH) and on lipid peroxidation was also investigated. Treatment of macrophages and skin cells with resveratrol (0-50 μ M) resulted in a time-dependent increase in GSH while levels of thiobarbituric acid-reactive substances (TBARS) remained stable. In contrast to unstimulated macrophages, stimulation with lipopolysaccharide (LPS) and zymosan conferred significant protection against resveratrol toxicity (LC50 >50 μ M). Piceatannol (3,3',4,5'-tetrahydroxy-trans-stilbene), a major metabolite of resveratrol, was also found to be toxic to macrophages, with cell viability decreasing in a dose-dependent manner (LC50 ~ 35 μ M; 48hr). As observed with resveratrol, the cytotoxicity of piceatannol was reduced by zymosan stimulation (LC50>45 μ M). The present results demonstrate resveratrol and piceatannol to be cytotoxic. Furthermore, stimulation of macrophages with zymosan or LPS results in attenuation of resveratrol toxicity.

1766 EFFECTS OF FIVE HERBAL EXTRACTS ON CELLULAR PROLIFERATION IN CHO-K1-BH4 CELLS.

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It is commonly believed that herbal supplements are an effective way to improve health, and the dietary supplement industry is a \$30 million business in the U.S. As such, there have been countless studies performed on the therapeutic benefits and applications of various herbs and herbal extracts. However, safety evaluations on herbal products are rare. In this study, 5 commonly used herbs: chaste tree berry (*Vitex agnus-castus* L.), flaxseed (*Linum usitatissimum*), ginseng (*Panax quinquefolius*), soy bean (*Glycine max*) and wild yam (*Dioscorea villosa*), were investigated for their cytotoxic activity. Dry herbal products were ground and extracted in cell culture medium (ratio of 50mg powder/ml medium) for 24h followed by centrifugation and filter sterilization of the supernatant. Then serial dilutions were prepared and exposed to CHO-K1-BH4 cells for 48 hours in 96 well plates. The concentration of the tested herb extract that suppressed the cell numbers by 50% in comparison to the untreated controls was determined (IC50) and used as an index of cytotoxic activity. CHO-K1-BH4 cells were seeded at a density of 4×10^4 cells/ml for 24 hrs before treatment. Then, the culture medium was replaced with a new medium containing the tested extract with different concentrations and incubated at 37°C for 48 hours. The medium without extract was used as the untreated control and ethanol with different concentrations correspondingly served as positive control. A minimum of two independent assays was performed with each herb in duplicate for each concentration. The results showed that the IC50 of ethanol was 1.41%, wild yam 0.69%, ginseng 4.29%, chaste tree berry 6.62%, flaxseed and soy bean >10%, the maximum test concentration. This suggested wild yam had a greater inhibitory effect on cell proliferation than other tested herbs in the present experiment.

1767 CHEMOPREVENTIVE EFFECTS OF CITRUS AURAPTENE IN N-METHYLNITROSOUREA INDUCED RAT MAMMARY CARCINOGENESIS.

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Auraptene, a citrus coumarin derivative, has been studied for its chemopreventive effects in rodent models of skin, tongue, esophagus and colon carcinogenesis. We investigated the chemopreventive effect of auraptene in N-methylnitrosourea (MNU)-induced mammary carcinogenesis in female Sprague-Dawley rats. At 42 days of age the rats were divided into 4 groups of 24-26 each and were fed a test diet as follows. Groups 1 and 2 were fed Teklad (powdered diet) only. Groups 3 and 4 received auraptene (200 ppm and 500 ppm, respectively) mixed in the test diet for the duration of the study. At 50 days of age Groups 2-4 received an intraperitoneal injection of MNU (50 mg/kg body weight in sterile acidified saline). Group 1 received vehicle only. The rats were palpated for tumors twice a week starting at 3 weeks after MNU administration. Rats were weighed once a week. At the end of 16 weeks, the average percentage of rats with palpable mammary tumors (incidence, means $\pm$ SE) in Group 2 (Teklad/MNU) was 84.6 ± 7.2 , and the average number

of palpable mammary tumors per rat (e.g., multiplicity, means $\pm$ SE) in Group 2 was 1.96 ± 0.28 . In Group 3 (auraptene 200 ppm/MNU) incidence and multiplicity were 73.1 ± 9.0 and 1.65 ± 0.28 , respectively. In Group 4, incidence and multiplicity were reduced to 50 ± 10 and 1.33 ± 0.38 , respectively. There were no palpable tumors in Group 1 (vehicle only). Trend analysis confirmed a dose-dependent effect of auraptene. There was a mild but significant decrease in body weight gain in all the MNU-treated rats compared to the saline control (Group 1). However, there were no differences in body weights between Groups 2-4. These results point to the potential chemopreventive effects of auraptene in mammary carcinogenesis. Further studies are required to understand the mechanism of action of auraptene. (Supported by 1K22CA102005-01A2)

1768 POLYPHENOLIC FRACTION OF PHYLLANTHUS MADERASPATENSIS AMELIORATES CHEMOTHERAPY-INDUCED NEPHROTOXICITY AND GENOTOXICITY.

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The polyphenolic fraction of *Phyllanthus maderaspatensis* (PPM) was studied on cisplatin and induced nephrotoxicity and genotoxicity in Swiss albino mice. The treatment of mice with different doses of PPM (50 and 100 mg/kg body weight) for seven days before the administration of a single i.p. dose of cisplatin (5mg/kg), exhibited significant chemoprotective activity. The single dose of cisplatin significantly elevated the levels of blood urea nitrogen, serum creatinine, and the kidney to body weight ratio, but pretreatment with PPM (100mg/kg/day) for seven days significantly attenuated the cisplatin induced nephrotoxicity. The frequency of micronucleated polychromatic erythrocytes (MNPCEs) in the bone marrow were determined at 24 h after the administration of cisplatin. After administration of cisplatin, the frequency of MNPCEs distinctly increased. In mice treated with PPM before cisplatin application, there is a decrease in the number of MNPCEs, when compared to mice injected with only cisplatin. PPM showed a marked free radical scavenging effect in the kidney, indicating its antioxidative property. Quercetin, one of the active compound in the PPM was identified and quantified with HPLC. The results suggest that PPM has a protective effect against cisplatin induced toxicity through its antioxidant property.

1769 THE EFFECT OF TWO COMMONLY USED HERBAL SUPPLEMENTS, GLYCINE MAX (SOY) AND LINUM USITATISSIMUM (FLAXSEED) ON RNA ISOLATION IN HEPG2 CELLS.

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The herbal supplement industry has been growing over the last twenty years; however, the safety assessment of these products is still in its infancy. There are several herbs marketed towards women for relief of menopausal symptoms. Soy and Flaxseed are predominant in this group of herbs. The objective of this study was to monitor the effect of these herbs, in unprocessed form, on RNA isolation from cells treated *in vitro*. HepG2 cells were grown in a maintenance media of MEM (with Earle's and nonessential amino acids without L-glutamine) and 10% Fetal Bovine Serum, supplemented with L-glutamine, Sodium Pyruvate, Nystatin, and Penicillin/Streptomycin. 7.134×10^7 cells were plated for Negative, PGE₁, Soy and Flaxseed treatments in 6 well plates, with 3 wells dedicated to each. Extracts of soy and flaxseed were made using the maintenance media solution at the concentration of 50 mg finely milled herb powder per 1 mL HepG2 maintenance media; the suspensions were put in a shaker for 24 hours before they were filter sterilized. 10% v/v extract/media was used for both herbs, and treatment time in a 37°C incubator was 48 hours. The cells were then isolated and subjected to a Bio-Rad™ AquaPure® RNA Isolation kit. A NanoDrop® 1000 was used for quantification of the resulting RNA. The treatments did have an effect on amount of RNA isolated. All treatments showed comparable 260/280 ratios. The soy showed a 48.6% increase whereas the flaxseed showed a 162.4% increase over untreated cells. These findings demonstrate that herbal supplements do affect RNA isolation in this cell line.

1770 ANALYTICAL METHOD DEVELOPMENT AND METHOD VALIDATION FOR THE QUANTITATION OF BETA-MYRCENE IN FISCHER 344N RAT PLASMA.

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The monoterpene, β -myrcene, is found in essential oils isolated from the small tree *Xylopia Aromatica*, lemon grass and other plants. Because of its use in food products, cosmetics and perfumes, β -myrcene is under toxicological evaluation by the

NIEHS. In this work, a gas chromatographic method using flame ionization detection (GC/FID) was developed and validated for the analysis of β -myrcene in F344 rat plasma. An eight-point spiked standard curve was prepared in Fischer 344N male rat plasma, the β -myrcene concentration ranged from -0.06 to -51 $\mu\text{g/mL}$. To prepare each spiked standard, proteins were precipitated using acetonitrile followed by extraction with 400 μL of hexane containing 1-undecene as the internal standard. The analysis method was linear (correlation coefficient > 0.999), accurate (%RE of -18.7 to 12.0), and precise ($\leq 11.1\%$ RSD). The within-day variability and instrument drift was minimal with precision of 11.1% RSD for the low concentration spiked matrix standard. Between-day variability, evaluated by comparing precision of each validation day ($\leq 11.1\%$), met criteria of $\leq 15\%$ RSD. The mean percent recovery was 74.9 to 91.1% relative to the corresponding solvent standards and β -myrcene was not detected in any control blanks. The experimental limit of quantitation (ELOQ) was -0.06 $\mu\text{g/mL}$, with a limit of detection (LOD) of 0.01329 $\mu\text{g/mL}$ and a limit of quantitation (LOQ) at 0.0443 $\mu\text{g/mL}$. A short stability study evaluated three β -myrcene concentrations at -0.2 $\mu\text{g/mL}$, -2 $\mu\text{g/mL}$, and -20 $\mu\text{g/mL}$ β -myrcene in male rat plasma. After short-term storage under ambient, refrigerated (-5 $^{\circ}\text{C}$) and freezer (-20 $^{\circ}\text{C}$ with 3 freeze/thaw cycles) conditions, the percent recoveries ranged from: 60.9 to 74.0% ; 91.5 to 105.0% ; and 98.7 to 109.8% , respectively. The method was cross validated and shown to be transferable to Fischer 344N female rat plasma over a concentration range of -0.06 to -50 $\mu\text{g/mL}$ β -myrcene in matrix.

1771 NATURALLY OCCURRING SMALL MOLECULE INHIBITOR FOR INDUCIBLE NITRIC OXIDE SYNTHASE AND CYCLOOXYGENASE-2 EXPRESSION MEDIATED THROUGH THE DOWN-REGULATION OF NF- κ B, AP-1, AND JNK IN LPS-STIMULATED RAW264.7 CELLS.

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In the present study, we investigated the effect of the plant-derived small molecules on inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression in RAW264.7 murine macrophages stimulated with lipopolysaccharide (LPS). Of the small molecules tested, IMB-112 from *Petroselinum sativum* markedly inhibited production of nitric oxide and prostaglandins, which were found to be correlated with decreases in the protein synthesis and mRNA expression of iNOS and COX-2, respectively. To further characterize the inhibitory mechanism of IMB-112 at the transcriptional level, we examined the DNA binding and transcriptional activity of NF- κ B and AP-1. We found that IMB-112 inhibited NF- κ B- and AP-1-dependent transcriptional activities as well as DNA binding activities of both transcription factors. IMB-112 also suppressed the phosphorylation of c-Jun N-terminal kinase (JNK). These results suggest that IMB-112, a naturally occurring small molecule derived from plant source, inhibits iNOS and COX-2 expression mediated through the down-regulation of NF- κ B, AP-1, and JNK signaling pathway, which may explain its potential for immune modulation. Supported by a grant from MOCIE and ITEP through the BPRC of Inje University.

1772 INHIBITION OF T-CELL PROLIFERATION AND IL-2 EXPRESSION BY PLANT-DERIVED NATURALLY OCCURRING SMALL MOLECULE IMB-112 IS MEDIATED THROUGH THE DOWN-REGULATION OF NF- κ B AND AP-1.

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In the present study, we show the effect of IMB-112, a naturally occurring small molecule derived from *Petroselinum sativum*, on T-cell function. IMB-112 produced an inhibition of concanavalin A (ConA)-induced lymphoproliferation in B6C3F1 mouse splenocytes. In light of the fact that IL-2 is responsible for the clonal expansion of T-cells, effect of IMB-112 on IL-2 expression was determined using ELISA and real-time RT-PCR. Mouse splenocytes and EL-4 T-cells exhibited IL-2 gene expression in response to ConA or phorbol-12-myristate-13-acetate (PMA) plus ionomycin (Io), which were inhibited by IMB-112 in a dose-dependent manner. No effect was observed on the treatment with IMB-111, a structural analogue to IMB-112. Electrophoretic mobility shift assay was performed to evaluate the effect of IMB-112 on binding activity of transcription factors for IL-2 gene expression in PMA/Io-stimulated EL-4 cells, and IMB-112 decreased the binding activity of NF- κ B and AP-1. These results suggest that the IMB-112 has the poten-

tial for immune modulation, which is due to the inhibition of NF- κ B- and AP-1-mediated IL-2 expression. Supported by a grant from MOCIE and ITEP through the BPRC of Inje University.

1773 PLANT-DERIVED SMALL MOLECULE INHIBITORS FOR T-CELL PROLIFERATION AND INTERLEUKIN-2 EXPRESSION MEDIATED THROUGH THE DOWN-REGULATION OF NF-AT, NF- κ B, AND ERK1/2.

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In the present study, we investigated the effect of plant-derived small molecules, IMB-104 and its analogue IMB-105, on T-cell function. IMB-104 and IMB-105 from *Syzygium aromaticum* produced inhibition of concanavalin A (ConA)-induced lymphoproliferation in B6C3F1 mouse splenocytes. In light of the fact that IL-2 is responsible for the clonal expansion of T-cells, effect of IMB-104 and IMB-105 on IL-2 expression was determined. Phorbol-12-myristate-13-acetate (PMA) plus ionomycin (Io) as well as ConA induced IL-2 mRNA expression and protein secretion in mouse splenocytes, thymocytes, and EL-4 T-cells, which were inhibited by IMB-104 and IMB-105 as determined by real-time RT-PCR and ELISA, respectively. Electrophoretic mobility shift assay was performed to evaluate the effect of the small molecules on binding activity of transcription factors for IL-2 gene expression. IMB-104 and IMB-105 decreased the binding activities of NF-AT and NF- κ B in PMA/Io-stimulated EL-4 cells. No effect was observed on the binding activity of AP-1 or Oct. Western blot analysis showed that IMB-104 and IMB-105 decreased the phosphorylation of ERK1/2 as well as the protein levels of NF-AT and NF- κ B p65 in nucleus. These results suggest that IMB-104 and IMB-105, naturally occurring plant-derived small molecules, inhibit IL-2 expression mediated through the down-regulation of NF-AT, NF- κ B, and ERK1/2 signaling pathway, which may explain their potential for immune modulation. Supported by a grant from MOCIE and ITEP through the BPRC of Inje University.

1774 PROTECTIVE EFFECTS OF THE SAPONINS DERIVED FROM ROOTS OF PLATYCODON GRANDIFLORUM ON THE CADMIUM-INDUCED CYTOTOXICITY.

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Cadmium is a ubiquitous toxic metal which is toxic to humans. Cadmium-induced cytotoxicity has been implicated in reactive oxygen species (ROS) generation due to the depletion of glutathione and protein-bound sulfhydryl groups, demonstrated in experiments involving several antioxidants and antioxidant enzymes. In this study, the effects of saponins isolated from the roots of *Platycodon grandiflorum* A. DC (Campanulaceae), Changkil saponins (CKS), on the cadmium-induced cytotoxicity were investigated in human hepatoma cell line HepG2 cells. Pretreatment with CKS significantly inhibited cadmium-induced cellular toxicity, suggesting that CKS might protect cells against cadmium toxicity by inducing the synthesis of a certain protein or proteins. The synthesis of metallothionein, a protein that is known to reduce the toxicity of heavy metals, was induced by CKS. Furthermore, CKS significantly inhibited the cadmium-induced production of intracellular ROS. These results suggested that CKS might reduce cadmium toxicity by promoting the synthesis of metallothionein that protect cells against the toxic effects of cadmium.

1775 IDENTIFICATION AND INTERPRETATION OF TREATMENT-RELATED EFFECTS IN DEVELOPMENTAL NEUROTOXICITY TESTING.

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Expert working groups were convened by ILSI Research Foundation/Risk Science Institute to assess the knowledge and experience gained from the implementation of the USEPA Developmental Neurotoxicity Guideline (OPPTS 870.6300) and to provide guidance for future use. Manuscripts were prepared in public health protection, use of positive controls, understanding normal variability, appropriate statistical techniques, and data interpretation. This paper presents the key findings

that provide a framework for the integration of the available information to properly interpret the results of DNT studies. The reliable detection, measurement and interpretation of treatment-related effects depend on appropriate study design and execution, using scientifically established methodologies, with appropriate controls to assist with interpretation. Statistical analysis should be optimized for the specific endpoints in advance, analyzing results across time and functional domains, as far as possible. The findings should be interpreted in light of available information from historical and positive controls, maternal and offspring systemic toxicity and other relevant data from the study, using "weight of evidence" principles to aid the assessment of the biological significance of statistically significant differences from concurrent controls. Case studies are used to illustrate the application of these principles. This work was supported by USEPA Cooperative Agreements X-82916701 (OPPT) and R-83049601 (OPP) and the ILSI/RF Risk Science Institute. This abstract does not necessarily represent EPA policy.

1776 EVALUATION AND INTERPRETATION OF NEURODEVELOPMENTAL ENDPOINTS FOR HUMAN HEALTH RISK ASSESSMENT – POSITIVE CONTROL STUDIES, NORMAL VARIABILITY, AND STATISTICAL ISSUES.

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ILSI Research Foundation/Risk Science Institute convened an expert working group to assess the lessons learned from the implementation of the EPA Developmental Neurotoxicity (DNT) Guideline and provide guidance for future use. The group prepared manuscripts in five areas: public health protection, use of positive controls, understanding normal variability, appropriate statistical techniques, and data interpretation. This poster will present the results from the positive control, variability, and statistical reports. Positive control data are instrumental in evaluating laboratory proficiency in detecting chemical-induced changes in measured endpoints. This review is a practical guide for the selection, use, and interpretation of positive control studies in DNT. To evaluate data quality and interpret results of treatment, normal levels of variability in measured parameters must be understood. This report presents a framework for investigators and regulators to use in comparing achieved versus expected levels of variability in DNT study data, as well as a discussion of factors contributing to inappropriate levels of variability. Appropriate statistical analyses of the behavioral data collected in DNT studies are imperative, including attention to within-litter correlations, gender and repeated testing as factors, and approaches specific for the different data types. This report presents a discussion of statistical approaches and makes general recommendations. This work is supported by EPA Cooperative Agreements X-82916701 (OPPT) and R-83049601 (OPP) and the ILSI/RF Risk Science Institute. This abstract does not necessarily represent EPA policy.

1777 ADMINISTRATION OF 3, 3'-IMINODIPROPIONITRILE (IDPN) IN JUVENILE RATS: EVALUATION AS A POSITIVE CONTROL FOR THE MOTOR ACTIVITY TEST IN THE DEVELOPMENTAL NEUROTOXICITY STUDY (DNT) AND STATISTICAL CHARACTERIZATION OF EFFECTS.

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Positive control data are a required component of DNT studies submitted to the USEPA. Administration of IDPN on postnatal days (PNDs) 5, 6 and 7 (day of birth = PND 0) has been shown to induce dyskinesia and hyperactivity that persists into adulthood (Crofton KM et al. *Neurotoxicol Teratol* 1993;15:117-29), and in this study the effects of that dosage regimen were evaluated using a different means of measuring motor activity and alternative statistical analyses. IDPN, or the saline vehicle alone, was administered intraperitoneally at dosages of 0, 75, 150, 225 or 300 mg/kg/day (10 mL/kg) to one Crl:CD(SD) rat/sex in each of 15 litters. On PNDs 13, 17, 21 and 60 the rats were transported to a separate testing area and the number of movements and time spent in movement were monitored for six 10-minute intervals using individual passive infrared sensors on a cage rack. These data were evaluated using separate repeated measures analyses of variance (ANOVA) for each sex and postnatal day. A second, comprehensive approach evaluated dose-related trends in a RANOVA model that included both sexes and all sessions. Approximately 10% and 30% of the rats at 225 and 300 mg/kg, respectively, died prior to scheduled necropsy. Body weights at PND 71 were reduced 7.4, 7.6, 14 and 32% in the male rats at 75, 150, 225 and 300 mg/kg, and the corresponding

reductions in the female rats were 7.0, 7.2, 14 and 30%. The IDPN treatment affected motor activity at every age except PND 14. Both statistical approaches identified significant increases in activity at 300 mg/kg on PNDs 18, 21 and 60 as well as the increases at 225 mg/kg on PND 18; however, the analysis that included both sexes and evaluated dose-related trends also identified significantly increased activity at 150 mg/kg on PND 18.

1778 A RETROSPECTIVE REVIEW OF THE USE OF THE DEVELOPMENTAL NEUROTOXICITY STUDY IN PESTICIDE RISK ASSESSMENTS.

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As of August 2006, guideline developmental neurotoxicity (DNT) studies (OPPTS 870.6300) for 68 pesticide active ingredients had been submitted by registrants to the US Environmental Protection Agency (US EPA) Office of Pesticide Programs (OPP) in support of product registration. These studies, conducted in rats, include detailed neurobehavioral and neuropathological assessments, following in utero and postnatal exposures to pesticide chemicals. OPP assesses risk for various routes and durations of human exposure to pesticide chemicals. To determine the influence of DNT studies in OPP risk assessment decisions, NOAELs and LOAELs from the DNT studies (maternal and offspring) were compared to the doses and endpoints used in the most recently published OPP risk assessments. The impact or potential impact of the DNT study on those risk assessments was determined. Of the submitted studies, reviews were available for 58 pesticide chemicals. For 8 of those 58, the DNT study was selected as the hazard value for regulatory purposes in at least one exposure scenario. For 18/58, the review indicates potential regulatory impact since these studies were received after the current regulatory decisions had been finalized. (This abstract does not necessarily reflect the policy of the US Environmental Protection Agency.)

1779 ASSESSMENT OF CHEMICAL EFFECTS ON NEURONAL DIFFERENTIATION USING THE ARRAYSCANTM HIGH CONTENT SCREENING SYSTEM.

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The development of alternative methods for toxicity testing is driven by the need for scientifically valid data that can be obtained in a rapid and cost-efficient manner. In vitro systems provide a model in which chemical effects on cellular events can be examined using techniques that are amenable to high-throughput analysis. We used the new technology of high content screening (HCS) to examine the effect of chemicals on differentiation and neurite outgrowth in Neuroscreen-1 cells (NS-1, a PC12 cell clone). HCS was performed using the Cellomics ArrayScan VTITM, an automated epifluorescence microscope and image analysis system using cells in a microtiter plate format. To establish the parameters for differentiation, NS-1 cells were grown on a 96-well plate in the absence (negative control) or presence (positive control) of increasing concentrations of nerve growth factor (NGF). Morphological measures indicative of NS-1 cell differentiation and growth (cell size, neurite elaboration, neurite length) were assessed after fixation and staining for Hoechst (nucleus) and beta-tubulin (cell body and neurites). Treatment with NGF caused a concentration-related increase in cell body area, average number of neurites per cell, and neurite length. Maximal effects of NGF occurred at 30-100 ng/ml. Differentiation of NS-1 cells induced by NGF was observable at 48 hr and reached maximum at 72 hr. All subsequent studies used 100 ng/ml NGF to induce differentiation and included non-NGF treated wells as negative controls. NGF-treated cells were exposed to chemicals 2 hr after plating, and differentiation was assessed 72 or 96 hr later. DMSO (vehicle) did not effect differentiation at a concentration of 0.1%. A MAP kinase inhibitor (U0126) and a PKC inhibitor (Bis I) both inhibited differentiation at concentrations that were not cytotoxic. These studies demonstrate the utility of HCS to assess chemical effects on neuronal differentiation and growth in an in vitro model. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

1780 DEVELOPMENT OF AN *IN VITRO* ES-D3 STEM CELL ASSAY FOR QUANTITATIVE DEVELOPMENTAL NEUROTOXICITY TESTING.

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Effects of chemicals on any developmental process like proliferation, migration, differentiation or apoptosis will likely have neurotoxic consequences for the CNS as an organ. In search for an in vitro screening test for developmental neurotoxicity we

have developed an assay in which ES-D3 stem cells differentiate into neurons, astrocytes and oligodendrocytes. We hypothesize that the in vitro neuronal development from stem cells mimics the in vivo differentiation to such extent that chemicals that interfere with any process in in vivo neuronal development also interfere with the neuronal development in this in vitro system. To improve the assay, variations were made 1) in culturing embryonic bodies in order to achieve uniform and reproducible embryonic bodies, 2) in the transfer from Petri dish to flask inducing cell migration, 3) in cell numbers replated prior to differentiation and 4) in additions to promote differentiation after replating. As an endpoint in the assay, immunohistochemical staining is used to distinguish different cell types in the culture. Treatment of the test system with the thyroid hormone T3 leads to an increase in oligodendrocytes. However, quantitative analysis of results needs further effort in order to overcome misinterpretation of observed differences in cell-ratios. For that purpose, the use of the computer aided stereological toolbox (CAST) system in in vivo developmental neurotoxicity testing and the translation to its possible use in quantification of in vitro results is explained. Further validation should be done by testing a selection of chemicals for their effects on individual developmental processes in relation to a shift in neuronal cell types arising after occurrence of these effects.

1781 USING ZEBRAFISH TO SCREEN FOR NEUROBEHAVIORAL TOXICITY.

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Zebrafish models can help in toxicant discovery with high through-put, low-cost assays. Neurobehavioral function can be efficiently and reliably determined in zebrafish with appropriate test methods. These can complement classic mammalian models of toxicant effects on behavioral function including learning and memory as well as locomotor activity and stress response. In addition, molecular mechanisms of the drug and toxicant effects can be effectively studied in zebrafish with the wide variety of mutants available. We have developed tests of learning and memory, stress response and locomotor activity for zebrafish, which are sensitive to drug and toxicant effects, which resemble those seen in classic rodent assays. Nicotine in adults improves both learning and memory in zebrafish much as in rats. Developmental exposure to the organophosphate pesticide chlorpyrifos impairs learning in zebrafish as it has been found in rats. Anxiolytic effects of nicotine have been documented in studies with rodents and humans. We have documented a similar effect in adult zebrafish with nicotine significantly reducing the diving response that zebrafish show in novel environments. This is at the same dose that we earlier found to significantly improve learning and memory. Zebrafish models can usefully complement mammalian investigations by providing a high through-put front-end to the investigatory sequence to triage the chemicals for further testing. In addition, molecular tools and reporter techniques for continuous visualization of the developmental process can help determine mechanisms of toxicity. The development of a neurobehavioral test battery for zebrafish will bring these advantages to bear for functional neurotoxicity. Zebrafish can provide useful starting points for the full characterization of toxicity with classical mammalian models. (Supported by the Duke University Superfund Basic Research Center ES010356).

1782 LOW LEVEL ORGANIC MERCURY EXPOSURE DISRUPTS OLIGODENDROCYTE PRECURSOR CELL FUNCTION.

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Methylmercury (MeHg) and thimerosal (THIM) (an ethylmercury derivative) are two major sources of human organic mercury exposure. Although the developing central nervous system (CNS) is a primary target of organic mercury toxicity, there is limited information regarding whether MeHg or THIM interferes with neural precursor cell function. During CNS development, oligodendrocyte-type-2-astrocyte precursor cells or oligodendrocyte progenitor cells (O-2A/OPC), undergo self-renewal and differentiation. Both intracellular redox status and platelet-derived growth factor (PDGF)-AA regulate O-2A/OPCs maturation. This study tested the hypothesis that organic Hg disrupts O-2A/OPCs function by interfering with PDGF-mediated signaling pathways. Therefore, experiments determined whether MeHg or THIM exposure, at environmentally relevant levels, impacted survival, proliferation, and intracellular signaling in O-2A/OPCs both in vitro and in vivo. Cell survival assays showed that O-2A/OPCs are extremely sensitive to nanomolar (nM) levels of MeHg or THIM. Sublethal MeHg or THIM concentrations (20-30nM) also compromised O-2A/OPCs self-renewal and differentiation by altering cellular redox status. These observations were accompanied by a downregulation of

the PDGF signaling pathway and reduced PDGF receptor α (PDGFR α) expression in O-2A/OPCs following exposure in vitro. Furthermore, N-acetyl-L-cysteine, a glutathione precursor, protected O-2A/OPCs from MeHg or THIM toxicity. Additional results showed that perinatal exposure to low levels of MeHg or THIM reduced O-2A/OPC division in postnatal SJL mice. PDGFR α expression was also decreased in multiple CNS regions. The adverse effects of organic Hg exposure were dependent upon mouse strain, since CBA mice were less sensitive. These findings suggest that developmental exposure to low levels of organic Hg may interfere with myelination in the CNS. Moreover, impaired O-2A/OPC development following organic Hg exposure could depend upon gene environment interactions. Supported by NIH RO1 ES 012708 & P30 ES 01247.

1783 METHYLMERCURY (MEHG) INDUCED CELL CYCLE ARREST AND APOPTOSIS IN NEURAL PRECURSORS ARE PREVENTED BY CASPASE INHIBITION.

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Recent studies show that MeHg, a neurotoxicant, inhibits DNA synthesis in hippocampus of newborn rat pups (Burke et al, 2006). Mechanistic studies in cerebral cortical precursor neurons in culture suggested that initial inhibition depends on a blockade in cell cycle progression, whereas later reductions were consequent to apoptosis. Further, inhibition of DNA synthesis and S phase entry were accompanied by reduced levels of cyclin E, a cell cycle regulator required for G1/S phase transition. Since the apoptosis-associated caspases cleave proteins, including cyclin E, we examined the role of the enzymes by blocking their activity using the pan-caspase inhibitor, Z-VAD-FMK (R&D System).

Primary cultures of S-D E14.5 cortical precursors were incubated in defined medium or medium containing 1.5uM MeHg with or without 60uM Z-VAD for 24h and assessed for [3H]thymidine incorporation, protein levels, and cell numbers. MeHg reduced DNA synthesis by 27%. In the presence of the Z-VAD, MeHg reduced DNA synthesis by only 9%, suggesting that caspase activity contributes to inhibition of DNA synthesis. The inhibitor alone had no effect. In addition, while cyclin E levels were reduced by 40% after MeHg exposure, the Z-VAD virtually prevented cyclin E degradation. Finally, MeHg also induced mild cell loss, reducing numbers by 12%, an effect partially reversed by Z-VAD at 24h.

These data indicate that MeHg exposure reduces DNA synthesis, cyclin E levels and neuronal numbers after 24h, and that a pan-caspase inhibitor protects against these losses. Results further suggest that activation of the caspase cascade contributes to both cell loss as well as cell cycle arrest. Specifically, caspases may not only initiate cell death but also block the G1/S phase transition through degradation of cyclin E. Further studies at earlier timepoints will clarify relationships between caspase activation and inhibition of DNA synthesis. Support: NIH: ES07148, ES11256; USEPA R829391; FRM SPE2006.

1784 GESTATIONAL LEAD EXPOSURE (GLE) INCREASES CELL PROLIFERATION AND NEUROGENESIS IN THE DEVELOPING MOUSE RETINA.

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Postnatal lead exposure decreases the scotopic electroretinogram (sERG) a- (rod photoreceptors) and b-wave (bipolar cells) in man and rodents and produces rod-selective apoptosis. In contrast, GLE increases these sERG amplitudes in children, monkeys and rodents. To determine the molecular mechanism underlying these novel supernormal ERGs, a murine model of GLE was established. Adult retinas from control and GLE mice were immunostained with molecular markers for different retinal cells and retinal immunoblots were run. To assess kinetics of retinal cell proliferation, developing retinas were immunostained for cell cycle markers (BrdU & phosphohistone 3 [PH3]) and apoptotic cells. Retinal sections were examined by light or confocal microscopy and stereological techniques determined cell counts. Affymetrix mouse genome microarrays were run with developing retinas from control and GLE mice. Real-time PCR validated the results. In adult mice, GLE produced a dose-dependent selective increase in rods and bipolar cells as horizontal, amacrine, ganglion and Müller glial cells counts were unchanged. Immunoblots confirmed these results. In retinas from GLE mice, BrdU- and PH3-positive cell numbers increased, retinal progenitor cell (RPC) proliferation was prolonged, and developmental apoptosis decreased. Microarray data analysis followed by functional classification revealed that genes associated with cell cycle and cell fate determination were up-regulated in GLE mice. Thus, GLE in mice produced a

novel phenotype characterized by an increased proliferation and genesis of late-born neurons, but not glial cells. The persistent increase of these neurons likely produces the lead-induced supernormal ERGs. Upregulation of cell growth genes appears to underlie the increased/prolonged RPC proliferation and rod/bipolar cell neurogenesis. These findings have relevance for retinal development and the permanent cognitive and behavioral deficits observed in GLE children. NIH Grants ES012482, EY007551, EY007024, EY11115 and Sramek Foundation.

1785 DIFFERENTIAL SUSCEPTIBILITY OF ROD PHOTORECEPTOR SYNAPTIC AND NON-SYNAPTIC MITOCHONDRIA TO LEAD EXPOSURE: NEUROPROTECTION BY BCL-XL OVEREXPRESSION.

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Rod inner segment (RIS) mitochondria are considered primary target sites of action during inherited and chemical-induced retinal degenerations. To date, rod synaptic terminal (spherule) mitochondria have not been examined. To determine and compare the long-term effects of low-level postnatal lead (Pb) exposure on RIS and rod spherule mitochondria, we used wild-type and transgenic mice overexpressing Bcl-xL in photoreceptors (Tg). Newborn C57BL/6 mice were raised by dams drinking water (Con) or Pb solution throughout lactation. Immunocytochemistry and confocal microscopy, electron microscopy (EM) and three-dimensional electron tomography (ET) studies used adult retinas from all four groups: Con, Tg, Pb and Tg+Pb. Molecular markers of mitochondrial compartments revealed retinal lamination and cellular localization of mitochondria. Bcl-xL localized to RIS and rod spherule mitochondria. Each rod spherule had one large mitochondrion compared to ~5/RIS cross section. Pb induced mitochondrial swelling and decreased crista/mitochondria volume, crista/mitochondria surface area and crista junction size. In contrast, the same Pb exposure did not alter these measures in RIS mitochondria, although it increased contact sites and activated a cytochrome c-caspase apoptotic cascade. Bcl-xL prevented the Pb-induced alterations in RIS mitochondria. In contrast, persistent Pb-induced structural and substructural alterations remained in the rod spherule mitochondria of Tg+Pb mice. These results reveal the importance of compartmental analysis/differences between RIS and rod spherule mitochondria and in their differential sensitivity to divalent cation-induced overload. These findings have relevance for exocytosis/endocytosis, cell injury, neuroprotection and visual function. They also expand lead's retinal sites of action and demonstrate the importance of retinal compartmental analysis and their relation to therapies for mitochondrially-mediated retinal degenerations. NIH Grants RR04050, ES10337, ES012482, EY007551.

1786 KETAMINE-INDUCED OXIDATIVE STRESS AND DNA REPAIR IN MONKEY FRONTAL CORTICAL CULTURE DURING DEVELOPMENT.

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This study was designed to determine whether ketamine-induced neuronal cell death correlates with oxidative DNA damage and altered DNA repair system. Frontal cortical cultures were incubated for 24 hrs with 10 μ M ketamine or normal culture medium (control). After washout of ketamine, cultures were kept in serum and glutamate-containing medium for 2, 6 and 24 hrs. In this study, 8-oxoguanine, a modification of guanine induced by endogenous and exogenous reactive oxygen species (ROS) to nucleic acids, was used as a biomarker for oxidative DNA damage. Antibody to human 8-oxoguanine DNA glycosylase (hOGG1) that catalyzes the removal of the 8-oxoG through cleavage of the DNA phosphodiester bond, was applied to explore the potential regulatory DNA repair systems. Immunostaining data indicate that 8-oxoguanine in both neurons and glia was primarily localized in the nucleus and mitochondria. Ketamine administration remarkably upregulated 8-oxoguanine expression and withdrawal time-dependently decreased hOGG1 expression that is responsible for repair of oxidative DNA damage. Meanwhile, ketamine (10 μ M) caused a marked reduction in immunostaining for PSA-NCAM, a substantial increase of lactate dehydrogenase (LDH) release, increased TUNEL-positive cells, and a reduction in mitochondrial metabolism of 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT). Western analysis showed that co-administration of NR1 antisense oligonucleotide prevent the synthesis of NMDA NR1 subunit protein and effectively block the neurotoxic effects induced by ketamine. These data suggest that Ketamine-induced neurotoxic effect is associated with an up-regulation of NMDA receptor function. ROS have been associated with ketamine-induced oxidative DNA damage as indicated by increased 8-oxoguanine expression and decrease DNA repair enzyme (hOGG1) expression in developing monkey. Supported by FDA/NCTR E-7189, and NICHD.

1787 MATERNAL-FETAL DISTRIBUTION OF PCB 153 VERSUS PCB 169 IN CYP1A2(-/-) KNOCKOUT COMPARED WITH CYP1A2(+/+) WILD-TYPE MICE.

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Polychlorinated biphenyls (PCBs) are persistent organic pollutants that occur in complex mixtures, with dozens of individual congeners identifiable in common food products. PCBs cause CNS damage, immunosuppression, endocrine disruption, and cancer in lab animals. Human exposures are well documented, based on samples taken from blood, breast milk, and tissues from stillborn infants. Children exposed in utero and via mother's milk are known to be at highest risk for PCB-induced neurotoxicity. Previously this lab has demonstrated the critical importance of maternal liver CYP1A2 levels in protecting fetuses from prenatal exposure to TCDD (dioxin); CYP1A2 appears to act as a "sink" to sequester planar TCDD. We have extended this work by comparing Cyp1a2(-/-) knockout with Cyp1a2(+/+) wild-type mice, following in utero exposure to an environmentally relevant mixture of eight PCB congeners spiked with either ¹⁴C-labeled PCB 153 or the PCB 169 isomer. PCB 153 is a non-planar hexachlorobiphenyl unable to bind the aryl hydrocarbon receptor (AHR); PCB 169 is a planar isomer with high affinity for the AHR. Pregnant dams were given a single gavage dose at gestational day 10 (GD10), and tissues were harvested at GD18. There were no significant differences in the distribution of PCB 153 from dams to fetuses; highest levels were found in dam adipose tissue followed by dam mammary tissue. In contrast, liver levels of PCB 169 were >5 times higher in Cyp1a2(+/+) dams than Cyp1a2(-/-) dams (p < 0.001), and fetuses carried by Cyp1a2(-/-) dams had PCB 169 levels 54% higher than fetuses carried by Cyp1a2(+/+) dams (p < 0.01). Our findings further support the role of maternal hepatic CYP1A2 levels in protecting the developing embryo/fetus by sequestering planar hydrophobic environmental toxicants in the maternal liver.—Supported, in part, by NIH grants P30 ES006096, T32 DK059803, and R01 ES014403.

1788 DEVELOPMENTAL EXPOSURE TO POLYCHLORINATED BIPHENYLS REDUCES INFARCT SIZE IN THE ADULT RAT FOLLOWING ISCHEMIC STROKE.

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Stroke outcome is a sexually dimorphic response and there is evidence suggesting that sexual dimorphism in stroke is shaped by exposure to sex steroids during development of the brain. Polychlorinated biphenyls (PCBs) are environmental contaminants that have been identified as endocrine disruptors, and experimental evidence suggests that PCBs interfere with sexual differentiation of the brain. Moreover, PCBs have been linked to increased incidence of risk factors associated with stroke such as vascular disease and a recent epidemiological study reported a positive association between PCB exposure and increased stroke incidence. Thus we tested the hypothesis that developmental exposure to PCBs would alter stroke outcome in adults in a sex-specific manner. Female Wistar rats were dosed daily with vehicle or Aroclor 1254 (0.1 mg/kg or 1 mg/kg) beginning 2 weeks prior to conception and continuing throughout gestation and lactation. Exposure to the pups ended upon weaning at postnatal day 21. At 8-10 weeks of age, male and female adult rats that had been exposed to PCBs developmentally were subjected to 2-hour middle cerebral artery occlusion (MCAO), followed by 22 hours of reperfusion. Body and head temperatures, blood gases and laser-Doppler cortical perfusion were monitored during MCAO and early reperfusion, and infarct size was measured by TTC staining. Unexpectedly, developmental exposure to Aroclor 1254 significantly decreased striatal infarct in both males and females at 1 mg/kg and 0.1mg/kg respectively. A similar trend, which was not statistically significant, was observed with cortical infarcts. We conclude that PCB exposure during development alters the brain's susceptibility to stroke in adult life in a brain region specific manner, and that males and females may exhibit differential sensitivity. This work supported by the NIH grants ES010338 (PJL), HD40936 (PJL), NS49210 (PDH) and NS33668 (PDH).

1789 EFFECTS OF DEVELOPMENTAL EXPOSURE TO CHLORPYRIFOS OR METHYL PARATHION ON BRAIN-DERIVED NEUROTROPHIC FACTOR EXPRESSION DURING SPATIAL MEMORY FORMATION.

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Brian-derived neurotrophic (BDNF) is a member of the neurotrophin family whose expression is believed to be necessary for spatial memory formation. In the present study, we investigated the involvement of BDNF mRNA expression in

working and reference memory formation following developmental exposure to methyl parathion or chlorpyrifos in rats. The 12-arm radial maze was used to assess working and reference memory. Working memory was assessed using the total number of accurate choices within the first eight arm visits and entries to repeat. On the final day of training in the 12-arm radial maze, males exposed to the medium and high dosage regimen of methyl parathion (MPS) reduce significantly ($p < 0.05$), the total number of accurate choices within first eight arms visits. In contrast, entries to repeat and reference memory was not significantly affected by either chlorpyrifos or methyl parathion exposure for both males and females. To assess the involvement of BDNF mRNA expression in working and reference memory formation, the hippocampus of male and female rats were dissected 30-minutes post radial arm maze training. The radial arm maze training paradigms significantly ($p < 0.05$) increase the expression of BDNF mRNA expression in control male and females when compared to naive controls. However, the exposure of males to the CPS medium and high dose regimen, decrease significantly ($p < 0.05$), the expression of BDNF mRNA hippocampal expression. This reduction of BDNF gene expression due to treatment resulted in the impairment in working memory formation. Interestingly, females exposed developmentally to CPS or MPS resulted in a significant ($p < 0.05$) reduction in hippocampal BDNF mRNA gene expression at all exposure levels but these reductions did not correlate significantly with impairment in memory formation. Finally, these data seem to suggest that developmental exposure to CPS or MPS impair BDNF mRNA expression and these impairments sex-selectively correlated to working memory formation.

1790 SHIPPING STRESS DURING PREGNANCY AFFECTS THE NEUROTOXIC EFFECTS AFTER PRENATAL EXPOSURE TO VALPROATE.

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Developmental neurotoxicity tests are important to protect the pediatric brain from chemically-induced tragedies. Prenatal stress is well known to affect the development of the central nervous system. However, numerous developmental neurotoxicity studies published recently have used pregnant rodents purchased from a breeder. The pregnant rodents purchased definitely sustain shipping stress. In the present study, we investigated the effect of shipping stress on the neurotoxicity induced by valproate (VPA) exposure (a proposed rat model of autism). Two groups of pregnant rats, purchased (P) and mated at our animal facility (M), were established. These rats were exposed to VPA (800 mg/kg) orally on gestation day (GD) 9 or 11 (VPAE9 or VPAE11), and the fetal brain was observed on embryonic day 14 by serotonin and TuJ1 (a marker of immature neurons) immunohistochemical staining. Fetal brains were carefully observed using serial coronal and sagittal sections. The VPA exposure affected the number of dead fetuses and fetal body weight only in the P group. Development of the serotonin nervous system was not affected by VPA. VPA on both exposure days induced an altered structure in the thalamus, which was rounder with an enlarged third ventricle, and diminished development of TuJ1 positive neurites in the cerebral cortex. The size of the forebrain was also affected by VPA. Two factor ANOVA analysis demonstrated that the P group was much more sensitive to these toxic effects. The findings of this study strongly suggest that purchasing pregnant rats or mating rats at the test facility is a very important issue in risk assessment. (Supported in part by a grant for Long-range Research Initiative from the Japan Chemical Industry Association).

1791 CONCURRENT EXPOSURE TO PERFLUOROOCTANE SULFONATE (PFOS) AND RESTRAINT STRESS DURING PREGNANCY IN MICE: NEUROBEHAVIORAL EFFECTS ON THE OFFSPRING.

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Perfluorooctane sulfonate (PFOS) is an organic persistent pollutant used in a great variety of commercial products. Concern about this chemical is due to its detection in human tissues and wildlife samples on a global scale. Studies performed in mammals show that PFOS exposure can cause adverse effects on adults. However, only few studies have provided information concerning the effects of PFOS on development. Data on the development of the offspring after weaning are particularly scarce. On the other hand, several investigations have demonstrated that stress can enhance the developmental toxicity of some chemicals. The aim of this study was to assess the combined effects of maternal restraint stress and PFOS on the behavior of the offspring of mice at three months of age. Thirty-four plug positive females were divided in two groups). Animals were given by gavage 0 and 6 mg PFOS/kg/day on gestation Days 12-18. One-half of the animals in each group was subjected to restraint stress (30 min/session, three sessions per day) during the same period. At

three months, mice were evaluated for general activity in an open-field, and for learning and memory in a water maze task. The group prenatally exposed to PFOS and restraint presented a reduced mobility in the open-field, whereas the group exposed to restraint performed a greater number of rearings. In the water maze, an interaction between sex and restraint was observed. Delayed task learning was detected in females prenatally exposed to PFOS and restraint. An overall effect of restraint was observed in mice on retention of the task, suggesting a better retention in restrained animals. Corticosterone levels were lower in animals prenatally exposed to restraint. The current results suggest that prenatal PFOS exposure induced long lasting effects in mice. Interactive effects between PFOS and maternal stress were also observed.

1792 HIGHLY BROMINATED DIPHENYL ETHERS (PBDE 209) INTERACT WITH THE PERFLUOROOCTANOIC ACID (PFOA) DURING NEONATAL BRAIN DEVELOPMENT TO ENHANCE DEVELOPMENTAL NEUROBEHAVIOURAL DEFECTS.

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Polybrominated diphenyl ethers (PBDEs) and perfluorinated compounds (PFCs) have been identified as emerging classes of persistent environmental compounds, present in humans as well as wildlife. A concern is that these compounds are present at a higher level in the children compared to the mother's and grandmother's generation. PBDEs are used in large quantities as flame-retardant in polymers, especially in electric appliances. PFCs are found in consumer applications such as stain resistant treatment coatings for clothing fabrics, carpets, as well as foam fire extinguishers. We have earlier reported that neonatal exposure to PBDE 209 on PND 3 cause developmental neurotoxic effects, but not when exposed on PND 10. Neonatal exposure to PFOA causes developmental neurotoxic effects when given on PND 10 to mice. The present study was carried out to see whether PBDE 209 and PFOA interact during neonatal brain development to enhance neurobehavioural defects. Neonatal mice, 3 and 10 days of age (PND 3/PND 10), were orally exposed to PBDE 209 (1.4 or 8.0 $\mu\text{mol/kg}$ bw), PFOA (1.4 or 8.0 $\mu\text{mol/kg}$ bw), co-exposed to PBDE 209 and PFOA, or a vehicle (20 % fat emulsion). Neonatal exposure to single oral dose of PBDE 209 on PND 3 and a single oral dose of PFOA on PND 10 caused impaired spontaneous behaviour in 2- and 4-month-old mice, an effect significantly different from single exposure to PBDE 209 or PFOA. Furthermore, neonatal exposure to PFOA on PND 10, but not on PND 3, significantly impaired spontaneous behaviour in 2- and 4-month-old mice. There was no interaction between PBDE 209 and PFOA when both compounds were given on PND 3. This study suggests that highly brominated diphenyl ethers interact with perfluorinated compounds (PFOA). At low doses of these compounds enhance developmental neurobehavioural defects when present during a critical phase of neonatal brain development.

1793 NEONATAL DEVELOPMENTAL NEUROTOXICITY OF DECABROMINATED DIPHENYL ETHER, PBDE 209: A SUMMARY.

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Polybrominated diphenyl ethers (PBDEs) are flame-retardants used in order to reduce the risk of fire. PBDEs have been found to increase in the environment and in human mother's milk. In recent studies we have seen that neonatal exposure to several congeners of PBDEs (PBDE 47, 99, 153, 183, 203, 206, and 209), can cause permanent neurotoxic effects in mice and/or rats. Here follows a summary of the neonatal developmental neurotoxic effects of the decabrominated diphenyl ether, PBDE 209, the only PBDE congener still in use.

Study 1: Male mice were exposed to 2.22 or 20.1 mg PBDE 209/kg b.wt. on PND 3, 10 or 19 and tested in a spontaneous behaviour test at different ages. Exposure to 20.1 mg PBDE 209/kg b.wt. on PND 3 induced irreversible behavioural effects that got worse with age.

Study 2: Male mice, exposed to 1.34 to 20.1 mg PBDE 209/kg b.wt. on PND 3 showed significantly impaired spontaneous motor behaviour as adults. Animals exposed to PBDE 209 showed opposite reaction to the cholinergic agent nicotine, compared to the controls. These effects were dose-response related.

Study 3: Male rats, exposed to 6.7 or 20.1 mg PBDE 209/kg b.wt. on PND 3 showed significantly impaired spontaneous motor behaviour as adults. This effect was dose-response related. Animals exposed to 20.1 mg/kg b.wt. showed opposite reaction to the cholinergic agent nicotine compared to the controls.

Study 4: Male mice, exposed to 20.1 mg PBDE 209/kg b.wt. on PND 3 showed significant changes in the three structurally and functionally important proteins BDNF, CaMKII and GAP-43 in the brain 7 days after exposure to PBDE 209.

In conclusion, neonatal exposure to PBDE 209, during a restricted period of brain development, induces behavioural derangements in adult mice and rats, effects that are dose-response dependent and get worse with age. In addition, PBDE 209 affects the cholinergic system in both mice and rats. PBDE 209 also affects levels of BDNF, CaMKII and GAP-43 in the neonatal brain. These studies indicate that PBDE 209 has to be metabolized to exert its neurotoxic effects.

1794 POTENTIATION OF METHAMPHETAMINE NEUROTOXICITY IN ADULT RATS FOLLOWING POSTNATAL EXPOSURE TO DELTAMETHRIN.

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Methamphetamine (METH) abuse has become a serious public health issue in the United States. In addition to its addictive properties, METH has been demonstrated to be acutely toxic to both dopaminergic and serotonergic neurons. There is evidence in the human population and in animal studies that there are a number of modifying factors that influence the severity of METH's neurotoxicity. Among these factors is age (younger animals less susceptible than older), gender (males more susceptible than females), and potentially genetic differences that influence the outcome of METH exposure. However, little attention has focused on potential environmental factors that may influence the neurotoxicity of METH. Here, we demonstrate that exposure to the pyrethroid pesticide deltamethrin (DM) during postnatal development potentiates the neurotoxicity of METH administered in adulthood. Rats were administered corn oil (control) or DM (0.1 mg/kg) every day from postnatal day (PND) 1-21. Following weaning on PND22, rats were aged to adulthood (12 weeks of age) and then administered either saline or 4x5 mg/kg METH every two hours. Two days following the last injection, the striatum was dissected from these animals and assayed for dopamine (DA) and serotonin (5HT) by HPLC. METH exposure reduced striatal DA by 42% and 45% in control males and females, respectively. Reduction of DA by METH was greater in DM males (62%), with no potentiation observed in DM females (45%). METH reduced 5HT levels by 34% and 41% in control males and females. No potentiation of 5HT reduction was observed in DM males (34%) or females (44%). These data suggest that environmental exposures during development may render the dopamine system more susceptible to neurotoxic damage by METH in adulthood. Additionally, these data suggest that males are more sensitive to the potentiation of METH neurotoxicity following developmental exposure to deltamethrin. Supported in part by NIH R21ES013828 (JRR)

1795 GESTATIONAL POLYCYCLIC/HALOGENATED AROMATIC HYDROCARBON EXPOSURE EFFECTS ON HIPPOCAMPAL AND SOMATOSENSORY PLASTICITY MECHANISMS.

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To characterize the impact of gestational exposure to Benzo(a)Pyrene [B(a)P] and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on hippocampal and cortical plasticity mechanisms; pregnant Long Evans rats were exposed to either sub-acute doses of B(a)P (100ug/m3) on gestation day 14-17 (GD) and/or to a single oral dose of TCDD (700ng/kg BW) on GD15. When F1 generation animals reached maturity, long-term potentiation (LTP) was measured from the hippocampus and neuronal activity was recorded from neurons in the whisker representation of SI (barrel) cortex. LTP was significantly reduced as was spontaneous/evoked activity of cortical neurons in environmental contaminant-exposed F1 generation animals when compared to controls. To mechanistically ascertain the basis of environmental contaminant induced plasticity perturbations, total RNA from F1 animals was analyzed to determine mRNA profiles for molecules that are important for synaptic plasticity. Ionotropic glutamate receptor subunits were profiled for developmental mRNA and protein expression levels in offspring control and exposed rats using semi-quantitative PCR, western analysis and fluorescence microscopy. The results of the molecular studies revealed a significant down-regulation in the temporal developmental expression of glutamate receptor (NMDA and AMPA) subunits in the hippocampus and SI cortex. The results also demonstrate that exposed offspring animals present with deficiencies in behaviors that depend on normal hippocampal and cortical function and predict that deficits in behaviors will be present throughout life. The results from these studies suggest that gestational exposure results in plasticity deficits mediated through down-regulation of developmental glutamate receptor subunit expression and function at a time when excitatory synapses are being formed for the first time in the developing central nervous system. [Supported by NS041071 and 1S11ES014156].

1796 CORRELATION BETWEEN THE EVALUATION OF CORTICAL PLATE FORMATION AND HYPERACTIVITY INDUCED BY PRENATAL BRDU EXPOSURE IN RATS.

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Developmental neurotoxicity (DNT) tests have been developed to protect against developmental disorders in humans. In the current DNT guidelines, treatment covers a long period from prenatal to postnatal, so that the critical period of DNT has not been evaluated to date.

We reported that a genotoxic agent, 5-bromo-2'-deoxyuridine (BrdU) induced hyperactivity when 50 mg/kg of BrdU was administered intraperitoneally to rats on gestational day (GD) 9 to 15. In this study, the critical period for induction of hyperactivity by prenatal BrdU exposure was evaluated, and the relationship between findings obtained from fetal brain and postnatal behavioral abnormality (hyperactivity) was investigated. BrdU treatment was administered on GD9-10, GD11-13, or GD14-15, and behavior in the offspring and GD16 fetal brains were examined. In the GD 9-10 treated group, locomotion in the open field was not changed. In the GD11-13 and GD14-15 treated groups, locomotion was significantly increased but was less than that of GD 9-15 treated group (GD11-13 < GD14-15 < GD9-15). On examination of GD16 fetal brain, dysgenesis of the cortical plate was observed in the GD11-13 and GD14-15 (and GD9-15) treated groups, and grades were consistent with the induction of hyperactivity. Anti-BrdU immunoreactive cells were observed in sub- and cortical- plates in these groups, indicating that BrdU exposure timing, which induced hyperactivity coincided with cortical plate formation.

Thus, the critical period of hyperactivity induced by BrdU was estimated to begin after closure of the neural tube and continuing for a relatively long period. The findings in fetal brain (abnormal cortical plate) reflected the grade of hyperactivity in the offspring, suggesting that observations of the cortical plate in the fetal brain can be one of the useful endpoints supporting postnatal DNT test.

1797 THE EFFECTS OF LOW DOSE PTU ON ENDPOINTS OF THYROID HORMONE ACTION IN THE DEVELOPING BRAIN.

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Thyroid hormone (TH) is essential for normal brain development. Therefore, there is concern that any factor that reduces TH levels may permanently alter brain development. As part of an EPA Cooperative Agreement, the goal of this work was to characterize the degree to which circulating TH levels must decline to alter endpoints of TH action in the developing brain. We induced graded decreases in serum TH levels in timed pregnant rats by exposure to the goitrogen propylthiouracil (PTU; 0, 1, 2, and 3ppm). Measures of TH action included RC3 expression in the hippocampus and cingulate cortex, and the oligodendrocyte to astrocyte ratio in the corpus callosum (CC) and anterior commissure (AC). To characterize potential adaptive responses to TH insufficiency, we measured the expression of the deiodinase enzymes (D2 and D3), and the TH transporters MCT8 and OATP14 in the hippocampus. The results presented here are focused on postnatal day 15, a time when TH levels are at their peak during development. We report that PTU treatment induced a dose-dependent decrease in serum T4 levels. In addition, we observed a dose-dependent decrease in the oligodendrocyte to astrocyte ratio in the both the AC and CC. Two additional points are worth noting regarding this TH endpoint. Interestingly, we found that the dose of PTU necessary to significantly reduce T4 levels (1ppm), was less than the dose necessary to measure an altered glial ratio (2ppm) and second, the CC was more sensitive than the AC to TH insufficiency. These data will be evaluated from the perspective of finding the degree to which TH levels must decline before adverse effects result and, in addition, to identify adaptive mechanisms that may account for differences in the shape of the dose response observed between individual endpoints of TH action and different thyroid toxicants. This work is supported by an EPA STAR predoctoral fellowship (FP916424 to DSS) and an EPA STAR Grant (RD-83213701-0 to RTZ). Does not reflect US EPA policy.

1798 THYROID HORMONE INSUFFICIENCY AND BRAIN DEVELOPMENT - EXAMINING NEUROTOXICITY AT LOW LEVELS OF HORMONE DISRUPTION.

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Thyroid hormone (TH) deficiencies during development produce deleterious effects on brain structure and function. The degree to which TH must be perturbed to induce neurotoxicity remains unclear. The present study was conducted as part

of a Cooperative Agreement between US EPA, U Georgia, and U Massachusetts to define dose-response relationships between low level TH reductions induced by the goitrogen propylthiouracil (PTU) and brain dysfunction. Pregnant rats were administered PTU via the drinking water (0, 1, 2, 3ppm) from gestational day (GD) 6 until weaning of pups on postnatal (PN) day 23. Maternal weight gain was similar across dose groups. Pup weights were similar from birth to PN15, with a slight decrease in the 3ppm dose group on PN21. Eye opening was delayed by 3ppm PTU. Modest reductions in dam T4 levels were observed, with no change in T3 detected at any dose. Pups displayed dose-dependent reductions in T4 between PN4-22, with modest reductions in T3 limited to the high dose group. TH returned to control values by PN127. Cognitive function was assessed in adults using 2 fear conditioning (FC) paradigms. Trace FC was induced in male offspring by presenting a neutral stimulus (light/tone) paired 30s later (trace interval) with mild foot-shock. To increase task difficulty, a 2nd FC paradigm incorporated a flashing light randomly presented throughout training (Distract FC). Conditioning to context in trace FC was enhanced at 1 and 2ppm dose levels, with no effect at the high dose. Cue learning was impaired in the 3ppm dose group. Deficits were observed in Distract FC in context and cue learning at the high dose. Context learning in female offspring in Distract FC was impaired at all dose levels. These findings reveal learning deficits at low levels of TH insufficiency, potential gender differences in susceptibility, and the power of postnatal serum T4 levels to predict alterations in some but not all toxicological and functional endpoints. (Does not reflect US EPA policy)

1799 AMPHETAMINE EXPOSURE DURING THE BRAIN GROWTH SPURT PRODUCES BEHAVIORAL MODIFICATIONS IN ADOLESCENT RATS.

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Prenatal exposure to amphetamine has been shown to interfere with behavioral development in the affected offspring. This study was to examine whether exposure to d-amphetamine sulfate (AMPH) during the most dynamic brain growth spurt altered behavior during both adolescence and adulthood. Pups were intragastrically administered a milk formula containing 5, 15 or 25 mg/kg/day of AMPH from postnatal day (PD) 4-9. Additionally, an intubation control (IC; 0 mg/kg/day AMPH) and a normal control (SC; no experimental manipulation) were employed. Animals received two intubations each day spaced two hours apart, with each intubation consisting of half the daily dose. Pups were raised by dams until weaning on PD 21. On PD 24, offspring were subjected to open-field behavioral testing with 2 trials/day from PD 24-27. On PD 60, behavioral testing of the Morris water maze began. Each animal was given 5 daily training trials for six consecutive days with one probe trial occurring at the end of the sixth day, and another probe trial taking place 24 hr later. Results from these two behavioral tests revealed a main effect of AMPH in the open-field behavioral test, with animals in the AMPH 25 group having significantly lower levels of activity. However, while animals did learn to find the escape platform in the Morris water maze test, there were no changes in behavioral performance as a result of AMPH exposure. Since neonatal AMPH exposure altered the behavior of animals in the behavioral test employed during their adolescence (open-field) and not during adulthood (Morris water maze), these findings suggest that neonatal AMPH-induced alterations in behavior may be either transient in nature or behavioral task specific. (Supported by NIH grant DA018809)

1800 DEVELOPMENTAL EXPOSURE TO AMMONIUM PERCHLORATE ALTERS RUNNING WHEEL ACTIVITY AND BALANCE BEAM PERFORMANCE IN RATS.

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Ammonium perchlorate (AP) is a major chemical component in solid rocket fuels and fireworks and is reliably detected in soil and water throughout the U.S. Currently, the effects of developmental AP exposure on neurobehavioral function are largely unknown. The current experiment is part of a larger project designed to assess the ability of developmental AP exposure to alter neurobehavioral function and learning ability in rats. Female Long Evans rats (n=22 and n=20 for AP-exposed and control rats, respectively) were dosed daily with 0 or 30 mg/kg of AP (99.8% purity, Sigma-Aldrich, Milwaukee, WI) beginning 17 days prior to mating and continuing throughout the weaning of the offspring. One male and one female were randomly selected from each successful litter (n=15 and n=16 for AP-exposed and control litters, respectively) and began testing on the neurobehavioral test battery on postnatal day 70. The neurobehavioral battery included an assessment of spontaneous wheel running, balance beam performance, and open field locomotor activity. For spontaneous wheel running, a sex-specific effect was evident in which the AP-exposed females emitted significantly more wheel revolutions than the control females while the AP-exposed males were unaffected. Performance on the bal-

ance beam was also affected by developmental AP exposure with the AP-exposed rats slipping off the balance beam more rapidly than the non-exposed controls. Open field activity testing did not reveal an effect of AP exposure. The results of the current study indicate that developmental exposure to 30 mg/kg/day of AP is capable of increasing activity and altering balance beam performance in the exposed offspring. The effect on the balance beam could be an effect of AP on balance but could also be an artifact of elevated activity while on the balance beam, thus placing the rats at greater risk of slipping off. Lastly, the running wheel data suggest that females may be more susceptible than males to the neurobehavioral effects of developmental AP exposure.

1801 DEVELOPMENTAL NEUROBEHAVIOURAL EFFECTS AFTER CO-EXPOSURE TO GAMMA-RADIATION AND METHYL MERCURY DURING A CRITICAL PHASE OF NEONATAL BRAIN DEVELOPMENT IN MICE.

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In our environment mammals (including humans) are exposed to different types of ionizing radiation as well as persistent and nonpersistent chemicals. It is known that ionizing radiation, as well as methyl mercury, can give rise to neurotoxicological and neurobehavioural effects in mammals and that developmental neurotoxic effects can be seen after exposure during gestation. There is a lack of knowledge concerning effects and consequences from low-dose exposure during critical phases of perinatal and/or neonatal brain development and the combination of ionizing radiation and environmental chemicals. A recent study has also indicated that low doses of ionising radiation to the human brain during infancy influence cognitive ability in adulthood. Neonatal NMRI male mice, 10 days of age, were exposed to a single oral dose of MeHg (0.40 mg/kg bw). Four hours after the MeHg exposure the mice were irradiated with 60Co gamma radiation (The Svedberg Laboratory, Uppsala University). Mice were irradiated in plastic dishes with a surface dose-rate of around 0.070 Gy/min. The mice were irradiated at the doses of 0.2 and 0.5 Gy, respectively, given at one occasion. The animals were subjected to spontaneous behaviour test at ages of 2- and 4-months, and water maze test at an age of 5 months. Neither the single dose of MeHg (0.4 mg/kg bw) nor the radiation dose of 0.2 Gy affected the spontaneous behavior, but the co-exposure to external gamma radiation and MeHg caused developmental neurotoxic effects. The effects were also dose-response and time-response related. The study shows that co-exposure to ionising radiation and MeHg can interact and significantly enhance developmental neurotoxic effects, manifested as disrupted spontaneous behaviour, lack of habituation, and impaired learning and memory functions.

1802 TITLE: THE EFFECT OF CHRONIC ORAL ACRYLAMIDE EXPOSURE ON INCREMENTAL REPEATED ACQUISITION (LEARNING) PERFORMANCE IN FISCHER 344 RATS.

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Acrylamide (ACR) is a known neurotoxicant routinely found in our diet. At relatively high levels of exposure, it has been shown in rats and humans to produce effects on the nervous system. The possibility that chronic dietary exposure to ACR might produce effects on learning is a question of current concern. This research evaluated the effects of daily exposure to ACR beginning prenatally from gestation day 6 and continuing throughout the rats' lifespan. Plug-positive Fischer 344 dams (8-10 dams per treatment group) were gavaged daily with 0, 0.1, 0.3, 1.0 or 5.0 mg/kg/day ACR. On postnatal days (PNDs) 1-22, pups were gavaged with the same dose their dam had received. On PND 22, pups were weaned and pair-housed with a same-sex littermate with exposure continuing via drinking water. One male and one female pup per litter was behaviorally tested in an operant chamber for incremental repeated acquisition (IRA) performance beginning at approximately six weeks of age. Rats performing this task must learn a new sequence of lever presses each test day. Rats receive food pellets for correct responses. Over 28 testing sessions (from ~PNDs 36-140), a significant treatment effect was found on percent task completed (PTC), with Tukey's posthoc test (P<0.05) indicating a significantly lower PTC for the 5.0 mg/kg/day group compared to controls. No treatment effects were found for response accuracy (P=0.07) and response rate (P=0.07). For each of these measures, a significant effect of session was observed (P < 0.0001) demonstrating that performance improved overall through the 28 sessions, regardless of treatment group. The results suggest that chronic exposure to ACR can adversely affect learning performance albeit at only the highest exposure tested. Supported by ORISE (JG) and NTP 224-93-0001.

1803 CARDIOVASCULAR EFFECTS OF INHALED ULTRAFINE (NANO) PARTICLES IN HUMAN VOLUNTEERS.

E. Cassee¹ and R. Devlin². ¹Centre for Environmental Health Research, National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands and ²Human Studies Division, EPA, Research Triangle Park, NC.

Particulate matter (PM) is any material that exists as solid or liquid droplets in the atmosphere that are less than 10 microns. PM may be in the form of fly ash, soot, dust, fog, fumes etc. Sources of particulate matter include traffic and power plants. Although PM is currently regulated by the mass concentration in ambient air, particles in the nanometer size range, also known as ultrafines, hardly contribute to the total mass. Yet, these ultrafine particles are of major concern because they will penetrate deep into the lung, are likely to escape normal defense mechanism and may end up in the systemic circulation. In addition, nanoparticles are also found to be translocated to the brain through the olfactory nerve system. Technologies that allow inhalation exposure to this complex mixture of gases and particles have now been applied by the scientific community for several years. In addition controlled studies with for instance diesel soot or carbon particle have been performed. Many different biological endpoints have been investigated to determine whether or not PM can cause health effects at levels that are not unheard of in real life.

This session aims to present:

- (a) different approaches used to study ultrafine PM health effects in humans to understand the epidemiological observations.
- (b) the potential biological mechanisms by which ambient nano-sized PM may lead to pulmonary and also cardiovascular effects.
- (c) the representativeness of the health endpoints in experimental particulate matter studies in relation to the epidemiological observations (premature death, exacerbation of respiratory diseases)

This session will narrow the gap between exposure, toxicology and epidemiology.

This abstract does not necessarily reflect RIVM or EPA policy.

1804 HUMAN CLINICAL STUDIES OF ELEMENTAL CARBON ULTRAFINE PARTICLE INHALATION: EFFECTS ON VASCULAR FUNCTION.

M. W. Frampton, M. J. Utell and G. Oberdörster. *Medicine & Environmental Medicine, University of Rochester, Rochester, NY.*

Ultrafine particles (UFP) may contribute to the cardiovascular effects of exposure to particulate air pollution, in part because of their relatively efficient alveolar deposition, potential to enter the pulmonary vascular space, and ability to deliver reactive molecular species as part of their surface chemistry. We have tested the hypothesis that inhalation of elemental carbon ultrafine particles alters cardiovascular homeostasis in healthy and compromised human subjects. Inhalation of ultrafine carbon particles (25 to 50 µg/m³, count median diameter ~27 nm) for 2 hours, with intermittent exercise, caused alterations in peripheral blood leukocyte phenotype consistent with increased transit time through the pulmonary circulation, reduced diffusing capacity for carbon monoxide (DLCO), and blunted increases in flow-mediated vasodilatation in the forearm. These findings suggest that short-term inhalation of ultrafine carbon particles alters vascular function, both systemically and in the lung. Data from these human clinical studies of exposure to air pollutants help to elucidate the mechanisms responsible for pollutant health effects, and assist in establishing rational air quality standards.

1805 BIOLOGICAL RESPONSES OF HEALTHY HUMAN VOLUNTEERS FOLLOWING EXPOSURE TO CONCENTRATED AMBIENT COARSE, FINE, AND ULTRAFINE PARTICLES.

R. Devlin¹, J. Samet¹, D. Graff¹, A. Ghio¹ and W. Cascio². ¹Human Studies Division, EPA, Research Triangle Park, NC and ²Department of Medicine, East Carolina School of Medicine, Greenville, NC.

Particle size has been implicated by epidemiological and toxicological studies as an important determinant of the toxicity of ambient particulate matter (PM). In an effort to characterize the cardiovascular, hematological and pulmonary effects of different PM size fractions in humans, we have conducted controlled human exposures of normal volunteers to ultrafine, fine, and coarse fraction PM concentrated from ambient air in Chapel Hill, NC. Healthy non-smoking male and female subjects between the ages of 18 and 35 participated in these studies. Exposures were undertaken with the use of particle concentrators fitted with size-selective outlets. These devices are capable of generating concentration factors between 10- and 20-fold over ambient levels. Lung function was assessed and bronchoalveolar lavage performed to measure lung inflammation and injury. Holter monitoring was used to characterize changes in heart rate variability and repolarization. Blood was obtained to characterize inflammation, acute phase response, and changes in clotting/coagulation factors. The design of these studies permits direct comparison of

the effects of concentrated ambient PM as a function of particle size. The results of these studies show reveal modest size fraction-dependent effects of concentrated PM exposure on cardiovascular, pulmonary and hematological parameters in normal adult human subjects. These findings have relevant implications for the design of future controlled exposure studies and the role of particle size fraction in the adverse health effects of PM exposure in humans. This abstract does not necessarily reflect EPA policy.

1806 THE EFFECTS OF COMBUSTION DERIVED NANOPARTICULATE ON VASCULAR AND ENDOGENOUS FIBRINOLYTIC FUNCTION IN MAN.

N. L. Mills¹, F. R. Cassee², D. E. Newby¹ and K. Donaldson³. ¹Centre for Cardiovascular Sciences, University of Edinburgh, Edinburgh, United Kingdom, ²Centre for Environmental Health Research, National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands and ³Centre for Inflammation Research, University of Edinburgh, Edinburgh, United Kingdom.

Increased exposure to particulate air pollution PM is a risk factor for death and hospitalization with cardiovascular disease. It has been suggested that the nanoparticulate component of PM is capable of translocating into the circulation with the potential for direct effects on the vasculature. This paper will focus on recent findings from inhalation exposures to filtered and unfiltered diesel engine exhaust, nano-sized carbon black and ambient fine+ultrafine particles in healthy non-smoking volunteers. The effects of exposure on vascular and endothelial function, endogenous fibrinolysis and ex vivo thrombosis were assessed. At levels encountered in an urban environment, inhalation of dilute diesel exhaust impairs two important and complementary aspects of vascular function in humans: the regulation of vascular tone and endogenous fibrinolysis. These important findings provide a potential mechanism that links air pollution to the pathogenesis of atherothrombosis and acute myocardial infarction.

1807 DO CLINICAL FINDINGS WITH PARTICLE EXPOSURES (INCLUDING AMBIENT PM FRACTIONS, CARBON, AND DIESEL EXHAUST) EXPLAIN EPIDEMIOLOGICAL RESULTS?

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During the past decade, acute adverse health effects of particulate matter (PM) pollution have been evaluated by:

- (a) epidemiologic time series studies of daily morbidity and mortality;
- (b) short-term observational epidemiologic studies of volunteer panels;
- (c) controlled exposures of lab animal or human volunteer subjects to artificially generated pollutant particles;
- (d) partly controlled exposures of subjects to concentrated ambient particles (CAPs) with differing size ranges (coarse, fine, ultrafine) and composition; and
- (e) field exposures of human volunteers with well characterized PM sources and monitored ambient emissions.

Current air quality standards are primarily based on epidemiologic (mostly time series) studies, which find adverse biological and clinical effects in a variety of urban PM environments. This research has found considerable evidence that combustion-derived PM elicits health effects, most notably systemic inflammation and cardiovascular morbidity and mortality. Mechanistic pathways have been explored in animal toxicology and human clinical studies. The results from these disciplines have been enlightening and yet not fully corroborative, consistent, or concordant with those from epidemiological studies. Nonetheless, further research may reveal missing links and ultimately show that multiple pathways exist, perhaps determined by different ambient PM modes (ultrafine, fine, and coarse), mixtures with different PM sizes and/or copollutant gases, and by susceptibility characteristics of the exposed population. Gaps in our knowledge should foster healthy skepticism and further scientific investigation.

1808 INTRODUCTION: CURRENT ISSUES IN DEVELOPMENTAL TOXICOLOGY.

S. L. Makris. *ORD/NCEA, U.S. EPA, Washington, DC.*

The maturing field of developmental immunotoxicology (DIT) is an area of interest and concern for children's health risk assessment. A strong body of evidence has demonstrated that the developing immune system is more susceptible to some chemicals (including some drugs and environmental contaminants) than the adult immune system, and that toxicant exposure during critical periods of immune system development can result in an adverse response that is quantitatively or qualitatively different from the adult response. Treatment-related effects can be morphological and/or functional, can exhibit evidence of greater severity or persistence following developmental exposures, and may be observed near the time of exposure

or expressed later in life. Recent research in the area of developmental immunotoxicity, the focus of this symposium, covers a broad range of immunotoxicological responses including hypersensitivity, autoimmunity, and immunosuppression following developmental exposures to chemical toxicants. Studies that consider critical processes and interactions in the developing immune system, explore alternative animal models, and characterize differential DIT responses and susceptible life stages/populations, contribute information that will be useful in developing a regulatory testing paradigm (i.e., DIT study protocol and test strategy) for characterizing immunotoxic hazard and potential risk to children from chemical exposures.



1809 AUTONOMIC AND NEUROENDOCRINE CROSS-TALK WITH THE IMMUNE SYSTEM: RELEVANCE TO EARLY LIFE EXPERIENCES, CHRONIC DISEASE, AND AGING.

D. L. Beller. *Pathology & Human Anatomy, Loma Linda University School of Medicine, Loma Linda, CA*. Sponsor: S. Makris.

Bi-directional communication exists between the nervous and immune systems. Both the hypothalamo-pituitary-target organ systems and the autonomic nervous system are major sources of neural-immune signaling, while cytokines act as signaling molecules for immune-neural signaling. Sympathetic nervous system (SNS) activation of the immune system via beta-adrenergic receptor (AR) stimulation can alter functional activities of neutrophils, antigen-presenting cells, T and B lymphocytes, and natural killer (NK) cells. In vitro and in vivo studies examining the role of SNS and AR signaling in immune regulation after immune challenge and under disease conditions suggest that the SNS can: (1) limit the magnitude of acute and chronic inflammatory responses; (2) promote Th2-driven antibody (Ab) response; (3) enhance cell-mediated responses; and (4) differentially affect Ab responses. In contrast, exposure to stressors or agents that activate the SNS (increasing circulating catecholamines) reduces T cell responses, anti-viral immune reactivity, and NK cell activity, reflecting the importance of the SNS in maintaining immune homeostasis. Similarly, hypothalamo-pituitary-adrenal cortical (HPA) activation modulates the immune system to eliminate pathogens and restore homeostasis. Studies have shown that HPA and SNS dysregulation affects severity and expression of viral and bacterial infections, wound healing, autoimmune reactivity, tumor growth, proliferation and spread, and immune senescence. During development, environmental stimuli can influence the maturation of stress pathways that modulate immune function with long term functional consequences. Because the SNS and neuroendocrine systems play an important role in numerous adult diseases, early life stressors affecting SNS and neuroendocrine development may contribute to the etiology of common disorders, such as obesity, hypertension, insulin resistance, and host resistance to infectious diseases, cancer and autoimmunity.



1810 AIR POLLUTANT EXPOSURE DURING PREGNANCY INCREASES NEONATAL SUSCEPTIBILITY TO ASTHMA.

L. Kobzik. *Environmental Health, Harvard School of Public Health, Boston, MA*. Sponsor: S. Makris.

In utero exposure to environmental agents can cause significant abnormalities in the developing immune system, but the mechanisms remain poorly understood. Asthma exemplifies the need for new models to study how environmental exposures in utero may alter immune responses. Epidemiologic and experimental data indicate that air pollution components, e.g., diesel exhaust particles (DEP), can promote allergic responses. We have adapted a previously developed mouse model of the maternal transmission of asthma risk to study exposure to air pollutants during pregnancy. The key finding is that baby mice show increased susceptibility to asthma after exposure of normal pregnant mice to diesel exhaust particles or aerosols of residual oil fly ash (ROFA) leachates. The increased susceptibility is manifest as allergic airway inflammation and airway hyper-responsiveness in response to aerosolized allergen (OVA, ovalbumin), after a suboptimal sensitization protocol that has minimal effects in normal baby mice. The potential of this model for evaluation of environmental exposures and for analysis of immune mechanisms will be discussed.



1811 DEVELOPMENTAL EXPOSURE TO TCDD INCREASES AUTOREACTIVE T CELLS IN SPLEEN OF C57BL/6 MICE AND IN LYMPH NODES OF SNF1 MICE AT 24 WEEKS OF AGE, AND CAUSES OTHER CHANGES CONSISTENT WITH INCREASED RISK OF AUTOIMMUNE DISEASE.

S. Holladay, R. Gogal, A. Mustafa and R. Kerr. *Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA*.

Developmental exposure of autoimmune-predisposed SNF1 mice to TCDD induces and exacerbates postnatal autoimmune lupus-like nephritis. Mechanisms that may singly or collectively underlie this environmental chemical effect on immune development were examined in these TCDD-insensitive SNF1 mice as well as

TCDD-sensitive C57BL/6 mice, exposed to vehicle or TCDD by maternal oral gavage on day 12 of gestation. In C57BL/6 mice, developmental TCDD exposure resulted in increased spleen cellularity in females but not males at 24 weeks of age. Numbers and percentages of CD4-CD8+ T cells, and of T cells expressing autoreactive CD4+ Vβ17a+ and CD3+ Vβ3+ TcR were significantly increased in both genders. Peripheral lymph nodes were hypocellular with histopathologic evidence of increased apoptosis. In the SNF1 mice, spleen weight was increased in males but not females at 24 weeks of age, such that the male spleen became equivalent in weight to the females. In these mice, the female spleen is normally significantly larger than the male, and females display more severe autoimmune disease and earlier lesions than males. Autoreactive CD4+ Vβ17a+ and CD3+ Vβ3+ TcR were not increased in SNF1 spleens at 24 weeks of age. However percentage expression of CD4+ Vβ17a+ T cells was increased by 70% and 110% in peripheral lymph nodes of TCDD-exposed males and females, respectively, compared to vehicle-exposed mice. Males, but not females, also showed increased lymph node expression of CD3+ Vβ3+ T cells, to 2.6 times percentages in vehicle-exposed mice. In both strains of mice, impaired deletion of autoreactive T cells was therefore suggested, as evidenced by increased peripheral numbers of these cells, and may relate to exacerbated postnatal autoimmune disease in the predisposed SNF1 mice. Supported by NIH R21-PAR-03-121.



1812 DEVELOPMENTAL IMMUNOTOXICOLOGY (DIT) AND LATER LIFE IMMUNE DYSFUNCTION.

R. R. Dietert. *Microbiology and Immunology, Cornell University, Ithaca, NY*.

The perinatal period of development is a time of key immune maturational events in the offspring. Additionally, it is a period of dramatic shifts in functional capabilities and balance within the immune system. In fact, exquisitely timed changes in fetal immune balance near birth are important for successful pregnancy maintenance and may influence parturition itself. In the fetus, certain immune capacities such as Th1-driven allogeneic responses need to be suppressed to avoid fetal-maternal rejection and miscarriage. However, for the neonate a significantly different immune balance, including enhanced dendritic cell maturation with Th-1 promotion, is needed to effectively meet postnatal disease challenges. Not surprisingly, many early-life immune maturational events are either exclusive to this period of development or have significantly different ramifications when they occur later in life. Given these life-stage associated differences in immune maturation, it is not surprising that a fully-matured immune system is an ineffective surrogate to model potential DIT risk. Results to date from DIT studies indicate that the developing immune system is far more sensitive to xenobiotic-induced immunotoxicity than that of the adult. This presentation will consider: 1) the immune developmental windows sensitive to immunotoxicants based on current data; 2) the types of errors encountered when adult-exposure modeling of DIT is employed; 3) the predisposition of the perinatal system for specific types of xenobiotic-induced immune dysfunction as related to risk of later life disease and 4) the benefits of DIT screening in safety assessment.



1813 DEVELOPMENTAL IMMUNOTOXICOLOGY AND RISK ASSESSMENT.

Y. G. Yang. *Office of Pesticides Program, U.S. EPA, Washington, DC*. Sponsor: S. Makris.

Recent research has demonstrated that the developing immune system is more susceptible to exposure of environmental chemicals and drugs than the fully mature immune system. There is a concern that immunotoxicity studies conducted exclusively in young adult animals would not detect this greater susceptibility. Several scientific workshops and roundtable discussions have been held to discuss a variety of aspects for developmental immunotoxicology assessment, including appropriate testing endpoints, methodologies and the evolving state of the science. This presentation will include an analysis of the current state of immunotoxicology testing for pesticides and other environmental chemicals, and a discussion of current policy and practice regarding the potential use of developmental immunotoxicity test data in risk assessment. (*Disclaimer: This presentation does not necessarily reflect the view or policies of the U.S. Environmental Protection Agency.)



1814 DEVELOPMENTS IN EXPOSURE-BASED QUANTITATIVE RISK ASSESSMENT (QRA) FOR INDUCTION OF DERMAL SENSITIZATION FOR FRAGRANCE INGREDIENTS.

A. Api¹ and P. M. McNamee². ¹Research Institute for Fragrance Materials, Inc., Woodcliff Lake, NJ and ²Procter & Gamble Company, Egham Surrey, United Kingdom.

Some of the chemicals in common use today may have the potential to cause dermal sensitization. However, the fact that a chemical is a skin sensitizer does not mean it cannot be formulated into consumer products at safe levels. This is also the

case for fragrance ingredients. Based on the chemical, cellular and molecular understanding of dermal sensitization, it is possible to conduct an exposure-based quantitative risk assessment (QRA) to determine safe levels of fragrance ingredients in different consumer product types. Key steps of the quantitative risk assessment process are determination of known safe benchmarks; application of sensitization assessment factors and calculation of consumer exposure through normal product use. Using these parameters, an acceptable exposure level (AEL) can be calculated and compared with the consumer exposure level (CEL). The ratio of the AEL to CEL must be favorable to support the safe use of the skin sensitizer. This ratio must be calculated for the skin sensitizer in each product type. This symposium provides an overall picture of how a recently developed exposure-based QRA approach is being newly applied to fragrance ingredients. Within the overall theme, each presentation addresses a specific element of this risk assessment methodology with the last presentation providing examples of implementing this QRA approach for induction of dermal sensitization to fragrance ingredients.

1815 AN INTRODUCTION TO QRA FOR INDUCTION OF DERMAL SENSITIZATION FOR FRAGRANCE INGREDIENTS.

P. A. Cadby. *Firmenich SA, 1211 Geneva 8, Switzerland.* Sponsor: [A. Api](#).

Since the 1960s, the fragrance industry has attempted to manage the risks of Type IV allergy to fragrance materials. Due to the paucity of reliable data on allergic reactions following the use of different types of perfumed articles, risk assessment has been typically based on predictive testing, most often on human subjects, to determine the potency of different substances in inducing allergic states. Earlier risk assessments by industry were based on the belief that passive absorption through the skin makes the concentration of a particular allergen on the skin the determinant metric of exposure. More recent evidence shows that this is not the case. Within certain limits, the quantity per surface area is a better measure of exposure. Furthermore, other factors previously ignored in risk assessment of fragrance materials must also be considered. These include the condition of the skin and body-region, the presence of other irritants, of other factors that increase penetration and residence time on the skin. For this reason, a new approach has been used for the risk assessment of fragrance allergens in different types of consumer products. A quantitative risk assessment (QRA) approach has been developed based on exposure and these other factors.

1816 TOOLS FOR THE IDENTIFICATION OF A MATERIAL AS A DERMAL SENSITIZER.

G. Gerberick. *Procter & Gamble, Cincinnati, OH.*

Different methods are available to identify a material as a dermal sensitizer. The different methods including their strengths and limitations will be described in this presentation. Since it is extremely important to understand allergenic potency in risk assessment, the recently approved LLNA will also be reviewed in terms of its ability to predict allergen potency in humans. Possible impact of future method developments e.g. in vitro assays will also be reviewed.

1817 THE IMPORTANCE OF USING DOSE/UNIT AREA AS THE DOSE METRIC IN QRA FOR INDUCTION OF SKIN SENSITIZATION.

I. Kimber. *Central Toxicology Laboratory, Syngenta, Cheshire, United Kingdom.*

Skin sensitization is acquired following topical exposure of inherently susceptible subjects to an amount of chemical allergen necessary to induce a cutaneous immune response of sufficient vigor, and of the appropriate quality. Of pivotal importance in the induction of skin sensitization are the roles played by cutaneous dendritic cells, and in particular epidermal Langerhans cells (LC). These cells are responsible for the recognition, internalization, processing, and subsequent presentation to T lymphocytes of allergen encountered at skin surfaces. There is now compelling evidence, deriving from both clinical and experimental studies, that the important exposure metric in the acquisition of skin sensitization is the dose of chemical per unit area of skin, rather than the total delivered dose. Thus, for instance, when a fixed amount of chemical allergen (2,4-dinitrochlorobenzene) was administered topically to human volunteers, then the effectiveness of sensitization was found to decrease as the area of skin over which the chemical was applied increased. The relationship between dose per unit area and the effectiveness of sensitization applies under most circumstances of exposure, although when chemical is administered over a very small area then total dose assumes greater importance. It is assumed that the relationship between the acquisition of sensitization and the dose of chemical per unit area of skin surface is a reflection of the requirements for inter-

action of allergen with epidermal LC. Specifically, it is proposed that the amount of allergen displayed per presenting cell directly influences the effectiveness with which T lymphocytes will be activated, and the vigor of the cutaneous immune response.

1818 THE PRINCIPLES OF QRA FOR INDUCTION OF DERMAL SENSITIZATION FOR FRAGRANCE INGREDIENTS.

P. M. McNamee. *Procter & Gamble Company, Egham Surrey, United Kingdom.* Sponsor: [A. Api](#).

This presentation describes the principles of an exposure-based QRA for induction of dermal sensitization as applied to fragrance ingredients. It specifically addresses the elements of this exposure-based risk assessment approach that are unique to the induction of dermal sensitization, while being consistent with the principles of general toxicology risk assessment. The key steps of the QRA process: 1) determination of benchmarks (No Expected Sensitization Induction Level or NESIL); 2) application of sensitization assessment factors (SAF); 3) calculation of consumer exposure (CE) through product; 4) calculation of an acceptable exposure level (AEL) 5) comparison with the consumer exposure level (CEL) and 6) comparison of the AEL to CEL and the impact of the AEL to CEL ratio are described for fragrance ingredients.

1819 APPLICATION OF QRA FOR INDUCTION OF DERMAL SENSITIZATION OF FRAGRANCE INGREDIENTS.

[A. Api](#). *Research Institute for Fragrance Materials, Inc., Woodcliff Lake, NJ.*

Using the principles of quantitative risk assessment for dermal sensitization, it is possible to determine acceptable exposure levels for fragrance materials in a variety of consumer products. No Expected Sensitization Induction Levels or NESILs for a group of fragrance allergens, categorized in potency classes, were based on a weight of evidence approach, which considers all data including guinea pig, mice (LLNA) and human predictive tests. In addition, sensitization assessment factors specific for fragrance materials in the Research Institute for Fragrance Materials, Inc., (RIFM) scientific program, were determined in different product types and supported by scientific data. Examples will be presented using fragrance ingredients in various consumer product types.

1820 THE SAFETY ASSESSMENT OF IMPROVED FOOD AND FEED CROPS DEVELOPED WITH GENE SUPPRESSION TECHNOLOGY.

B. G. Hammond. *Product Safety Center, Monsanto Company, St. Louis, MO.*

RNA interference (RNAi) has allowed agricultural researchers to develop new crop varieties such as Flavr Savr tomatoes, high amylose potatoes and even blue roses. RNAi is a promising tool for development of other new and improved plant varieties using agricultural biotechnology. Suppression of endogenous plant gene expression by RNAi is achieved through the introduction of a construct that produces double stranded RNA (dsRNA) in the plant. Plant RNAi machinery converts this larger dsRNA to 19-24 base pair small interfering RNAs (siRNAs). These siRNA molecules subsequently target complementary mRNA molecules for nuclease degradation, resulting in gene suppression. To date, there has been limited discussion in public scientific forums regarding appropriate safety assessment considerations for products derived using this technology. The speakers in this symposium will address topics to foster greater understanding of the use of this technology and of the safety considerations for products derived using this technology. The opening presentation will focus on the mechanisms of RNAi and on manufacture and chemical modification of synthetic siRNA molecules for in vivo use, from a leading commercial siRNA supplier. In the second presentation, the use of siRNA to treat human disease and the relevant safety considerations for siRNA use in therapeutics will be addressed by a representative of a leading company specializing in siRNA-based therapeutics. The third presentation will include a discussion of the function of RNAi in plants, applications of gene suppression technology in agricultural biotechnology, and possible applications for RNAi in future development of improved plant varieties. The closing talk will present a safety assessment strategy for using RNAi in agricultural products, building upon the current understanding of gene suppression in animals and plants, coupled to what has been learned both in human pharmaceutical applications and in the safety assessment of agricultural products derived using modern biotechnology.



1821 THE BIOCHEMISTRY OF RNA INTERFERENCE (RNAi): IMPACT ON DESIGN, SYNTHESIS, AND MODIFICATION OF RNA DUPLEXES FOR *IN VITRO* AND *IN VIVO* USE.

M. Behlke. *Molecular Genetics and Biophysics, Integrated DNA Technologies, Inc., Coralville, IA.* Sponsor: B. Hammond.

RNA interference (RNAi) is an evolutionarily conserved pathway present in organisms ranging from plants to humans, in which double-stranded RNA (dsRNA) triggers a series of biochemical events culminating in sequence-specific suppression of gene expression. Within "RNAi", related but distinct biochemical pathways exist which target different steps of gene expression. MicroRNAs (miRNAs) comprise an endogenous gene-regulatory network which suppresses protein translation in the cytoplasm. Small interfering RNAs (siRNAs) direct mRNA degradation in the cytoplasm and may also be capable of suppressing transcription in the nucleus, either directly or via chromatin remodeling. Knowledge of RNAi biochemistry can be used to guide intelligent design of experiments, protocols and reagents. For the degradative RNAi pathway, long dsRNAs are cleaved by the RNase-III class endoribonuclease Dicer into 21-23 base duplexes having 2-base 3'-overhangs. These species, called siRNAs, enter the RNA Induced Silencing Complex (RISC) and serve as a sequence-specific guide to target degradation of complementary mRNA species. Synthetic siRNAs can be used as an experimental tool to selectively reduce expression of targeted genes. In addition to utility in research applications, several siRNA drugs are already undergoing clinical trials as therapeutic agents. As drugs, nucleic acids (both antisense oligos and siRNAs) can be administered by a variety of routes including IV, IP, SQ, intrathecal, direct local injection, or as a topical cream; oral bioavailability is generally poor. Chemical modification of the siRNA can confer nuclease stability and improve pharmacodynamic properties. Building upon historical work done in the area of antisense therapeutics, the medicinal chemistry of therapeutic siRNAs is already advanced and a number of effective chemical modification strategies have been described.



1822 SAFETY CONSIDERATIONS FOR THE USE OF SIRNA IN THE PHARMACEUTICAL INDUSTRY: DEVELOPMENT OF AN SIRNA FOR THE TREATMENT OF VIRAL HEPATITIS.

J. Lockridge, D. V. Morrissey, C. Vargeese and B. Polisky. *Sirna Therapeutics, Inc., Boulder, CO.*

Development of siRNAs as therapeutic agents will require improvements in not only the stability of siRNAs but also in the efficiency and specificity of tissue-targeted delivery in vivo. Considerable progress has been made in developing chemical modifications for synthetic siRNAs that provide resistance to nuclease degradation and are also compatible with proper recognition and function of the siRNA by cellular RNAi protein machinery. However, efficient delivery modalities will likely be needed in addition to stabilization chemistry in order to fully realize the therapeutic potential of siRNAs. A method for lipid nanoparticle (LNP)-mediated delivery of stabilized siRNA has been developed and initially characterized in animal models for HBV. Significant reduction of viral titer has been observed with this formulation at therapeutically relevant doses that correlate with improvements in pharmacokinetics. Both in vitro and in vivo models have been used to assess the toxicity profile and target organ toxicity of this LNP-formulated siRNA. The findings from this preclinical siRNA antiviral program will be used to discuss potential safety considerations for administering siRNA systemically to humans.



1823 THE USE OF GENE SUPPRESSION TECHNOLOGY TO DEVELOP IMPROVED CROPS.

W. Parrott. *Crop and Soil Sciences, University of Georgia, Athens, GA.* Sponsor: B. Hammond.

Gene suppression technology has been used successfully in agriculture since before WWII, long before its practitioners knew the biological basis of this phenomenon. The discovery that fruit trees could be protected from severe viral infection by intentional inoculation with a milder strain of the virus led to development of plants genetically engineered to express viral genes that confer resistance to the target virus. Recent investigations into the basis behind transgene-mediated virus resistance led to the discovery that plants possess a gene-silencing mechanism triggered by presence of double-stranded RNA. This phenomenon is now known as RNA interference (RNAi). The discovery of RNAi provided a unifying concept in plant genetics, and explained everything from developmental pathways, such as organ differentiation, to pigmentation patterns in flowers and seeds. Not only does RNAi

explain natural phenomena, it also explains the apparently random gene silencing patterns that were identified in various engineered plants, and clarifies why both over-expression and antisense expression of genes leads to silencing. In hindsight, biotech products such as the FlavrSavr tomato, and virus-resistant squash and papaya are examples crops that utilize RNAi technology. The advent of RNAi technology is timely and provides an effective means to overcome the lack of homologous recombination in plants, which has hindered previous efforts at gene suppression in plants. Now that the RNA-based gene silencing mechanism is well understood, it is possible to design vectors that readily generate double-stranded RNA that silence the target gene when engineered into plants. This approach is proving effective at achieving pest resistance, including resistance to viruses and nematodes. The use of RNAi also can alter composition of plant products, such as oil and protein, to enhance their nutritional quality or tailor them for specialty purposes. For example, silencing of genes involved in production of linolenic acid results in increased content of oleic acid. Thus, RNAi will be a key technology for future development of improved agricultural products.



1824 SAFETY ASSESSMENT STRATEGY FOR FOOD AND FEED FROM BIOTECHNOLOGY-DERIVED CROPS DEVELOPED USING GENE SUPPRESSION TECHNOLOGY.

J. Petrick. *Product Safety Center, Monsanto Company, St. Louis, MO.*

RNA interference (RNAi) is an endogenous process mediating transcriptional and translational silencing in plants and animals. Plant breeders are using RNAi to develop new plant varieties that have improved nutritional properties or enhanced pest or viral resistance. A safety assessment approach is proposed that ensures new crop varieties developed using RNAi are safe to consume. This strategy includes: (1) Establishing a history of safe consumption: Plants possess endogenous dsRNA-mediated gene-silencing mechanisms, and thus, small interfering RNAs (siRNAs) and other dsRNAs are widely consumed in the diet. These endogenous small dsRNAs will be identified in plants to demonstrate their presence in the food/feed. (2) Assessing the potential digestibility of siRNAs and dsRNAs in simulated digestive fluids containing nucleases will be confirmed experimentally. Based on available literature, siRNAs and dsRNAs are likely to be readily digested by endogenous nucleases. (3) Confirmatory animal studies will test whether oral administration of naked siRNAs or food containing dsRNAs or siRNAs exerts any adverse effects. Existing animal studies show that effective intravenous or intraperitoneal administration of naked siRNAs requires large doses and may require carrier agents or stabilizing modifications. This observation indicates that naked siRNAs in food or feed are unlikely to be orally active. The dietary exposure of laboratory animals to dsRNA or siRNA will be considerably in excess of potential human exposure levels, thus providing a large margin of safety. (4) The absence of unintended changes in food/feed will be tested using comprehensive agronomic assessment and compositional analyses for key nutrients and antinutrients, if present. In light of the specificity of RNAi, the proposed lack of oral activity of siRNA and dsRNA, and the history of safe RNA consumption, this adaptation of the current safety assessment paradigm for biotechnology-derived food and feed crops is considered suitable for evaluation of those crops produced using gene suppression technology.



1825 TARGET-BASED TOXICITY OF NOVEL PHARMACEUTICAL AGENTS.

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The toxicity of a pharmaceutical compound can be due to its interactions with an unanticipated molecule in the body (off-target) or the direct consequence of inhibiting or stimulating the molecular target (target-based). Target-based toxicities are especially likely when the target has many roles in multiple tissues. In some situations, target-based toxicity can be accepted as an unpleasant but inevitable side effect of a life-saving drug, but in other situations, target-based toxicity is unacceptable. In those cases, early recognition of target-based toxicity enables a company to avoid further investment in a program that cannot achieve success. This early recognition enables pharmaceutical companies to focus on more viable therapeutic targets and increase the likelihood of success to market. In other cases, target-based toxicity can be specific only to particular preclinical species and is not relevant to human toxicity. A variety of approaches have been used to identify target-based toxicity and to put issues into an appropriate risk-assessment perspective. This session will explore these approaches and give a flavor of how scientists are integrating such approaches at earlier phases of drug discovery and development.

1826 USE OF TRANSGENICS, KNOCKOUTS, AND CONDITIONAL KNOCKOUTS IN TARGET DISCOVERY AND VALIDATION.

E. J. Galbreath. *Discovery Biology Research and Clinical Investigation, Lilly Research Laboratories, Indianapolis, IN.* Sponsor: G. Cantor.

Each completed genome provides vast sequence and variation data that pharmaceutical discovery research is actively translating into functional biologic assessments. The ultimate goal is to understand the therapeutic potential of gene function and validate targets in the context of the whole animal. Strategies to elucidate function include genetic overexpression of proteins of interest, elimination or knock-down of target expression and tissue-specific or time-course regulatory control of gene expression. This overview will review the more common approaches and provide examples of how in vivo research of gene function and target validation has led to new understanding of gene function in the whole animal, including the potential for exuberant pharmacology and identification of potential detrimental effects.

1827 MECHANISM-BASED HEPATOTOXICITY OF TUMOR NECROSIS FACTOR α (TNF α) CONVERTING ENZYME INHIBITORS.

L. Lehman-McKeeman. *Discovery Toxicology, Bristol-Myers Squibb, Princeton, NJ.*

TNF α is an important mediator of inflammatory diseases, and numerous agents have been developed to neutralize its activity or suppress its production. Chemical inhibitors of TNF α converting enzyme (TACE) reduce the processing and release of mature TNF α and are effective in animal models of inflammatory diseases. However, TACE inhibitors were also hepatotoxic in several species, producing early liver lesions manifested as intra-sinusoidal aggregates of mononuclear cells that progressed to large mononuclear cell aggregates, Kupffer cell hypertrophy and hyperplasia and hepatocellular necrosis. To evaluate the mechanisms underlying the development of lesions, studies were carried out to determine whether hepatotoxicity was caused by unique properties of the compounds, by unexpected off-target effects or by untoward effects associated with TACE inhibition. Results indicated that adverse effects in liver were directly associated with TACE inhibition. Evidence for mechanism-based toxicity included: (1) elimination of gut-derived endotoxin did not reduce the severity of hepatotoxicity suggesting that toxic effects were observed in the absence of an endogenous signal for hepatic TNF α secretion, and (2) inactive enantiomers of TACE inhibitors were not hepatotoxic. The uncleaved, transmembrane proTNF α is biologically active, and may accumulate when TACE is inhibited. Furthermore hepatotoxicity was reported in transgenic mice engineered to overexpress the TNF-R2 receptor, which is also a substrate for TACE. Studies conducted to determine whether hepatic levels of pro-TNF α and/or TNF receptors increased during TACE inhibition indicated that proTNF α increased to 4-times control, whereas TNF-R2 increased approximately 2-fold over control levels. Collectively, these studies demonstrate that the hepatotoxicity of TACE inhibition is a mechanism-based effect resulting from decreased shedding of the TACE substrates proTNF α and TNF-R2. These studies also provide a general experimental strategy for determining whether toxicity is directly related to the mechanism of drug action.

1828 ANGIOGENIC INHIBITORS: TARGET-RELATED EFFECTS AND RISK ASSESSMENT USING A MULTISYSTEM PHYSIOLOGIC APPROACH.

R. A. Westhouse. *Discovery Toxicology, Bristol-Myers Squibb Co., Princeton, NJ.* Sponsor: G. Cantor.

Because angiogenesis plays a significant role in the pathogenesis of numerous diseases, such as cancer, wet macular degeneration and inflammation, the inhibition of this process is commonly considered a lucrative therapeutic target. Angiogenesis is a critical factor for a number of normal physiologic processes, such as wound healing, endochondral ossification related to bone growth, estrous cycling, and fetal development. The inhibition of angiogenesis will directly impact these processes. A number of angiogenic inhibitors, either already marketed or in development, target the vascular endothelial growth factor (VEGF) pathway by various mechanisms (antibodies to the receptor or ligand, small molecule receptor tyrosine kinase inhibitors, ligand traps, etc). Angiogenic inhibitors acting through the VEGF pathway, regardless of the specific interventional mechanism, have been confirmed to affect these processes and also cause VEGF target specific effects, such as hypertension, in nonclinical and clinical studies. A multisystem physiology-based mechanistic approach was used to associate various findings in preclinical models for the purpose of a broad-based robust risk assessment and management. This type of risk assessment for VEGF inhibitors has revealed the utility of using one of these target-related effects, specifically, hemodynamic changes, as an indicator of pharmacodynamic effect and as a surrogate biomarker for efficacy in clinical studies. The use of

these biomarkers in clinical development allowed critical decisions to be made early, instead of having to rely on time to tumor progression data, which would come much later in time.

1829 ANDROGEN DEPENDENT MAMMARY GLAND VIRILISM IN RATS GIVEN THE SELECTIVE ESTROGEN RECEPTOR MODULATOR LY2066948 HYDROCHLORIDE.

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In the rat, male and female mammary glands exhibit striking morphologic differences that can be altered secondary to disruption of the normal hormonal milieu. Mammary glands from female rats given the Selective Estrogen Receptor Modulator (SERM) LY2066948 hydrochloride (LY2066948) for one month had morphologic alterations characterized by intralobular ducts and alveoli lined by multiple layers of vacuolated, hypertrophied epithelial cells. This altered mammary gland ductal and alveolar morphology in SERM-treated female rats resembled the morphology of these structures in the normal male rat mammary gland. We will describe investigative studies that demonstrated the mechanism and species-specific nature of these changes and will discuss how these data impacted the nonclinical risk assessment of the mammary gland finding in rats.

1830 INTRODUCTION TO TOXICOGENOMICS IS A TEAM SPORT.

G. A. Boorman. *Environmental Toxicology Program, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC.*

There is increasing use of toxicogenomic studies by research groups in academic, government, pharmaceutical and chemical settings. Toxicogenomic data is beginning to be submitted in support of product development to government regulatory agencies. Geneticists, molecular biologists and bioinformaticists often conduct the studies but toxicologists and pathologists are often approached to become involved with study interpretation. While the amount of data generated by these studies is massive, toxicologists and pathologists are crucial in putting the genomic data in context. Toxicologists and pathologists can make their results more assessable to the bioinformaticists by transforming the diagnoses into a digital format. They also need to effectively communicate the importance and significance of the toxicological data endpoints. The most effective toxicogenomic studies utilize the expertise of toxicologists and pathologists in formulating the research question, study design, selection of appropriate animal models, animal care issues, nutrition, tissue and data collection all of which can have significant impact on the resulting data. Toxicogenomics is a team sport that to be successful requires the active participation of pathologists and toxicologists.

1831 VISUALIZATION OF HISTOPATHOLOGICAL DATA ENHANCES BIOLOGICAL INSIGHT AND INTERPRETATION.

E. K. Lobenhofer. *Investigational Genomics, Cogenics, a Division of Clinical Data, Morrisville, NC.* Sponsor: G. Boorman.

Gene expression profiling, metabolomic screens, and other high-dimensional methods have become an integral part of many biological investigations. To facilitate interpretation of these data, it is important to have detailed phenotypic data – including histopathology – to which these data can be associated, or “anchored”. However, as the amount of phenotypic data increases, associations within and across these data can be difficult to visualize and interpret. We have developed an approach for categorizing and clustering biologically related histopathological diagnoses to facilitate their visualization, thereby increasing the possibility of identifying associations and facilitating the comparison with other data streams. To demonstrate the utility of this approach, a case study will be presented in which histopathological data generated as part of a standardized toxicogenomics compendium study was used to categorize and compile histopathological diagnoses in order to develop visualizations that facilitate biological insight that were further supported by gene expression profiling data.

1832 INTERPRETATION OF TOXICOGENOMICS DATA AND CONFOUNDING VARIABLES, WITH SPECIAL REFERENCE TO FASTING AND THE HEPATIC TRANSCRIPTOME.

K. T. Morgan and Z. Jayyosi. *Drug Safety Evaluation, Sanofi-Aventis, Bridgewater, NJ.*

The liver plays a central role in many aspects of whole-body physiology, including regulation of energy metabolism, immune and endocrine functions. Toxicogenomics (TGx) studies, with their large-scale view of the hepatic transcriptome(s), provide an opportunity to explore many aspects of liver physiology and pathophysiology. A transcriptome is 'considered to be the entire population of mRNA transcripts in a cell or cell population at a given moment in time.' A whole-liver TGx study provides a measure of a mixture of such transcriptomes, each present in proportion to the population size of their cell type of origin. Cell type is but one of many confounding variables in hepatic TGx studies. Others include sexual cycles, strain and age of animals and their husbandry, circadian rhythms, inter-individual animal variation, the presence of pathophysiological events in extra-hepatic sites, and pharmacologic action of test articles. Fasting has the potential to influence a large proportion of TGx studies because (a) fasting is often selected in toxicology study protocols, (b) toxic responses to high doses of test articles can cause test subjects to go off of their food, and (c) a broad range of hepatic functions are altered during the fasting state. Thoughtful examination of TGx data, with due consideration of confounding variables, can generate a rich source of insights for the design of critical follow-up studies.

1833 DIFFERENTIAL RESPONSE OF THE LEFT AND MEDIAN LIVER LOBES TO TOXIC CHALLENGE.

R. Irwin¹, G. A. Boorman², J. S. Parker³ and E. K. Lobenhofer⁴. ¹NIEHS, Durham, NC, ²NIEHS, Durham, NC, ³Constella Group, Durham, NC and ⁴Congenic, Durham, NC.

Although the liver in most species of mammals is composed of four separate lobes, it is generally assumed that the lobes are functionally equivalent. However several liver disorders exhibit a distinct lobe preference, including Wilson's disease (right lobe), progression of cirrhosis (right lobe), and iron storage disease (left lobe), suggesting that lobes may respond differentially to the challenge posed by these diseases. We hypothesized that functional heterogeneity among lobes might be revealed in their response to a toxic challenge. To explore this possibility we examined the gene expression profiles of the left and median lobes of male F344 rats administered toxic and non toxic doses of acetaminophen. Using a variety of analytical tools we find significant time and dose related differences in the response of the left and median lobes to acetaminophen exposure.

1834 PATTERNS OF DIFFERENTIAL GENE EXPRESSION IN LIVER FOLLOWING HEPATOTOXICANT EXPOSURE.

K. E. Gerrish and R. S. Paules. *NIEHS, Research Triangle Park, NC.*

Our studies are designed to gain insight into mechanisms of liver injury or disease and to link gene expression alterations to specific parameters of well-defined indices of injury and disease. One central component of this research strategy is the concept of phenotypic anchoring in which alterations in gene expression are correlated with specific adverse effects of environmental stresses defined by conventional parameters of toxicity and pathology. To accomplish this task, we have utilized classical hepatotoxicants at multiple doses and times of treatment in order to study the biological responses to these agents. Clinical chemistry and histopathological analysis indicated a dose and time-dependent increase in hepatotoxicity. Analyses of differentially expressed genes suggest that many biological pathways play a critical role in regulating the liver's response to these agents. These include processes that disrupt normal liver function as well as those that promote cellular survival in response to stress. Results suggest that translational and transcriptional programs known to regulate bile acid homeostasis, drug metabolism and the unfolded protein response, play an important role in the liver's response to xenobiotic exposure. In summary, gene expression measurements in liver have the potential to yield a comprehensive insight into cellular pathways that are activated following exposure to hepatotoxicants.

1835 KIM-1, CLUSTERIN AND HO-1 AS POTENTIAL BIOMARKERS FOR NEPHROTOXICITY TESTING IN VITRO.

E. Rached, W. Dekant and A. Mally. *Department of Toxicology, University of Würzburg, Würzburg, Germany.*

The kidney is one of the main targets of xenobiotic induced toxicity, but early detection of renal damage is often difficult. Recently, several novel biomarkers of nephrotoxicity have been identified in vivo by transcription profiling, including

heme oxygenase-1 (HO-1), clusterin, and kidney injury molecule-1 (KIM-1). Changes in the expression of these markers were frequently observed before nephrotoxicity was evident by traditional endpoints, suggesting that they may serve as sensitive endpoints for nephrotoxicity detection in vivo. However, it is not known if these cellular marker molecules may also be useful for detection of nephrotoxic effects in vitro.

The aim of this study was to determine if changes in the expression of HO-1, clusterin and KIM-1 occur in proximal tubule cell cultures in response to sub-lethal concentrations of model nephrotoxins. Normal rat kidney epithelial cells (NRK-52E) were treated with CdCl₂, cisplatin, KBrO₃, and ochratoxin A (OTA) for 24-48h, and expression of HO-1, clusterin and KIM-1 was analyzed by qRT-PCR and immunoblotting. Treatment with CdCl₂ or KBrO₃ for 24 and 48h resulted in concentration-dependent increase in HO-1, whereas even cytotoxic concentrations of OTA and cisplatin had little or no effect. Expression of clusterin was induced following treatment with 5 µM cisplatin for 48h, but inconsistent changes were observed by immunoblotting and qRT-PCR in response to CdCl₂, KBrO₃ and OTA. Although KIM-1 was previously shown to be up-regulated in various models of nephrotoxicity in vivo, modulation of kim-1 gene expression was only observed after treatment with cytotoxic concentrations of CdCl₂, OTA, and KBrO₃ in NRK-52E cells, indicating that KIM-1 may not be a sensitive marker in vitro.

In summary, altered expression of HO-1 and clusterin, but not of KIM-1, was detected in NRK-52E cells treated with non-cytotoxic concentrations of model nephrotoxins. However, responses varied between compounds and may, therefore, depend on the mechanism of toxicity.

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1836 EVALUATION OF KIDNEY TOXICITY BIOMARKERS IN HUMAN KIDNEY CELLS.

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Renal toxicity is one of the common side effects that can occur on administration of pharmaceutical agents. Traditional markers of nephrotoxicity, like BUN and Creatinine, are not very sensitive and are expressed when two-thirds of the kidney is damaged. The goal of this study was to evaluate the expression of four more recently discovered nephrotoxicity biomarkers, clusterin, alpha-GST, osteopontin and neutrophil gelatinase-associated lipocalin (NGAL) in human proximal tubular kidney (HK2) cells. In this study, cisplatin was selected as the model nephrotoxin while transplatin was selected as negative control. The cells were treated with various concentrations of the toxin ranging from 0-150 mM. After 24hrs, ATP levels were measured in control and treated cells as an indicator of cytotoxicity while expression of biomarkers was measured using ELISA assays using commercially available kits. Dose dependent cytotoxicity was seen in HK2 cells with cisplatin while transplatin treated cells did not exhibit toxicity at any concentration in the dosing range. In cisplatin-treated cells, an increase in NGAL, osteopontin, alpha GST and clusterin was noted at concentrations lower than when significant cytotoxicity was observed. This indicates that these biomarkers can be used as early biomarkers of kidney toxicity in HK2 cells. On the other hand, the levels of the four biomarkers remain unchanged at all concentrations in transplatin-treated cells, corroborating that non-nephrotoxic compounds do not induce these biomarkers in HK2 cells. Further studies have shown that NGAL levels were increased in cells treated with other nephrotoxins like amphotericin B, cyclosporin A and puromycin at concentrations lower than needed to achieve significant cytotoxicity. The results imply that in an in vitro kidney cell model, these biomarkers provide a sensitive and predictive assessment of cytotoxicity and can be used for screening compounds early in drug development for potential proximal tubular toxicity.

1837 EVALUATION OF NOVEL BIOMARKERS OF NEPHROTOXICITY IN RATS TREATED WITH CISPLATIN.

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Cisplatin is an anticancer agent that induces renal proximal tubule lesions in rats. Studies were conducted in Sprague-Dawley (SD) and Han-Wistar (HW) rats to evaluate the utility of novel preclinical biomarkers of nephrotoxicity with this compound. Groups of 10 males were given a single intraperitoneal injection of 0.3, 1 and 3 mg/kg cisplatin and were sacrificed at Days 2, 3 and 5. The novel biomarkers GSTα (for tubular proximal lesions), GSTμ (for tubular distal lesions), clusterin (for general kidney lesions) and RPA-1 (for papilla/collecting ducts lesions) were

measured in urines using enzyme immunoassays. Histologically, degeneration and necrosis of the S3 segment of the renal proximal tubule were observed with 1 and 3 mg/kg cisplatin at Day 2 (HW), Day 3 (both strains) and Day 5 (both strains). Serum creatinine and BUN were significantly increased with 3 mg/kg cisplatin in SD rats (1.7 and 1.6-fold controls, respectively) and HW rats (2.5 and 3.5-fold, respectively) at Day 5 only. The novel biomarker GST α was clearly increased at Day 3 and Day 5 in SD rats (2.9 and 3.6-fold, respectively) and HW rats (3.1 and 20-fold, respectively) given 3 mg/kg. Clusterin was strongly increased at Day 5 in SD rats (4.5-fold) and HW rats (23-fold) given 3 mg/kg. An increase of clusterin was also observed at Day 3 in SD rats treated with 3 mg/kg cisplatin (2.2-fold) and at Day 5 in HW rats given 1 mg/kg (3.2-fold). These results suggest that GST α and clusterin are more sensitive than the traditional biomarkers tested. The novel urinary biomarkers GST μ and RPA-1 were increased at Day 5 in SD rats (1.8 and 1.5-fold, respectively) and HW rats (2.6-fold for both) given 3 mg/kg, and RPA-1 was increased at Day 3 in SD rats at this dose (2.6-fold). These changes may indicate concomitant damage of the distal tubules and/or the collecting ducts.

1838 EVALUATION OF NOVEL BIOMARKERS OF NEPHROTOXICITY IN RATS TREATED WITH GENTAMICIN.

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As part of HESI Nephrotoxicity Biomarker Initiative, male Sprague Dawley rats (n=10-20/group) were treated orally for 7 or 14 days with a proximal tubular toxicant, gentamicin sulfate, at doses of 0, 5, 25, 50 or 100 mg/kg/day. Urine collected after 3, 7 and 14 days of dosing was analyzed for traditional biomarkers and by EIA for α - and μ -GST (proximal and distal tubule injury markers, respectively), clusterin (general renal injury marker) and RPA-1 (renal papillary antigen-1; collecting duct injury marker). Clinical pathology and histopathology assessments were performed on a set of control and high dose rats dosed for 7 days and for all groups after 8-14 days (10 moribund high dose animals euthanized after 8 and 10 days of dosing). Moderate to severe proximal tubular necrosis and regeneration were significant changes in high dose rats after 7, 8, or 10 days. Dose-dependent proximal tubular necrosis, regeneration and tubulointerstitial nephritis were significant changes in the 5, 25 and 50 mg/kg groups after 14 days. Increases in RPA-1 and clusterin excretion were first detected at day 3 in high dose rats and at day 7 in other groups. At day 3 urine NAG (N-acetyl glucosaminidase) activity, urine protein, granular casts were increased in high-dose rats, and at day 7 in 50 mg/kg group. Increases in excretion of α -GST and μ -GST were first detected at day 7 in 50 and 100 mg/kg groups. Excretion of α -GST in 5 and 25 mg/kg groups increased by day 14, but excretion in 50 mg/kg group was less at day 14, likely due to severe necrosis with loss of tubules in these rats. Urinary μ -GST peaked on day 14 in 5, 25, 50 mg/kg groups and was highest on day 10 in surviving high-dose rats. Serum BUN and creatinine were increased at day 7 in high dose rats and at day 14 in only 50 mg/kg group. In summary, tubular injury had associated increases in both traditional (protein, casts, NAG) and novel biomarkers (clusterin, RPA) at 3 days. Additional study is needed to define the relationship between increases in RPA, clusterin, NAG and chronology of morphologic injury.

1839 EVALUATION OF NOVEL MARKERS OF NEPHROTOXICITY IN A RATS TREATED WITH NPAA.

G. R. Betton¹, D. T. Davies¹, T. Lambert², D. Ennulat², S. Gould¹ and C. HESI Biomarkers³. ¹AstraZeneca, Macclesfield, Cheshire, United Kingdom, ²GlaxoSmithKline, King of Prussia, PA and ³HESI, Washington, DC. Sponsor: S. Pettit.

As part of the ILSI-HESI nephrotoxicity initiative, Han Wistar (HW) and Sprague Dawley (SD) rats (n=15/group) were treated orally for 14 days with N-phenylanthranilic acid (NPAA) at toxic (350-500mg/kg/day, mkd) and non-toxic (50mkd) doses. Urine collected overnight on days 4, 8 and 15 was analysed for standard markers and by EIA for α - and μ -GST (for proximal and distal tubule, respectively), clusterin (general renal injury marker) and RPA-1 (renal papillary antigen-1, for collecting duct injury). Papillary necrosis occurred in 8/15 HW and all high dose SD rats on day 15 and in a subset of high dose HW and SD rats on day 8. Other findings included cortical tubular degeneration and/or basophilia, localized by immunohistochemistry to cortical collecting duct, distal tubule and thick ascending limb. Rats given 50 mkd NPAA had no treatment-related findings. Standard urinalysis was generally unremarkable. Paradoxical decreases in α - and μ -GST excretion (61-74%) occurred in both strains. RPA-1 and clusterin were maximally increased in HW and SD rats on day 4 or 8 and were decreasing on Day 15, with the largest increases in both RPA-1 (24.8 fold) and clusterin (12.4 fold) in HW rats. Clusterin increases were greatest in HW rats with full necrosis and were highly variable in SD rats with papillary necrosis. In the NPAA model, degenerative

changes affecting the collecting duct in both cortex and papilla commence on day 7, and only a proportion of animals progress to full papillary necrosis with irreversible loss of the papillary tip. RPA-1 increases were observed prior to necrosis from day 4 onwards in both rats with full papillary necrosis and those with earlier pathologies. As RPA-1 is a specific collecting duct marker, early (day 4) appearance of RPA-1 in the urine prior to full papillary necrosis supports its utility in early collecting duct injury.

1840 A HESI EVALUATION OF NOVEL BIOMARKERS OF NEPHROTOXICITY.

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This work was conducted as part of a HESI collaborative program with the goal of evaluating new bridging biomarkers (BMs) of toxicity in order to establish their scientific and regulatory acceptance. There is agreement among industrial, academic and regulatory professionals on the need to develop better renal BMs that can report toxicity before the occurrence of irreversible damage, or that can help monitor low-grade nephrotoxicity in the clinic. Better understanding of the pathophysiology of nephrotoxic processes, combined with information arising from studies using 'omic-based approaches, has led to the identification of promising new BMs. Following adequate validation, these have the potential to serve as sensitive, site-specific BMs of nephrotoxicity with application in both the preclinical and clinical setting. Four candidate BMs were selected by the HESI consortium to enter the experimental program of acute and chronic rodent studies using region-specific kidney toxicants. These 4 novel BMs are: α - and μ -GST, localized in the proximal and distal tubules respectively, renal papillary antigen-1 (RPA-1), specific to the collecting duct, and clusterin, which is more generally distributed in the nephron. Enzyme immunoassays (EIAs) were either available (the GSTs) or were developed and their performance characteristics assessed (RPA-1 and clusterin). Six laboratories have contributed to the analytical validation of the EIAs that included assessment of linearity, recovery, repeatability (intra-run precision), intermediate precision (intra-lab precision) and reproducibility (inter-lab precision). The assays performed very well with respect to linearity ($r^2 > 0.98$) and recovery (90 – 110%). The acceptance criteria for repeatability and intermediate precision were pre-set at < 25% and were generally <10%. It is concluded that the EIAs for these BMs are fit for use in biological qualification studies.

1841 NEPHROTOXICITY BIOMARKER DEVELOPMENT: SUCCESSFUL TRANSFER FROM A DISCOVERY PLATFORM TO A SCREENING PLATFORM, I.E. MICROARRAY TO MULTIPLEX.

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Microarrays have successfully been utilized in the area of biomarker discovery providing useful tools to probe the whole genome during this phase of the biomarker development process. Once putative biomarker(s) are identified, there is a need for a higher throughput platform to determine the expression level of these genes in multiple samples. Currently low complexity real time assays are developed for each biomarker and used to monitor the gene expression of each biomarker separately. This is not optimum because of the cost of each individual assay, as well as the large sample requirement for multiple reactions, may provide significant hurdles. We have addressed these challenges by developing a multiplex RT-PCR assay incorporating genes that may be candidate biomarkers of nephrotoxicity and re-screening the samples that were used in the initial discovery process.

Rats were treated with model nephrotoxicants mercuric chloride, 2-bromoethylamine, and puromycin at two different doses, and tissue samples were collected 1, 3, and 7 days post dosing. Gene expression profiles were generated from kidney RNA using rat microarrays and analyzed in conjunction with histopathology. A set of potential biomarkers showing a time and dose response with respect to the proximal progression of proximal tubular toxicity were identified. A subset of these genes, were selected to be included in a multiplex assay to confirm concordance of data generated from the two platforms and to develop an assay to screen samples for monitoring the expression of nephrotoxicity biomarkers (Thukral et al., 2005). Data generated from the RT-PCR assay correlated to microarray data which in turn was reflective of the pathology seen at each time point. In summary, microarray discoveries can be successfully transferred to a higher throughput gene expression platform for sample analysis and evaluation.

References:

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1842 QUALIFICATION OF BIOMARKERS FOR REGULATORY DECISION MAKING – A KIDNEY SAFETY BIOMARKER PROJECT.

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In drug development, the use of early, sensitive and reliable predictors of safety and efficacy is primordial. In the last years, several "Exploratory Biomarkers" have been identified by a number of groups. It is expected that some of them can become appropriate for regulatory decisions as "Probable Valid Biomarkers" or "Known Valid Biomarkers" after submission to and approval by regulatory authorities.

Following a Cooperative Research and Development Agreement (CRADA), the FDA and Novartis initiated a collaboration to qualify kidney safety biomarkers for preclinical use and further clinical applications. The lessons learned and the process map proposed in this project will support the development of a draft guidance.

Here we present the results and milestones on the way to regulatory approval of up to 14 biomarkers for monitoring kidney safety in rats, such as:

- Selection of appropriate model compounds to cover different compartments of the target organ(s) and identification of specific biomarkers to monitor different modes of toxicity
- Design of toxicity studies in rats for 8 nephrotoxicants and 2 hepatotoxicants
- Evaluation of toxicity by standard methods including in-life observations, clinical biochemistry and histopathology
- Validation of RT-PCR low density arrays for the quantification of the biomarkers on a gene expressions level in kidney, liver and blood samples
- Development and validation of multiplexed protein assay panels to quantify the biomarkers on a protein level in urine, plasma and organ homogenates
- Statistical data mining focusing on diagnostic predictivity of markers and signatures versus histopathology and clinical biochemistry

The sensitivity and specificity of these biomarkers and signatures thereof in urine, blood and tissue will be presented discussed, and compared to the current gold standards of kidney function/toxicity (i.e. histopathology, creatinine, BUN). Finally, the status of this prototype submission will be communicated with respect to regulatory approval.

1843 4-HYDROXY ESTRADIOL-INDUCED ROS MEDIATED ACTIVATION OF CELL CYCLE GENES, CDC2 AND ITS REGULATORS CDC25C AND PRC1, CONTROLS CELL TRANSFORMATION.

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There is considerable experimental and epidemiological evidence that 4-hydroxy estradiol (4-OH-E2), a catechol metabolite of 17 β estradiol (E2) plays a critical role in breast carcinogenesis; however, this effect cannot be fully accounted for by the mitogenic effects induced by estrogenic stimulation of its cognate receptor (ER). We have recently shown that reactive oxygen species (ROS) are involved in the regulation of E2 induced growth of breast cancer cells. In this study we have investigated whether 4-OH-E2-induced ROS through activation of nrf1-1 signaling is involved in the transformation of normal breast epithelial cells to malignant cells. Using a normal cell line (MCF-10A) that develop preneoplastic foci in response to 4-OH-E2, we measured the effects of this metabolite on nrf-1 interaction with cell cycle genes by ChIP assay and the expression of cell cycle genes cdc25c, cdc2, nrf-1 and prc1 by real time RT-PCR. After exposure of 8h following two 48 hrs treatments with 4-OH-E2; we observed an approximately 1.14, 1.55, 1.57, 1.81 and 1.0 fold increase in the expression of prc1, cdc2, nrf-1, cdc25c, and TFAM respectively. Overexpression of MnSOD markedly decreased 4OHE2 induced cdc2(-.45), cdc25c(-.86), and TFAM(-.39) expression compared to normal cells. Overexpression of catalase also decreased 4-OH-E2-induced expression of prc1(-.50), cdc2(-.39), and nrf1(-0.4). We further examined whether nrf-1 interacts with potential binding sites that regulate transcription of target cell cycle genes cdc2, cdc25, and prc1 in cells exposed to 4-OH-E2. We found a greater rate of nrf-1 binding to chromatin in cells exposed to 4-OH-E2 compared to unexposed cells. In conclusion, these results indicate distinct altered expression of cell cycle genes following exposure to 4-OH-E2. We postulate that 4-OH-E2-induced ROS through nrf-1 mediated transcriptional activation of cell cycle genes, cdc2 and its regulators cdc25C (critical for mitotic entry) and prc1, controls 4-OH-E2-induced cell transformation. Supported by DoD grant BC051097.

1844 ACTIVATION OF PI3K/AKT SIGNALING PATHWAY IS INVOLVED IN 4-HYDROXY-ESTRADIOL-INDUCED CELL TRANSFORMATION.

V. O. Okoh. *Environmental and Occupational Health, Florida International University, Miami, FL.* Sponsor: D. Roy.

The involvement of estrogen in the etiology of human breast cancer is supported by a large body of epidemiologic and experimental evidence. However, the mechanisms by which estrogen initiate or stimulate the progression of cancer remain the subject of a long-standing controversy, because we have not been able to resolve whether estrogen or their metabolites are procarcinogenic. Thus, in order to obtain novel and reliable insights into dysregulation of signaling networks associated with breast carcinogenesis, we have recently developed an in vitro model for estrogen-induced cellular transformation. The ER alpha negative, normal human breast epithelial MCF-10A cells when exposed to 17 beta estradiol (E2) or 4-hydroxyestradiol (4-OH-E2) produced increased reactive oxygen species formation, DNA synthesis and cell transformation. When these cells were pre-treated with ebselen, N-actylcystine or catalase before 4-hydroxy-estrogen exposure, reactive oxygen species, DNA synthesis and cell transformation were abrogated. In addition, treatment of cells with LY294002, a PI3K inhibitor, prevented AKT phosphorylation and cell transformation. In conclusion, the exposure of 4-OH-E2 or E2 to normal human breast epithelial MCF-10A cells produced transformed phenotypes. The 4-OH-E2-induced cell transformation was attenuated by the antioxidants. Overexpression of catalase and MnSOD also reduced the extent of 4-OH-E2-dependent increased cell transformation and AKT activation. Taken together, these data indicate that 4-OH-E2-induced ROS activate the AKT pathway in MCF-10A cells, which, in turn, supports the growth of anchorage independent cells. Supported by DoD grant BC051097.

1845 RESVERATROL PREVENTS THE FORMATION OF DEPURINATING DNA ADDUCTS AND NEOPLASTIC TRANSFORMATION IN HUMAN BREAST EPITHELIAL CELLS.

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Prolonged exposure to estrogen is a major known risk factor for breast cancer. Specific estrogen metabolites may contribute to estrogen carcinogenicity. Genotoxicity is caused by cytochrome P450 (CYP)-mediated oxidation of estrogens to quinones that react with DNA to form depurinating DNA adducts and initiate cancer. CYP1B1 favors quinone formation by catalyzing estrogen 4-hydroxylation, whereas Quinone reductase (NQO1) catalyzes the reduction of quinones back to catechol estrogen (CE). Resveratrol has anti-cancer effects in diverse in vitro and in vivo systems. TCDD and estradiol (E₂) induce CYP1B1 expression through the AhR. Resveratrol is an AhR antagonist that decreases the CYP expression, whereas it induces NQO1 expression. We hypothesize that resveratrol may prevent estrogen genotoxicity and neoplastic transformation via blocking the formation of CEs and DNA adducts.

We used MCF-10A cells to investigate the chemopreventive effect of resveratrol in breast cancer initiation. The profile of estrogen metabolites and DNA adducts in resveratrol-treated cells co-treated with TCDD and/or E₂ was analyzed by HPLC. The expression and activity of CYP1B1 and NQO1 were determined by immunoblotting and HPLC, respectively. The anti-transformation effects of resveratrol were determined by an anchorage-independent growth assay. Resveratrol induced apoptosis in a dose-dependent fashion in both normal and tumor cells. Resveratrol (25 μ M) completely blocked the formation of DNA adducts in MCF-10A cells treated with E₂ or TCDD plus E₂. Resveratrol suppressed TCDD and/or E₂-induced cell transformation by decreasing E₂ metabolism and preventing DNA adduct formation. We conclude that TCDD induction of CYP1B1, enhancing estrogen metabolism and formation of DNA adducts, plays a major role in breast cancer initiation, and resveratrol acts as a potential chemopreventive against estrogen-induced breast cancer by blocking multiple sites of the estrogen genotoxicity pathway (Supported by U.S.P.H.S grant P01 CA49210).

1846 POTENTIAL SYNERGISM OF P-GLYCOPROTEIN MODULATING PHYTOCHEMICALS AND TAXANES IN SUPPRESSION OF POLYCYCLIC AROMATIC HYDROCARBON INDUCED MAMMARY DNA ADDUCTS AND TUMORS.

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Each year breast cancer is diagnosed in 910,000 women worldwide, and 376,000 women die from the disease. Polycyclic aromatic hydrocarbons (PAHs) are commonly found in our environment and appear to be carcinogenic by genotoxic mechanisms. Some of these mimic the properties of estrogen and it has been proposed that they may exert a hormonal influence on breast cancer.

Drug metabolizing enzymes (DMEs) play central roles in the metabolism, elimination and/or detoxification of xenobiotics. P-glycoprotein (P-gp), Pregnane X receptor (PXR), and CYP3A4 are associated with multi drug resistance (MDR) in breast cancer chemotherapy. Taxanes are among the most unique, and successful, chemotherapeutic agents used for the treatment of breast and ovarian cancer. Taxanes induce CYP3A by activating PXR-mediated transcription and enhance P-gp mediated drug clearance in human mammary tumor cells. The abundant and inexpensive chemopreventive dietary phytochemicals are being explored as P-gp modulators for the reversal of MDR to breast cancer chemotherapeutic drugs.

In the present study, we evaluated the effects of PAHs and chemopreventive dietary phytochemicals on the function of P-gp and reversal of multidrug resistance induced by taxanes. We also assessed the effects of dietary phytochemicals and drugs, alone or in combination, on Cyp3a11 and PXR activities, and studied the mechanism of regulation using transgenic mice. Chemotherapeutic drugs, paclitaxel and docetaxel induced hepatic CYP3A11 mRNA in female mice compared to controls. The mammary P-gp expression was elevated in mice treated with paclitaxel and docetaxel compared to controls. Administration of dietary chemopreventive phytochemicals inhibited the P-gp expression significantly showing a potential to reverse multi drug resistance.

These studies will help to develop rational strategies for the combination drug development for the successful chemotherapy of environmental and hormonal induced human breast cancer.

1847 ALTERED DIFFERENTIATION AND ACTIVATION OF GROWTH FACTOR SIGNALING PATHWAYS IN 2D AND 3D CULTURES OF PRIMARY HUMAN MAMMARY EPITHELIAL CELLS (HMEC) PRODUCED BY ENVIRONMENTAL POLYCYCLIC AROMATIC HYDROCARBONS (PAHS).

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There is great interest in determining mechanisms by which environmental agents contribute to the increasing incidence of human breast cancer. Benzo(a)pyrene (BaP) is a complete carcinogen that is present in emissions from the burning of fossil fuels, woodsmoke, tobacco smoke, and in the diet where it is associated with charcoal-broiled foods. BaP exerts important genotoxic and non-genotoxic actions, but its mechanism of tumor promotion is poorly understood. Recent work in the PI's lab has shown that BaP exerts novel actions on the epidermal growth factor receptor (EGFR) and insulin-like growth factor (IGF) signaling pathways. Metabolites of BaP, most notably BaP-quinones (BPQs), stimulate cell proliferation through an oxidant stress and redox-cycling mechanism [Burdick, et al (2003) Cancer Res 63, 7825-7833]. Stimulation of cell proliferation and inhibition of apoptosis are both important non-genotoxic mechanisms of tumor promotion and progression. We have found using mRNA arrays that BPQs activate AhR and anti-oxidant response element (ARE) associated pathways in MCF-10A cells. Studies performed in the human mammary epithelial cell line MCF-10A were further validated in primary cultures of normal human mammary epithelial cells (HMEC). In 3D cultures of HMEC, we found that low concentrations (.01-.1 μ M BPQs) produce: 1) an increase in acinar size, 2) a loss of cell-cell junctions with a decrease in β -catenin expression, and 3) a decrease in expression of E-cadherin. These results suggest that BPQs may induce an epithelial mesenchymal transition (EMT). In addition, we have detected an increase in the expression of CXCR4 in these normal HMEC. Thus, treatment of primary HMEC in 3D cultures with BPQs produces changes in surface markers that are associated with breast cancer phenotypes. Supported by NIEHS RO1 ES-07259 and P30 ES-012072

1848 OVEREXPRESSION OF LIPOCALIN 2 IN COP RAT MAMMARY GLAND AND ITS RELATIONSHIP WITH DIFFERENTIAL GENETIC SUSCEPTIBILITY TO MAMMARY CARCINOGENESIS BETWEEN COP AND F344 RAT.

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Rat strains differ dramatically in their susceptibility to mammary carcinogenesis following exposures to chemical, hormones or ionizing radiation. The H-ras oncogene, activated by a G→A transition at the codon12, is found in the majority of NMU-induced rat mammary carcinomas, as well as some spontaneous mammary tumors. This mutation event was thought to be the most important initiating event during the development of rat mammary carcinogenesis. However, although H-ras

mutation was also found in Copenhagen (Cop) rat, Cop rats are completely resistant to mammary carcinogenesis. Further study showed the resistance of the Cop rat to mammary carcinogenesis is not due to a defect in initiation but rather appears to be due to suppression of the growth of preneoplastic cell. In the current study, we compared the transcriptomes in mammary tissue from the resistant Cop rat and the susceptible Fischer 344 (F344) rat at different time points following exposure to a single, carcinogenic dose of NMU. Among the genes differentially expressed between Cop and F344 rat strains, the steady state levels of Lipocalin-2 (Lcn2) mRNA levels were 30 to 70-fold higher in Cop rat mammary gland vs. in F344 rat. Our further study showed that highly expressed Lcn2 could inhibit Ras-transformed cell independent growth by regulation of the Erk 1 and 2 phosphorylation. It suggests that Lipocalin2 is a mammary tumor suppressor that, highly expressed Lcn2 in Cop rat contributed, at least in part, to the genetic resistance of the Cop strain to mammary carcinogenesis by suppressing the growth of preneoplastic cells that carries the activated NMU induced H-ras oncogene. (This study was supported by grants U19-ES-011387 & P30-ES-07033 from the NIH/NIEHS.)

1849 MOLECULAR BIOMARKERS OF MAMMARY CANCER IN THE ACI RAT: A MODEL FOR CHEMOPREVENTION.

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The ACI rat is susceptible to mammary cancer induction by estradiol (E2) and is an outstanding model of estrogen receptor positive (ER+) human breast cancer (BC). Potential biomarkers for ER+ BC were identified by differential proteomic and genomic expression in E2-induced tumors compared to normal mammary tissues. Rats were implanted with 2 mg E2 pellets sc., for up to 46 wks resulting in 70% tumor incidence. Utilizing an 8K rat oligo library DNA microarray, 7137 genes were detected: 610 genes showed a 2-fold increase and 487 genes a 2-fold decrease (tumor vs. normal). Ten of 19 genes representing the Luminal type A of human BC were detected. Six of these 10 (GATA6, ER α , protein tyrosine phosphatase 4a1, COX, neuregulin 1, GST-mu1) exhibited at least 2-fold changes up or down. DNA-microarray results were supported by proteomic analysis from 2-D gels. 60+ spots were significantly different between normal and tumor tissues. Selected spots were identified by LC-MS/MS after trypsin digestion. 12 of 26 proteins identified are associated with human cancers. Five were upregulated in tumor samples: glutamate dehydrogenase, chloride intracellular channel 1, cytokeratin19 and nucleolar phosphoprotein B23.1; seven were downregulated: transaldolase 1, β -galactoside-binding lectin, heart-type Fatty Acid Binding Protein (hFABP), GDP-dissociation inhibitor, glycosylation dependent CAM1, annexinV, tropomyosin 1 and calreticulin. Cytokeratin 19 (CK 19), whose human ortholog (CK18) is upregulated in the ER+, Luminal type A human BC, was increased 26-fold in ACI tumors. We also found a marked suppression of hFABP, a postulated tumor suppressor gene down-regulated in some human BC. These results are the first reported expression analysis for E2-induced ACI mammary tumors. The constellation of CK19 and GATA-6 up-regulation along with hFABP down-regulation in breast tumors is a biomarker for the detection of BC. (Supported by CINJ Breast Cancer Research Program Award, Rutgers Busch Award and NIEHS ES05022)

1850 A NOVEL MECHANISM FOR THE CHEMOPROTECTION OF TCDD FOR BREAST CANCER.

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Although it is causative for many types of cancers, experimental and epidemiological evidence suggest that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) may in fact protect against breast cancer. The mechanism(s) for this protection remain unclear, although an anti-estrogen effect has been implicated. In an attempt to further elucidate this mechanism, we performed a microarray experiment in order to identify genes that were modulated in MCF-7 breast cancer cells upon TCDD treatment. We found that TCDD down-regulated the G-protein coupled receptor, CXCR4, as well as its unique chemokine ligand, CXCL12. The interaction between CXCR4 and CXCL12 is known to play a central role in the metastasis of breast cancer. Organs to which breast cancers preferentially metastasize, namely the lung, liver, and bone, secrete high levels of CXCL12, which binds to CXCR4 expressed on the primary cancer cells. This subsequently stimulates the invasive properties of the primary tumor cells and attracts them to the preferred organ sites of metastases. Utilizing an in vitro chemotaxis assay, we demonstrate that TCDD inhibits the migration of MCF-7 cells towards a CXCL12 gradient. Additionally, we show that TCDD inhibits the inductive effects of estradiol on CXCL12. As the CXCR4/CXCL12 axis is also implicated in the progression of numerous other

types of cancer, we screened several cancer cell lines for a similar response to TCDD and found that CXCR4 and CXCL12 are indeed modulated in some of these lines. Finally, we demonstrate that 3,3'-diindolylmethane (DIM), a selective aryl hydrocarbon modulator (SAHRM) and nontoxic AHR ligand currently in clinical trials for the treatment of breast cancer, down-regulates CXCR4 and CXCL12 in a dose-dependent manner. We therefore propose that TCDD protects against breast cancer metastasis via down-regulation of the chemokine CXCL12 and its receptor CXCR4. Further, other less toxic ligands for the AHR (ShARMs) may exert their protective effects by a similar mechanism.

1851 THE EFFECT OF REPEATED LOW LEVEL SARIN EXPOSURE ON BRAIN MUSCARINIC RECEPTOR SITES IN F344 RATS.

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The objective of the study was to address whether Gulf War veterans returning from duties in a hot desert region where low levels of nerve gas might have been present during destruction of munitions, could have suffered persistent adverse health effects. In earlier studies, we reported that 5 days of exposure of F344 rats to low levels of sarin gas resulted in alterations in muscarinic receptor sites in the brain. This report is on the effects of continuing those exposure for 10 days to determine if the effects were enhanced or if adaptation occurred. F344 male rats were exposed nose-only for 60 min/day to 0.2 or 0.4 mg/m³ sarin for 5 days/wk for 2 weeks under normal or heat-stressed conditions. The levels of sarin represent 1/20th and 1/10th, respectively, of the expected LC50 for rats. The heat stress was sufficient to raise the core temperature of the rats by 1 degree C. Control rats were exposed to vehicle under the same conditions as the sarin-exposed rats. Brains of the rats were observed 30 days after the end of the exposures to look for persistent changes. The findings confirmed our earlier results following 5 days of exposure and showed an enhanced response. The M1 receptor sites were decreased in a dose-dependent manner in all areas of the brain tested (frontal cortex, olfactory region, hippocampus and striatum). In contrast to the 5-day studies, these changes occurred under both normal and heat-stress conditions. There was little change in the M2 receptor site densities with the exception of an increase in sites in the frontal cortex at the higher exposure level under heat stress condition. As in the earlier studies, the M3 receptor sites were increased only when the exposures were done under heat stress conditions. These findings demonstrate that the rat model of sarin exposure is an important first step in identifying brain systems involved in the development of persistent effects following subclinical sarin exposures. Research supported by US Army Medical Research and Materiel Command, Contract DAMD17-97-C-7054.

1852 GENOMIC ANALYSIS OF RAT BRAIN FOLLOWING EXPOSURE TO SOMAN.

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Soman (O-Pinacolyl methylphosphonofluoridate) is a potent neurotoxicant. Acute exposure to soman causes profound inhibition of the critical enzyme acetylcholinesterase, resulting in excessive levels of the neurotransmitter acetylcholine. Excessive acetylcholine levels cause convulsions, seizures, and respiratory distress which are fatal without treatment. We performed gene expression profiling of rat hippocampus following soman exposure to gain greater insight into the mechanism of neurodegeneration following soman poisoning. Male Sprague-Dawley rats were pretreated with oxime HI-6 (125 mg/kg, ip) 30 min prior to challenge with soman (1.6xLD50 or 180 ug/kg, sc). One minute after soman challenge, animals were treated with atropine methyl nitrate (2.0 mg/kg, im). Hippocampal tissue was harvested 1, 3, 6, 12, 24, 48, 72, 96, and 168 hr post exposure. RNA was extracted from the hippocampal tissue and used to generate oligonucleotide microarray probes for gene expression profiling using Affymetrix Rat 230 2.0 microarrays. Principal component analysis of the gene expression data revealed a progressive alteration in gene expression profiles beginning at 1 hr post exposure and continuing through 24 hr post exposure. At 48 hr to 168 hr post exposure, the gene expression profiles clustered nearer to controls, but did not completely return to control profiles. Based on the principal component analysis, analysis of variance was used to identify the genes most significantly changed as a result of variation over time and soman exposure. These genes were rank ordered by p-value and categorized using gene ontology-based algorithms into molecular functions and biological processes significantly affected by soman poisoning, which include immune response, metabolic activity, and cell cycle regulation. These data provide important insights into the molecular pathways involved in soman-induced neuropathology.

1853 MICROARRAY ANALYSIS OF MURINE GENE UPREGULATION AFTER CUTANEOUS SULFUR MUSTARD EXPOSURE.

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Skin exposure to sulfur mustard [bis-(2-chloroethyl)-sulfide; SM] results in characteristic blister formation in the exposed skin. Skin from mice exposed to a high level (0.08 mg) of SM was collected at early (6, 12, and 24 h post exposure) or late (72 and 168 h post exposure) time intervals, the RNA isolated, and analyzed for gene expression analysis by the Affymetrix Murine high-density oligonucleotide array (46K genes and ESTs). Expression of greater than 1000 genes was upregulated greater than 2-fold in SM-exposed tissue compared to control skin for a minimum of one of the five timepoints. Data was organized into three major groups based on cluster analysis: 1) those with a 2-fold increase in early genes that stayed upregulated; 2) those with a 2-fold increase in early genes that became down-regulated in the late timepoints; and 3) those with a 2-fold increase in late genes only. Group 1 was mostly cytokines, growth factors, and transcription factors. Group 2 was mainly families of cellular activators, and cellular regulators. Families included eukaryotic translation factors, MAP kinases, prostaglandins, nuclear receptors, ribosomal proteins, and zinc finger proteins. Group 3 contained families of genes involved in inflammatory and immune responses including the cathepsins, various CD antigens, interleukin receptors, lymphocyte antigens, toll-like receptors, TNF superfamily members and individual inflammation inducing genes such as caspase 1 and TGF- α and TGF- β 1. Selected genes were analyzed by quantitative PCR to verify our observations. Histopathology of tissue sections furthermore supported the data. The results provide a further understanding to cutaneous SM exposure and enable diagnostic markers and therapeutic targets to be identified for treating exposure to SM.

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1854 ESTIMATING MIOTIC, SEVERE AND LETHAL TOXIC EFFECTS IN GOTTINGEN MINIPIGS FOLLOWING INHALATION, INTRAVENOUS AND SUBCUTANEOUS EXPOSURES TO VX.

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Constriction of the pupils (miosis) is often identified as the first noticeable sign of exposure to vapor nerve agents. In rats, VX vapor has been shown to be more potent at causing pupil constriction than GB or GF (VX>GB>GF). We have previously identified that the miotic potencies of GB and GF are roughly equal in minipigs. In the current studies sexually mature male Gottingen minipigs were exposed to various concentrations of VX vapor in order to determine the effective concentrations (ECT50s) for pupil constriction over exposure durations of 10, 60 and 180 minutes. Initial studies show that in minipigs VX is less potent than either GB or GF at causing pupil constriction. In practical terms the concentrations of VX vapor that elicit pupil constriction are much closer to concentrations that result in severely toxic or lethal signs than are seen for either GB or GF vapors. Additionally, male Gottingen minipigs were exposed to liquid VX via intravenous (IV) or subcutaneous (SC) injections. Signs of nerve agent exposure were classified as lethal, severe, or moderate. Maximum likelihood estimation using a probit model was used to calculate the median effective (severe effects) and lethal doses (LD50) for IV and SC exposures. For intravenously injected VX, the LD50 was 11.8 μ g/kg (9.7 – 14.5 μ g/kg) and the ED50 (severe) was 6.6 μ g/kg (4.8 – 9.0 μ g/kg). For subcutaneously injected VX, the LD50 was 16.1 μ g/kg (12.9 – 19.9 μ g/kg) and the ED50 (severe) was 12.8 μ g/kg (10.1 – 16.2 μ g/kg).

1855 DIFFERENTIAL BRAIN ACETYLCHOLINESTERASE INHIBITION FOLLOWING MICROINSTILLATION INHALATION EXPOSURE TO SOMAN IN GUINEA PIGS.

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We assessed acetylcholinesterase activity following inhalation exposure to the chemical warfare nerve agent soman (GD) in different brain regions of guinea pigs. Guinea pigs were exposed to GD by a newly developed microinstillation inhalation exposure technique. This technique delivers aerosol with a particle size less than 2 microns and bypasses the degradation of GD by carboxylesterase present in the airway of guinea pigs. The technique mimics human inhalation exposure. Guinea pigs

were exposed to GD, 0.3LCt50 at a rate of 40 pulses/min during inspiration for 4 min. Since the animals exposed to GD die 10-15 min after exposure, methyl atropine nitrate (2.5 mg/ml) was delivered immediately after GD exposure, (40 pulses/min, 1 min). Treatment with methyl atropine nitrate, which does not cross the blood brain barrier, restored the respiration of the animals. The animals were euthanized 24 h after exposure and the brain removed and placed immediately on ice. Different regions of the brain were dissected, and the samples were suspended in 7-volumes of tissue protein extraction reagent (T-PER) (wt/vol), and homogenized on ice using an Omni microhomogenizer. The supernatant was separated after centrifugation and assayed for cholinesterase activity and protein levels. Acetylcholinesterase was specifically assayed by samples incubating with tetraiso-propyl pyrophosphoramidate (iso-OMPA, specific BChE inhibitor). In brain from guinea pigs 24 h after GD exposure, there was a significant inhibition of AChE in the cortex ($p=0.034$). Other regions exhibited less inhibition than cortex. These data suggest that AChE in the cortex is inhibited more due to the soman induced aging of the enzyme and poor blood circulation to the deeper areas of the large cortex. In other brain regions, AChE inhibition was lower probably due to quicker recovery and higher vasculature and blood circulation.

1856 CORRELATION OF VAPOROUS VX EXPOSURE WITH BIOMARKERS AND EFFECTS BY MEANS OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC/PHARMACODYNAMIC MODEL (PBPK/PD).

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A challenge in the use of animal studies of chemical warfare nerve agent (CWNA) toxicology is extrapolation of the results to risk estimates of human response. Most mechanistic animal studies with CWNAs have been at near lethal to supra-lethal doses, by dosing routes (i.e. subcutaneous, intramuscular) that are less applicable to likely human exposure scenarios (i.e. inhalation, dermal), and in animal models that may be compromised (atropine pretreatment). A previously developed PBPK/PD model for GB (sarin) that allows the quantitative extrapolation of exposure effects in one species and dose route, to other species by other dose routes, was parameterized to simulate the kinetics and the dynamic effects of VX vapor exposure in the Gottingen minipig. Biomarkers of VX exposure simulated were the fluoride ion regenerated VX G-analog (VX-G) in red blood cells, blood plasma and tissues (Jakubowski 2006), as well as dynamic effects like miosis. The PBPK/PD model was used to simulate agent exposure profiles from serial blood samples taken before, during, and after whole body VX vapor exposures of Gottingen minipigs. Simulation of these VX biomarker data were compared to those available in the literature for other species. The apparent species differences in response were addressed with regards to the different PK profiles of VX. Simulation of VX in the present PBPK/PD model, initially using parameters from GB simulations of multiple dose routes and levels of dose, required decreasing the plasma enzymatic hydrolysis parameters by 7x, while increasing the affinity of VX for the lipid tissues to 4x that of sarin. The model simulations were also used to explain the differences in VX-G kinetics versus that of the freely extractable VX found in the plasma. Previously published in vivo and in vitro estimates of VX hydrolysis were confirmed as a necessary model parameter requiring adjustment to reproduce VX pharmacokinetics and pharmacodynamics in the minipig.

1857 PRALIDOXIME INDUCES A SUSTAINED CHOLINESTERASE REACTIVATION BUT ONLY TRANSIENT NORMALIZATION OF PARAOXON-INDUCED RESPIRATORY DISTRESS.

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Objective: The efficiency of oximes in organophosphate poisoning is a matter of debate. In rats poisoned with paraoxon (PO), we studied the effects of pralidoxime (PX) methylsulfate on respiratory distress and blood cholinesterase (ChE) activities. Methods: we tested in whole blood in vitro the efficiency of various PX concentrations to reactivate PO-induced ChE inhibition. Male Sprague-Dawley rats were treated with PX (base: 10, 25, 50 mg.Kg-1) using IV and IM routes to assess the dosage regimen providing plasma PX > 4 mg/l during the longest period of time. PO (0.215 mg.Kg-1 i.e. 50% LD50) was administered subcutaneously. Rats were treated with PX 30 min post injection of PO. Respiratory function was assessed using whole body plethysmography. ChE activities were measured in whole blood. Results are expressed as mean $\pm$ SEM. Statistical analysis used Student's t test and ANOVA tests with $p < 0.05$. Results: The 50 mg.Kg-1 IM dose resulted in plasma PX concentrations > 4 mg.L-1 during 35 min post infusion. PO induced an in-

crease in total time, expiratory time and tidal volume, and a decrease in respiratory rate. In the same time, whole blood ChE activities were decreased to 40% of the basal value. Thirty minutes after PO administration, PX (50 mg.Kg-1, IM) induced: 1-) a fast, total and prolonged ChE reactivation (> 180 min), 2-) a rapid (< 5 min), complete but only transient (< 30 min) reversal of PO-induced respiratory effects. Discussion : A 50 % LD50 of PO induced respiratory distress and decrease in ChE activity. A single dose of PX rapidly corrected both effects which were dependent on PX concentrations. Thereafter, normalization was sustained for ChE activity but only transient for respiratory distress. Our data suggest that ChE reactivation is a necessary but not sufficient condition for the antidotal effect of PX. ChE reactivation cannot be used alone to assess the antidotal efficiency of oximes.

1858 MEDICAL COUNTERMEASURES USING ATROPINE IN COMBINATION WITH OTHER DRUGS FOR VX INHALATION EXPOSURE ACUTE RESPIRATORY TOXICITY, AND LUNG INJURY.

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The efficacy of atropine, administered in combination with other drugs (dexamethasone, N-acetylcysteine, Syk antisense oligonucleotides, surfactant and liqui-vent), was evaluated against lung injury and respiratory toxicity caused by VX exposure. Guinea pigs were exposed to saline or 27 mg/m³ VX for 4 min using a microinstillation inhalation exposure technique. Exposure to this dose of VX resulted in a 24 h survival rate of 52%. A significant increase in bronchoalveolar lavage (BAL) protein, total cell number, and cell death were observed in the VX-exposed animals compared to the saline controls. Surprisingly, direct pulmonary treatment via instillation 2 minutes post-exposure with pulmonary therapeutics did not increase the survival rate of VX-exposed animals. VX-induced viscous mucous secretions were exacerbated by the aerosolized treatments leading to further blocking of the airway. To overcome this effect, we developed a strategy to combine the individual treatments with atropine. Atropine inhibits muscarinic stimulation and markedly reduced the copious airway secretion following vx exposure. When atropine methyl bromide, which acts peripherally and does not penetrate the blood brain barrier, was administered in conjunction with the other pulmonary treatments the result was 100% survival of VX-exposed animals at 24 h. Atropine treatment in combination with pulmonary therapeutics dexamethasone and antisense Syk also reduced the VX-induced increase in bronchoalveolar lavage protein levels, cell number, and cell death. These results demonstrate that post-exposure nasal treatment with atropine in combination with pulmonary therapeutics inhibits mucous secretion for survival and also protect against lung injury and respiratory toxicity from inhalation exposure to VX.

1859 FETAL MOUSE PHTHALATE EXPOSURE SHOWS THAT GONOCYTE MULTINUCLEATION IS NOT ASSOCIATED WITH DECREASED TESTICULAR STEROIDOGENESIS.

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The rat has been explored in detail for its in utero susceptibility to male reproductive tract malformation following phthalate exposure. Few other species have been studied in detail. We investigated the response of the fetal mouse testis to phthalate exposure and compared these results with those previously obtained from the rat. Initial experiments using a variety of phthalate congeners (monobutyl phthalate, di-(n-butyl) phthalate, or mono(2-ethylhexyl) phthalate) and exposure paradigms did not reduce fetal mouse testis testosterone levels. Pharmacokinetic data after a single 500 mg/kg DBP exposure on mouse gestation day (gd) 18 demonstrated that the concentrations and kinetics of the active metabolite (MBP) in fetal and maternal plasma were similar to the rat. After a single 500 mg/kg or multiple day 250 mg/kg fetal mouse DBP exposure, rapid and dynamic changes in testis gene expression were observed, including induction of immediate early genes. Unlike the rat, expression of genes involved in cholesterol homeostasis and steroidogenesis were not decreased. Similar to the rat however, a 250 or 500 mg DBP/kg/day mouse exposure from gd 16 through 18 significantly increased seminiferous cord diameter, the number of multinucleated gonocytes per cord, and the number of nuclei per multinucleated gonocyte. Together, these results demonstrate that fetal mouse and rat phthalate exposure both induce immediate early gene expression and disrupt of seminiferous cord development. This response in the mouse occurs without a measurable decrease in testicular steroidogenesis, suggesting that altered seminiferous cord formation is not mechanistically linked to lowered steroidogenesis.

1860 IDENTIFICATION OF REGION-SPECIFIC GENE EXPRESSION CHANGES AND SIGNALLING PATHWAYS AFFECTED BY DI(2-ETHYLHEXYL)PHTHALATE (DEHP) IN FETAL RAT TESTES.

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Previous studies have shown di(2-ethylhexyl)phthalate (DEHP) to elicit a testicular dysgenesis in rats following in utero exposure. This study was performed in order to identify signalling pathways associated with DEHP-induced testicular dysgenesis and to determine the region-specificity of the gene expression alterations. Pregnant Wistar rats were administered DEHP (500 mg/Kg body weight daily by gavage) from gestation day (GD) 12 to GD 19. At GD 19, fetal testes were harvested and interstitial (INT) and tubular (CHORD) regions isolated by laser capture microdissection. RNA was prepared from the isolated INT and CHORD cells and high density microarray analysis (Agilent rat whole genome array) performed. Comparison of the INT and CHORD signature gene lists ($p < 0.01$) identified genes that were uniquely or commonly regulated by DEHP in the two regions. In the INT region down-regulation of genes involved in sterol and fatty acid biosynthesis and steroidogenesis was seen (eg. hydroxymethylglutaryl coenzyme A reductase and synthase, farnesyl diphosphate synthase, isopentenyl diphosphate delta isomerase, mevalonate decarboxylase, CYP17, scavenger receptor class B member 1). Several genes that are regulated by insulin like growth factor 1 (IGF1) such as cathepsin D, solute carrier family 2 member 1, and ribosomal protein S6 kinase, were also uniquely down-regulated in the INT (Leydig cell region), correlating with repression of IGF1 in this region. By contrast, genes involved in regulation of androgen and estrogen receptors were strongly represented in the CHORD 'unique' signature gene list. These results indicate that DEHP-induced testicular dysgenesis involves region- and cell type-specific effects on a number of genes, many of which are involved in sterol biosynthesis and steroidogenesis.

The Phthalate Esters Panel of the American Chemistry Council funded this study

1861 STRUCTURE ACTIVITY RELATIONSHIP OF PHTHALATE ESTERS TO INHIBITED FETAL TESTICULAR TESTOSTERONE PRODUCTION IN THE SPRAGUE DAWLEY RAT.

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Several of the phthalate esters (widely used as plasticizers of polyvinyl chloride and other applications) have been shown to inhibit fetal testicular testosterone (T) production and *Ins3* mRNA in the laboratory rat. The current study was designed to define the dose response of 7 individual phthalates with respect to their impact on fetal T production. Time-pregnant Sprague Dawley female rats were administered oral doses from gestation day (GD) 8 through 18 with a broad dose range (25-900 mg/kg/d) of the following phthalates: benzyl butyl phthalate, BBP; dibutyl phthalate, DBP; diisobutyl phthalate, DiBP; diisooctyl phthalate, DiHP; dipentyl phthalate, DPP; diethyl phthalate, DEP; and di-n-heptyl phthalate, DnHP. On GD18, fetal testes were collected and incubated ex vivo for fetal T production. We report that fetal T production was suppressed by phthalates with monoester metabolites of 4 to 6 carbons in the ortho position, whereas those with longer or shorter side chains (or in the para-position) were inactive. BBP, DBP and DiBP were of similar potency to the known reproductive toxicant diethylhexyl phthalate (DEHP). DiHP and DPP were 2-fold and 5-fold more potent than DEHP, respectively. As expected based on structure, a range of 100 to 900 mg/kg/d DEP and DnHP did not significantly reduce fetal T production. The same phthalates that inhibited fetal T production also caused mid-pregnancy fetal loss, albeit at higher dose levels. The fetal resorptions are consistent with previously reported phthalate-induced suppression of progesterone during pregnancy. In conclusion, the ability of phthalates to suppress fetal T production is determined by their monoester metabolite structure and the SAR of our study was the same as that reported in pubertal male rats. We plan to use the relative potency data of the bioactive phthalates to design mixture studies measuring the effects of phthalate mixtures on fetal T production and *Ins3* gene expression. (This abstract does not necessarily reflect EPA policy.)

1862 CUMULATIVE REPRODUCTIVE EFFECTS OF IN UTERO ADMINISTRATION OF MIXTURES OF ANTIANDROGENS IN MALE SD RATS: SYNERGY OR ADDITIVITY?

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In 1996 the USEPA was charged under the FQPA to consider the cumulative effects of chemicals in their risk assessments. Our studies were conducted to provide a framework for assessing the cumulative effects of antiandrogens. Toxicants were

administered individually or as mixtures to rats on GD 14-18 and the development of the male offspring evaluated. Data were analyzed to determine if the chemicals behaved in a response- or dose-additive fashion. When seven antiandrogenic chemicals were combined at doses that individually produce little or no hypospadias or epididymal agenesis 100% of the male offspring were malformed (vinclozolin (V-15 mg/kg/d), procymidone (P-15 mg/kg/d), linuron (L-20 mg/kg/d), prochloraz (PZ-35 mg/kg/d) and three phthalate esters (DBP, DEHP, and BBP-150 mg/kg/d/phthalate)). Dose addition was the only model that accurately predicted the malformations induced by this complex mixture. When pairs of chemicals that act via similar and dissimilar mechanisms of action were combined, we found that dose addition was the appropriate model to predict hypospadias and other malformations. For example, mixtures of androgen receptor antagonists with phthalates that reduce fetal testosterone produced effects consistent with dose- but not response- additivity. Individually, the chemicals induced low rates of hypospadias P (1.5%), and L, BBP and DBP (0%) as compared to L plus BBP (56%) or P plus DBP (49%). The observation that dose- but not response-addition accurately predicts the effects of chemical mixtures that act via diverse mechanisms suggests that synergistic interactions may commonly occur among chemicals that disrupt the same signaling pathway at different molecular sites during sexual differentiation. This abstract does not necessarily reflect EPA policy.

1863 THE GENOMIC RESPONSE OF ISHIKAWA CELLS TO 17 α -ETHYNYL ESTRADIOL AND BISPHENOL A: A POTENTIAL ALTERNATIVE TO ANIMAL TESTING FOR ESTROGENS.

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The establishment of in vitro systems to determine the potential estrogenic activity of chemicals of interest is indispensable in order to develop reliable high-throughput screening assays and to minimize animal testing studies for this mode of action. With this goal in mind, we have determined the genomic response of the Ishikawa cells to estrogen exposure in a time and dose dependent manner, using two estrogen receptor agonists: 17 α -ethynyl estradiol (EE) and bisphenol A (BPA). The concentrations tested were: 1pM, 100pM, 10nM, and 1 μ M, for EE and 1nM, 100nM, 10 μ M and 100 μ M for BPA. The time points were 8, 24 and 48h. The transcript profiles were evaluated using Affymetrix Human Genome U133plus2.0 arrays. The analysis of the data indicates that the genomic response of the Ishikawa cells to a potent and a weak ER agonist exposure is time and dose dependent, following a pattern of a monotonic response for both parameters. Similar characteristics of the genomic response are elicited by EE and BPA in an in vivo model (the juvenile rat uterus). We determined by statistical analysis of the two experiments separately, that more than 1200 genes changed their expression by exposure to EE or BPA. Further, the change in the expression of 389 genes is identical in the direction of the change, although the magnitude of the change of some genes is different in the Ishikawa cells exposed to these two ER agonists. Using the annotation available for the genes responding to EE and BPA, we determined that the response of the Ishikawa cells involves cellular processes relevant for cell proliferation, differentiation and tissue remodeling. Many of these genes are also involved in organogenesis in the rat uterus, a process that is also affected by exposure to potent estrogens. These data indicate that the Ishikawa cells are capable of mounting a proper estrogenic response and they seem to offer a robust in vitro system to evaluate the potential estrogenicity of chemicals of interest.

1864 ROLE OF THE ESTROGEN RECEPTOR IN THE DIETHYLSTILBESTROL-INDUCED DISRUPTION OF THE EXPRESSION OF WNT5A AND WNT7A IN HUMAN ENDOMETRIAL (ISHIKAWA) CELLS.

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Genes of the WNT family are involved in signal transduction cascades important for development and carcinogenesis. WNT5A is supposed to be an upstream regulator of WNT7A expression in a signaling pathway that transmits information among stromal and epithelial cells of the endometrium. Disruption of this pathway is associated with morphological abnormalities of the female reproductive tract, and mice treated neonatally or in utero with the trans-placental acting carcinogen diethylstilbestrol (DES) show the same abnormalities, whereas estrogen receptor (ER) α knockout mice are insensitive towards DES.

The aim of the present study was to determine the influence of the synthetic estrogen DES on the mRNA expression of WNT5A and WNT7A in a human endometrial adenocarcinoma cell line (Ishikawa cells) by reverse transcription/competitive PCR. The role of the ER was examined by (i) the determination of the mRNA expression and the enzyme activity of the alkaline phosphatase (ALP), which is regulated by the ER in Ishikawa cells, (ii) the use of the ER antagonist ICI 182,780 (ICI), and (iii) silencing of the ER α by RNA interference using siRNA.

After treatment of Ishikawa cells with DES at concentrations that stimulated the expression of the ALP for 6-48 h, a significant decrease in the mRNA levels of WNT7A was observed reaching maximum reduction after 24 h. DES-induced reduction of WNT7A mRNA levels was prevented by the ER antagonist ICI as well as by knock down of ER α by RNA interference. After treatment with DES for 24 h and longer, mRNA levels of WNT5A correlated with those of WNT7A; however, there were pronounced differences after treatment periods of 6 h and less. In conclusion, when challenged with DES, the Ishikawa cell line reacts similar to the endometrial epithelium of mice exposed to DES in utero or neonatally, thus providing a powerful tool for the investigation of endocrine disrupting of the female reproductive tract.

1865 INVOLVEMENT OF THE C-KIT SIGNALING PATHWAY IN 4-VINYLCYCLOHEXENE DIEPOXIDE-INDUCED FOLLICLE LOSS IN RAT WHOLE OVARIAN CULTURES.

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The occupational chemical, 4-vinylcyclohexene diepoxide (VCD), selectively destroys small pre-antral (primordial and primary) ovarian follicles after repeated daily dosing of postnatal day (PND) 28 rats and following *in vitro* exposure of PND4 rat ovaries in culture. From previous studies, expression of the tyrosine kinase receptor, c-Kit, in ovarian cultures was reduced ($p < 0.05$) by VCD exposure in ovarian mRNA (0.45 ± 0.31 relative to control; $n = 4$) and protein ($78.64 \pm 0.91\%$ of control; $n = 3$). Interactions between c-Kit and its ligand, Kit Ligand (KL), play a crucial role in primordial follicle recruitment and follicle survival. Additionally, growth and differentiation factor-9 (GDF-9) and bone morphogenetic protein-4 (BMP-4), also play important roles in folliculogenesis. Therefore, the current study was designed to investigate a possible role of KL, GDF-9, and BMP-4 in VCD-induced ovarian follicle loss. Ovaries from PND4 Fischer 344 rats were excised and cultured in control or VCD-containing (30 μ M) medium with or without KL (25, 50, 100 ng/ml), GDF-9 (50, 100, 200 ng/ml), and BMP-4 (50, 100, 200 ng/ml). Following incubation (8d), ovaries were collected and prepared for histological evaluation. When co-incubated with VCD, KL at 50 and 100 ng/ml prevented ($p < 0.05$) VCD-induced small pre-antral follicle loss (Primordial: VCD 225 ± 31 ; 50 ng/ml KL+VCD 341 ± 33 ; 100 ng/ml KL+VCD 342 ± 28 follicles counted/ovary; Primary: VCD 39 ± 6 ; 50 ng/ml KL+VCD 65 ± 6 ; 100 ng/ml KL+VCD 76 ± 5 follicles counted/ovary; $n = 8-9$). Conversely, GDF-9 and BMP-4 had no effect on VCD-induced follicle loss. These data support that ovarian c-Kit/Kit Ligand signaling is disrupted by VCD, and likely plays a role in the mechanism by which VCD induces ovarian small pre-antral follicle loss. Whereas, two other signaling pathways associated with small pre-antral follicle survival/recruitment (GDF-9, BMP-4) appear to not be involved. (ES09246 and Center Grant 06694)

1866 *IN VITRO* EFFECTS OF COMMERCIAL HEXABROMOCYCLODODECANE (HBCD) AND ITS INDIVIDUAL STEREO-ISOMERS ON AROMATASE ACTIVITY.

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Hexabromocyclododecane (HBCD) is a brominated aliphatic cyclic hydrocarbon used as flame retardant in polystyrene foam and textile coating. The commercial HBCD mixture contains 3 stereo-isomers (α , β and γ). Several recent environmental studies indicated a sharp increase in HBCD concentrations from 2001 onwards, possibly due to an increase in production.

During the last years, we focused in our laboratory on environmentally relevant brominated flame retardants BFRs including HBCD and their possible effects on sex steroidogenesis. Aromatase (CYP19) is one of the key enzyme in the pathway. In the present study, the human H295R adrenocortical carcinoma cell line has been used to assess the effects of HBCD on aromatase activity. Concentrations of HBCD ranged from 0.01 to 100 μ M. Aromatase was measured using the tritiated water-release assay. Inhibition of aromatase activity was only found after a 24h exposure although with cytotoxic effects above 10 μ M, while IC50 values for the reference compound 4-hydroxyandrostendione was 0.3 μ M.

The inhibitory properties of HBCD, commercial mixture and individual stereo-isomers were then further studied using microsomes of human placenta for possible interaction with aromatase enzyme. Concentrations of commercial HBCD and its stereo-isomers ranged from 0.04 to 400 μ M. All compounds showed aromatase inhibition with IC50 values around 5 μ M, 50 times higher than the IC50 of the reference compound 4-hydroxyandrostendione (80 nM). Based on concentrations used in these *in vitro* experiments and those observed in humans and wildlife a preliminary risk assessment was done for the relevance under environmental conditions.

1867 THE PHOSPHOINOSITIDE 3-KINASE RELATED KINASE HSMG1 ACTIVATES AN EARLY GENOTOXIC STRESS RESPONSE TO HYPEROXIA.

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While clinically important, hyperoxic ventilation results in the production of reactive oxygen species which damage macromolecules and promote cell death. Prolonged exposure to hyperoxia has also been used as a model to study signaling pathways activated by chronic exposure to pro-oxidants. In response to genotoxic stress caused by hyperoxia, the p53-dependent expression of p21 inhibits proliferation and protects against cell death. Given the importance of p53-mediated expression of p21 in promoting cell survival, this study examines activation of this pathway in hyperoxia. Our laboratory has demonstrated a role for the wortmannin-sensitive phosphoinositide 3-kinase related kinases (PIKKs) in regulating hyperoxia-dependent phosphorylation of p53 on Ser15, a critical site in activating p53 in response to damage. Here, genetic and pharmacologic approaches were utilized to examine the contribution of PIKK family members in stimulating the p53 pathway in hyperoxia. To explore the role of the PIKK ATM in regulating this pathway, ATM wild-type or deficient lymphoblasts were treated with hyperoxia for various lengths of time. Interestingly, ATM was not required for Ser15 phosphorylation at early time points in hyperoxia and activation of p53 was abrogated by wortmannin treatment in both wild-type and ATM-deficient cells. In human adenocarcinoma A549 cells exposed to hyperoxia, siRNA targeting of the newly identified PIKK hSMG1 but not ATR reduced p53 phosphorylation relative to control treated cells demonstrating that hSMG1 contributes to p53 activation in hyperoxia. In addition, siRNA knockdown of ATM in A549 cells resulted in the loss of Ser15 phosphorylation only after 48 hours of hyperoxia while targeting of hSMG1 attenuated the p53 response at earlier time-points. These results suggest that hSMG1 activates an early damage or redox-sensitive p53 response to hyperoxia and indicate that hSMG1 may play a pivotal role in regulating checkpoints and other pathways that determine cellular outcome to genotoxic stress.

1868 ARSENIC INDUCES APOPTOSIS BY INCREASING PHOSPHORYLATION OF LCK AND FYN IN MOUSE LYMPHOCYTES.

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Arsenic is a widely distributed human carcinogen with various toxic mechanisms that interferes with DNA repair enzymes, microtubule and cytoskeletal assembly. Arsenic can produce chromosomal aberrations, cell cycle delay and differential toxicity depending on the gender and on the cell type evaluated. It has been reported that arsenic presents a differential toxicity on cell type regarding the immune system. We postulate that this differential toxicity is related with the capacity of arsenic to induce apoptosis and cell cycle arrest on T helper cells by increasing the phosphorylation rate of several molecules on the signal transduction pathway of interleukin 2 receptor-mediated activation such as lck and fyn.

In order to elucidate the possible mechanism by which arsenic can induce apoptosis in immune cells, we determine the effects of sodium arsenite on lck and fyn phosphorylation status and the induction of apoptosis on male C57BL6N mouse spleen cells *in vivo* and *in vitro*. Animal studies were carried out in accordance with the Society's criteria for the care and use of animals in research. Animals were exposed orally to arsenic for 15 days and sacrificed by CO₂ inhalation. Spleens were collected and cells were washed and incubated with a mitogen (Concanavalin A or antiCD3/CD28 antibodies). The proliferative response, as tritiated thymidin incorporation, and IL-2, IL-4 and IL-10 secretion by ELISA-sandwich, were evaluated as activation parameters. The phosphorylation of lck and fyn was evaluated by flowcytometry as well as the viability, necrosis and apoptosis rate. This same parameters were evaluated in spleen cells *in vitro* with different exposure times.

Arsenic exposure and *in vitro* treatment increased phosphorylation of lck and fyn in a dose- and time-dependent manner in spleen cells, this effect was also observed on mitogen-stimulated cells. Arsenic exposure also caused an increase in apoptotic cells and an inhibition on cell proliferation and IL-2 secretion, both effects probably due to the induction of phosphorylation of lck and fyn.

1869 MODULATION OF LPS-INDUCED CYTOKINE AND TISSUE FACTOR EXPRESSION IN MACROPHAGES BY THE PI3K-AKT PATHWAY.

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Lipopolysaccharide (LPS) stimulation of monocytes/macrophages activates various intracellular signaling pathways, including the mitogen-activated protein kinases (MAPKs; e.g., ERK1/2) and the phosphatidylinositol-3-kinase (PI3K)-Akt path-

way. In addition, LPS induces the expression of a variety of genes, such as the cytokines TNF α , IL-6 and IL-10, as well as tissue factor (TF), the principal activator of blood coagulation. At present, the role of the PI3K-Akt pathway in the regulation of LPS-induced gene expression is controversial. The activation of Class IA PI3K requires both a 110kD catalytic subunit (α , β , or δ) and a 85kD regulatory subunit (α or β). PI3K generates PIP3, a lipid second messenger required for the activation of Akt. PIP3 levels are counterbalanced by the phosphatase PTEN. We tested the hypothesis that activation of PI3K-Akt signaling modulates LPS induction of cytokines and TF in peritoneal macrophages (PMs). First, we determined the effect of genetically reducing PI3K activity on LPS induction of cytokines and TF expression using PMs from p85 $\alpha^{-/-}$ mice. LPS activation of Akt was reduced and ERK1/2 activation was enhanced in PMs from p85 $\alpha^{-/-}$ mice compared with PMs from wild type mice. These p85 α -deficient PMs exhibited enhanced LPS induction of IL-6, TNF α and TF, whereas expression of the anti-inflammatory cytokine, IL-10, was reduced. Next, we determined the effect of genetically increasing Akt activity on LPS induction of cytokine and TF expression using PMs from PTEN^{fllox/lox}/LysMCre mice. LPS-stimulated Akt activity was enhanced in PTEN^{fllox/lox} PMs, whereas LPS activation of ERK1/2 was reduced. This change in intracellular signaling was associated with reduced LPS induction of IL-6, TNF α and TF expression and increased expression of IL-10 in PTEN^{fllox/lox} PMs. Taken together, our results suggest that the PI3K-Akt pathway inhibits proinflammatory gene expression in macrophages by inhibiting LPS activation of MAPKs, but is required for IL-10 expression.

1870 DIFFERENTIAL REGULATION OF CYP1A1 AND MUC GENE EXPRESSION BY TCDD IN HUMAN AIRWAY EPITHELIAL CELLS.

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RATIONALE: 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been identified to be one of the most potent toxicant in the environment. It has been shown that TCDD is associated with chronic obstructive pulmonary disease, although the exact mechanism is still unknown. **METHODS:** Primary human tracheobronchial epithelial (TBE) cells and HBE1 cell line were cultured under the air-liquid interface condition and treated with various amounts of TCDD and vehicle (DMSO). For EGFR and MAPK pathway inhibitor studies, the inhibitor was added 30 min prior to the TCDD treatment. At various times after the treatment, cultures were harvested for RNA isolation. Quantitative real-time PCR was used to quantify the levels of CYP1A1, MUC5B, MUC5AC and control genes (b-actin and GAPDH). **RESULTS:** We have observed a 3 to 6 fold stimulation of CYP1A1, MUC5B, and MUC5AC messages in these human TBE cells after 24 hrs treatment. The stimulation was time- and dose-dependent on TCDD. Inhibitor studies have showed that TCDD-induced CYP1A1 was JNK inhibitor sensitive, but insensitive to inhibitors of the other two MAPK pathways, ERK and p38, and EGFR signaling pathways. In contrast, TCDD-induced MUC5B and MUC5AC expressions were both sensitive to the ERK and p38 inhibitors and the inhibitor of EGFR signaling, but insensitive to the JNK inhibitor. **CONCLUSION:** We have demonstrated that TCDD is a potent inducer for MUC gene expression, a clinic hallmark associated with the pathogenesis of COPD. The inhibitor studies suggest that TCDD induces the expression of MUC5AC and MUC5B, not through conventional aryl-hydrocarbon receptor, but through EGFR, ERK and p38 MAPK pathways.

1871 REDUCTIONS IN THE LEVEL OF AHR PROTEIN IN CELLS LINES HAS A DRAMATIC IMPACT ON AHR-MEDIATED GENE REGULATION.

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The Ah receptor (AHR) is rapidly degraded both in vivo and in vitro following binding to halogenated aromatic hydrocarbons (HAH) typified by 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD). Since several studies have shown that the overall level of endogenous AHR in cells and tissues from different species can vary over an order of magnitude, it was of interest to evaluate the impact of reducing the level of endogenous AHR on AHR-mediated gene regulation in cell culture lines derived from mouse or human tissues. It was also of interest to evaluate whether there would be differences in the level of gene regulation when different ligands were utilized. Mouse Hepa-1 and human retinal epithelial cells (hRPE) were used for these studies. Both cell lines are responsive to AHR ligands and induce endogenous CYP1A1. To quantitatively reduce the level of endogenous AHR in the cells, siRNA transfection was used. This method could consistently achieve >90% transfection of the cell populations and by using graded levels of siRNA, the level of endogenous AHR could be reduced from 30-95%. The results show that reduction in the level of AHR has a profound impact on the dose-response curve for the induction of endogenous CYP1A1. In Hepa-1 cells, a reduction of as little as 30% of the

AHR level shifted the dose-response curve for TCDD induction of CYP1A1 to the right and reduced the overall magnitude of the response. At 70% AHR reduction, the EC50 for TCDD was increased by an order of magnitude and the maximal level of CYP1A1 induction was reduced by >80%. The changes in dose-response were even more profound when less potent AHR ligands were used, and modest reductions in the level of AHR resulted in complete loss of CYP1A1 induction in some cases. Collectively, these studies suggest that modulation of the level of AHR protein can have a dramatic impact on the magnitude of AHR-mediated gene regulation and suggest that the concept of "spare receptors" may not hold for the AHR signaling pathway. Supported by ES10991.

1872 CROSSTALK BETWEEN THE AHR AND WNT SIGNALING PATHWAYS DURING TISSUE REGENERATION.

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Inhibition of early life stage zebrafish caudal fin regeneration by aryl hydrocarbon receptor (AHR) activation has been developed as an in vivo platform to define the cross talk between AHR and other molecular signaling pathways. Using antisense repression approach, we have previously identified that AHR2 and ARNT1 are necessary and sufficient for TCDD to impair regenerative growth. Global gene expression analysis performed using RNA isolated from the regenerating tissue obtained from the control or TCDD exposed larvae revealed that a cluster of genes involved in extracellular matrix remodeling, cell migration and cell adhesion were misregulated by AHR activation. Using pathway analysis, we identified more than 20 Wnt target genes differentially expressed in regenerating tissue suggesting misregulation of Wnt signaling by AHR activation. Distinctively, R-Spondin 1, a novel ligand for the Wnt receptor was one of the most highly TCDD-induced genes at all the time points analyzed. Since R-Spondin-1 is an upstream modulator of Wnt signaling, we proposed that AHR-dependent misregulation of R-Spondin-1 may dictate the downstream gene expression changes that collectively result in impaired extracellular matrix remodeling and cellular migration. If the induction of R-Spondin 1 is required for TCDD to block regenerative growth, antisense repression of R-Spondin 1 should permit regeneration even in the presence of TCDD. In support of this hypothesis, partial suppression of R-Spondin1 using splice blocking morpholinos abrogated the TCDD-dependent block of regeneration, suggesting that inappropriate induction of R-Spondin 1 is required for TCDD to impair regeneration. These studies for the first time identify functional cross talk between Wnt and AHR signal transduction pathways and indicate the power of zebrafish model to discover and define molecular pathways that regulate complex in vivo biological responses. Supported by NIEHS grants ES10820, ES00210, and #ES03850.

1873 INHIBITION OF BLADDER CANCER CELL GROWTH BY GLYCYRRHETINIC ACID DERIVATIVES.

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Bladder cancer incidence has been increasing and, even after chemotherapy with highly cytotoxic drugs, the prognosis for disease-free survival in most patients is only 1 to 2 years. 2-Cyano-3,11-dioxo-18 β -oleana-1,12-dienoic acid (CDODA) and its methyl ester (CDODA-Me) are synthetic analogs derived from glycyrrhetic acid (GA), a triterpenoid natural product found in licorice. While GA and several unsubstituted analogs exhibited minimal cytotoxicity to Ku7 and 253J-BV bladder cancer cells, IC50 values for growth inhibition by CDODA were < 5 μ M (both cell lines) and, by CDODA-Me, were 1.0 and < 0.5 μ M, respectively. Both cell lines were used as models for investigating the effects of CDODA-Me, the most active analog, on inducing growth inhibitory and proapoptotic pathways in bladder cancer cells. CDODA-Me activated peroxisome proliferator activated receptor γ (PPAR- γ) in transactivation assays; however, most of the responses induced by CDODA-Me were not inhibited by PPAR γ antagonists. CDODA-Me did not induce expression of p21 or p27 whereas cyclin D1 expression was downregulated in both cell lines. The tumor suppressor gene caveolin-1, which is induced in colon cancer cells by PPAR γ agonists, was decreased in 253J-BV cells treated with 0.1-1.0 μ M CDODA-Me and the high constitutive expression of caveolin-1 in this cell line was totally suppressed in cells treated with 1.0 μ M CDODA-Me. In contrast, there was neither induction nor suppression of caveolin-1 in Ku7 cell lines. CDODA-Me also induced the proapoptotic nonsteroidal anti-inflammatory drug activated gene-1 (NAG-1) and caspase-dependent PARP cleavage in both cell lines, and downregulation vascular endothelial growth factor (VEGF) was also observed. The mechanisms associated with these proapoptotic/antiangiogenic effects of CDODA-Me in bladder cancer cells is currently being investigated. (Supported by NCI CA112337)

1874 GLYCYRRHETINIC ACID DERIVATIVE INHIBIT PANCREATIC CANCER CELL GROWTH THROUGH ACTIVATION OF MULTIPLE PATHWAYS.

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Glycyrrhetic acid (GA), a triterpenoid found as a glycoside conjugate in licorice, exhibits a broad spectrum of therapeutic properties. GA exhibited minimal cytotoxicity to pancreatic cancer cells. The IC₅₀ values for inhibition of Panc1 pancreatic cancer cell growth by a synthetic derivative 3,11-dioxo-18 β -oleana-1,12-dienoic acid (DODA) and its methyl ester (DODA-Me) were >40 and >15 μ M, respectively. In contrast, IC₅₀ values for 2-cyano-3,11-dioxo-18 β -oleana-1,12-dienoic acid (CDODA) and its methyl ester (CDODA-Me) were 2.5 and 1.0 μ M in Panc1 and 5.0 and 1.0 μ M in Panc28 cells, respectively. The growth inhibitory effects of CDODA and CDODA-Me were paralleled by their proapoptotic activity which included activation of caspase-dependent PARP cleavage. We also observed that both CDODA-Me and CDODA, but not DODA or DODA-Me, activated peroxisome proliferator-activated receptor γ (PPAR γ) in pancreatic cancer cells transfected with a PPAR γ -GAL4 chimera and a pGAL4-Luc construct which contains 5 yeast GAL4 response elements linked to luciferase. Using the more potent CDODA-Me as a model, the proapoptotic and growth inhibitory effects and the role of PPAR γ in mediating these responses was further investigated. CDODA-Me induced p21 and p27 and downregulated cyclin D1 protein expression in Panc28 cells; however, cotreatment with PPAR γ antagonists did not affect these responses. We also observed that CDODA-Me affected additional receptor-independent responses associated with apoptosis and these included induction of glucose related protein 78 (endoplasmic reticulum stress), non-steroidal anti-inflammatory drug activated gene-1 (NAG-1), and activating transcription factor 3 (ATF3). This study has identified a novel cytotoxic triterpenoid acid (CDODA) derived from GA that activates PPAR γ and induces a diverse spectrum of receptor-independent proapoptotic and growth inhibitory pathways. (Supported by NCI CA112337)

1875 CAN A SINGLE EXPERIMENTAL MODEL PREDICT THE RESPIRATORY SENSITIZATION POTENTIAL OF CHEMICALS AND PROTEINS?

J. Hotchkiss. *Respiratory Toxicology, The Dow Chemical Company, Midland, MI.*

The prevalence of asthma in the United States appears to have substantially increased during the past two decades. While progress has been made in the control of asthma, complete remission is uncommon. One potential control strategy, therefore, is to minimize exposure to xenobiotics with the potential to induce allergy and asthma. Implicit in the concept of reducing exposure is the ability to identify the proteins and chemicals with the potential to induce asthma and airway hyperreactivity in humans. The development of animal models of asthma has paralleled our interest in and our understanding of the pathogenesis of the human disease. Each model has its strengths and weaknesses and many were developed to examine specific aspects of the human disease by exploiting unique characteristics of the experimental species. This roundtable will examine current in vitro and in vivo experimental models in light of their ability to identify chemicals and proteins with the potential to induce asthma and respiratory sensitization in humans. The goal of the roundtable is to identify the key attributes that a robust, predictive, experimental model of respiratory sensitization must have and use this framework to evaluate current models, identify knowledge gaps, and propose a path forward to a consistent, predictive testing strategy. This roundtable should be of interest to experimental respiratory- and immuno-toxicologists investigating asthma and respiratory sensitization induced by chemicals and proteins as well as individuals involved with pharmaceutical, agrochemical, and biotechnology development, human risk assessment, and product regulation and registration.

1876 K-12 EDUCATION: INVESTING IN THE FUTURE OF TOXICOLOGY.

S. C. Fitzpatrick¹ and D. Cragin². ¹Office of the Commissioner, FDA, Rockville, MD and ²Merck, Whitehouse Station, NJ.

Today's young students face a world of increasing global competition. To maintain the US pre-eminence in science, scientific organizations such as the Society of Toxicology must foster innovation in K-12 science. Experienced scientists wish to get involved in enhancing opportunities for young students to actively participate in toxicology but often they don't know where to begin. This symposium will focus on the many opportunities and resources available for toxicologists to enable them to make a difference in our future generation of scientists by introducing them to the exciting world of toxicology.

1877 THE IMPORTANCE OF K-12 ACTIVITIES TO THE FUTURE OF SOT.

K. Wallace. *University of Minnesota, Duluth, MN.*

SOT's goals is to be the leading global force in enhancing human, animal, and environmental health through the science of toxicology. To help achieve this goal, SOT must look at new and creative ways to enhance opportunities for young students to actively participate in toxicology. Engaging students early in their K-12 years will help recruit talented students into the field of toxicology.

1878 OUTSOURCING, OFFSHORING, AND OCCUPATIONS IN A GLOBAL ECONOMY: THE CASE FOR IMPROVING K-12 SCIENCE EDUCATION.

B. Fuchs. *National Institutes of Health, Bethesda, MD.* Sponsor: S. Fitzpatrick.

To remain competitive in the 21st century, science education will become increasingly important to America. International surveys show that middle school and high school students in America rank near the bottom in terms of math and science knowledge. Yet, the creation of new scientists and engineers is key to the future growth of the American economy. Motivating middle school and high students to become scientists will help meet this need.

1879 PLANNING K-12 OUTREACH EVENTS - 'NUTS AND BOLTS' OF WHAT YOU CAN DO.

S. Wild. *Amgen, Inc., Thousand Oaks, CA.*

The K-12 committee will share its experiences in planning a K-12 Outreach Event with practical tips, lessons learned, and ideas for future outreach. The committee will share experiences from the 2006 SOT "Paracelsus Explores the Genome: Toxicology Advances Health" and provide information on partnering with existing institutions, utilizing their expertise, and planning a successful event.

1880 TIPS FOR KEEPING YOU COOL IN A K-12 CLASS: DO, DON'TS, AND TOOLS.

S. Gilbert. *Institute of Neurotoxicology & Neurological Disorders, Seattle, WA.*

Volunteering to share your knowledge of toxicology with K-12 students is one of the personally most rewarding endeavors we can do and it serves to increase science education. Obviously there are different requirements depending on the grade level but all the students want to hear what it is like to be a scientist. It is important to check with the teacher on how to fit your material into the topics they are covering. This presentation will cover some practical do and don'ts in K-12 teaching and review some useful tools.

1881 INTRODUCTION.

T. T. Kawabata. *Pfizer Research and Development, Groton, CT.*

You've completed your post-doctoral training and landed your first job. After many dedicated years in graduate school and as a post-doctoral fellow, you now have the scientific knowledge and technical skills needed to get the job done and you are ready to hit the ground running. However, there are many other important skills you need to learn. Whether your new job is in academia, government or industry, you just can't focus on the science. You need to be aware of the many planning and people skills needed to be successful in your new job.

1882 PLANNING FOR SUCCESS AND CAREER SATISFACTION.

H. M. Mehendele. *Department of Toxicology, University of Louisiana at Monroe, Monroe, LA.*

Regardless of whether one chooses a career in the private sector, academia or government, planning and preparing for success and career satisfaction must begin very early. First, you need to identify your strengths, weaknesses, likes and dislikes in order to plan your career path and to assess the skills that need to be developed. For example, if you like to work with and develop people, you may direct your career toward management or as a research advisor. Secondly, one needs to consider the

practical aspects of career choices. Should one specialize in a specific area or be broader for greater flexibility and employability? Should you stay with one employer or move between them? Personal factors such as geography, work-life balance and salary should also be taken into consideration. Third, when developing these plans, it is important to seek advice from others. This includes networking with colleagues and working with mentors. Finally, changes that alter your career path are to be expected. Those with personal resilience and flexibility will be able spot opportunities, recharge, and develop necessary new skills, thereby maintaining a high level of career satisfaction and employability.

1883 THE IMPORTANCE OF PEOPLE AND LEADERSHIP SKILLS IN CAREER SUCCESS.

W. D. Pennie. *Drug Safety Research and Development, Pfizer Research and Development, Groton, CT.* Sponsor: T. Kawabata.

To be successful in your career you must develop skills to lead and work with people in many different situations. For many people, the development of these skills are pushed aside for higher priority issues. However, they soon encounter difficult situations with their staff, colleagues and supervisors that they are not equipped to handle. One of the most important skills that should be a key focus is effective communication. This involves developing listening and interactive approaches. A good understanding of individual and cultural differences will help increase collaborations. One must also learn how to assert yourself in certain situations and be able to resolve conflicts. These techniques will be very important when working in teams and facilitating meetings throughout your career. Leadership of technical staff and projects should also be a key development area for all scientists throughout their career. A good leader provides vision and goals, energizes the team, knows how to best utilize each individuals abilities and is willing to change the status quo when needed. It is not true that certain people are just born with these skills, these skills can be developed and must be reinforced throughout your career.

1884 CHEMICAL MIXTURES: IS THE 'PROBLEM' SOLVABLE?

J. Simmons. *NHEERL/ORD, U.S. EPA, Research Triangle Park, NC.*

The potential human health risk(s) associated with exposure to multiple chemicals, either concurrently or sequentially, has been an open question for many years. Solving the mixtures problem is of considerable importance as there are economic costs of assuming increased risk due to chemical mixtures when none exists and human health costs of assuming no increased risk if, in fact, such risk exists. Historically, advances in understanding mixtures has been limited by a variety of factors, including the astronomical number of combinations of chemicals and the inability to conduct the extremely large experiments required by traditional full-factorial designs for mixtures of several chemicals. Progress has been made with regard to experimental designs that require fewer dose groups, detection of departure from dose- and response- additivity, and methods for analyzing and modeling mixture response. This Roundtable will address whether recent advances have given us the necessary tools and the knowledge base needed to 'solve' the mixtures problem. The purpose is to bring together experts to examine important questions whose answers will advance our understanding of the potential human health risk posed by exposure to mixtures and recent advances in knowledge gained by use of techniques, methods and models developed and applied over the past decades. The objective is open discussion aimed at stimulating thinking in this very important area of science. (This abstract may not reflect EPA policy.)

1885 HOW DO WE EVALUATE AND IMPROVE NONMECHANISTIC MIXTURE RISK FORMULAS?

R. Hertzberg. *Consultant, Atlanta, GA.* Sponsor: J. Simmons.

Formulas for mixture risk assessment in use by regulatory agencies and related groups based on the mixture component chemicals are conceptually simple and require few data resources. For example, both the standard and interaction-based hazard index formulas of the U.S. EPA are roughly based on the classical formula of dose addition. Attempts to support dose addition with biological arguments such as Finney's concept of simple similar action have advanced the understanding of mixture risk formulas only to a limited degree. This presentation will examine quantitative methods for direct evaluation of existing risk formulas that involve tight definitions, bounding simplifications, statistical applications with nested mixture data, and comparison with more biologically based mixture models.

1886 HOW CAN PHARMACOKINETIC AND PHARMACODYNAMIC MODELING BE USED TO UNDERSTAND OBSERVED INTERACTIONS?

N. J. Waker. *NTP, NIEHS, Research Triangle Park, NC.*

The dioxin Toxic Equivalency Factor (TEF) approach is currently used worldwide for assessing and managing the risks posed by exposure to mixtures of polychlorinated dibenzodioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs). While the TEF approach is only applied to dioxin-like PCBs (non-ortho and mono ortho substituted PCBs), previous studies have shown the potential for interactions between different classes of PCBs for nonneoplastic responses. Analyses from studies conducted by the National Toxicology Program evaluating dose additivity of effects for dioxins and interactions between dioxin-like and nondioxin-like PCBs for carcinogenic responses support the TEF methodology for additive risk when based on administered dose. Given the use by the USEPA of body burden as a dose metric for dioxin like compounds, there is a need to evaluate this approach using internal dose metrics. Using tissue data from the NTP studies we have developed an integrated pharmacokinetic and pharmacodynamic model for dioxin like compounds for the assessment of the relative potency of these compounds using different internal dose metrics.

1887 ARE GENOMIC DATA USEFUL FOR UNDERSTANDING CHEMICAL INTERACTIONS IN TOXICITY STUDIES?

J. Groten. *TNO, Zeist, Netherlands.* Sponsor: J. Simmons.

In the area of combination toxicology, toxico-genomics enables the study of the combinatorial effects of chemicals at the molecular level without a priori knowledge on the possible mechanisms of action. Although the number of published toxicogenomics studies on single compounds outweighs that on chemical mixtures or combined exposures, the first studies on complex chemical mixtures using genomics approaches have appeared in the literature. This talk summarizes results of studies with mixtures of chemicals to compare toxicity end-points or biomarkers in laboratory animals making use of a systems toxicology approach. One of the research projects deals with hazard identification and risk assessment of a quaternary mixture of pollutants (methyl mercury, lead, benzene, and trichloroethylene). PBPK-models of the four individual compounds are used to translate the dosing regime in experimental animals and this modeling approach will be used to distinguish interactions at the toxicokinetic level from interactions at the toxicodynamic level and to compare conventional toxicological assessment with toxicogenomic parameters.

1888 IS CHEMICAL MIXTURE TOXICOLOGY PREDICTABLE?

R. S. Yang. *Colorado State University, Colorado Springs, CO.*

Predicting the toxicity of chemical mixtures is one of the most important issues facing toxicologists today. This issue is significant because there are not enough resources to keep on conducting toxicity testing on the single chemicals in commerce, let alone chemical mixtures. Further, the number of deaths due to adverse drug reactions from multiple drug treatments is alarmingly high and we currently have limited means to deal with it. Developed and advanced in our laboratory, biochemical reaction network modeling, and its possible integration with physiologically based pharmacokinetic modeling, provides a ray of hope in helping us to address the complex issue of toxicity prediction. This presentation will briefly outline our approach, the progress, and future perspective.

1889 IMPLICATIONS OF JOINT TOXICITY AND RISK ASSESSMENT TO PUBLIC HEALTH.

M. Mumtaz. *Division of Toxicology and Environmental Medicine, ATSDR, Atlanta, GA.*

Ideally, toxicity assessments must represent summation and conclusion of all available epidemiological, experimental, animal and human data of a chemical or its mixtures and the potential risk such exposures pose to the public. Qualitative, and if data lend themselves, quantitative conclusions are made through the process of exposure assessment, hazard identification, dose response assessment, leading to risk characterization. In turn, such conclusions serve as the basis of decision making for controlling releases of contaminants in the environment. This process has served well for the protection of general populations. However, because of lack of data, certain uncertainties are part of these toxicity estimates. Apart from these known uncertainties, a major challenge is to address the issue of susceptible populations that are age, body burden or polymorphisms dependent. A renewed interest

in the process of hormesis and the role of tissue repair/injury pose additional challenges to this practice of toxicology. We have to prioritize research to diminish the uncertainties and increase the accuracy in such toxicity estimates. The evaluation of these processes and their role at low level exposures will be discussed in this talk.

1890 UROPORPHYRIN ACCUMULATION IN MOUSE LIVER EXPRESSING EITHER MOUSE OR HUMAN CYP1A2: RELATIONSHIP OF CYP1A2 LEVELS TO HUMAN PORPHYRIA CUTANEA TARDA.

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Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants that cause cancer, immunosuppression, and act as endocrine disruptors. PCB-induced CYP1A2 results in inhibition of uroporphyrinogen decarboxylase and oxidation of uroporphyrinogen to uroporphyrin (URO); the result is an increased rate of URO formation (uroporphyria) and hepatic tumors in laboratory animals. In mice, CYP1A2 is absolutely required for developing uroporphyria; *Cyp1a2*(-/-) knockout mice treated with PCBs, polycyclic aromatic hydrocarbons, or dioxin never show any detectable URO accumulation in the liver, blood or urine. Humans show >60-fold genetic differences in liver basal CYP1A2; however, the involvement of CYP1A2 in clinical uroporphyria (porphyria cutanea tarda) is unclear. We compared two BAC-transgenic mouse lines, carrying hCYP1A2 and hCYP1A1 in the absence of mCYP1A2 [*hCYP1A1_1A2/mCyp1a2*(-/-)] or carrying hCYP1A2 and hCYP1A1 in the absence of mCYP1A1 [*hCYP1A1_1A2/mCyp1a1*(-/-)], with wild-type mice [*mCyp1*(+/+)]. We induced uroporphyria by pretreatment with Fe-dextran, a 75 µg/kg PCB126 injection, and 5-aminolevulinic acid in the drinking water for 4 and 8 weeks. The *mCyp1*(+/+) and *hCYP1A1_1A2/mCyp1a1*(-/-) mice accumulated URO at 4 and 8 weeks, whereas *hCYP1A1_1A2/mCyp1a2*(-/-) showed no detectable URO levels at 4 or 8 weeks. On the other hand, hepatic acetanilide 4-hydroxylase activity and hCYP1A2 mRNA and protein were 1.5- to 2-fold higher in *hCYP1A1_1A2/mCyp1a2*(-/-) than in *hCYP1A1_1A2/mCyp1a1*(-/-) mice. We conclude that, in the absence of mouse CYP1A2, human CYP1A2 appears to be normally expressed yet, in the protocol used here, did not support URO accumulation. —Supported in part by NIH grants R01 ES008147, R01 ES014403, and P30 ES06096; and Dept of Veteran's Affairs.

1891 TRANSGENIC MICE OVEREXPRESSING THE HUMAN CYTOCHROMES P450 (CYP)1A1 OR 1A2 GENE PROMOTERS DISPLAY ENHANCED SUSCEPTIBILITIES TO HYPEROXIC LUNG INJURY *IN VIVO*.

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Supplemental oxygen therapy is often required for the treatment of infants having pulmonary insufficiency. However oxygen therapy contributes to bronchopulmonary dysplasia (BPD) in infants. Recent studies have suggested a role for cytochrome P450 (CYP)1A enzymes in hyperoxic lung injury in rodents. Here, we tested the hypotheses that (i) induction of CYP1A1/1A2 expression by hyperoxia *in vivo* is mediated by transcriptional activation of the corresponding genes; and (ii) transgenic overexpression of CYP1A1 or 1A2 gene promoters in mice would alter their susceptibility to oxygen-mediated lung injury *in vivo*. Twelve week-old male wild type (WT) (CD-1) or transgenic mice expressing the human CYP1A1 (CYP1A1-luc) or 1A2 (CYP1A2-luc) gene promoters and the reporter luciferase gene were exposed to hyperoxia (greater than 95% O₂) or room air for 24-72 h. Luciferase expression was determined *in vivo* by bioluminescent imaging. Endogenous pulmonary and hepatic CYP1A1/1A2 enzyme activities, apoprotein contents, and mRNA levels were also determined. The CYP1A2-luc as well as CYP1A1-luc mice were more susceptible to lung damage than similarly exposed WT animals, with 1A2-luc mice being the most sensitive. Forty-eight hours of hyperoxia caused significant (2-4-fold) increases in luciferase expression in the CYP1A1-luc and CYP1A2-luc animals, compared to WT animals. Luciferase induction declined by 72 h. The transgenic animals were refractory to induction of endogenous CYP1A1/1A2 expression by hyperoxia. Our results indicate that induction of CYP1A1/1A2 expression by hyperoxia *in vivo* is mediated by transcriptional activation of the corresponding genes. The lack of induction of endogenous CYP1A1/1A2 by hyperoxia in the CYP1A1-luc and 1A2-luc mice may have contributed to the increased susceptibilities of these mice to hyperoxic lung injury, suggesting that these enzymes play protective roles against oxygen-mediated injuries. (Supported by NIH grant ES009132 and HL070921.)

1892 CYTOCHROME P450 1 GENE EXPRESSION IN ZEBRAFISH, *DANIO RERIO* ADULTS AND EMBRYOS.

E. E. Jönsson, B. R. Woodin, J. V. Goldstone and J. J. Stegeman. *Biology, WHOI, Woods Hole, MA.* Sponsor: M. Hahn.

Effects of planar halogenated AH receptor agonists (e.g., non-ortho-substituted polychlorinated biphenyls - PCBs; polychlorinated dibenzo-*p*-dioxins) largely involve AHR regulated genes. Fish exhibit greater sensitivities to developmental defects caused by these chemicals than do mammals, which may involve differences in expression of multiple *CYP1* genes (*CYP1A*, *1B1*, *1C1*, and *1C2*). We cloned *CYP1C2* and examined expression of the *CYP1* complement in zebrafish, *Danio rerio*. Cloned *CYP1C2* exhibits a 78% amino acid identity with zebrafish *CYP1C1* and has several dioxin response elements in the proximal promoter region. Adult and embryonic (8 hpf) zebrafish were exposed to 100 nM 3,3',4,4',5-PCB (PCB126) or 20 ppm acetone (vehicle control) for 24 h and then held in clean water for 24 and 48 h, respectively. The *CYP1* genes were expressed in control embryos and in all examined organs (eye, gill, heart, liver, kidney, brain, gut, and gonads) in control adults. The highest basal expressions in adults were observed in liver (*CYP1A*) and in heart and eye (*CYP1B1*, *1C1*, and *1C2*). PCB126 induced *CYP1A* in all organs, *CYP1B1* in eye, gill, heart, liver, kidney, and brain, and *CYP1C1* in eye, gill, heart, liver, and kidney, whereas *CYP1C2* was not induced significantly in any organ examined. In embryos (80 hpf) both *CYP1C1* and *1C2* tended to have a higher basal expression than *CYP1A* and *CYP1B1*, and all four genes were strongly induced by PCB126. The results indicate that the two *CYP1C* genes are AHR regulated. Patterns of *CYP1C* expression suggest that these genes play roles in zebrafish during their whole lifespan, as both genes were present in embryo controls and in all organs examined in control fish. The two genes may have distinctive functions, however, as *CYP1C1* was induced by PCB126 in several organs, while *CYP1C2* was not induced in any adult organ. Furthermore, the difference in inducibility of *CYP1C2* in embryos and adults suggests that *CYP1C2* may play different roles in embryonic and adult fish. (The Swedish Research Council Formas; NIH P42-ES07381; EPA R827102; NIH 1 F32 ES012794)

1893 COMPARISON OF *IN VITRO* PHASE I AND II METABOLIC ACTIVITY ASSAYS BETWEEN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AND HUMAN HEPATIC MICROSOMES AND S9 FRACTIONS.

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Phase I (e.g. cytochrome P450-mediated oxidation and demethylation) and Phase II (e.g. sulfotransferase and UDP glucuronosyltransferase) drug metabolizing enzymes mediate the biotransformation of xenobiotics in both mammalian and aquatic species (i.e. fish). Assessment of the bioaccumulation of environmental contaminants can be achieved utilizing metabolism in model organisms such as rainbow trout. Metabolic turnover can be predicted by *in vitro* assays with isolated liver fractions. However, metabolic enzymes in trout liver are not well characterized. The goal of this study is to compare Phase I and II drug metabolizing activities in liver microsomes and S9 fractions from rainbow trout (*Oncorhynchus mykiss*) and humans. Microsomes and S9 fractions were prepared from both isogenic and hatchery-reared rainbow trout livers (age: two years) using standard protocols. The human liver subcellular fractions were pooled from 15 donors. Phase I drug metabolizing enzymes were measured for EROD (ethoxresorfin deethylase) and testosterone hydroxylation activities and Phase II drug metabolizing enzyme activities were measured for sulfotransferase (ST) and UDP glucuronosyltransferase (UGT). The incubations were performed at pH 7.8 and 25 °C, conditions which are optimal for *in vitro* metabolic activity for trout liver fractions. Metabolic activities for isogenic and hatchery-reared trout liver fractions showed similar activities which were lower (>2 fold) than corresponding human activities. These results will aid in the development of standard conditions for assessment of *in vitro* metabolism in trout and in the application of trout metabolic activities for the assessment of bioaccumulation potential of environmental compounds.

1894 GENETIC MAP OF THE CYTOCHROME P4501A5 GENE ASSOCIATED WITH AFLATOXIN B1 HYPERSENSITIVITY IN THE TURKEY (MELEAGRIS GALLOPAVO).

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Turkeys are the most susceptible animals to the toxic effects of the mycotoxin aflatoxin B1 (AFB1). This was graphically demonstrated by "Turkey X Disease" which caused widespread deaths of turkeys and other poultry throughout Europe in the

1960s, later shown to be caused by AFB1-contaminated feed. For toxicity, AFB1 requires metabolic activation by hepatic cytochromes P450 (CYP) which convert it to the reactive and electrophilic intermediate, the AFB1-8,9-epoxide (AFBO), which then binds to DNA and other critical macromolecules. We recently cloned CYP1A5 from turkey liver, the heterologously-expressed protein from which had high AFB1 bioactivation activity (high V_{max} , low K_m), which is inhibited by prototype CYP1A inhibitors, and by phenolic antioxidants. This supports our previous work showing that the mechanism of aflatoxicosis chemoprevention in turkeys by dietary antioxidants is by inhibition of the hepatic form of this enzyme. Comparative genomic approaches were used to selectively amplify and sequence the introns and 3' flanking region of CYP1A5. The architecture of CYP1A5 was found to be equivalent to human CYP1A1 with seven exons of 38, 858, 127, 90, 124, 87 and 307 bp, respectively, and six introns. A single nucleotide polymorphism in the 3' untranslated region (UTR) was used to genetically map CYP1A5 to turkey linkage group M16 (equivalent to chicken chromosome 10). The results of this study provide the framework for identifying allelic variants of this important CYP gene in poultry, and may represent a useful strategy to identifying AFB1-resistance traits in poultry.

1895 BRAIN CYTOCHROME P450 ISOFORMS EXPRESSION IN RATS EXPOSED TO DDT.

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DDT is an endocrine disruptor and a cytochrome P-450 (CYP) inducer. CYP-dependent metabolism is regulated by age, gender and tissue. Information about the effect of DDT on expression CYP isoforms in extrahepatic tissues is limited. The objective of this study was to determine the effect of DDT on CYP isoforms levels in rat brain. Wistar adult male rats (200±20 g) were administered with single oral doses of DDT technical grade (0.1, 1, 5, 10 and 100 mg/kg) dissolved in corn oil. Animals were sacrificed 24 h later and brain removed and processed to obtain microsomes. CYP1A1, 2B, 2A, 2C11, 2E1, 3A2 and 4A1 were assessed by Western blot analysis. DDT produced a biphasic dose-dependent response in brain CYP levels. An inductive effect in the majority of the isoforms was observed in the range of 0.1 to 5 mg/kg. At the lowest dose of DDT (0.1 mg/kg) the maximum inductive effect (70%) was detected in CYP1A1, 2A and 4A1 levels. CYP2B, 2E1 and 3A2 levels increased in a dose-dependent manner and the maximum effect was at 5 mg/kg (2-fold). On the contrary, at 10 and 100 mg DDT/kg CYP isoforms expression levels drastically decreased, representing less than 40% of levels in the non-treated group. CYP2C11 levels decreased dose-dependent in a manner at all DDT doses. These results indicate that exposure to single DDT doses may modify brain metabolism of xenobiotics and endogenous substrates, and that such effects on CYP expression are dose-dependent. They also suggest that the selective effect on brain CYP expression may represent an endocrine disruption mechanism.

1896 MODIFICATION OF CYTOCHROME P450 3A4 METABOLISM OF NAPHTHALENE AND 17 β -ESTRADIOL BY ORGANOPHOSPHORUS COMPOUNDS.

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Cytochrome P450 (CYP) 3A4 is the most abundant monooxygenase in human liver and small intestine, and is the primary contributor to the phase I metabolism of both endogenous and exogenous substrates. Due to its abundance and broad spectrum of metabolic activity, CYP3A4 frequently causes a significant impact on the bioavailability of clinical drugs, thus a modification of the metabolic activity of the enzyme by inhibitors or activators may affect the safety and efficacy of such drugs or the toxicity of other xenobiotics. In the current studies, we examined the metabolic modification of CYP3A4 metabolism of naphthalene and 17 β -estradiol (E_2) by chlorpyrifos-oxon (CPO) and chlorpyrifos (CPS). CYP3A4 metabolism of naphthalene was significantly activated by preincubation of the enzyme with CPO, in a dose-dependent manner. V_{max} and K_m for naphthalene metabolism by CYP3A4 were significantly increased and decreased, respectively as the concentration of CPO preincubated with the isoform was increased, indicating that CPO bound to the enzyme appears to facilitate the binding of naphthalene to CYP3A4. This activation was independent of cytochrome b_5 , indicating that the metabolic activation was probably facilitated by conformational changes of the enzyme rather than stimulation of electron transfer from cytochrome b_5 . The CYP3A4 metabolism of E_2 was significantly inhibited by CPS in a concentration- and time-dependent manner. The inactivation rate constant (k_{inact}) and the inhibitor concentration required for half-maximal rate of inactivation (K_i) were 0.3 min⁻¹ and 4.9 μ M, respectively. This irreversible inhibition of E_2 metabolism by CYP3A4 is believed to be due to the sulfur atom released from CPS during CPO formation. These studies

support the hypothesis that the binding site of CYP3A4 can hold multiple substrates and that the metabolic activity of the enzyme can be modified by the first molecule bound to the enzyme.

1897 EFFECTS OF PUERARIA MIRIFICA SUBCHRONIC EXPOSURE ON HEPATIC CYTOCHROME P450 IN RATS FED WITH NORMAL- AND HIGH CHOLESTEROL DIET.

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Pueraria mirifica Airy Shaw and Suvatabandhu, known locally as White Kwao Keur, is a plant in family Leguminosae. In this study, effects of P.mirifica on hepatic cytochrome P450 (CYP) were investigated in male Wistar rats. Rats were randomly divided into four treatment groups as following: normal diet-fed group; normal diet-fed supplemented with P.mirifica group; high cholesterol diet-fed group; high cholesterol diet-fed supplemented with P.mirifica group. Each group comprised 10 rats. P.mirifica was administered orally at a dosage of 100 mg/kg/day for 90 consecutive days. At the end of the treatment, animals were anesthetized. Microsomes were prepared from the livers for enzyme assays. The results showed that P.mirifica significantly inhibited CYP2B1&2B2 in either normal diet or high cholesterol diet-fed rats. Its inhibitory effect of CYP1A2 and CYP2E1 was found only in normal diet-fed rats. No effect of P.mirifica was found on CYP1A1 and CYP3A. Inhibitory effects of P.mirifica on CYP2B1&2B2 and CYP2E1 were also found in the in vitro study. Inhibitory effects of P.mirifica on CYP1A2, CYP2B1&2B2 and CYP2E1 indicated a beneficial potential of this plant regarding the chemical-induced carcinogenesis while providing a possible potential information of this plant regarding drug-drug interaction with other medicines that are metabolized by these CYPs. Effects of P.mirifica at various doses as well as the mechanism of these effects should be further investigated. Effects of P.mirifica on human CYP should also be explored.

1898 CYP2E1 KNOCKOUT MICE ARE PROTECTED FROM THIOACETAMIDE-INDUCED LIVER INJURY.

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Thioacetamide (TA), a model hepatotoxicant, is bioactivated to TA sulfoxide (TASO), and further to the highly reactive sulf dioxide (TASO₂), that initiates hepatic necrosis by covalent binding. Although the mechanism by which TA initiates liver injury has been well understood, the specific enzymes responsible for the bioactivation of TA in rats had not been identified for a long time. Studies by Wang et al. (2001) using specific CYP2E1 inducer, isoniazid, and inhibitor diallyl sulfide, suggested a major involvement of CYP2E1 in TA bioactivation. The objective of the current study was to further investigate the role of CYP2E1 in TA bioactivation. We employed male cyp2e1 knockout mice (KO) in a 129SV background for this purpose. Liver injury was assessed over a time course (0 to 48 h) in wild type (WT) and cyp2e1 KO mice after administration of a LD100 dose (500 mg TA/kg dose, ip) in WT mice. The cyp2e1 KO mice were completely protected from TA liver injury as assessed by ALT, AST and liver histopathology when compared with the WT mice. The cyp2e1 KO mice exhibited neither initiation nor progression of any liver injury. As expected, the WT mice (Cyp2e1^{+/+}) showed marked liver injury leading to liver failure and mortality. These findings provide confirming evidence in support of previous conclusions that CYP2E1 is the enzyme responsible for bioactivation of TA.

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1899 MECHANISMS OF ETHANOL-MEDIATED INDUCTION OF THE RENAL 1, 25-DIHYDROXYVITAMIN D3-24-HYDROXYLASE (CYP24A1).

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Chronic ethanol (EtOH) consumption via total enteral nutrition leads to the disruption of vitamin D3 (VD) homeostasis. Serum levels of 1,25-(OH)₂VD are in part regulated by the 1,25-(OH)₂VD-24-hydroxylase (CYP24A1) which mediates its conversion into inactive calcitriol. EtOH consumption induced the mRNA, protein and gene transcription (hnRNA) of CYP24A1. In the present work, we explore the underlying mechanisms of how EtOH mediates the induction of

CYP24A1 using *in vitro* systems. Dose (50 – 100 mM EtOH) and time-course (0-48 h) studies with rat primary renal proximal tubule cells (RPTC) revealed ~6-fold induction of the CYP24A1 mRNA levels at 24 h with 50 mM EtOH. Both hnRNA and protein levels of CYP24A1 were also increased following EtOH treatment. Induction of CYP24A1 was only partially blocked by 4-methyl pyrazole suggesting that at least part of the induction may be directly mediated by EtOH. However, CYP24A1 induction by EtOH was completely abolished by cycloheximide suggesting the requirement of new protein synthesis. Further, we investigated the roles of specific MAP kinases in EtOH mediated induction of CYP24A1. Both p38 MAPK inhibitor (SB203580) and MEK1 inhibitor (U0126) but not the MAPKK inhibitor (PD98059) completely mitigated EtOH effects on CYP24A1. EtOH treatment also increased production of reactive oxygen species (ROS) in RPTCs and treatment of cells with different doses of H₂O₂ also led to induction (4 to 10-fold) of CYP24A1 mRNA. Finally, we examined the influence of EtOH on VD mediated induction of CYP24A1. We observed dramatic synergism in the induction of CYP24A1 (~125-fold) with co-treatment with VD and EtOH that themselves only caused 6 and 10-fold induction of the enzyme, respectively. In conclusion, our data show a novel regulation of CYP24A1 by EtOH presumably via increased p38 MAPK activation subsequent to EtOH-induced ROS production. Supported in part by R01AA012928 to MJJR.

1900 INHIBITION OF THE HUMAN METABOLISM OF FIPRONIL AND NONANE BY CHLORPYRIFOS.

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Based on our previous studies, it has been established that chlorpyrifos (CPS), fipronil and n-nonane can all be metabolized by human liver microsomes (HLM) and a number of cytochrome P450 (CYP) isoforms. However, metabolic interactions between these three substrates have not been described. In this study the effect of either co-incubation or pre-incubation of CPS with either n-nonane or fipronil was investigated. In both co- and pre-incubation experiments CPS significantly inhibited the metabolism of n-nonane or fipronil by HLM although CPS inhibited the metabolism of fipronil more effectively than that of n-nonane. CPS significantly inhibited the metabolism of fipronil by CYP3A4 as well as the metabolism of n-nonane by CYP2B6. In both cases pre-incubation with CPS caused greater inhibition than co-incubation, suggesting that the inhibition is mechanism based.

1901 EFFECT OF CYTOCHROME P450 INHIBITOR 1-AMINOBENZOTRIAZOLE (ABT) ON THE PHARMACOKINETICS OF NIMODIPINE IN MALE SPRAGUE-DAWLEY RATS.

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The purpose of this study was to evaluate the effect of broad spectrum and isozyme specific cytochrome P450 (CYP) inhibition on the pharmacokinetic parameters of nimodipine in male Sprague-Dawley rats. Nimodipine (NIM) is an L-type calcium channel antagonist known to undergo CYP-mediated oxidative metabolism in rat, presumably via isozymes CYP3A and CYP2D. Pretreatment with 50 mg/kg po ABT 2 hr prior to administering 10 mg/kg po NIM to fasted male Sprague-Dawley rats resulted in a greater than 200-fold increase in AUC_{0-24 hr} and 50-fold increase in C_{max} compared with sham pretreatment + NIM. Pretreatment with selective CYP3A inhibitors ketoconazole and bergamottin produced a more modest increase in AUC_{0-24 hr} (2-4 fold) and C_{max} (2-fold) of NIM. Pretreatment with 50 mg/kg po ABT 24 hr prior to and concurrent with 10 mg/kg po NIM to fed male Sprague-Dawley rats resulted in a greater than 40-fold increase in AUC_{0-24 hr} and 10-fold increase in C_{max} compared with sham pretreatment + NIM. ABT pharmacokinetic parameters were consistent with previously published reports and were not altered by NIM co-administration. Pre-dose and concurrent ABT treatments were also shown to significantly increase NIM in brain tissue concentration 24 hr post-administration. These data suggest that this model can be used to increase plasma exposure in pharmacokinetic and drug safety assessment studies of other dihydropyridines that undergo extensive CYP-mediated metabolism in rodents.

1902 TESTOSTERONE HYDROXYLASES IN EVALUATING INDUCTION OF CYTOCHROME P450 ISOENZYMES BY PERMETHRIN.

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Pyrethroid insecticides are potent neurotoxicants to both insects and mammals. Although their acute neurotoxicity has been well characterized, information regarding the potential interaction with liver drug-metabolizing enzymes of this class of

compounds is limited. In this work, the effect of the pyrethroid Type I permethrin on hepatic cytochrome P-450 (CYP) activities was investigated. Steroid hydroxylase reactions, particularly reactions utilizing testosterone as substrate, have proved useful in characterizing the individual catalytic capacity of CYP. Testosterone hydroxylation at the 2 α -, 16 α -, 6 β -, 7 α -, and 16 β - positions was chosen as marker for CYP2C11, CYP3A1/2, CYP2A/2 and CYP2B1/2 activity. Groups of 6 male Wistar rats weighing 200 g were deprived of food for 6 h before the single daily oral dose of permethrin (180 mg/kg for 6 days). Permethrin- and corn oil-treated animals were killed 24 h after the last dose by cervical dislocation and decapitation. The livers were removed, individually homogenized and microsomal pellets prepared and stored at -90C prior to use. The microsomal hydroxylation of testosterone was determined as described (1). Testosterone metabolites were analyzed by HPLC assay utilizing a reverse phase column and a mobile phase consisting of a methanol:water:acetonitrile gradient. Column effluents were monitored at 254 nm. Metabolites were quantitated by comparison of their peak areas with those of reference standards. Permethrin induced some members of CYP2C, CYP3A, CYP2A and CYP2B subfamilies. Permethrin treatment resulted in an increase in the formation of 2 α -hydroxytestosterone (37%, p<0.01), 6 β -hydroxytestosterone (103%, p<0.05), 7 α -hydroxytestosterone (327%, p<0.001) and 16 β -hydroxytestosterone (176%, p<0.001). Further studies of the oxidative metabolism of pyrethroid insecticides can strengthen the scientific basis for risk decisions. (1) Wood, AW et al. (1983) J. Biol. Chem. 258(14), 8839-8847.

1903 SUPPRESSION OF 3-METHYLCHOLANTHRENE-INDUCED CYP1A1 EXPRESSION BY 1-FURAN-2-YL-3-PYRIDIN-2-YL-PROPENONE IN MOUSE HEPA-1C1C7 CELLS.

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1-Furan-2-yl-3-pyridin-2-yl-propenone (FPP-3) is a chemically synthesized novel propenone compound with anti-inflammatory activities, without showing any ulcerogenic effects. In the present study, we investigated the effect of FPP-3 on 3-methylcholanthrene (3-MC)-inducible cytochrome P450 (CYP) 1A1 gene expression in mouse hepatoma Hepa-1c1c7 cells. 3-MC-induced CYP1A1-specific 7-ethoxyresorufin O-deethylase (EROD) activity and CYP1A1 mRNA level were markedly reduced in the concomitant treatment of 3-MC and FPP-3 in a dose dependent manner. A transient transfection assay using dioxin-response element (DRE)-linked luciferase revealed that FPP-3 reduced transformation of the aryl hydrocarbon receptor (AhR) to a form capable of specifically binding to the DRE sequence in the promoter of the CYP1A1 gene. These results suggest the down regulation of the CYP1A1 gene expression by FPP-3 in Hepa-1c1c7 cells might be antagonism of the DRE binding potential of nuclear AhR.

1904 EXPRESSION OF HEPATIC CYP2E1, CYP1A1/2 AND CYP2B1/2 PROTEIN IN DEVELOPING SPRAGUE DAWLEY RATS.

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Cytochrome P450s (CYP450s) are Phase I enzymes that metabolize endogenous as well as exogenous compounds. They play a key role in the metabolic activation of a number of potentially carcinogenic halocarbons such as trichloroethylene, methylene chloride and perchloroethylene. The ontogeny of a number of CYP450 isozymes has been recently delineated in human liver. There is relatively little such information about immature rats, a primary animal model for infants and children. The overall objective of our project has been to characterize the ontogeny of several toxicologically-relevant CYP450 isozymes (CYP1A1/2, CYP2B1/2 and CYP2E1) in Sprague-Dawley (S-D) rats. Previous data from our laboratory generally indicates an age-dependent increase in the activity of CYP2E1, CYP2B1/2 and CYP1A1/2 in unsexed preweanling S-D rats. We did see a decrease in CYP2E1 activity after 10 days of age, and peak CYP1A1/2 and CYP2B1/2 activity prior to adulthood. The aim of the current study was to determine whether the increase and decrease in CYP450 activities coincided with changes in protein expression through the use of the Western blot technique. A Fotodyne Inc. instrument and software were used to attain the protein images, while Kodak 1D Image Analysis system was used to quantify the protein. Liver microsomes were prepared from naïve rats on days 5, 10, 15, 21, 30, 40, 50, 60 and 90 after birth. Day 5, 10 and 15 samples were pooled and analyzed as single samples. Day 21-60 samples were sexed and day 90 males were used as the adult. Primary antibodies to CYP2E1, CYP1A1/2 and CYP2B1/2, along with the appropriate secondary antibody, were used to quantify 10 ug of microsomal sample from each animal. The expression of CYP2E1, CYP1A1/2 and CYP2B1/2 protein levels followed a pattern very similar to the ac-

tivities measured previously. Characterizing the maturation of hepatic CYP450 isozymes in rats may prove useful in assessing their applicability as models for children in the investigation of halocarbons and other xenobiotics which undergo metabolic activation.

1905 CYP3A4, CYP2B6 AND CYP1A2 mRNA INDUCTION IN RAT, PIG, AND HUMAN HEPATOCYTES.

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The induction of drug metabolizing enzymes by xenobiotics can lead to drug-drug interactions, autoinduction, and toxicity. Traditional pre-clinical animal studies are generally not sufficient to determine the induction potential of a new compound, as induction effects are often species-specific. Consequently, in vitro assays in primary human hepatocytes are currently used to assess clinical induction potential. Human hepatocytes are quite expensive and often difficult to obtain, however, so an alternative cell type that is predictive of human induction would be advantageous for early screening of compounds. In this study, we treated rat, human and Gottingen minipig hepatocytes with a variety of human CYP3A4, CYP2B6, and CYP1A2 inducing and non-inducing compounds, and we compared the inductive responses to each compound in all cell types. We found that rat hepatocytes respond differently to inducers of CYP3A4 and CYP2B6 than pig or human hepatocytes, but responded similarly to CYP1A2 inducers. Pig hepatocytes, however, responded to inducers of all three enzymes in a very similar manner to human hepatocytes. These data suggest that pig hepatocytes may be useful in early screening for induction potential, and the Gottingen minipig may be a useful species for predicting clinical induction potential.

1906 TEMPORAL CHARACTERIZATION OF CYP1A2, 2B6 AND 3A4 INDUCTION IN PRIMARY CULTURES OF HUMAN HEPATOCYTES BY RT-PCR, ENZYME ACTIVITY AND WESTERN BLOT.

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Cytochrome P450 (CYP) induction potential of drug candidates is commonly assessed during drug discovery and development. Recent guidance from the FDA recommends the exposure time of test article for in vitro induction experiments with primary cultured hepatocytes to be 2 to 3 days. In this study, we evaluated the time course for the induction of CYP1A2, CYP2B6 and CYP3A4 by prototypical inducers in human hepatocytes with three endpoints: 1) enzyme catalytic activity, 2) protein expression by Western blotting and 3) mRNA expression by real time RT-PCR. These enzymes were chosen as they are regulated by distinct but overlapping mechanisms involving nuclear receptors Ahr, CAR and PXR. Hepatocytes from two donor livers were treated with 20 μ M β -naphthoflavone (BNF), 2 mM Phenobarbital (PB) or 20 μ M rifampicin (RIF). At each time point (6, 24, 48 and 72 hours) catalytic activity of CYP1A2, CYP2B6 and CYP3A4 was determined in situ by measuring acetamidophenol, nirvanol and 6 β -hydroxytestosterone formation from corresponding probe substrates. The hepatocytes were then lysed and homogenate was prepared for Western blotting assays. In addition, replicate plates were used for preparation of RNA for real time RT-PCR analysis. The results showed that mRNA expression for all three enzymes was induced as early as 6 hours. The fold induction for CYP1A2, CYP2B6 and CYP3A4 mRNA averaged from these two donors were 6.9 (9.3 and 4.4), 14 (21 and 7.4) and 7.5 (2 and 13), respectively. The mRNA induction increased further with exposure time and was maximal at 48 or 72 hours. Induction of catalytic activity and protein of each enzyme was not observed at 6 hours but a robust induction response was found for each enzyme at 24 hours and subsequent time points. In conclusion, the mRNA expression was the most sensitive measure of induction and as expected provided the earliest indicator of response. Exposure time for optimal sensitivity of CYP induction potential for these three enzymes was 48 to 72 hours in the two donors tested.

1907 EFFECTS OF DIELDRIN AND PHENOBARBITAL ON THE SPATIAL DISTRIBUTION OF CYP2B1/2 MESSENGER RNA WITHIN THE LIVER ACINUS.

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Traditionally the liver has been considered a homogeneous, well-mixed organ. The literature, however, suggests that cytochrome P450 is differentially distributed within the hepatocytes lining the sinusoids of the liver acini and that this distribution is altered by various xenobiotics. The mRNA levels of CYP2B1/2 within the liver acinus were compared following treatment of rats with two P450 inducers, phenobarbital or dieldrin (a persistent organochlorine insecticide). Adult male Sprague Dawley-derived rats were treated with either i.p. phenobarbital in saline at

80mg/kg/day for 5 days or dieldrin in corn oil p.o. at 2.5 mg/kg/day for 13 days. Control rats received i.p. saline or p.o. corn oil for the same length of time. Laser capture microdissection (LCM) and real-time reverse transcriptase duplex-PCR (QPCR) were used to measure the CYP2B1/2 mRNA produced in bands of hepatocytes isolated from 3 different locations along the path of blood flow from the hepatic portal vein (periportal area) to the central vein (centrilobular area). Mean QPCR critical threshold (CT) differences and fold changes were determined. All LCM band samples showed significant increases in CYP2B1/2 mRNA following treatment. Dieldrin increased CYP2B1/2 mRNA values as blood flows through the acinus from the periportal region (320 fold increase) to the midzonal (538 fold increase) to the centrilobular region (1,418 fold increase). A different pattern resulted following phenobarbital exposure where the largest increase was present in the midzonal area (4,973 fold increase) with lower expression in both the periportal (1,031 fold increase) and the centrilobular areas (1,136 fold increase). These results reinforce the concept of a highly variable liver microenvironment in which CYP2B1/2 expression changes both temporally and spatially in response to different xenobiotics. Supported by NIH P20RR017661

1908 PHENOBARBITAL EFFECTS ON GENE EXPRESSION IN HUMAN HEPARG CELLS.

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The effects of phenobarbital were studied in the human hepatoma cell line, HepaRG. When maintained at confluence, this cell line differentiates into both biliary-like cells and hepatocyte-like cells and these latter become able to express many phase I and phase II enzymes, influx and efflux transporters. Therefore, this cell line could be considered as a relevant model for *in vitro* drug metabolism and toxicity studies. Differentiated HepaRG cells were treated with phenobarbital at 8 mM for 20h through 4 different experiments (intra- and inter-laboratory reiterations). Their functional capacity was investigated using 22k or 44k AgilentTM whole human genome microarrays (two-color). The cytotoxicity levels varied from 53% to 79% between experiments. Data sets were analysed using Feature ExtractionTM 8.5 and ResolverTM 6.0 (Rosetta Biosoftware) to build Venn diagrams and unsupervised clustering with a dose-related effect. These studies allowed to identify a reproducible set of about 1200 signature genes (p-values $\leq 4.5 \times 10^{-7}$) in response to phenobarbital treatment on 22k microarrays. This gene set included about 50% up-regulated and 30% down-regulated genes showing at least a 2-fold change. Approximately 90% of this gene set was retrieved on the 44k microarrays. Major networks hold the scales between proliferation, cell growth and apoptosis (Ingenuity Pathway AnalysisTM). Our results showed the interest of gene expression profile analysis for elucidating mechanism of action of phenobarbital in human liver cells.

1909 PERSISTENT INDUCTION OF HEPATIC CYP1A1 AND 1A2 GENE EXPRESSION BY 3-METHYLCHOLANTHRENE IS MEDIATED BY SUSTAINED TRANSCRIPTIONAL ACTIVATION OF THE CORRESPONDING PROMOTERS.

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Humans are constantly exposed to polycyclic aromatic hydrocarbons (PAHs), many of which are carcinogens. Cytochrome P4501A (CYP1A) enzymes play important roles in the activation of PAHs such as 3-methylcholanthrene (MC) to genotoxic metabolites, which could in turn lead to carcinogenesis. We reported earlier that exposure of rats to MC leads to persistent induction of hepatic microsomal cytochrome P450 (CYP)1A enzymes for up to 45 days (B. Moorthy, J. Pharmacol. Exp. Ther, 294: 313-322, 2000). In this study, we tested the hypothesis that persistent induction of CYP1A1 and 1A2 gene expression by MC is mediated by sustained transcriptional activation of the corresponding promoters. Twelve week-old female wild type (WT) (CD-1) or transgenic mice expressing the human CYP1A1 (CYP1A1-luc) or 1A2 (CYP1A2-luc) gene promoters and the reporter luciferase gene were treated with MC (100 μ mol/kg, i.p.), once daily for 4 days, and luciferase expression was determined in vivo by bioluminescent imaging of the mice at 1, 8, 15, and 28 days after the last treatment. Endogenous expression of CYP1A1 and 1A2 catalytic activities, apoprotein contents and mRNA levels was also determined in the liver tissues. MC elicited a 12- and 7-fold augmentation in luciferase expression in the CYP1A1-luc and 1A2-luc mice, respectively at the 1 day time point, compared to vehicle treated controls. This induction persisted for up to 28 days. On the other hand, MC caused only a modest induction in the endogenous expression of CYP1A1 and 1A2 in the 1A1-luc and 1A2-luc mice, respectively. These results support the hypothesis that persistent induction of CYP1A1 and 1A2 by MC is mediated by sustained transcriptional activation of the corresponding gene promoters. Future studies on the detailed mechanisms of persistent induction of CYP1A by MC could lead to the development of rational strategies in the prevention and/or treatment of humans cancers induced by PAHs. (Supported by NIH grant ES009132.)

1910 CHROMIUM INTERFERES WITH THE EPIGENETIC REGULATION OF BENZO[A]PYRENE-INDUCIBLE EXPRESSION OF MOUSE CYP1A1 GENE IN HEPA-1 CELLS.

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Carcinogenic metals, such as chromium (Cr), and polycyclic aromatic hydrocarbons (PAH), such as benzo[a]pyrene (B[a]P), co-exist naturally and may lead to multiple adverse health effects. In mouse hepatoma Hepa-1 cells, Cr affects the B[a]P-inducible expression of numerous genes including Ah receptor (AHR)-dependent CYP1A1 activation, due to the inhibition of both the release of histone deacetylase (HDAC) 1 and the recruitment of p300 to the CYP1A1 promoter. Concomitantly, binding of the AHR to the xenobiotic response elements is unaffected by Cr. Since change in histone modifications is a major event in the critical steps of chromatin structure remodeling necessary for gene transactivation, we tested the hypothesis that Cr interferes with the epigenetic control of B[a]P-induced CYP1A1 gene expression. We performed chromatin immunoprecipitation assays with cells treated with B[a]P in the presence or absence of Cr to compare the profile of histone modifications at the CYP1A1 promoter. Our results show that Cr leads to an inhibition of B[a]P-induced histone 3 (H3) S10 phosphorylation, H3K4 tri-methylation, and both H3 and H4 acetylation. Furthermore, sodium butyrate and 5-aza-2'-deoxycytidine (azaC) decreased Cr repression of CYP1A1 induction by B[a]P. However, these treatments did not affect the constitutive and inducible expression level of CYP1A1. In addition, we found that DNA methyl transferase (DNMT) 1 is associated with HDAC1 on the CYP1A1 promoter. Although, no DNA methylation was detected in the CYP1A1 promoter CpG island, azaC is able to reduce the association of DNMT1 and HDAC1 on the promoter. These data helps develop an understanding of the molecular mechanisms underlying the toxicity associated with environmental mixtures. Supported by NIEHS grants P30 ES06096, 2R01 ES06273 and 1R01 ES10807.

1911 EFFECTS OF HISTONE ACETHYLATION AND DNA METHYLATION ON THE mRNA EXPRESSIONS OF CYP1A AND 3A IN HEPG2 CELLS.

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The human cytochrome P450 (CYP) plays a key role in metabolizing various endogenous compounds and xenobiotics including therapeutic drugs, and CYP enzymes exhibit inter-individual variation at the activity level. Single nucleotide polymorphisms (SNPs) of CYP genes have been analyzed but only few SNPs have been associated with observed inter-individual differences. Nuclear receptor pathways have been reported to participate in the regulation of CYP enzymes. Roles of other regulating factors, however, have been suggested, which ultimately result in the inter-individual variation of CYP expression. In this study, effects of inhibitors of histone deacetylation and DNA methylation on the constitutive and induced CYP 1A and 3A mRNA levels were studied using HepG2 cells.

Cells were pretreated with trichostatin A (TSA) or 5-aza-2'-deoxycytidine (AzaC) and then treated with or without CYP inducers, omeprazole (OME) or rifampicin (RIF). The mRNA expression levels of CYP1A1, 1A2, 3A4, 3A5 and their related receptors, arylhydrocarbon receptor (AHR), pregnane X receptor (PXR) were measured by the real-time reverse-transcription polymerase chain reaction method (RT-PCR) with specific TaqMan probes.

TSA increased the constitutively expressed levels of CYP1A2 and 3A4 by 5- and 11-fold, respectively. Much more remarkable increase was observed in the induced expression levels of CYP1A2 and 3A4 by 22- and 13-fold. Similar results were observed in the case of AzaC. On the other hand, AHR and PXR expressions were changed no more than 2 fold with TSA and AzaC. These results show that TSA and AzaC influenced the constitutive and induced expressions of CYP1A and 3A. The inhibitions of histone deacetylation and DNA methylation seemed to affect the constitutive expression more profoundly than induction of CYP3A4 gene in HepG2 cells.

1912 REGULATION OF TRANSCRIPTIONAL ACTIVITY OF HUMAN PREGNANE X RECEPTOR BY HISTONE METHYLTRANSFERASE PRMT1.

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Histone modification is an important mechanism for the epigenetic regulation of gene expression. Protein arginine methyltransferase 1 (PRMT1), which methylates arginine 3 at the N-terminal tail of histone H4, has recently been shown to play an

important role in the functions of nuclear/steroid receptors. The pregnane X receptor (PXR) regulates the expression of a set of genes involved in phase I, II xenobiotic/drug metabolism as well as transporters. Cytochrome P450 3A4 (cyp3a4) is regulated by PXR at transcriptional level and is responsible for the first pass biotransformation of over 50% prescription and non-prescription drugs. Using coimmunoprecipitation/Western analysis with Flag-tagged human PXR, we have found that PRMT1 associated with PXR complex in human hepatoma (HepG2) cell line. The immunoprecipitated complex contained methyltransferase(s) that specifically methylated arginine 3 of histone H4 as determined by N-terminal sequencing analysis, suggesting PRMT1 may be the primary methylating enzyme. Using GST-pulldown and mammalian two hybrid assays, we found that PRMT1 interacts with PXR directly. Using the chromatin immunoprecipitation (ChIP) assay, we detected a ligand (rifampicin)-dependent, transient recruitment of PRMT1 to cyp3a4 regulatory regions concomitant with methylation of arginine 3 in histone H4. Histone methylation by PRMT1 is a critical step for subsequent histone modifications (e.g. acetylation). Our results suggest that PRMT1 plays important roles in regulating PXR-mediated gene expression (Supported in part by NIEHS Grant ES09859, ES09106 and American Heart Association Grant 0355131Y).

1913 CCR4-NOT COMPLEX REGULATES THE TRANSCRIPTIONAL ACTIVITY OF PREGNANE X RECEPTOR.

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Pregnane X receptor (PXR) is an orphan nuclear receptor that regulates the metabolism and disposition of various xenobiotics and endobiotics. PXR has been considered as the xeno-sensor because of its remarkable ability to bind a wide range of structurally diverse ligands and coordinately regulate the Phase I and Phase II drug metabolizing enzymes as well as the "Phase III" transporters. PXR plays an important role in drug-drug interactions, adverse drug responses, cholestatic liver diseases, and colon cancer. In order to understand the mechanisms of transcriptional regulation of PXR, we performed yeast two-hybrid screening to identify the interactive proteins in human liver cDNA library, using PXR ligand binding domain as a bait. More than one million independent clones were screened; the known PXR interactive partners were among the positive clones, including NcoR2 and RXR β . One positive clone was a partial cDNA of CNOT2 (amino acids 183-540). CNOT2 is a component of Ccr4-NOT which is multi-subunit protein complex highly conserved from yeast to human. Ccr4-NOT complex plays critical roles in various aspects of transcriptional regulation, including initiation, and mRNA degradation. Using mammalian two-hybrid assay in CV-1 cells and GST-pulldown assay, we confirmed the direct interaction between PXR and CNOT2. In HepG2 cells, overexpression of CNOT2 suppressed the PXR-regulated luciferase reporter gene activity. These preliminary results strongly suggest that Ccr4-NOT complex regulates the transcriptional activity of PXR. (Supported in part by NIEHS Grants ES09859).

1914 RETINOIDS ACTIVATE THE RXR/RXR AND RXR/VDR-MEDIATED PATHWAY AND INDUCE CYP3A4 IN HEPG2 CELLS.

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Retinoids are frequently used as food supplements or as drugs to treat cancer or dermatological diseases. Retinoids exert their effects through retinoic acid receptor (RAR) and retinoid x receptor (RXR). 1 α , 25-dihydroxyvitamin D3 (D3), can activate RXR/vitamin D receptor (VDR) heterodimers and regulate transcription of hepatic cytochrome P450 3A4 (CYP3A4). However, the role of retinoids in regulation of CYP3A4 through the RXR/VDR signaling pathway is currently unknown. We therefore tested a panel of retinoids as potential ligands for RXR/VDR, using transient transfection assay. Cells were co-transfected with reporter, tk-(3A4)3-Luc, which contains RXR/SXR binding site (ER6) of CYP3A4 promoter, and expression plasmids. In CV-1 cells, of 13 retinoids screened, 9-cis-retinoic acid (9-cis RA) and all-trans-retinoic acid (all-trans RA) activated reporter tk-(3A4)3-Luc (3-fold induction). In HepG2 cells, 7 out of 13 retinoids activated reporter activity, among them, 9-cis-retinal (9.8-fold), 9-cis RA (23.9-fold), and all-trans RA (5.1-fold) showed the highest level of induction. To discern whether the retinoid mediated tk-(3A4)3-Luc induction occurs via RXR homodimers or RXR/VDR heterodimers, a mutant RXR α (Tyr402) expression plasmid with impaired VDR heterodimerization capability and enhanced homodimerization capability was employed for transfection. The results suggest that retinoic acids activate both RXR α homodimers and RXR α /VDR heterodimers and induce tk-(3A4)3-Luc. Among the three RXR isoforms (α , β , and γ), the β isoform was less effective than α or γ in inducing tk-(3A4)3-Luc. The induction of reporter luciferase activity by 9-cis-RA was dose-dependent and low dose (0.1 μ M) 9-cis RA induced reporter activity.

Neither additive nor synergistic effects were noted when retinoids and D3 were used simultaneously. Furthermore, 9-cis RA and all-trans RA induced CYP3A4 activity by 2.9- and 2.6-fold, respectively, in HepG2 cells. In conclusion, certain retinoids, 9-cis RA and all-trans RA, can induce CYP3A4 activity through the RXR/RXR and RXR/VDR-mediated pathway in HepG2 cells.

1915 INDUCTION OF CYP3A4, PXR AND RXR ALPHA BY SODIUM ARSENITE AND DIMETHYL ARSENIC IN THE SMALL INTESTINE OF A CYP3A4 TRANSGENIC MICE.

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Inorganic arsenicals are widely distributed in the environment. Exposure of humans, experimental animals, and cultured cells to arsenic results in a variety of health effects, including cancer. Changes in the liver morphology, and a decrease on cytochrome P-450 (CYP) content have been reported in rats treated with sodium arsenite. Moreover, it has been shown that arsenite decreases induction of CYP1A1, 1A2, 2B1 and 3A23 in cell cultures. These effects may modify the metabolism of several compounds, thus affecting human health. CYP3A4, the predominant CYP expressed in human liver and intestine, contributes to the metabolism of approximately half of the drugs in clinical use today. CYP3A4 catalyzes the 6 beta-hydroxylation of a number of steroid hormones, and is involved in the bioactivation of environmental pro-carcinogens. In the present study we determined the effect of sodium arsenite and dimethyl arsenic on CYP3A4 expression as well as on PXR, RXR alpha, and C/EBP beta expression, all of them involved in the activation of CYP3A4 transcription. Transgenic mice expressing intestinal CYP3A4 were treated with a single dose of 0.05, 0.5, or 5 mg per kg body weight of sodium arsenite or 0.05 mg per kg body weight of dimethyl arsenic. Twenty four h later animals were sacrificed by cervical dislocation and the small intestine of the mice were removed. CYP3A4, PXR, RXR alpha, and C/EBP beta mRNA levels were evaluated by Real Time PCR. Our results indicate that both, sodium arsenite and dimethyl arsenic increase CYP3A4, PXR, and RXR alpha mRNA levels whereas no effect was observed in C/EBP beta transcript levels. In conclusion, the present study revealed that sodium arsenite and dimethyl arsenic increase CYP3A4 levels by inducing PXR and RXR alpha transcription, and suggest that arsenic compounds may induce other genes that are under PXR and RXR alpha regulation.

1916 BROMINATED FLAME RETARDANTS AS INDUCERS OF CYTOCHROME P450 3A.

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Brominated flame retardants (BFRs) are added to plastics, textiles and electronic equipments to reduce the risk of fire. The group of BFRs comprises, e.g., polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD), tetrabromobisphenol A (TBBPA) and polybrominated biphenyls (PBBs). Many of these substances are persistent and lipophilic, and accumulate in the environment, in wildlife, and humans. Some BFRs have been shown to be active as endocrine disrupters, and to exert developmental toxicity. The induction of drug metabolism may play a role in such effects.

In this study HBCD and a cleaned-up commercial PBDE mixture ('Pentamix') mainly containing BDE 99 (2,2',4,4',5-pentabromo-DE) were used. A previous study in rats has shown induction of hepatic CYP3A after treatment with HBCD and Pentamix. In rat hepatocytes in primary culture, both compounds also induced CYP3A mRNA and protein. Furthermore, reporter gene expression in H4IIE (rat) and HepG2 (human) hepatoma cells, transiently transfected with an XREM-driven reporter gene construct was also increased significantly. Co-transfection of the human pregnane-X-receptor (PXR) further enhanced the response in HepG2 cells. In summary, our findings demonstrate that HBCD and Pentamix have the potential to induce CYP3A, a group of key enzymes in drug metabolism of humans and rodents. Thus these compounds may cause adverse effects by affecting the metabolism of exogenous and endogenous substrates.

1917 GLUCOCORTICOID REGULATION OF PXR INFLUENCES CYP3A4 INDUCTION BY DEHP AND THE METABOLITE MEHP

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Di (2-ethylhexyl) phthalate (DEHP) is a widely used phthalate ester plasticizer found in polyvinyl chloride medical devices. High level DEHP exposure to hospital patients occurs as it leaches from medical tubing, blood bags and IV fluid bags.

Phthalates such as DEHP are ubiquitous environmental contaminants that act as developmental and reproductive toxicants. DEHP is rapidly metabolized to mono (2-ethylhexyl) phthalate (MEHP). DEHP and MEHP are ligands for the pregnane X receptor (PXR), a key regulator of xenobiotic metabolism, and induce CYP3A4 (3A4) which is known to metabolize pharmaceuticals and endogenous steroids. The magnitude of CYP3A induction by phthalates may be dependent on PXR protein levels. PXR expression has been shown to be regulated by glucocorticoids and many hospital patients exposed to DEHP are also administered glucocorticoids. The purpose of these studies was to examine the effects of glucocorticoid and phthalate co-exposure on 3A4 expression. The rat liver cell line H4IIE-C3 was transiently transfected with a CYP3A4-luciferase reporter and treated with dexamethasone (Dex) and 10 µM DEHP. CYP3A4 promoter activity was highly induced by DEHP and Dex co-administration over either agent alone. This induction was abrogated by administration of the glucocorticoid receptor (GR) antagonist RU486, suggesting a critical role for GR. Similar effects were observed with MEHP treatment. Western blots of H4IIE-C3 cells demonstrated a dose dependent induction of PXR in response to Dex. To examine the effect of Dex on PXR protein in a human model system, western blot analysis was performed on Dex treated primary human hepatocytes and revealed a Dex dose-dependent increase in PXR expression. Dex and DEHP or MEHP co-treated hepatocytes demonstrated a synergistic induction of 3A4 protein expression. These data suggest that PXR is regulated by glucocorticoids and induction of PXR protein contributes to synergistic induction of 3A4 expression in response to DEHP or the metabolite MEHP.

1918 REGULATION OF CONSTITUTIVE ANDROSTANE RECEPTOR (CAR) BY SILENCING MEDIATOR OF RETINOID AND THYROID RECEPTORS (SMRT).

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Nuclear receptors (NRs) are ligand-activated transcription factors regulating metabolism, development, reproduction, and other biological processes. The NR constitutive androstane receptor (CAR) is expressed predominantly in the liver and its activity is induced by a number of xenobiotics and endogenous chemicals, resulting in activation of the hepatic drug clearance pathway that consists of cytochrome P450 enzymes, conjugating enzymes, and drug transporters. In this study, we find that the over expression of the NR corepressor silencing mediator of retinoid and thyroid receptors (SMRT) inhibits CAR transactivation of the CYP2B promoter. Using in vitro and in vivo assays, we show that the interaction between the ligand-binding domain of CAR and SMRT is dependent upon a few critical amino acid residues in the SMRT nuclear receptor-interaction domain (RID). Most importantly, we found that a mouse strain harboring point mutations in the SMRT RID shows increased expression of CAR target genes. As a result, the mutant mice have a higher level of drug metabolism when challenged with multiple chemicals. Taken together, we propose that SMRT is a key regulator of hepatic drug metabolism.

1919 RETINOIC ACID REGULATION OF THE PULMONARY EXPRESSED CYTOCHROME P450 2S1.

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Cytochrome P450 2S1 is one of the few P450 enzymes that exhibit both selective expression in extrahepatic tissues, including the lung, and constitutive expression throughout development of the fetus. Although there is conflicting evidence in the literature as to the nature of its substrate(s), the fact that this enzyme is highly conserved indicates that it may play an important metabolic role. Previous studies have demonstrated CYP2S1 induction by all-trans retinoic acid in human skin biopsies. Retinoic acid (RA) is an important signaling molecule, derived from the metabolism of Vitamin A (retinol). RA signaling plays an important role in development by regulating cell growth, differentiation and proliferation in numerous cell types including the lung. Deregulation of RA signaling has been implicated in tumorigenesis. The CYP2S1 promoter contains a number of potential Retinoic Acid Response Element (RARE) half-sites. Using CYP2S1 promoter luciferase constructs and real-time PCR, we wanted to determine 1) which promoter regions are important for the lung-selective expression of CYP2S1 by comparing human lung aveolar A549 with human liver HepG2 cells, and 2) determine whether retinoic acid induces CYP2S1 expression in A549 cells. Results from these studies showed that A549 cells supported a robust (125-fold) enhancement of reporter gene activity and RA significantly induced both reporter luciferase activity and CYP2S1 transcription. The human liver HepG2 cells only moderately (25-fold) supported luciferase activity and RA did not induce the promoter-driven reporter gene. This research was supported by NIH grant #HL60143.

1920 ALTERNATIVE PROMOTERS DETERMINE TISSUE-SPECIFIC EXPRESSION PROFILES OF HUMAN MICROSOMAL EPOXIDE HYDROLASE (EPHX1).

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Microsomal epoxide hydrolase (EPHX1) catalyzes hydration reactions that determine the cellular disposition of reactive epoxide derivatives derived from xenobiotic exposures. Whereas the previously defined human EPHX1 exon 1 sequence, E1, is derived from a gene promoter immediately proximal to exon 2 of the EPHX1 coding region. Remarkably, the E1 promoter directs expression only in the liver. We have identified an alternative EPHX1 promoter that is localized ~18.5 kb upstream of exon 2. Northern hybridizations demonstrated that the E1b promoter functions as the primary driver of EPHX1 expression in human extrahepatic tissues. Using a rolling circle amplification-RACE technique, together with EST database analyses, we have identified 2 distinct EPHX1 transcripts in extrahepatic tissues and cell lines that are derived from the E1b promoter region. We term the transcripts E1b and E1b'. The E1b transcript includes a noncoding 46 bp exon 1 in its 5'-untranslated region. In contrast, the length of the E1b' 5'-UTR is extended, and includes 2 upstream AUG codons together with high GC content. Analyses of the human E1b gene promoter region indicated the presence of multiple transposable elements, including Alu repetitive elements of the AluSp, AluSx, AluJo and AluYa5 classifications. The AluYa5 elements are relatively recent in evolutionary history, and their presence in the EPHX1 promoter was associated with significant reduction in EPHX1 transcriptional activity following transfection experiments conducted in transformed human cell lines derived from multiple tissues, including kidney 293A, lung A549, hepatoma HepG2, and breast cancer MCF7. Further investigations are underway to assess the regulatory features of this promoter that direct its transcriptional activity in human tissues.

1921 ACQUIRED ANDROGEN INDEPENDENCE DURING CADMIUM-INDUCED MALIGNANT TRANSFORMATION OF HUMAN PROSTATE EPITHELIAL CELLS.

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Early human prostate cancer (PCa) is typically androgen-dependent but can progress to an aggressive androgen-independent (AI) state that is frequently lethal. AI growth often involves changes in the androgen receptor (AR). Cadmium (Cd) is a possible prostate carcinogen potentially linked to aggressive PCa. Thus, acquisition of AI growth was studied in normal prostate epithelial RWPE-1 cells malignant transformed by chronic low-level Cd exposure (CTPE cells). CTPE cells were hyperproliferative compared to control, and androgens or antiandrogens had little effect on this proliferation. Prostate specific antigen (PSA) was markedly increased while the tumor suppressor gene *p27* was reduced in CTPE cells, changes typical in AI PCa. AR levels were similar in control and CTPE cells but androgen actually induced less AR-related gene expression in CTPE cells. Thus, this AI growth is unrelated to changes in AR levels or sensitivity. AI growth can involve estrogen signaling via the estrogen receptor (ER). In fact, after exposure to estradiol (E2) CTPE cell proliferation greatly increased while control cells showed little response. ER α transcript was elevated in CTPE cells, while ER β transcript was distinctly lower. Antiestrogen treatment nearly abolished E2-induced CTPE cell growth. CTPE cells had increased 5 α -aromatase (5 α -A), which metabolizes testosterone to estrogen. Disruption of IGF signaling is frequently found in AI PCa and IGF expression is frequently controlled by estrogen. Although basal levels were similar, E2 markedly (6.5-fold) increased in IGF-1 in CTPE cells but not in control, indicating IGF-1 is a key factor in E2-induced CTPE cell proliferation. Thus, Cd-induced malignant transformation of human prostate cells precipitates AI growth, unrelated to AR expression or activity. Increased ER and 5 α -A expression and IGF-1 responsiveness suggests estrogen signaling may be critical to Cd-induced acquired AI growth, which fortifies their emerging role in PCa development and progression.

1922 EUKARYOTIC TRANSLATION ELONGATION FACTOR 18 OVEREXPRESSION CELLS ARE RESISTANT TO CADMIUM-INDUCED TOXICITY AND APOPTOSIS.

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The role of Eukaryotic translation elongation factor 18 (eEF18) in cadmium-induced cytotoxicity and apoptosis was investigated. Transgenic cell line overexpressing the eEF18 gene were generated by transfecting HeLa cells with the expression vector pcDNA 3.1 containing human eEF18 cDNA. Stable transfectant cell line was developed using blasticidin (5mg/mL medium). Expression of eEF18 in the

transgenic cell line was determined by real-time PCR and Western blot analysis. Cell line exhibiting 2 fold (eEF184) and 3 fold (eEF1811) increases in eEF18 expression were developed. The vector alone transfected cell line served as the control. The transgenic cell line exhibited enhanced cell proliferation, overexpression of eEF18, eIF4E and eEF2 genes compared with the control cell line. Cadmium-induced cytotoxicity was significantly lower in the eEF18 overexpressing cell line compared with the control. Cadmium induced apoptosis was also studied using the control and eEF18 overexpressing cell lines. The eEF18 overexpressing cell line exhibited significantly higher resistance to cadmium-induced apoptosis compared with the control cell line. Real-time PCR analysis of eEF18 overexpressing cell line exposed to cadmium showed significant induction of anti-apoptotic genes like Bcl-2, with a concurrent decrease in the expression of the pro-apoptotic gene, p53 compared with the control cell line. In summary, the eEF18 gene overexpressing cell line exhibited significant resistance to cadmium-induced cytotoxicity and apoptosis.

1923 TOXICOGENOMIC ANALYSIS OF CADMIUM RESPONSIVE TRANSCRIPTION IN CAENORHABDITIS ELEGANS REVEALS NOVEL GENES AND PATHWAYS INVOLVED IN HEAVY METAL RESISTANCE.

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The transition metal cadmium is a known human carcinogen, and exposure to this metal is associated with a variety of human diseases. Although individuals are continuously exposed to this metal, pathologic effects are prevented by intracellular defense and repair systems. In the present study, we used *C. elegans* as a model system to identify (a) genes that are induced by a sub-lethal level of cadmium; (b) biological pathways and functions that are affected by cadmium, and (c) genes that are induced by cadmium to protect nematodes against metal toxicity. The global transcriptional changes after 4h and 24h exposure to a sub-lethal concentration of cadmium (100 μ M) were measured using *C. elegans* whole genome arrays. Gene ontology analysis indicated that *C. elegans* metabolism and transport pathways were significantly enriched with differentially expressed genes following both 4h and 24h exposure, while proteolysis and fatty acid metabolism pathways were affected only in 24h exposure. The relationship between cadmium-induced expression changes and protection against cadmium toxicity was investigated by quantifying growth and reproduction after RNA interference. We identified a list of cadmium-inducible genes that were related to cadmium sensitivity in *C. elegans* and many of them have catalytic or binding activities. Protein interaction analysis also revealed that *kel-8*, which is a signaling molecule expressed in neurons, was required in the defense response against cadmium toxicity. Further, the protective effect of *kel-8* depends on normal function of *mek-1*, suggesting that a novel pathway is involved in the metal response. We are also applying the information obtained from *C. elegans* to the mammalian system by studying the homologue of a novel cadmium-responsive *C. elegans* gene in human HEPG2 cells.

1924 LOSS OF GLUTAMATE-CYSTEINE LIGASE MODIFIER SUBUNIT SENSITIZES MICE TO CHRONIC CADMIUM TOXICITY.

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High concentrations of Cd cause toxicity in several organs including liver, lung, bone and kidney. Reduced glutathione (GSH) is one of the cell's major defenses against oxidative stress and has also been shown to possess a protective role against Cd damage, acting as a "first line of defense" in both animals and cultured cells. Glutamate-cysteine ligase modifier subunit (GCLM) has the overall effect of increasing the efficiency of GSH synthesis. *Gclm*(-/-) knockout mice generated in our laboratory show GSH levels that are 15% (liver) and 25-30% (kidney) of that in *Gclm*(+/+) wild-type littermates. Numerous studies have demonstrated Cd as being a toxic metal that causes oxidative stress. Cd toxicity, in the context of chronic GSH depletion, has not been studied-most likely because of complications in interpreting data from animals chronically treated with chemicals that deplete GSH, such as buthionine sulfoximine. To examine the role of chronic GSH depletion in chronic Cd toxicity, we compared *Gclm*(-/-) mice and WT littermates. The effects of repeated subcutaneous injections (6 per week) of CdCl₂ at a dose of 10 μ mol/kg vs isotonic saline (0.9% NaCl) given to both genotypes were compared. This dose of Cd is not acutely lethal, even to sensitive strains. All mice were given 1 mL of water by gavage once a week, urine was collected for 2 h in metabolic cages, and blood collected via saphenous vein. Increases in weight loss, plasma ALT and AST, blood urea nitrogen, and urinary *N*-acetyl- β -D-glucosaminidase (NAG), suggesting liver and kidney failure preceding mortality, occurred in both genotypes. Fifty percent mortality with CdCl₂ treatment occurred in 28 days for *Gclm*(-/-) mice vs 40 days

for WT littermates. No mortality was observed in saline-treated groups. The lower GSH levels present in *Gclm*(*-/-*) mice led to a dramatic sensitization of the animal to chronic Cd toxicity, suggesting that the protective role of GSH is important not only in acute Cd toxicity but also in chronic Cd toxicity. **Supported in part by NIH grants R01 ES012463 and R01 ES010416**

1925 THE MOLECULAR CHARACTERIZATION OF A CADMIUM INDUCIBLE TRANSFORMER-2-LIKE PROTEIN FROM THE NEMATODE CAENORHABDITIS ELEGANS.

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Cadmium is a known human carcinogen and was recently ranked number eight on the hazardous substance priority list. It is not completely clear how cadmium can affect signaling pathways to induce specific changes in gene expression. The underlying mechanisms that modulate cellular response to metal exposure are highly conserved. To understand and describe more fully the molecular basis for cadmium response in *Caenorhabditis elegans*, whole genome analysis was used to identify genes responsive to cadmium exposure. In this analysis, we identified a novel gene, F08F8.5, whose transcript level showed a 7.1-fold induction after 24 hr exposure to cadmium. The amino acid sequence for F08F8.5 has a 50% identity to a transformer-2-like serine/arginine-rich protein. Quantitative real time PCR was used to further quantify the response of F08F8.5 to cadmium and seven other metals. Heat shock was also examined to assess the effects of a more general stressor on F08F8.5 transcript levels. The strongest induction of F08F8.5 occurred from treatment with cadmium, followed by copper. Heat shock did not result in an increase in the mRNA abundance of F08F8.5, indicating F08F8.5 is a metal-specific responsive gene, with cadmium being the strongest inducer. Transgenic *C. elegans* containing promoter-GFP fusions of F08F8.5 were generated. Constitutive expression of the F08F8.5 promoter was observed in the AWA sensory neurons. Cadmium treatment resulted in the rapid synthesis of detectable levels of GFP expression in the intestine and pharynx; where as copper exposure resulted in high levels of GFP only in the pharynx. We are also conducting RNAi screens to gain a better understanding of the biological relevance of F08F8.5 in mediating a response to cadmium exposure in *C. elegans*. The identification and characterization of cadmium-responsive genes will facilitate an understanding of how cadmium exposure results in specific cellular responses.

1926 CHARACTERIZATION OF ZIP14: MEMBER OF THE VERY ANCIENT SOLUTE-CARRIER-39 (SLC39) METAL-TRANSPORTER FAMILY.

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Our lab [PNAS 2005; 102: 3401] has shown that the mouse *Slc39a8* gene, encoding the ZIP8 metal transporter, is responsible for Cd-induced testicular necrosis. Sensitive inbred strains have much greater ZIP8 mRNA and protein accumulation in endothelial cells of the testis vasculature than resistant strains, leading to higher Cd uptake and damage. ZIP8-containing cells in culture show >10-fold increased Cd uptake and >30-fold risk of Cd-induced cell death than control cells. The endogenous function of ZIP8 is that of a Mn/HCO₃⁻ (perhaps also Zn/HCO₃⁻) co-transporter and highly expressed in testis, kidney and lung. All 14 *SLC39* family members (mouse, human) encode for Zrt-like Irt-like Proteins (ZIP), which possess a characteristic ZIP domain by which divalent metals influx across membranes. These very ancient transporters exist in prokaryotes, yeast, plants and all animals. The gene having greatest evolutionary relatedness to mouse *Slc39a8* is *Slc39a14* (similar number of amino acids; highest percent identity). We found two functional isoforms of ZIP14, due to alternative splicing of exons 4: ZIP14A, ZIP14B. We generated stable retrovirally-infected ZIP14A and ZIP14B cDNA into mouse fetal fibroblast (MFF) cultures to make rvZIP14A and ZIP14B cell lines. We established that ZIP14A and 14B, just like ZIP8, are rogue Cd transporters showing a pH optimum of 7.5, temperature optimum of 37 °C, and inhibition by CN⁻. Both also require HCO₃⁻ and, like ZIP8, are Mn, Zn and Cd/HCO₃⁻ co-transporters. Consistent with their "influx" role, ZIP14 proteins are located on the plasma membrane of MFF cells, as demonstrated by Western blot and immunofluorescence microscopy, and are located on the apical side of polarized MDCK epithelial cells, implicating important roles in Cd uptake and disposition. We found that Zn is much more effective in preventing Cd uptake by rvZIP14A or rvZIP14B cells, compared with that by rvZIP8 cells.—**Supported in part by NIH grants R01 ES010416, R01 ES012463, and P30 ES06096.**

1927 MODULATION OF PROTEIN EXPRESSION IN RAT KIDNEY PROXIMAL TUBULAR EPITHELIAL CELLS BY CADMIUM.

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Renal impairment is the main toxic effect observed upon chronic cadmium (Cd) exposure and proximal tubular epithelial cells are the primary target. We hypothesized that Cd induced cytotoxicity in these cells may involve a changes in expression of certain biologically important proteins. Therefore, the present study was designed to elucidate protein expression in a normal rat kidney epithelial cell line, NRK-52E. The cells were incubated with 5, 10, 15, or 20 µM CdCl₂ for 6, 12, 18, or 24 h. At the conclusion of the exposure, soluble proteins were extracted and separated by two-dimensional polyacrylamide gel electrophoresis. With the aid of PD-Quest software, a total of ten protein spots exhibiting changes in their expression patterns were identified. Nine of these proteins were under-expressed and only one was over-expressed. The approximate molecular weights and isoelectric points (PI) of these proteins were determined by using proteins of known molecular weight and PI. The expression of three protein spots (39 kD, PI 5.3; 36 kD, PI 5.4; 21 kD, PI 6.2) was suppressed after exposure to 5 µM Cd for 6 h and of one (40 kD, PI 5.4) after 12 h exposure. The fifth protein spot (47 kD, PI 6.9) did not show any decrease in staining intensity until the Cd concentration was increased to 10 µM and the cells were exposed for 12 h. Protein spots six (51 kD, PI 6.7) and seven (50 kD, PI 6.3) were under-expressed after 12 h exposure to 15 µM Cd. As the concentration of Cd was raised to 20 µM, protein spots eight (36 kD, PI 4.9) and nine (26 kD, PI 5.2) were under-expressed following 12 and 24 h exposures, respectively. In contrast, protein spot ten (9 kD, PI 4.6) increased in intensity in a concentration- and time-dependent manner. In summary, exposure to Cd causes changes in the expression patterns of specific proteins. Further identification of these proteins may give us more insights into their roles in the Cd-induced renal toxicity.

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1928 RAPID ACTIVATION OF ERK1/2 AND AKT IN HUMAN BREAST CANCER CELLS BY CADMIUM: ROLE OF MEMBRANE ESTROGEN RECEPTOR ALPHA.

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Cadmium (Cd), an estrogen disruptor, can rapidly induce a variety of signaling events including the activation of ERK1/2 and AKT. In this study, the involvement of membrane estrogen receptor alpha (ERα) in these events was evaluated in three human breast cancer cell lines (MCF-7, MDA-MB-231, and SKBR-3) following exposure to Cd. In the MCF-7 cells, that express both ERα and ERβ, exposure to 10 µM Cd caused maximum activation of ERK1/2 within 2.5 min and the level of phosphorylated ERK1/2 gradually decreased during the next 60 min. Cd also triggered a gradual increase and sustained activation of AKT during this period. These activation patterns of Cd were similar to those exhibited by the MCF-7 cells in response to 17β-estradiol. A transient phosphorylation of ERα was also observed within 2.5 min of Cd exposure. In contrast to the MCF-7 cells, Cd was unable to induce rapid activation of either ERK1/2 or AKT in MDA-MB-231 cells that express only ERβ. Similarly, in SKBR-3 cells, that express neither ERα nor ERβ, while Cd induced a transient activation of ERK1/2 at 7.5 min, it failed to induce activation of AKT. Next, an estrogen receptor antagonist, ICI 182780, was utilized to investigate the role of membrane ERα in Cd-induced rapid signaling. This specific receptor antagonist did not prevent the Cd-induced activation of ERK1/2 and AKT. Moreover, their activation was not blocked by knockdown of ERα expression by siRNA. We conclude from these observations that Cd induces rapid signaling in the MCF-7 human breast cancer cells through mechanisms that do not seem to require membrane ERα.

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1929 THE PROTECTIVE EFFECTS OF LYCOPENE ON CADMIUM INDUCED GENE EXPRESSION AND LIPID PEROXIDATION IN RATS.

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It has been demonstrated that changes in the expression of c-fos, c-jun, bcl-2 and MT-I genes occurred in rats exposed to cadmium to cause carcinogenesis in testes and ventral prostate. To study the protective effect of lycopene on cadmium induced gene expression and lipid peroxidation, Male Wistar rats were divided into three groups and were pretreated by gavage with lycopene doses of 0, 6, or 30 mg/kg body weight (bw) for three weeks. These animals were further divided into a total of 9 subgroups and were given a single (s.c.) injection of CdCl₂ doses of 0, 2, 5 µmol/kg bw. Animals were then sacrificed 5 days after the treatment. RNA was made

from testes and prostate tissues using a Trizol kit from Gibco. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) analysis for gene expression was performed using an Access kit from Promega. Lycopene concentrations at blood and tissues were quantitated by HPLC. Lycopene pretreatment significantly reduced cadmium-induced mRNA expression of *c-fos* and *c-jun* in the testes and ventral prostate as compared to the cadmium treated control, but the expressions of *bcl-2* and *MT-I* mRNA were not affected. Lycopene pretreatment inhibited cadmium induced lipid peroxidation in blood, testes and prostate. Formation of lipid peroxide (LPO) was reduced up to 45.31% in 30 mg/kg lycopene pretreated group (33.02 nmol/g) as compared to 5 μ mol/kg CdCl₂ treated control group (60.38 nmol/g, $p < 0.05$). Averaged lycopene concentrations were 0.38 μ mol/L in blood, 47.61 nmol/g in the liver, 0.49 nmol/g in the testes, and 0.25 nmol/g in the prostate tissue after 3-week pretreatment with 30 mg/kg lycopene. These results supported epidemiological findings that a lower risk of prostate cancer was found in men frequently consuming tomato products and may contribute to the understanding of the protective effects of lycopene against cadmium-induced toxic effects.

1930 THE CRITICAL ROLE OF THE CELLULAR THIOL HOMEOSTASIS IN CADMIUM PERTURBATION OF THE LUNG EXTRACELLULAR MATRIX.

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Cadmium (Cd) enhances levels of cellular thiols providing the first defense line against heavy metal toxicity. Lysyl oxidase (LO), a copper (Cu)-dependent enzyme secreted by fibrogenic cells, stabilizes the lung extracellular matrix (ECM) by crosslinking of collagen and elastin. As shown here, CdCl₂ (0-6 μ M) exposure to rat lung fibroblasts (RFL6) for 24 h induced upregulation of metallothionein (MT) and glutathione (GSH), two metal scavenging agents, and gamma-glutamylcysteine synthetase (γ -GCS), a key enzyme for GSH biosynthesis, which was associated with downregulation of LO at the mRNA, protein and catalytic levels. To gain insight into the possible role of cellular thiol homeostasis in LO regulation, we further assessed effects of elevated levels of cellular thiols on LO expression at mRNA levels. Interestingly, increased cellular MT induced by scrape loading and elevated levels of cellular GSH by treatment of cells with GSH monoethyl ester (GME), a GSH delivery system, both significantly inhibited the expression of LO mRNAs. Notably, downregulation of LO in Cd treated cells was closely accompanied by inhibition of the synthesis of collagen type I, a major component of the lung ECM. Furthermore, using rats repeatedly instilled with CdCl₂ (30 μ g, once a week) intratracheally as an animal model, we demonstrated that Cd exposure induced a biphasic response in the lung LO activity and collagen synthesis, i.e., an initial increase (at 2 weeks) followed by a steady decrease (at 4-6 weeks) upon long-term exposure. However, the lung MT levels increased almost linearly over the 6-week exposure and emphysematous lesions were found. In view of regulatory effects of cellular thiols on the metabolism of essential metals such as Cu, a cofactor of LO, these results suggest that upregulation of cellular thiols as an important cellular event plays a critical role in LO regulation. This may be a potential mechanism for Cd-induced lung injury (Supported by NIH grant R01-ES11340).

1931 ROLE OF METALLOTHIONEIN(I, II, III) IN CADMIUM-TRANSFER FROM UTERUS-PLACENTA TO FETUS.

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The role of metallothionein(MT) for maternal to fetal and to new born rat of cadmium(Cd) transfer was investigated.

Mother rats were given Cd orally(1,2,5 mgCd/kg).Immunohistochemical localization and protein and gene expression of iso-MT(I,II,III) in the uterus and placenta were determined. Cd accumulation and MT concentration increased in uterus and placenta, and in intestine, kidney and stomach milk of offspring rats.

MT level in placenta increased depending on increase of Cd level, but the increased MT protein level in uterus depending on the increase of Cd accumulation was not found.

An increased gene expression of iso-MT (I, II, and III) were found in uterus of 2 and 5mgCd/kg group, but MT protein induction corresponding to gene expression of iso-MT-III was not found in uterus. It was suggested that iso-MT-III may not participate actively for the Cd transfer from mother to fetus in comparison with the cases of MT-I and MT-II. Because of more Cd was accumulated into liver rather than that in kidney of new born rats, it was suggested that Cd in fetus was transported or leaked from uterus and placenta by the form of non-MT-Cd. As meaning the dysfunction of placenta, the immunohistochemical staining showed the atrophy of syncytiotrophoblast cells and decreased positive-stain of MT in placenta tissues of 2 and 5 mgCd/kg groups depending to increasing Cd dose.

From these results, it was considered that MT is participating to trap Cd in uterus and placenta to protect Cd transport to fetus, but for trace amount of Cd to leak by dysfunction of syncytiotrophoblast cell into fetus.

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1932 RESISTANCE OF METALLOTHIONEIN TO PROTEOLYTIC DIGESTION AS A PROBE OF THE METAL BINDING STATUS OF THIONEINS: A LIQUID CHROMATOGRAPHY AND TANDEM MASS SPECTROMETRY ANALYSIS.

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Proteomics typically involves proteolytic digestion followed by mass spectrometric analysis of proteins. The metal binding protein, Metallothioneins (MTs) were observed to be resistant to proteolytic digestion by trypsin, the commonly used proteolytic agent in proteomics. The resistance of MT to tryptic digestion was further investigated using a model thionein, MT-3, which is a thionein fully saturated with 7 transition metals. It was shown that fully metal saturated MT-3 was completely resistant to proteolytic digestion. In contrast, metal free thionein-3, apoMT3 or MT3 fully depleted of metals with EDTA, was able to undergo complete digestion. When thionein-3 containing less than 4 metal ions was treated with trypsin, the thionein-3 was digested but displayed primarily peptides resulting from missed cleavages. When thionein-3 bound to either 4 or 5 metal ions was treated with trypsin, digestion occurred only at the hinged regions of the protein (Lysine 26, 31 and 32) located between the C-terminal α -domain and N-terminal β -domain. Trypsin was unable to digest thionein-3 once it was bound with 6 or more metal ions. A mechanism to explain these observations would be that the cleavage sites in thionein are located next to, or within one amino acid of, the conserved cysteine residues rendering these sites inaccessible to the relatively larger trypsin molecule (>20 kDa) due to space hinderance. In addition, when metal saturated, MT3 is folded into a compact and rigorous structure, shielding the cleavage sites from trypsin. This study demonstrated that proteolytic digestion followed by LC-MS/MS is a fast and sensitive method to distinguish among metal free, partially metal-bound, and metal-saturated thioneins. The combination of proteolytic digestion and LC-MS/MS analysis was able to identify thioneins as well as provide information regarding metal binding status of the molecule.

1933 UNAMBIGUOUS IDENTIFICATION OF METALLOTHIONEIN ISOFORMS BY NANO-FLOW LIQUID CHROMATOGRAPHY AND TANDEM MASS SPECTROMETRY: APPLICATION TO RABBIT LIVER AND HUMAN PROSTATE EPITHELIAL CELLS.

X. Lu, R. Wang, A. Albrecht, S. H. Garrett, S. Somji, M. A. Sens and D. A. Sens. *Pathology, University of North Dakota, Grand Forks, ND.*

Metallothioneins (MTs) are a family of low molecular weight, cysteine-rich metal binding proteins, whose sequences are highly conserved. There are six MT members in rabbits and more than ten in humans; the homology of protein sequence is greater than 90% among human or rabbit MT members. These proteins play an important role in the homeostasis of essential metals such as Zn⁺² or Cu⁺² during growth and development as well as in the detoxification of heavy metals such as Cd⁺² and Hg⁺², rendering the MTs important mediators and attenuators of heavy metal-induced. One of the major difficulties is to unambiguously identify individual MTs involved in a specific biological process. In this report, we described an automated proteomic method based on nano-flow liquid chromatography and tandem mass spectrometry using a high resolution quadrupole time-of-flight mass spectrometer. The method integrated top-down and bottom-up proteomic approaches where the individual MTs were identified by their unique full-length protein sequences as well as their accurate masses (mass accuracy < 50 ppm). The applicability of the method was first demonstrated by the analysis of commercial MT preparations from rabbit liver. Three MTs were identified including MT-1A, MT-2D and MT-2E, majority of which were N-terminal acetylated with a minor amount in unmodified (native) forms. The method was further applied to MT crude fractions prepared from cultured RWPE cells, a human prostate epithelial cell line. Six human MTs were identified and are currently under further investigation. The results were consistent with independent RT-PCR experiments which detected the expression of 1E, 1X and 2A isoforms in RWPE cells. The current proteomic method is advantageous in its abilities of profiling MT protein isoforms with high speed and characterizing the post-translation modifications of MTs.

1934 INDUCTION OF METALLOTHIONEIN IN OREOCHROMIS MOSSAMBICUS EXPOSED TO CADMIUM STRESS.

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Metals are known to induce oxidative stress in aquatic organisms. To date numerous studies have implicated metallothionein (MT) formation in the: a) detoxification of heavy metals, b) regulation of metal-distribution and c) the tissue damage due to generation of reactive oxygen species (ROS). MT is an ubiquitous, cysteine-rich metal binding protein and its synthesis is induced by cadmium (Cd) mediated-oxidative stress, glucocorticoids and anticancer agents. In the present study, the MT in liver, kidney, brain, gill and muscle of the edible fish *Oreochromis mossambicus* (Tilapia) exposed to Cd (5mg/l) for 30 days was quantified by cadmium-saturation method. The induction of MT was in the order of kidney (97.3 µg/g) > liver (63.5 µg/g) > gill (17.2 µg/g) > brain (7.4 µg/g) > muscle (6.8 µg/g). The data show that the Cd-concentration as determined by the DEAE cellulose ion exchange chromatography was higher in kidney (11.4 ppm) followed by liver (6.6 ppm), gill (2.8 ppm), brain (2.3 ppm) and muscle (1.98 ppm). The relative migration of MT protein was measured as 0.67 for liver, kidney, gill and muscle suggesting a molecular weight of 10-12 KD whereas for brain it was found to be 0.7 suggesting 10 KD. In general, the purification of brain MT protein showed the same elution pattern as that of hepatic and renal MTs. The present study suggests that the occurrence of MT-III protein in brain of piscine model could be a growth-inhibitor factor similar to the one reported in mammalian brain.

1935 VERY LOW DOSE MERCURY MODULATES CYTOKINE SECRETION BY HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS *IN VITRO* WITHOUT CHANGING CELL POPULATIONS.

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Mercury (Hg) is implicated as an immunotoxicant by studies in humans, animals, and in vitro cell culture systems. However, most studies have been conducted with exposures in the micromolar range, far above that found in blood under environmental exposure situations. To investigate Hg's immunotoxic effects under more environmentally relevant conditions, we investigated immune function in primary cultures of human peripheral blood mononuclear cells (PBMCs) exposed to a concentration of HgCl₂ up to 200 nM (which roughly corresponds to a whole blood concentration of 50 µg/L, at the upper range of occupationally exposed and fish eating populations). These concentrations, well below those in the published literature, do not induce overt cytotoxicity (as measured by MTT and trypan blue exclusion assays). Parameters of immune function were measured in the presence and absence of 50 ng/ml LPS to model Hg's effects on both resting and activated immune system. Cells were harvested after 48 hours in culture and immediately stained for flow cytometry. Cell culture supernatants were analyzed by ELISA for cytokine concentrations. In PBMCs from both male and female volunteers, dose-dependent increases in both TNFα and IL-6 were observed, while no changes were observed in IFNγ levels. These changes in cytokine production were detectable under conditions where no changes were detected in cell populations as measured by flow cytometry (CD19+ B cells, CD11b+ NK cells and monocytes, and CD3+/CD4+ or CD3+/CD8+ T cells and CD4+/CD8+ T cell ratios). Additionally, there were no changes in markers of activation (CD25, CD80, CD86, CD69) for any of the subpopulations studied over the dose range of Hg used. This study shows that Hg can exert significant effects on cytokine production at levels that are both non-cytotoxic and environmentally relevant, indicating that Hg effects at low concentrations are likely reflective of changes in signal transduction pathways rather than in cell viability or subsets.

1936 AN INTEGRATED FUNCTIONAL GENOMICS APPROACH TO ASSESS THE EFFECTS OF DIETARY EXPOSURE TO METHYLMERCURY AND SELENIUM IN JUVENILE AND ADULT MICE.

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Seafood is a good source of nutrition, yet may also be a route of exposure to contaminants such as methylmercury (MeHg). Epidemiological studies implicate the consumption of MeHg-contaminated seafood with effects on the nervous system, particularly during development. Micronutrients normally found in high levels in seafood, such as selenium have been shown to modify the toxicity of methylmercury. Selenium counteracts the toxic effects of methylmercury, possibly due to the

formation of stable adducts. A study was conducted to establish the importance of MeHg speciation, and identify the mechanisms of MeHg toxicity in a murine model. Female mice were fed MeHg (as either MeHgCl or MeHgCys) and in a separate study MeHgCys and/or selenium before mating, during gestation, and for two weeks following parturition. Neurobehavioural indices such as stress/anxiety response, reflex behaviour and locomotion were monitored in pups. Behavioural impacts were also determined in adult mice. An increased latency to movement in an elevated plus maze in MeHgCl- but not MeHgCys-exposed adults were discerned. Brains of these pups were then subjected to microarray analysis to monitor changes in gene expression. Results highlighted perturbations in a number of biological functions, befitting the widespread toxic effects of methylmercury noted in more restricted studies. Of note were alterations in genes relating to cell cycle control, cell signalling, calcium metabolism, cell adhesion, apoptosis, and oxidative stress. A number of genes were also identified that are associated with neural degenerative disorders such as Alzheimers disease, suggesting that mechanisms of neural impairment may be conserved, independent of the causative agent. The 5 mg kg⁻¹ methylmercurycysteine treatment induced the greatest number of significantly differentially expressed genes, contrary to expectations based on previous literature findings of lowered toxicity of this methylmercury species.

1937 NON-LINEAR EFFECTS OF METHYLMERCURY ON PRIMARY RAT CORTEX ASTROCYTES AND NEURONS: ROLE OF GRP78 AND GRP94.

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Methylmercury (MeHg) is a well-known environmental neurotoxicant. It has been suggested that MeHg exerts its neurotoxicity via the generation of reactive oxygen species and depletion of intracellular thiols. While preferentially accumulating in astrocytes, neurons are significantly more sensitive to MeHg's effects. However, the patterns and underlying mechanisms of low-level effects of MeHg in astrocytes and neurons remain unclear. Due to the crucial roles of endoplasmic reticulum (ER) chaperone proteins in protein folding, refolding and trafficking, the objective of this work was to define the differential vulnerability of astrocytes and neurons to low-level MeHg exposure with emphasis on the effects of GRP78 and GRP94, markers of ER. Primary cortical cultures of astrocytes and neurons were exposed for 0.5, 1, 2, 6, 12 and 24 hrs to 10⁻¹⁷ to 10⁻⁴ M MeHg. Cellular viability and cytotoxicity were determined by MTT and LDH leakage assays. The expression levels of GRP78 and GRP94 were assessed by western blotting. MeHg (>10⁻⁴ M) was toxic to both astrocytes and neurons at all time points. However, at lower concentrations (10⁻¹³-10⁻⁷ M MeHg), an opposite trend associated with cell protection, namely increased cellular viability (~120% of controls; p<0.05), was noted. Neurons and astrocytes exhibited a similar pattern of GRP78 and GRP94 protein expression levels. Significant induction of these proteins was noted at low-level (10⁻¹⁶-10⁻¹⁰ M) MeHg exposure, and inhibition was associated with exposures to high-levels (10⁻⁷-10⁻⁴ M) of MeHg at 6, 12 and 24 h. Our results demonstrate that MeHg induces non-linear effects in astrocytes and neurons potentially via the induction and inhibition of ER stress proteins. Accordingly, the role of ER stress signal pathways in MeHg neurotoxicity represents a fruitful area for future study (Acknowledgement: This work supported by Public Health Service of National Institute of Health Grant NIEHS ES07331).

1938 INVESTIGATION OF CADMIUM-INDUCED OXIDATIVE STRESS AND CHANGES IN RUNX2 mRNA EXPRESSION IN THE HUMAN OSTEOBLAST-LIKE CELL LINE, SAOS-2.

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The heavy metal cadmium is an environmental and occupational toxin. Notable sources include electronic waste and cigarettes. Human exposure to cadmium has been associated with the development of bone diseases including osteoporosis, but the mechanisms by which cadmium exerts a direct effect on bone are unclear. Oxidative stress has been implied in the pathogenesis of osteoporosis, therefore one possibility is that cadmium induces oxidative stress in Saos-2 cells. Furthermore, we explore whether cadmium-induced oxidative damage leads to a decrease in the osteoblast transcriptional factor, RUNX2. This transcriptional factor is a key modulator of osteoblastogenesis and has been reported to play a protective role against osteoporosis in postmenopausal women. We hypothesize that cadmium exposure induces oxidative stress which leads to a decrease in RUNX2 mRNA expression. Saos-2 cells were treated with or without 10 µM CdCl₂ for 0-24 hours. Oxidative stress induced by CdCl₂ was assessed by measuring glutathione (GSH) depletion. RUNX2 mRNA expression was determined by RT-PCR. Cadmium exposure induced oxidative stress, in part, by a depletion of the antioxidant GSH. Ongoing oxidative stress experiments are evaluating reactive oxygen species formation and lipid

peroxidation. Exposure to CdCl₂ also resulted in a significant decrease in RUNX2 mRNA expression, about 40% at 6 and 12 hours, compared to untreated controls. To show that cadmium-induced oxidative damage occurs upstream from changes in RUNX2 expression, cells will be pretreated with antioxidants to control the level of oxidative stress. We predict antioxidants will prevent cadmium-induced decrease in RUNX2 expression. This study will provide insight into the mechanisms underlying cadmium-induced alterations in bone function.

1939 A N-TERMINAL MUTANT FORM OF MT-3 AFFECTS THE GROWTH RATE, VECTORIAL ACTIVE TRANSPORT AND EXPRESSION OF CADHERIN MOLECULES IN MCF-7 BREAST CANCER CELLS.

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The metallothioneins (MT) are well-known for their role as metal-binding proteins and for their protective effects against heavy metal induced toxicity. MT-3 possesses neuronal growth inhibitory activity, a characteristic feature not shared by any other member of the MT family. Previous studies from this laboratory have shown that MT-3 can inhibit the growth of the PC-3 prostate cancer cell line or the MCF-7 and Hs578T breast cancer cell lines. Furthermore, studies from this laboratory also suggested that MT-3 might have a role in cell differentiation. This was suggested by studies which demonstrated that the stable expression of MT-3 restored active vectorial ion transport to a proximal tubule cell line that had lost this differentiated function. Active vectorial ion transport was monitored by the formation of out-of-focus areas of the cell monolayer known as domes, a hallmark of cultured renal epithelial cells that retain the in situ property of vectorial active transport. In the present study, a site directed mutant of MT-3 was generated in an attempt to determine which epitope of the MT-3 protein was responsible for the growth inhibition noted when MCF-7 cells were stably transfected with the MT-3 gene. The MT-3 mutant possessed alteration in the beta domain of the protein, specifically, proline 7 and 9 were mutated to threonines. Transfection of the MT-3 mutant into MCF-7 cells abolished the growth inhibitory activity of the protein. Furthermore, the cells formed domes in culture indicating a gain-of-function for the differentiated property of vectorial active ion transport. It was also demonstrated that these cells expressed high levels of E-cadherin compared to the parent MCF cells as well as the MCF cells expressing the MT-3 gene, suggesting that MT-3 may be involved in the regulation of junctional molecules.

1940 MOLECULAR AND TOXICOLOGICAL RESPONSES TO MERCURIALS IN C. ELEGANS.

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Humans are typically exposed to three different species of mercury: inorganic, methyl and the mercury-containing compound thimerosal. While these compounds are toxic, their mechanisms of toxicity are poorly understood. We hypothesize that different mercurial species modulate the activity of unique and overlapping intracellular signal transduction pathways to affect transcription. Using the nematode *C. elegans*, we have investigated the mechanisms of toxicity of these compounds. After determining an equitoxic dose for each compound, microarrays were run to determine changes in gene expression resulting from mercury exposure. Bioinformatics analysis was then used to predict which signaling pathways are affected by the different mercury species. To assess the potential roles of these pathways, siRNA used to knock-down genes of interest, and *C. elegans* was subsequently exposed to the different mercurials to test for a mercury-sensitive or mercury-resistant phenotype. The localization and induction of novel genes will be investigated by integrating GFP-tagged genes into *C. elegans*. Through this combination of toxicology, bioinformatics and molecular biology techniques, we hope to elucidate the mechanisms by which the different mercurials exert their respective toxicities.

1941 DOSE RESPONSE EFFECTS OF MUTANT KI-RASG12C IN MOUSE LUNG.

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Our laboratory has developed a bitransgenic mouse lung tumor model that, upon treatment with 500ug/ml of doxycycline (DOX) for 12 months, allows for the maximal expression of the human Ki-rasG12C allele in the lung, resulting in the development of benign focal hyperplasias and adenomas. We determined if different levels of mutant RAS expression would influence the phenotype of the lung le-

sions. Treatment with 25 and 100ug/ml of DOX resulted in dose-dependent increases in tumor multiplicity. At the 500ug/ml dose, all of the lesions were <1mm; however, in mice that received the lower doses of DOX, there was a significant number of lung lesions that were ≥1mm, with some reaching up to 4mm in size. Interestingly, there was a dose-dependent difference in the morphology of the proliferative lesions. The 25 and 500ug/ml treated bitransgenic mice exhibited hyperplasias and relatively benign adenomas, whereas the 100ug/ml treated mice also exhibited more severe, high grade adenomas with atypical features of AC. Immunohistochemical analysis of signaling pathways suggested similarities and differences in the expression and/or phosphorylation of specific signaling molecules. Increased expression of p19ARF, along with the concomitant activation of the p53 pathway, was only seen at the 500ug/ml dose. Elevated levels of Ki-67 staining were found at all 3 levels of transgene expression. Interestingly, increased caspase-3 activity was noted at the 25 and 100, but not the 500ug/ml, dose. Increased expression of survivin was seen at the 100ug/ml and 500ug/ml doses but not at the 25ug/ml dose. Using a phospho-specific antibody, we found an increase in phosphorylated JNK at the 25ug/ml dose, but not at the two higher doses. Our results suggest that the molecular alterations driving tumorigenesis may differ at different levels of transgene expression, and this should be taken into consideration when inducible transgene systems are utilized to promote tumorigenesis in mouse models. (Supported by NCI grant CA91909)

1942 SILICA CARCINOGENICITY IN A SUSCEPTIBLE MOUSE MODEL.

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In 1997 IARC classified crystalline silica as a Group I human carcinogen despite disagreements in the scientific community related to the lack of a mouse model for silica-induced carcinogenicity. Since mutations of the p53 tumor suppressor gene are the most frequently observed genetic changes in human and animal cancers, this study was designed with mice deficient in p53 to evaluate the potential carcinogenicity of crystalline silica. Specifically, these experiments examined the effects of pharyngeal aspiration of freshly fractured silica (0 or 2mg) on various biochemical, molecular and genomic changes and incidence of preneoplastic lesions in the lungs of wildtype, heterozygous and homozygous mice after 2 and 6 months. Analysis performed on bronchoalveolar lavage fluid markers (albumin, lactate dehydrogenase), cells (differential cell counts, apoptosis, cell cycle analysis), and lung tissue (microarray, DNA damage) have shown differences related to exposure in various markers of toxicology and oxidative stress. Histopathologic alterations associated with silica exposure included alveolar epithelial cell hyperplasia, peribronchiolar bronchiolization and lipoproteinosis in the lungs of silica-exposed animals. Microarray analysis demonstrated alterations in genes related to cell cycle control, DNA damage repair and apoptosis, consistent with alterations seen in the cellular analysis. Preliminary microarray analysis also suggests a variation in activated signaling pathways in the wild-type animals as compared to the heterozygous animals. The data described here shows an effect of p53 status on response to silica exposure as early as 2 months following the initial exposure. This susceptible mouse model may provide insights into the critical role of p53 in silica-induced lung injury. The findings and conclusions of this abstract are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

1943 USE OF GENETICALLY MODIFIED C3B6F1 TRP53(+/-) P53 HAPLOINSUFFICIENT MICE FOR SHORT-TERM CANCER BIOASSAYS OF ANTIRETROVIRAL (ARV) DRUGS.

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The advent of ARV drugs revolutionized the treatment of HIV/AIDS and has allowed pregnant HIV-positive women to give birth to uninfected babies. However, ARV therapy is not without side-effects and as new ARV drugs become available it is necessary to test them in combination with existing drugs. The ARV, zidovudine (AZT) is genotoxic in fetal mice and monkeys and carcinogenic in mice. We assessed a new C3B6F1 trp53(+/-) (designated C3B6-trp53tm1Brd[N12]F1) p53 haploinsufficient, genetically modified mouse model for use in short-term cancer bioassays. These mice are produced by mating Taconic C3H females with C57Bl6(N12)trp53(-/-) males. They were dosed with 0, 80, 160, 240 mg/kg/day AZT, by gavage in aqueous methylcellulose/ polysorbate 80 (0.2/0.1%), transplacentally (via maternal gavage) from GD12 to GD18 then postnatally from PND1 until 45 weeks old. The dose and dose volume was reduced to 50% from PND1-10 to reduce acute toxicity. During the initial postnatal period (PND1- PND28), survival was >95% and >82% for the control and dosed groups respectively. The AZT treatment produced only small (<10%) reductions in body weight gain. AZT at all 3 concentrations increased blood reticulocyte micronuclei formation at PND1, 10 and 28. AZT treatment did not significantly reduce hepatic mitochondrial gene ex-

pression or increase serum lactate levels, but did increase liver tumor incidence at 45 weeks in male mice: Incidence of hepatocellular adenoma plus carcinoma was 3/27 (12.5%), 3/27 (13.6%), 6/27 (22.2%) & 9/27 (33.3%) in the 0, 80, 160, 240 mg/kg/day dose groups respectively. Thus, unlike previous p53(+/-) mouse models which use a C57Bl6 background, this mouse model develops liver tumors within a 45 week period and has a similar tumor type profile to the B6C3F1 mouse. The C3B6F1 trp53(+/-) mouse may therefore be a good model for evaluating cancer risk of ARV drugs.

1944 APPLICATIONS AND UTILITY OF THE ACCELERATED CANCER BIOASSAY (ACB).

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The ACB is a versatile mechanistic initiation/promotion chronic toxicity and carcinogenicity bioassay designed to assess potential carcinogenic activity of a test substance (TS) in 6 critical organs and tissues of rodents in which human carcinogens are active, i.e. liver, lung, kidney, urinary bladder, stomach and mammary gland.

ACB is capable of identifying both putative genotoxic initiators and epigenetic carcinogens (promoters). The standard protocol involves 2 segments; a first in which the TS is administered as an initiator at an appropriate high dose for 16 weeks, followed by promoters for the target organs, and a second segment in which the test substance is administered in a promotion segment for 24 weeks after administration for 16 weeks of an initiator for the target organs. The TS is also given alone for 40 weeks. Thus, the effects of the TS are compared to those of 6 standard initiating agents and 6 standard promoting agents. Variations to the standard protocol can also be made to accommodate mechanistic needs, and other known toxicokinetic data. Age and gender considerations are also included. Post mortem markers to monitor tissue proliferation and differentiation, as well as during life markers measuring temperature and endocrine endpoints, are also routinely utilized.

Five chemically diverse TSs have been tested: (1) BAY12-9566 (BAY), a substituted 4-biarylbutyric acid, a matrix metalloproteinase (MMP) inhibitor (MMPi); (2) moxifloxacin (MOX), a novel bacterial gyrase-inhibitor fluoroquinolone antibiotic; (3) nifurtimox (NFX), a 5-nitrofuran derivative used in the treatment of American trypanosomiasis; (4) phentolamine (PHEN), an alpha-adrenergic receptor antagonist; and (5) Wingstay 100 (W100), a diaryl-p-phenylenediamine rubber antioxidant/antioxidant. We report that all 5 did not demonstrate carcinogenicity, although NFX is genotoxic. Rather, all 5 reduced initiator-induced or promoter-induced neoplasia or even spontaneous neoplasia. Thus, the utility and versatility of ACB is presented and discussed.

1945 A SHORT-TERM *IN VIVO* ASSAY FOR THE PREDICTION AND MECHANISTIC ASSESSMENT OF NON-GENOTOXIC HEPATOCARCINOGENS.

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Shorter term alternatives to the two year rodent bioassay for identifying carcinogenic chemicals would facilitate significant cost savings for development of therapeutics and speed the identification of potentially carcinogenic chemicals. However, existing short term assays demonstrate performance that renders them unsuitable for regulatory approval, and their utility as hazard identification tools for early stage screening is also in doubt. Using a 5 day repeat dose *in vivo* test in rats, a gene expression signature was derived using a training set of 100 structurally and mechanistically diverse non-genotoxic hepatocarcinogens and non-hepatocarcinogens. The multi-gene signature was then independently validated on 47 additional chemicals with resultant sensitivity and specificity of 85%. Alternate tests for hepatocarcinogenicity were evaluated for comparison, including liver weight, hepatocellular hypertrophy, hepatic injury and putative gene-based biomarkers such as cytochrome P450 genes, Tsc-22 and alpha2-macroglobulin. In contrast to these and other putative gene biomarkers, our signature was more accurate in predicting non-genotoxic hepatocarcinogens. Additionally, unlike existing tests, an understanding of potential modes of action for carcinogenicity can be derived through comparison of the signature profile of test chemicals to hepatocarcinogens of known mechanism. This was exemplified by the association of chloroform with the known carcinogens methapyrilene and thioacetamide, the association of betanaphthoflavone with known carcinogenic P450 inducers, and diethylstilbestrol with hormonal carcinogens. This signature is not only more accurate than current methods, but also facilitates the identification of mechanism of action to aid in the assessment of human cancer risk.

1946 PRENATAL AND NEONATAL MICE ARE MORE SENSITIVE TO MUTAGENICITY OF CARCINOGENS THAN ADULTS.

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The incidence of childhood cancer is increasing and recent evidence suggests an association between childhood cancer and environmental exposure to genotoxins. Genotoxic carcinogens generally induce more tumors in neonate mice than in

adults. To investigate the age-dependent mutagenic sensitivity to genotoxic carcinogens, neonatal and adult Big Blue mice were treated with three different genotoxic carcinogens, N-ethyl-N-nitrosourea (ENU), 4-aminobiphenyl (4-ABP) and aflatoxin B1 (AFB1). Liver samples from the ENU, 4-ABP and AFB1 treatments and brain samples from the ENU treatment were assayed for cII mutant frequency (MF). All three compounds resulted in much higher MFs from the neonatal treatment than from the adult treatment. ENU, a direct-acting mutagen, resulted in 3-fold and 4-fold higher MFs in neonates than in adults for brain and liver, respectively, while treatment with 4-ABP and AFB1, which are activated to genotoxic derivatives in the liver, produced 4-fold and 13-fold higher MFs in neonates than in adults. Molecular analysis of the mutants indicated that the higher MFs from the neonates treated with genotoxic carcinogens were not the result of clonal expansion and that the mutational spectra from the treated mice were, to a certain extent, age-dependent. The mutagenicity results indicate that the neonatal period is a life stage when some tissues are very sensitive to mutation induction by genotoxic carcinogens and that mutation is an important rate-limiting factor for age-related carcinogenesis.

1947 PRODUCTION OF GALL BLADDER AGENESIS, LIVER PRENEOPLASIA AND GENOTOXICITY IN TURKEY FETUSES ADMINISTERED DIETHYLNITROSAMINE.

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The *in ovo* carcinogenicity assay (IOCA) was used to examine the morphological changes in fetal turkey livers caused by the carcinogen diethylnitrosamine (DEN). Fertilized turkey eggs were divided into 3 groups: untreated control (UC), vehicle control (VC) and DEN-treated. At day 0, the eggs of the DEN group were injected with 4 mg/egg (50mg/kg) of DEN dissolved in 0.2 ml water. All eggs were allowed to incubate at 37°C and 60 % humidity for 24 days. The fetal livers were collected and processed for histopathological evaluation (H&E staining) and cell isolation for culture and comet analysis of DNA damage. Typical survival rates were 82 % for the UC, 50 % for the VC and 16 – 40 % for the DEN-treated fetuses. The fetal body weights in the DEN-treated group were about 10 % lower than in the UC, while the liver weights were similar in both groups. All DEN-treated fetuses exhibited absence of the gall bladder and the livers of these fetuses frequently were of greenish color.

All liver samples from both UC and VC groups demonstrated normal microscopic architecture reflecting their age. In the DEN group, there was hepatocellular cytomegaly, karyomegaly, anisokaryosis and assumption of a glandular pattern of the 2-cell thick plates. Microscopically, the agenesis of the gall bladder was accompanied by agenesis of the common bile duct and the 2 lobular ducts. Hepatocellular altered (basophilic) foci (FAH) were present in livers of most DEN-treated fetuses, resembling preneoplastic lesions of adult rat livers. An *ex vivo* model of cultured fetal turkey hepatocytes was also developed to study the morphology and growth regulation of initiated cells. Preliminary data reveal the presence of megalocytes (immunofluorescence) and increased levels of DEN-induced DNA damage (comet assay). The IOCA is a rapid and inexpensive short-term bioassay.

1948 HIGH INORGANIC PHOSPHATE STIMULATES LUNG TUMORIGENESIS THROUGH AKT PATHWAY IN K-RAS NULL MICE.

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Inorganic phosphate (Pi) plays a key role in diverse physiological functions. Most studies for Pi have been focused mainly on bone, because the bone is the major organ affected by Pi. Regulation of Pi balance is accomplished by the family members of sodium-dependent inorganic phosphate transporters (NPTs), which regulate entrance into the cellular membrane. The NPT2b is one of the NPT family, and expressed in lung, thus, suggesting that Pi may play an important role in lung homeostasis. In this study, we examined the effects of dietary high Pi on lung cancer development. Ten K-ras null mice were randomly allocated to two groups; one group fed normal diet containing 0.5% Pi, and other group fed high Pi (1%). The experiment period was four weeks. The serum Pi level was increased by high Pi diet; however, the serum calcium concentration remained unchanged. Dietary high Pi diet caused a significantly increase NPT-2b expression in lung of K-ras null mouse. Dietary high Pi stimulated lung cancer proliferation through increasing cyclin D3, CDK2 and PCNA expression. Additionally, the dietary high Pi decreased tumor suppressor gene PTEN and CTMP expression; and increased Akt phosphorylation at Ser473. High Pi increased the Akt-mediated cell signals including mTOR, 4EBP1 and p70S6K expression. Taken together our results indicate that dietary high Pi possibly stimulates the lung tumorigenesis in K-ras null mouse. Supported by KOSEF

1949 COMPLEX MIXTURES ALTER BENZO[A]PYRENE AND DIBENZO[A, L]PYRENE-INDUCED CARCINOGENESIS.

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The primary source of human exposure to the carcinogenic polycyclic aromatic hydrocarbons (PAHs) benzo[a]pyrene (B[a]P) and dibenzo[a,l]pyrene (DB[a,l]P) is through environmental complex mixtures, such as urban dust particulate matter (UDPM) and diesel exhaust (DE). To understand the influence of these complex mixtures on the carcinogenic potency of B[a]P and DB[a,l]P, we used both *in vivo* (SENCA mice) and *in vitro* (human breast epithelial cells) analyses on exposure to appropriate concentrations of PAH plus UDPM or DE. Results from the tumor initiation-promotion assay revealed not only decreased tumor formation but also a significant delay in the onset of tumors upon co-exposure to UDPM or DE plus PAH, compared to PAH alone. Tumor initiation results correlated with cytochrome P450 (CYP) enzyme activity, CYP1A1 protein expression and PAH-DNA adducts. *In vitro* experimentation indicated that UDPM and DE decreased the carcinogenic potency of B[a]P and DB[a,l]P by inhibition of CYP metabolic capacity and PAH-DNA adduct formation. Further, expression of aldo-ketoreductase (AKR) 1A1, 1C1, 1C2, and 1C3 genes were significantly upregulated (> 2-fold change) upon co-exposure to either mixture plus B[a]P or DB[a,l]P. Increased DNA strand breaks and oxidative damage, as measured by the presence of 8-oxo-dG, were also detected following exposure to PAH plus UDPM or DE, compared to PAH treatment alone. These findings suggest that complex mixtures alter the carcinogenic potency of PAH by inhibition of CYP metabolism and upregulation of the competing AKR1 metabolic pathway. In addition, the results from this study emphasize the need for in-depth assessments of mixtures, rather than individual compounds, in order to better predict human cancer risk.

1950 VALIDATION OF A HIGH THROUGHPUT GAP-JUNCTIONAL INTERCELLULAR COMMUNICATION (GJIC) ASSAY USING CIGARETTE SMOKE CONDENSATES.

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Cigarette smoke condensate (CSC) is known to inhibit cell communication in a dose-dependent manner. We developed and evaluated a new high throughput GJIC assay for reproducible dose response, accuracy, and precision. An automated fluorescent microscope (ArrayScan®, Cellomics), the rat liver epithelial cell line WB-F344, and CSC from the 2R4F Kentucky reference cigarette and two single-tobacco cigarettes (i.e., Bright and Burley) were utilized in the evaluation. Experimental: Cells were stained with the fluorescent dye Calcein. Stained cells were pipetted to unstained cells of the same type and incubated for 3 h with the test substance or solvent control (0.5% DMSO). After establishing the connexin channels, the transfer of the fluorescent dye to neighboring cells within 3 h was determined and expressed as "% stained cells" as a measure of gap-junctional intercellular communication. Phorbol-12-myristate-13-acetate (TPA), a well known inhibitor of GJIC, was used as a control. Results: The assay showed a reproducible dose response. The repeatability and reproducibility for the 2R4F were 3.7% and 6.9%, respectively; the minimal detectable difference was 5.7 µg/ml. The EC50 value for TPA was 0.34 ng/ml, which is in the range reported in the literature for other GJIC assay designs. The EC50 values for CSC from the three cigarette types were 45 µg/ml for Bright, 51 µg/ml for the 2R4F, which is a mixture of Bright and Burley tobacco, and 58 µg/ml for Burley, which had a shallower slope than the other two cigarette types. Conclusion: This cell-based assay can determine the GJIC-inhibitory activity of CSC, and meets ISO criteria for precision and accuracy. The assay can also discriminate between different CSCs, as reflected by the EC50 values for the three cigarette types. In conclusion, this screening assay is an adequate tool to determine GJIC, which has been proposed to play a role in tumor promotion.

1951 DEVELOPMENT OF AN ASSAY SYSTEM FOR SIMPLE AND EASY DETERMINATION OF CELL DIFFERENTIATION IN WELL-DIFFERENTIATED HUMAN COLON CANCER CELLS.

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Well-differentiated types of colon carcinoma account for 52.5% of the total number of colon cancer patients. However, very few *in vitro* studies have been performed with a well-differentiated colon cancer cell type, because the well-differentiated type of cells are generally difficult to culture *in vitro*. The goal of this study was to develop an assay system for a simple and easy determination of cell differentia-

tion in a well-differentiated human colon cancer cell type. Confluent RCM-1 cells (well-differentiated human colon cancer cells) spontaneously form some ducts resembling microvilli structures in plastic culture dish. When RCM-1 cells seeded in a 96-multi-well plate were cultured with kaempferol (a flavonoid mainly distributed in onion and broccoli) as an inducer of cell differentiation, duct formation was accelerated in the number and the size. Alkaline phosphatase activity, which is an early stage marker in cell differentiation, also increased. It is suggested that the real-time morphological observation of the duct formation can substitute for the alkaline phosphatase activity as an indication for differentiation. We thus developed the simple assay system that made possible speedy assessment for many samples in cell differentiation. Using this morphological differentiation assay, two well-differentiated human colon cancer cells (RCM-1 cells and KNC cells) were cultured to survey their potential involvement in differentiation. Forskolin accelerated the duct formation in RCM-1 cells, and AG490 changed the cell morphology in KNC cells. Thus, increasing intracellular cAMP and inhibition of Stat3 phosphorylation might be involved in the differentiation of RCM-1 cells and KNC cells, respectively. U0126 and wortmannin had no effects on either cell line. cAMP and Stat3 appeared to be involved in kaempferol-induced differentiation of RCM-1 and KNC cells, respectively.

1952 EVALUATION OF THE CYTOTOXICITY OF DRINKING WATER DISINFECTION BYPRODUCTS (DBPS): TRIHALOMETHANES (THMs), HALONITROMETHANES (HNMs), AND HALOACETIC ACIDS (HAAs) IN NORMAL HUMAN COLON CELLS.

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Epidemiological studies have linked the consumption of chlorinated surface waters to an increased risk of colorectal cancer. THMs and HAAs were found to increase cancer in laboratory animals, but no toxicity studies exist for the recently identified HNMs. Normal Human colonocytes (NCM-460) were plated at 7000 cells/well and incubated for 24 hours at 37°C/5% CO₂ after which the cells were exposed to THMs, HNMs, or HAAs at concentrations of 1.00E-07 M - 3.0E-03 M for 72-96 hours. Cell density was quantified using a crystal violet staining procedure. The concentration of a chemical that induced a 50% inhibition of cell growth (IC50) when compared to the untreated cells was calculated. The HNM IC50 ranged between 1.30E-05 - 1.0E-4 M. Only tribromo-methane and dibromochloromethane of the THM series exhibited cytotoxicity (~3.0E-03 M). Enzymatic analysis demonstrated that NCM460 cells possessed GSTT-1 and CYP2E1 activity similar to that measured in the large intestine and suggests a capability to activate THMs to cytotoxic intermediates. Prior treatment of the HNMs and THMs with human microsome preparations did not alter their cytotoxicity. The IC50 for the HAAs ranged between 1.48E-05 M - 3.41E-03 M. In general, the order of cytotoxicity was HNMs>HAAs>THMs. Within a series, the cytotoxicity increased with the degree of bromination and decreased with increasing molecular weight. The study demonstrated a cytotoxic response of NCM460 cells to DBPs similar to that of non-human mammalian cells (Plewa, MJ et al., Environ. Sci. Technol. 2004, 38, 62-68) and suggests that they might prove useful in determining a mechanistic potential for the carcinogenicity of unregulated DBPs. (This abstract has been reviewed and approved by the Environmental Protection Agency and does not necessarily reflect the views of the Agency)

1953 SIGNIFICANCE OF INFLAMMATION-ASSOCIATED REGENERATIVE MUCOSA CHARACTERIZED BY PANETH CELL METAPLASIA AND β-CATENIN ACCUMULATION IN THE ONSET OF COLORECTAL CARCINOGENESIS IN RATS INITIATED WITH 1, 2-DIMETHYLHYDRAZINE.

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It is known that a short-term dextran sodium sulfate (DSS) treatment strongly promotes colorectal tumor development in rats initiated with 1,2-dimethylhydrazine (DMH). In the present study, to clarify mechanisms for the DSS-associated tumor promotion, a time-course study for histopathological and immunohistochemical analyses and β-catenin gene mutations in colorectal mucosa at early stages of this model were conducted. F344 males were given three subcutaneous injections of DMH (40 mg/kg body weight) in a week, followed by free access to drinking water containing 1% DSS for a week. At 1 to 8 weeks after the DSS treatment, rats were euthanized and colorectal samples were collected. At week 1, the colorectal mucosa showed extensive loss of crypts/erosion along with significant inflammatory cell infiltration. Reactive hyperplasia was frequently accompanied in the neighboring mu-

cosa. At weeks 4 to 8, the pronounced inflammatory and hyperplastic lesions were mended, and regenerative mucosa with architectural alteration of crypts without significant cellular atypia were frequently observed. The regenerative mucosa was partly characterized by with Paneth cell metaplasia and altered subcellular localization of β -catenin along with some interstitial inflammatory cell infiltration. Dysplastic foci were frequently found in the regions with Paneth cell metaplasia, and adenomas/carcinomas also arose at this time point. Point mutations mainly in codons 34 and 41 of β -catenin gene were detected in the six of 27 samples of regenerative mucosa with Paneth cell metaplasia and four of 9 dysplastic foci/adenomas/carcinomas. These findings indicate that cell proliferation/inflammation-associated regenerative mucosa with Paneth cell metaplasia and alteration in APC/ β -catenin/Tcf signal transduction pathway is possibly involved in the acceleration of colorectal carcinogenesis in this DMH-DSS rat model.

1954 OCHRATOXIN A CARCINOGENICITY: TIME- AND DOSE-DEPENDENT INCREASE IN RENAL CELL PROLIFERATION IN MALE F344 RATS.

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The mycotoxin ochratoxin A (OTA) is one of the most potent renal carcinogens studied by the NTP to date. Male rats are most susceptible to OTA nephrotoxicity and carcinogenicity and chronic administration of OTA (70 and 210 μ g/kg b.w.) for two years has been shown to induce high incidences of adenomas and carcinomas. In contrast, treatment with a lower dose of 21 μ g/kg b.w. did not result in increased tumor rates. Since the mechanism of tumor formation by OTA is still largely unknown, this study was conducted to determine if sustained stimulation of renal cell proliferation may contribute to OTA carcinogenicity.

Male F344 rats were treated with OTA for up to 13 weeks under conditions of the NTP bioassay. Bromodeoxyuridine (BrdU) was administered via osmotic pumps implanted 7 days prior to necropsy and cell proliferation was quantified using BrdU immunohistochemistry. No signs of nephrotoxicity were evident by clinical chemistry, but relative kidney weight was significantly reduced in the mid and high dose group. Repeated administration of OTA at doses of 70 and 210 μ g/kg b.w. led to a marked dose- and time-dependent increase in renal cell proliferation. The pattern of labeling consisted of scattered individual or clusters of P3 tubules with multiple nuclei labeled, spreading from the medullary rays into the outer stripe of the outer medulla, with a clear increase related to time and dose. No significant effects were evident in the low dose group (21 μ g/kg b.w.) or in the liver, which is not a target for OTA carcinogenicity.

In summary, results from this study indicate that stimulation of cell proliferation may play an important role in OTA carcinogenicity. The apparent correlation between enhanced cell turnover and tumor formation induced by OTA provides further evidence for an epigenetic, thresholded mechanism.

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1955 HIGHER UPTAKE, CYTOTOXICITY, AND CELL TRANSFORMING ABILITY OF NICKEL SUBSULFIDE COMPARED TO NICKEL SULFATE IN C3H/10T1/2 MOUSE EMBRYO CELLS.

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Different nickel compounds have different carcinogenic potentials. To determine why nickel subsulfide (Ni3S2) was carcinogenic in animals and nickel sulfate (NiSO4) was not, we measured their cellular uptakes, cytotoxicities, and abilities to induce morphological transformation in cultured C3H/10T1/2 (10T1/2) mouse embryo cells. Ni3S2 (NiPERA, mean particle diam., 8.4 μ m) was phagocytosed into 10T1/2 cells at concentrations of 0.25 - 1.0 μ g/ml (60% of cells had phagocytic vesicles with Ni3S2 particles at 1 μ g/ml), and nickel ions were detected in the cytoplasm and nuclei of cells (Newport Green fluorescence method). Ni3S2 (48 hr exposure) induced strong, dose-dependent cytotoxicity in 10T1/2 cells (LC50 = 1.0 μ g/ml), and a linear, dose-dependent yield of morphologically transformed foci (slope = 3.2 foci/20 dishes/ μ g/ml compd.). NiSO4 hexahydrate (Sigma Chem. Co.) was not phagocytosed by 10T1/2 cells but did enter the cytoplasm and nucleus of cells, but only at high concentrations, 50 - 100 μ g/ml, and induced a dose-dependent but weak cytotoxicity (LC50 value = 50 μ g/ml). NiSO4 induced weak morphological transformation in three of four experiments, only at high concentrations, 25 - 100 μ g/ml (slope = 0.011 foci/20 dishes/ μ g/ml compd., p = 0.053). We

conclude: 1) Ni3S2 is a 50-fold stronger cytotoxin and at least a 291-fold stronger cell transforming agent than NiSO4 because Ni3S2 is taken up by cells at 0.25 - 1.0 μ g/ml, whereas NiSO4 is only taken up into cells at 50 - 100 μ g/ml; 2) NiSO4 is a weak cell transforming agent, only at much higher concentrations than nickel subsulfide (50 - 100 μ g/ml); and 3) the in vitro cell transforming activities of Ni3S2 and NiSO4, are consistent with the strong carcinogenicity of Ni3S2 and lack of carcinogenicity of NiSO4, by the inhalation route in rats. Supported by Contract from NiPERA to J. Landolph.

1956 GENE EXPRESSION ANALYSIS OF METAL-IMPLANTED LEG MUSCLE.

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Rhabdomyosarcoma is a rare childhood muscle cancer with an incidence of less than 1 per million per year and a poor prognosis, depending on the subtype. Though naturally-occurring rhabdomyosarcoma is rare, a similar disease has been shown to be highly penetrant in animals implanted with the transition metals nickel and cobalt, as well as nickel containing alloys. The applicability of this model to the human disease has not been investigated. In this work, microarray analysis was used to examine gene expression in rats implanted with rhabdomyosarcoma inducing alloys to see if gene expression could be linked with either mode of action or mechanism. Tissue samples surrounding implanted pellets were taken at necropsy for 1 and 3 month exposures; and from tumors (at 7 months) and tissue proximal to the tumor that appeared normal. Samples were flash frozen in liquid nitrogen. RNA was extracted from the tissue and used to generate oligonucleotide microarray probes for gene expression profiling using Affymetrix Rat 230 2.0 arrays. Results were analyzed using JMP Genomics and Genesifter, using PCA, ANOVA, and KEGG Pathways. The 1 and 3 month samples showed an immunologic response which was not evident in the tumors or control tissue. On the other hand the tumors showed cell cycle deregulation compared to normal tissue. These preliminary results represent the first analysis of gene expression in this type of model, and further work is proposed to examine the fundamental mechanism of this metal-induced sarcoma using selected time points on the pathway to tumor formation.

1957 FOLATE DEFICIENCY ENHANCES ARSENIC EFFECTS ON EXPRESSION OF GENES INVOLVED IN EPIDERMAL DIFFERENTIATION.

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Chronic arsenic exposure in humans is associated with cancers of the skin, lung, and bladder. There is evidence that folate deficiency may increase susceptibility to arsenic's effects, including arsenic-induced skin lesions. K6/ODC mice develop skin tumors when exposed to 10 ppm sodium arsenite for 5 months. In the current study, K6/ODC mice maintained on either a folate-deficient or a folate-sufficient diet were exposed to 0, 1, or 10 ppm sodium arsenite in the drinking water for 30 days. Total RNA was isolated from skin samples and gene expression analyzed using Affymetrix Mouse 430 2.0 GeneChips. Data from 24 samples, with four mice in each of the six treatment groups, were RMA normalized and analyzed by two-way ANOVA using GeneSpringTM. Top gene ontology (GO) categories for genes responding significantly to both arsenic treatment and folate deficiency include DNA metabolism, DNA replication, sulfur metabolism and epithelial cell differentiation. For these genes, folate deficiency magnifies the response to arsenic treatment. In particular, expression of markers of epidermal differentiation such as loricrin, small proline rich proteins, and involucrin was significantly reduced by arsenic in the folate-sufficient animals, and reduced further or at a lower arsenic dose in the folate-deficient animals. In addition, several genes involved in epidermal cell growth were up-regulated by arsenic treatment, but folate status did not consistently affect their expression. The suppressive effect of arsenic on epidermal differentiation marker expression in cultured human keratinocytes has been reported in the literature. This may point to a mechanism of neoplasia in which the cells are blocked from achieving the normal state of differentiation and remain capable of proliferation. Our *in vivo* results suggest that dietary folate deficiency may exacerbate this effect. *This abstract does not necessarily reflect EPA policy.*

1958 CENTROSOMAL AMPLIFICATION AND ANEUPLOIDY IN CULTURED HAMSTER CELLS EXPOSED TO ZIDOVUDINE (AZT).

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Alterations in chromosome number, or aneuploidy, play a pivotal role in malignant transformation, although it has not been established if they are the cause or the consequence of malignancy. The centrosome, an organelle that consists of two orthogonally-placed centrioles immersed in a matrix of proteins, directs chromosomal migration by a complex process of tubulin-chromatin binding. Here, alterations in these proteins, as well as centrosomal amplification, were explored in Chinese Hamster Ovary (CHO) cells exposed to 0, 200, 400 or 800 μ M AZT for 24 hours. Centrosomes, identified by a pericentrin antibody, and centromeric positive micronuclei, stained using a CREST antibody, were quantified in 500-1000 cells from each of three separate exposures. In addition, cell replication, but not centrosome replication, was arrested by hydroxyurea (HU), and genomic instability and aneuploidy were analyzed by immunofluorescence and confocal microscopy. Centrosomal amplification, evidenced by the percentage of cells containing ≥ 2 centrosomal positive bodies, occurred in 7.0, 10.5, 9.3, and 14.5% of cells exposed to 0, 200, 400 or 800 μ M AZT + 2mM HU, respectively. In parallel cultures, electron microscopy (EM) confirmed an increase in the number of centrosomes, and high resolution images revealed centrosomal fragmentation and electron-dense pericentriolar satellites. Centromeric positive micronuclei were analyzed in CHO cells exposed to 0, 200, 400 or 800 μ M AZT, and the percentage of 25 micronuclei with centromeric positive signals was 10, 8.0, 9.5, and 18.0% respectively. Therefore, genomic instability, driven by AZT genotoxicity and clastogenicity as well as alterations in proteins involved in centrosomal activation, may contribute to the carcinogenic potency of this compound.

1959 LINGERING OIL AND SEA OTTERS: DETECTION OF DNA ADDUCTS AS A BIOMARKER OF PAH EXPOSURE.

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Lingering oil from the Exxon Valdez oil spill has been known to persist in intertidal habitats in western Prince William Sound, particularly in bays and passages where oiling was most severe in 1989. Some of the strongest evidence of continuing effects of lingering oil spill comes from long term monitoring of sea otter populations and their exposure to polycyclic aromatic hydrocarbons (PAH). Sea otter population recovery remained incomplete as of 2003-2004 and individual sea otters continued to exhibit elevated levels of the cytochrome P450 1A biomarker in areas with greatest quantities of lingering oil. In order to understand the biological effects on individual animals, we used the detection of DNA adducts as a biomarker for PAH exposure. DNA was isolated from sea otter peripheral blood mononuclear cell (PBMC) samples collected from 1996-2005, including samples from otters recaptured in two different years, and subjected to DNA adduct analysis by 33P postlabeling and HPLC. Several of the archived samples revealed the formation of DNA adducts with a major adduct peak eluting at the same retention time as benzo[a]pyrene DNA adducts. The total DNA adducts formed ranged from 0.1-0.8 pmol/mg of DNA. Although there were no significant differences in DNA binding between samples from different years, low but detectable DNA adducts were exhibited by PBMC from several animals. The results from this study suggest that there may be biological effects of lingering oil on sea otters and that measurement of DNA adducts may be a strong indicator of deleterious effects from PAH exposure.

1960 REPLICATION OF N²-ETHYLDEOXYGUANOSINE DNA ADDUCTS IN HUMAN CELLS.

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The association between alcohol and the risk of cancer is well documented, but the exact mechanism of alcohol-related carcinogenesis is unknown. Ethanol is predominantly converted to acetaldehyde in vivo, which can react with DNA to form stable exocyclic alkylamino DNA adducts. The primary adduct formed by this reaction can be converted to N²-ethyldeoxyguanosine (N²-ethylGua). In vitro, N²-ethylGua blocks a variety of replicative DNA polymerases. Single chemically synthesized N²-ethylGua or O⁶-ethyldeoxyguanosine (O⁶-ethylGua) adducts were placed site specifically in the suppressor tRNA gene of the mutation reporting shuttle plasmid pLSX and replicated in human embryonic kidney 293 (HEK293)

cells. N²-ethylGua and O⁶-ethylGua were both minimally mutagenic in double stranded pLSX replicated in HEK293 cells. When deoxyuridines were placed on the complementary strand at 5' and 3' positions flanking the adduct, 5-fold (p = 0.02) and 22-fold (p = 0.00006) enhancements of the N²-ethylGua and O⁶-ethylGua-induced mutant fractions were seen in HEK293 cells, respectively. In the HEK293 cells, the mutation spectrum generated by N²-ethylGua consisted of 65% single base deletions, 25% adduct site-targeted transversions, and 18% transversions at the site of the adduct, in contrast to the exclusive production of adduct site-targeted transitions by O⁶-ethylGua. The yield of progeny plasmids after replication in HEK293 cells was reduced by the presence of N²-ethylGua in parental plasmids with or without deoxyuridine to 39% (p = 0.005) or 19% (p = 0.0004), respectively. Taken together, these data indicate that N²-ethylGua is a mutagenic DNA lesion, and that N²-ethylGua in DNA exerts its principal biological activity by blocking translesion DNA synthesis in human cells, resulting in either failure of replication or frameshift deletion mutations.

1961 DNA DAMAGE RESPONSE TO NAPHTHALENE METABOLITES IN DT40 MUTANT CELLS.

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Naphthalene is a ubiquitous environmental pollutant that has been associated with carcinogenesis in laboratory animals and humans. Reactive quinonoid and quinone intermediates, formed by biotransformation, may have a role in the mechanism of action of naphthalene toxicity. Three possible mechanisms by which the naphthalene metabolites cause deleterious mutations have been proposed: oxidative stress, abasic sites and bulky adducts. To better understand DNA damage response to the metabolites, we have used a series of isogenic DT40 cells deficient in the DNA damage response genes. Wild-type (wt) and mutant DT40 cell lines were exposed to 1,2-naphthalenediol (NCAT), 1,2-naphthoquinone (1,2-NQ), 1,4-naphthalenediol (NHQ) or 1,4-naphthoquinone (1,4-NQ) for ten cell replication cycles. Cell survival was determined by the XTT-based cell proliferation assay at the end of cultivation. The impact on the cell survival of the naphthalene metabolites in the wild-type (wt) DT40 cells was ranked in decreasing order as NHQ>1,4-NQ>NCAT>1,2-NQ. In contrast, the DNA damage response deficient cell lines displayed stronger sensitivity to the NHQ over 1,4-NQ. Cell survival in the mutant cell lines treated with NHQ was ranked as *msh3>fancd2>xpa=parp1>rev3>fen1>atm>brca1>blm>brca2>poβ>chk1=wt=wrn*. Cell death of 1,4-NQ in the same mutant cell lines was ranked as *fen1>brca1>fancd2>msh3>parp1>poβ>brca2>rev3>poβ>atm>wt=blm>xpa>chk1>wrn*. These results showed a distinct difference among the mutant cell lines in response to the 1,4 metabolites suggesting that exposure to NHQ and 1,4-NQ elicit different DNA damage responses. These DNA damage response data suggest that bulky adducts or oxidative DNA lesions, but not abasic sites, appear to play important roles in the DNA damage response to NHQ and 1,4-NQ. (Supported in part by P42-ES05948 and P30-ES10126 from the NIH; 5T32ES007126-22 and 5T32CA72319-08 from the NIEHS.)

1962 DNA DAMAGE RESPONSE TO FORMALDEHYDE AND ACROLEIN.

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This project has been undertaken to better understand cellular processes that respond to environmental and endogenous exposure to aldehydes. To date, we have focused on formaldehyde (FA) and acrolein (ACR) and the impact of each on excision repair processes, namely, mismatch repair (MMR), nucleotide excision repair (NER) and base excision repair (BER). Previous experiments have implied that NER may be the primary process that repairs DNA-protein cross-links (DPC) caused by FA, but the relative contributions of NER, MMR, and BER have not been characterized. DT40 cells utilized in this study provide a powerful system with advantages over either yeast or murine cells. There are a multitude of isogenic DT40 cell lines available which have been used primarily for genetic studies; however, we are aware of only limited use of these cells for studying cellular effects of environmental and endogenous toxins. To better understand the DNA damage response to FA and ACR, we have used a panel of isogenic DT40 cells deficient in the excision repair genes *poβ*, *fen1*, *parp1*, *xpa* and *msh3*. Our results show that cells deficient in *xpa* (NER), *msh3* (MMR) and *parp1* mutants are exquisitely sensitive to FA. *Poβ* (BER) showed moderate sensitivity to FA while *fen1* (BER) exhibited none. Thus, it is strongly suggested that FA-induced damage is repaired by both NER and MMR pathways. Parp1 has been reported to be an important protein in several DNA repair pathways (BER, SSB, perhaps homologous recombination

(HR) mediated double strand break repair). The hypersensitivity of *parp1* mutants along with moderate sensitivity of *poβ* mutants may imply a function of BER in DPC repair as well as in HR-mediated repair and translesion DNA synthesis. ACR exposure also resulted in strong sensitivity in *msh3*, and with moderate sensitivity in *xpa*, *fen1*, and *parp1* mutants indicating the importance of NER, MMR and BER. (This research was supported in part by P42-ES05948, ES11746, P30-CA16086, and P30-ES10126 from the National Institutes of Health.)

1963 THE ROLE OF HEMATOTOXICITY IN CHEMICALLY INDUCED ACUTE MYELOID LEUKEMIA.

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The vast majority of acute myelogenous leukemias (AML) are de novo, having no known cause. However, AML has also been reported following high dose exposure to benzene, certain classes of cytotoxic chemotherapy or ionizing radiation (s-AML). The cell of origin for AML and s-AML is believed to be a myeloid committed hematopoietic progenitor cell (HPC), historically found only in the bone marrow; thus, s-AML has traditionally been thought to require bone marrow toxicity. However, recent data reveal a small population of HPCs circulating in peripheral blood (pHPC), suggesting that s-AML could theoretically arise from a pHPC that is transformed in the periphery and migrates back into the bone marrow for subsequent proliferation and manifestation of AML. If true, chemicals (even those that lack systemic distribution following exposure, e.g. formaldehyde) could cause s-AML without bone marrow toxicity. We have evaluated the published literature to test these mutually exclusive hypotheses: 1) s-AML arises in bone marrow secondary to obligate bone marrow toxicity/involvement; and 2) s-AML arises in the peripheral circulation from a damaged pHPC. Literature was evaluated according to minimal criteria for establishing scientific evidence: a) measurements have been authentically identified and are stable within a known rate of experimental error; b) extraneous factors that could affect the measurements have been adequately controlled; c) the stable measurements have been independently replicated. Based on these criteria, there is considerable scientific evidence in support of hypothesis 1. However, data to support hypothesis 2 is lacking or falls short of constituting scientific evidence. Accordingly, peripheral transformation in the absence of bone marrow toxicity is not currently supportable based on existing scientific evidence.

1964 CAN INHALED FORMALDEHYDE RESULT IN HEMATOPOIETIC MALIGNANCIES?

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This poster specifically addresses the hypothesis that formaldehyde exposure is a potential etiological agent in the development of human hematopoietic malignancies. Proponents of this hypothesis have advanced the position that formaldehyde is a potential risk factor in the development of acute and chronic myeloid leukemia, Hodgkin's and non-Hodgkin's lymphoma (NHL), multiple myeloma and acute and chronic lymphocytic leukemia. If true, this would establish formaldehyde as an unprecedented hematopoietic toxicant as no single agent has ever been shown to increase the risks of all of these discrete diseases (including high dose benzene exposure, ionizing radiation exposure and myelotoxic doses of chemotherapeutic agents). Epidemiological evidence for the hematopoietic toxicity and carcinogenicity of formaldehyde is weak. As a result, attempts have been made to supplement the lack of strong epidemiological support with biological plausibility arguments. One hypothesized mechanism includes the potential for NHL to arise following formaldehyde exposure to susceptible cells located in the Nasal Associated Lymphoid Tissue (NALT). Experimental studies have demonstrated that blood concentrations of formaldehyde change very little following inhalation exposure to relatively high concentrations. Therefore, formaldehyde induced transformation must occur in peripheral lymphoid tissue and cannot be dependent upon systemic distribution. There is currently no evidence that chemically induced transformation of lymphoid tissue in the NALT can occur. Moreover, endogenous production of formaldehyde results in blood concentrations far greater than those resulting from occupational exposures. Therefore, it is difficult to understand how slight increases in blood levels of formaldehyde following inhalation could result in such an extraordinarily potent hematopoietic toxicant. The biological evidence pertaining to this hypothesis is discussed, particularly regarding NALT tissue as a potential target for chemical transformation.

1965 CYTOTOXICITY AND CYP MODULATION IN DELTAMETHRIN AND PERMETHRIN TREATED HUMAN HEPATOCYTES.

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Deltamethrin and permethrin are widely used in agriculture, public health and military deployments. Their toxicity is mediated by interference with nerve membrane sodium channels, GABA-receptor ionophore complexes and neurotransmitters. In this study the potential of deltamethrin and permethrin for cytotoxicity and induction of CYP isoforms in human hepatocytes was evaluated. Permethrin and deltamethrin showed dose-dependent effects on adenylate kinase activity in HepG2 cells, in which 50 and 100 μ M, respectively, induced ~3- and 5-fold, with no further increase above 200 μ M. Permethrin and deltamethrin likewise induced adenylate kinase in human hepatocytes. Approximately 3-fold induction was noted at 200 μ M deltamethrin and ~4-fold induction was noted at 100 μ M permethrin. Cytotoxicity was noted in HepG2 cells after 48 to 72 h exposure to 100 and 200 μ M deltamethrin [15-25%] or permethrin [15-20%]. Dose-dependent induction of caspase-3/7 by deltamethrin was initiated at 12.5 μ M and peaked at 100 to 200 μ M after 72 h. Permethrin showed induction of caspase-3/7 at 3.125 μ M, peaking at 100 μ M reaching a plateau at 200 μ M after 72 h. Maximal induction was ~2.5 fold by either pyrethroid. Actinomycin D induced caspase-3/7 ~5-7 fold whereas Z-DEVD-FMK completely abrogated the deltamethrin and permethrin induced caspase-3/7 activity. At 100 μ M deltamethrin 2-3 fold induction of CYP1A1 and CYP2B6 mRNA was noted while ~25-fold induction of CYP3A4 mRNA was noted. Permethrin-mediated CYP induction was much less potent, up to 4-fold or less for CYP1A1, CYP3A4, CYP3A5, CYP2B6, and CYP2A6. However, the variability in CYP mRNA induction was extensive making the induction potential of deltamethrin and permethrin difficult to evaluate. Overall, either pyrethroid showed mild induction of major CYPs but also caused cytotoxicity in human hepatocytes.

1966 METABOLISM OF CHLORPYRIFOS BY HUMAN HEPATOCYTES.

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The metabolism of chlorpyrifos (CPS) by human hepatocytes was investigated using LC-MS/MS. Cytochrome P450 (CYP)-dependent and phase II-related products were determined following incubation with CPS. The CYP-related products, 3, 5, 6-trichloro-2-pyridinol (TCP), diethyl thiophosphate (DETP) and dealkylated CPS were found. Diethyl phosphate (DEP) was not identified due to its high polarity and lack of retention with the chromatographic conditions employed. Phase II-related conjugates, including O- and S-glucuronides as well as glutathione (GSH)-derived metabolites were determined in CPS-treated human hepatocytes. CPS-SG and mono-O-deethyl CPS-SG, probable key intermediates of the GSH conjugation pathway were detected. CPS is likely to be biotransformed in the hepatocytes through four different pathways: dearylation (I), dealkylation (II), glucuronidation (III) and GSH conjugation (IV). However, chlorpyrifos oxon (CPO) was not found using either LC-MS/MS or HPLC-UV. Even though human liver CYP isoforms are known to activate CPS to CPO, these results suggest that human liver plays an important role in detoxification, rather than activation, of CPS.

1967 DIFFERENTIAL EXPRESSION OF RETINOIC ACID BIOSYNTHETIC AND METABOLISM GENES IN LIVERS FROM MICE TREATED WITH HEPATOTUMORIGENIC AND NON-HEPATOTUMORIGENIC CONAZOLES.

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Conazoles are fungicides used in crop protection and as pharmaceuticals. Triadimefon and propiconazole are hepatotumorigenic in mice, while myclobutanil is not. Previous toxicogenomic studies suggest that alteration of the retinoic acid metabolism pathway may play a key event in conazole-induced hepatotumorigenesis. We have used quantitative real-time polymerase chain reaction (qRT-PCR) to examine the expression of specific genes associated with the biosynthesis, and metabolism of all trans retinoic acid (RA) in the livers from male CD-1 mice (3 per group). The mice were exposed to triadimefon (1800 ppm), propiconazole (2500 ppm) or myclobutanil (2000 ppm) in the feed for 4, 30 or 90 days. The genes examined were RA biosynthesis genes *Bcmo1* (β -carotene 15, 15'-monooxygenase), *Aox1* (aldehyde oxidase), *Rdh9* (retinol dehydrogenase), and *Alldh1a7* (aldehyde dehydrogenase); the RA catabolism gene *CYP26a1* (retinoic acid hydroxylase); and the retinol transport and storage gene, *Lrat* (lecithin-retinol acyltransferase). *CYP26a1* mRNA had increased expression by all three conazoles at the 4 and 30

day exposure times. Triadimefon induced the greatest mRNA expression of *CYP26a1* at each time point (e.g. 9.6-, 11.6- and 2.1-fold induction vs. controls at day 4, 30 and 90, respectively). *Aldh1a1* was upregulated, and *Bco1*, *Rdh9*, and *Lrat* were down regulated by all three conazoles at selected time points. *Aox1* was unaffected by conazole treatment. Overall, the transcriptional activities of *Bco1*, *Rdh9*, and *Lrat* were correlated with temporal *CYP26a1* expression for triadimefon and propiconazole. The induction of *CYP26a1* and the associated down regulation of several RA biosynthesis genes and *Lrat* suggest that RA levels may be reduced in hepatic tissues by these conazoles. The consistent alteration of this pathway by the tumorigenic conazoles suggests that it might be a necessary event in conazole-induced hepatotumorigenesis. (This abstract does not reflect U.S. EPA Policy)

1968 O, P-DDT ENHANCES VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION THROUGH THE ACTIVATION OF HIF-1 α

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Dichlorodiphenyltrichloroethane (DDT) is used as an insecticide and prevents many people in the tropical zone from devastating malaria. On the other hand, a number of reports have indicated that it may act as an endocrine disruptor and also has possible carcinogenic effects. VEGF, a hypoxia-inducible endothelial cell mitogen, has been characterized as a potent vascular permeability critical factor in vasculogenesis and angiogenesis. In the present study, we investigated o,p-DDT activates HIF-1 α leading to VEGF expression in the human endothelial-like ECV304 cell line. o,p-DDT treatment increased the stability and activity of HIF-1 α protein. The expression of HIF-1 α by o,p-DDT was correlated with the activation of HIF-1 α DNA binding activity and the transactivation of a HIF-1 α -dependent VEGF reporter gene. o,p-DDT induced the phosphorylation of phosphatidylinositol 3-kinase (PI3K) and ERK in the regulation of HIF-1 α activation. These results demonstrate that o,p-DDT regulates HIF-1 α action through a PI3K and ERK dependent pathway, resulting in increased VEGF expression.

1969 UP-REGULATION OF MATRIX METALLOPROTEINASE-2 EXPRESSION BY O, P-DDT.

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Dichlorodiphenyltrichloroethane (DDT) is used as an insecticide and prevents many people in the tropical zone from devastating malaria. On the other hand, a number of reports have indicated that it may act as an endocrine disruptor and also has possible carcinogenic effects. In the present study, we investigated the effect of o,p-DDT on the expression of matrix metalloproteinase-2 (MMP-2) in human fibrosarcoma HT-1080 cells. o,p-DDT dose dependently increased MMP-2 mRNA and stimulated MMP-2 activity using reverse transcription-polymerase chain reaction and zymography assay, respectively. Transient transfection experiments also showed that o,p-DDT increased in MMP-2 promoter activity. MMP-2 secreted as a proenzyme, and activated by membrane-type 1 MMP (MT1-MMP). o,p-DDT treatment also increased the MT1-MMP expression. Taken together, these results demonstrate that o,p-DDT can stimulate the production of MMP-2 through the up-regulation of MT1-MMP expression in human fibrosarcoma HT1080 cells, which play an important cause of tumor-promotion.

1970 A PHYSIOLOGICALLY BASED PHARMACOKINETIC/PHARMACODYNAMIC (PBPK/PD) MODEL FOR ESTIMATION OF CUMULATIVE RISK FROM EXPOSURE TO THREE N-METHYL CARBAMATES: CARBARYL, ALDICARB, AND CARBOFURAN.

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A Physiologically Based Pharmacokinetic/Pharmacodynamic (PBPK/PD) model was developed using the Exposure Related Dose Estimating Model (ERDEM) system to estimate cumulative risk resulting from exposure to three N-methyl carbamate insecticides: carbaryl, aldicarb, and carbofuran. The three-chemical model parameters were derived from previous single-chemical models which were optimized

against controlled-study rat and human (where available) data. The model includes Michaelis-Menten metabolism kinetics and equations for possible metabolic interaction among the chemicals. Acetylcholinesterase (AChE) inhibition was described by competing bimolecular inhibition processes. Exposure to the three chemicals through the dietary pathway was based on outputs from the Stochastic Human Exposure Dose Simulation (SHEDS) model. Simulations from the cumulative PBPK model produced distributions of resulting concentrations of urinary biomarkers. These distributions were then compared with the available urinary biomarker concentration distributions from CDC's Third National Health and Nutrition Examination Survey, NHANES III. The cumulative model was also used to predict the distribution on red blood cell AChE activity. The simulated results from this human cumulative PBPK/PD model in ERDEM provide a good framework for future human risk assessment.

Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

1971 BIOMONITORING AND ENVIRONMENTAL MONITORING FOLLOWING INDOOR USE OF PYRETHROID PESTICIDES.

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Pyrethroid products are extensively used in residential pest management. Twenty families that used indoor pesticides were recruited from 5 states by word-of-mouth. Pyrethroid foggers and crack and crevice ("perimeter") sprays were included. Families included 46 children (< 12 years old) and 54 teens/adults (≥ 12). Commercially available indoor fogger (Raid Deep ReachTM Fogger) with 1.7 % cypermethrin and commercially available crack and crevice spray (Air DevilTM HPX) with 0.1 % cypermethrin active ingredient were utilized. These families were biomonitoring for at least 8 days by either 24-hour, a.m./p.m., or a.m. sampling strategies. Total $\mu\text{g/kg}$ pyrethroid equivalents following crack and crevice treatment were 1.5 ± 1.1 and 0.55 ± 0.21 $\mu\text{g/kg}$ for children and teens/adults after 8 days, respectively. Total $\mu\text{g/kg}$ pyrethroid equivalents following fogging were 11 ± 8.3 and 2.6 ± 1.65 for children and teens/adults after 8 days, respectively. Back-calculated exposures below known LOAELs and regulatory NOAELs for cypermethrin indicate about a 4-fold greater urine elimination of biomarkers by children than teens/adults. Deposition pattern, air level, total surface pesticide, and transferable surface residue from several common indoor surfaces were monitored after pesticide use. Following fogger use, floor, wall, and ceiling deposition coupons accounted for 75.0, 15.5, and 9.40 %, respectively, of the pyrethroid pesticide released while less than 0.01% was found in air. Transferability testing from several different flooring materials including wood, tile, carpet, and linoleum combined with floor plans for several of the residences involved in biomonitoring will be used for determination of the relationship between flooring materials and exposure in children and adults. Biomonitoring in conjunction with in-depth environmental monitoring could prove invaluable in characterizing indoor exposure.

1972 EFFECTS OF TYROSINAEMIA ON SELECTED ORGANS IN RATS.

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The compound 2-(2-Nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione (NTBC) is a potent inhibitor of 4-hydroxyphenylpyruvate dioxygenase (HPPDase), a key enzyme in mammalian tyrosine catabolism. In 28- and 90-day studies, we examined the effect of NTBC, administered alone or co-administered with diet enriched with 2% tyrosine, on plasma tyrosine concentration and on histopathological findings in selected organs in the rat. Co-administration of a low dose of NTBC (10 or 20 $\mu\text{g/kg}$ bw/day) with dietary tyrosine allowed maximal induction of tyrosinemia. In both studies, 2% dietary tyrosine increased plasma tyrosine concentrations only moderately over controls (by 4-fold in the 28-day study, by 7-fold in the 90-day study, with a plateau reached on day 1 or 2 and a decrease thereafter). Administration of NTBC alone also increased plasma tyrosine (by 3-fold only on day 28 at 10 $\mu\text{g/kg/day}$; by approximately 12-fold from day 1 to 90 at 20 $\mu\text{g/kg/day}$). Co-administration of NTBC and 2% dietary tyrosine had a much greater effect on plasma tyrosine concentrations (26-fold from day 21 up to day 28, as much as 43-fold at day 4 and decreased thereafter in the 90-day study) than did either treatment alone. Ophthalmological and histopathological examination revealed the expected increased incidence of corneal opacities and ocular histopathological findings only in the groups co-administered NTBC and tyrosine, where plasma tyrosine generally exceeded the known threshold value for corneal effects. In

animals co-administered tyrosine and NTBC for 28 or 90 days, or NTBC for 90 days, organ weight increases and histopathological findings were observed in the presumed target organs (kidney, liver, pancreas, thyroid). There were no effects observed in animals administered either tyrosine alone for either duration or NTBC alone for 28 days. From these data it is clear that histopathological findings observed in target organs after HPPDase inhibition are secondary to increased plasma tyrosine concentrations, and are not primary effects of the inhibition itself.

1973 EFFECT OF TYROSINEMIA ON PREGNANCY AND EMBRYO-FETAL DEVELOPMENT IN THE RAT.

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The relationship between increased plasma tyrosine concentrations, due to maternal 4-hydroxyphenylpyruvate dioxygenase (HPPDase) inhibition, and potential effects on embryo-fetal development was investigated in the rat. Tyrosinaemia in pregnant rats was induced by a co-administration of NTBC [2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione] at 10 µg/kg/day by gavage from gestation day (GD) 6 to 20 and tyrosine at 2% (w/w) in the diet from GD 6 to 21. NTBC is a triketone molecule which acts by inhibiting the HPPDase enzyme, i.e. the second step of the tyrosine catabolism in mammals. Co-administration of a low dose of NTBC with dietary tyrosine allowed maximal induction of tyrosinaemia. Similar groups of pregnant rats were co-administered 1) vehicle plus untreated diet, 2) NTBC plus untreated diet, or 3) vehicle alone plus 2% dietary tyrosine. The results of this study showed that tyrosinaemia was much more pronounced in the group where NTBC and tyrosine were co-administered: plasma tyrosine concentrations at necropsy were increased by 63-fold in pregnant rats co-administered NTBC plus tyrosine, by 5-fold in the group administered dietary tyrosine only, and by 8-fold in the group treated with NTBC alone. Tyrosinaemia as a result of co-administration of NTBC and dietary tyrosine produced minimal maternal toxicity in the form of slightly reduced mean food consumption, in association with a slight reduction of mean body weight gain and macroscopic findings (a few cases of mottled kidney and minimal corneal opacity). Fetal findings, induced by tyrosinaemia and observed only in the NTBC plus tyrosine co-treatment group, consisted principally of a generalized delay in ossification noted at the skeletal fetal examination, together with a higher incidence of short 14th rib and of extra ossification points on the 14th thoracic vertebra. In association, a reduction of mean fetal body weight of 7% was noted at cesarean section. In conclusion, a marked increase in plasma tyrosine concentration in pregnant rats induced a generalized delay in fetal skeletal ossification.

1974 IN VITRO METABOLISM OF TYROSINE IN VARIOUS SPECIES AFTER HPPDASE INHIBITION BY NTBC.

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The substance 2-(2-Nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione (NTBC), a potent inhibitor of the 4-hydroxyphenyl pyruvate dioxygenase (HPPDase) enzyme, was incubated with Liverbeads™ from rat, dog, rabbit, mouse and human, to determine the ability of these species to metabolize cellular tyrosine via a substitute enzymatic pathway producing hydroxylated metabolites including 4-hydroxyphenyl lactic acid (HPLA). The ability to produce such metabolites in vivo under HPPDase inhibition allows the reduction of plasma tyrosine concentrations to near-physiological levels and avoidance of the biological consequences of HPPDase inhibitor-induced tyrosinemia. In vitro measurement of HPLA production in the presence of an HPPDase inhibitor allows ranking of several species with regard to their ability to use an alternative pathway for tyrosine catabolism. Liverbeads™ contain hepatocytes in a 3-dimensional alginate matrix and were selected for their availability in all desired species. Liverbeads™ from all species were incubated in one of four conditions: 1) basal (tyrosine-free HBSS buffer), 2) basal + 100 mg/L tyrosine, 3) basal + 30 µM NTBC, or 4) basal + 100 mg/L tyrosine + 30 µM NTBC. Concentration of HPLA in the medium was measured by HPLC with fluorimetric detection at 0, 2, and 4 hours of incubation. In the rat, dog, and rabbit, HPLA was only produced in the basal + tyrosine + NTBC incubation, at low amounts and at late timepoints only. Mouse Liverbeads™ produced HPLA in all incubations and at all timepoints, with increasing production over time. Human Liverbeads™ produced HPLA in incubations with tyrosine, with NTBC, or with both tyrosine and NTBC. These results allowed us to classify the tested species into two main groups: 1) rabbit, dog, and rat, in which the alternative pathway of HPLA production is very inefficient under either physiological or stressed conditions; and 2) human and mouse, which efficiently produce HPLA via the alternative pathway. This ability to hydroxylate tyrosine prevents the toxicological consequences of HPPDase inhibition *in vivo*.

1975 LIVER-SPECIFIC EFFECTS OF CYPROCONAZOLE, A TRIAZOLE FUNGICIDE, ARE DEPENDENT UPON THE CAR NUCLEAR RECEPTOR.

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Cyproconazole is a triazole fungicide that causes liver tumors in mice at dose levels of 100 and 200 ppm via a non-genotoxic mode of action. Cyproconazole-induced enzyme induction and other phenotypic changes to the liver resemble those of phenobarbital, a known agonist of constitutive androstane receptor (CAR). *Car*-null and wild type (C3H/HeNClrBR background) male mice were administered cyproconazole (200 or 450 ppm) or phenobarbital (850 ppm) in the diet for up to 7 days. In wild type mice, 200 ppm of cyproconazole caused liver hypertrophy, increases in liver weight and cell proliferation, single cell necrosis and fat vacuolation – effects generally similar to those caused by 850 ppm of phenobarbital. The higher dose (450 ppm) of cyproconazole caused similar liver changes, but was also accompanied by liver damage as evidenced by large increases in serum transaminases. Induction of both mRNA and protein levels for *Cyp2b10* (187-258 fold) and mRNA for *Gadd45β* (2-7 fold) was observed in cyproconazole- and phenobarbital-treated wild type mice. *Gadd45β* is an anti-apoptotic gene that is also associated with stimulation of S-phase and cell proliferation. Coumarin 7-hydroxylase activity, a marker of *Cyp2a5*, was also increased by cyproconazole and phenobarbital. In *Car*-null mice, the effects noted with either cyproconazole or phenobarbital were absent or greatly diminished. These experiments demonstrate that the short-term liver effects of cyproconazole in mice are CAR nuclear receptor-dependent and similar to those of phenobarbital, a known non-genotoxic rodent liver carcinogen.

1976 THE TYPE II PYRETHROID PESTICIDE ESFENVALERATE LOWERS SERUM ESTRADIOL AND DELAYS ONSET OF PUBERTY.

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Concern has risen that alterations in the reproductive system by exposure to endocrine disruptive chemicals such as pesticides may be adversely impacting human reproduction. Organochlorine pesticides, such as p,p'-dichlorodiphenyl trichloroethane (DDT) have been reported to possess estrogenic activity; while exposure to organophosphates such as chlorpyrifos have been associated with altered thyroid function in humans. Because exposure to these pesticides may be affecting the endocrine system, less toxic alternatives, such as synthetic pyrethroids, are increasing in usage. Pyrethroids are used worldwide to control both agricultural and household pests, and exposure is occurring in children as well as adults as evidenced by metabolites found in urine from the general population. Even though pyrethroids have been considered relatively safe, recent in vitro studies have indicated that they too may be estrogenic. Therefore, we wanted to determine the effects of juvenile exposure to a type II pyrethroid, esfenvalerate (® Asana), on puberty-related hormones and the onset of female puberty.

To assess pubertal effects, female Sprague Dawley rats were gavaged once daily from postnatal day 22 until vaginal opening (VO) with esfenvalerate (ESF) at 12, 8, and 5 mg/kg. Controls received corn oil. Animals treated with ESF showed a delay ($p < 0.05$) in age at VO for all doses administered as compared to controls. A second group of rats was gavaged once daily from day 22 until day 30 with the 5.0 mg/kg dose, and serum levels of estradiol (E2) and luteinizing hormone (LH) were measured by radioimmunoassay. E2 levels were found to be two times lower in the pesticide treated animals ($p < 0.05$) while LH levels were the same in both groups. This data would indicate that the pesticide is not acting as an environmental estrogen. While the mechanism for how ESF caused a decrease in E2 is unknown, a direct effect on the ovary seems plausible. Also, the fact that lowered serum E2 should cause an increase in serum LH suggests a potential deficit at the hypothalamic-pituitary level as well.

1977 INHIBITION OF HUMAN RECOMBINANT ACETYLCHOLINESTERASE BY CHLORPYRIFOS OXON OR METHYL PARAOXON DISPLAYS CONCENTRATION-DEPENDENT KINETICS.

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The capacity of the anticholinesterase organophosphates to inhibit acetylcholinesterase is often quantified by the inhibitory constant k_i . In the current study, the k_s for both chlorpyrifos oxon (*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphate) and methyl paraoxon (*O,O*-dimethyl *O*-(4-nitrophenyl) phosphate) towards

human recombinant acetylcholinesterase changed as a function of inhibitor concentrations below 100 nM. For example, at 37° the k_i for 100 nM chlorpyrifos oxon was 2.95 nM⁻¹h⁻¹, while that for 2.5 nM chlorpyrifos oxon was 270.72 nM⁻¹h⁻¹. Similarly the k_i for 100 nM methyl paraoxon was 0.07 nM⁻¹h⁻¹, while that for 2.5 nM was 8.24 nM⁻¹h⁻¹. These results indicate that the interactions of acetylcholinesterase with chlorpyrifos oxon or methyl paraoxon cannot be adequately described by simple Michaelis-Menten kinetics. Co-incubation of chlorpyrifos oxon and *p*-nitrophenyl acetate with acetylcholinesterase gave a K_i and k_i for chlorpyrifos oxon of 126.69 nM and 13.93 h⁻¹, respectively. Interestingly, co-incubation of chlorpyrifos oxon and acetylthiocholine with acetylcholinesterase resulted in a reduced K_i compared to that determined with *p*-nitrophenol acetate. Acetylthiocholine and acetylcholine are known to be ligands for the peripheral anionic binding site on acetylcholinesterase, and their occupation of this site might alter the binding affinity of the enzyme for chlorpyrifos oxon. These data suggest that the inhibitory capacity of chlorpyrifos oxon towards acetylcholinesterase *in vivo* might continually change depending on oxon levels, as well as acetylcholine levels. (Supported by Grant ES012648 from NIEHS)

1978 PHARMACOKINETICS AND MILK TRANSFER OF A SINGLE ORAL DOSE OF CHLORPYRIFOS IN LACTATING RATS.

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Although chlorpyrifos has been banned as a household insecticide in the US, its ongoing use on golf courses and other settings may continue to expose potentially sensitive individuals such as pregnant or lactating women. In this study, the pharmacokinetics and milk transfer of a single oral 5.0 mg (7 uCi)/kg dose of ring-labeled [¹⁴C]chlorpyrifos were investigated in lactating Sprague-Dawley rats 6 days after delivery. Three dams and five pups per dam were sacrificed at 2, 4, 8, 12, 18, 24, 36, 48, 72, and 96 hr after dosing. Milk was also collected from the breast of each dam for each time point. Radioactivity and 3,5,6-trichloropyridinol (TCP) were determined using scintillation counting and high performance liquid chromatography (HPLC), respectively. Both were detected in all tissues and milk at 2 h after dosing. The peak concentration of radioactivity in maternal plasma was 3.3 ug chlorpyrifos equivalents/ml at 2 hr, in milk 1.6 ug chlorpyrifos equivalents/ml at 4 hr, in pups 0.15 ug chlorpyrifos equivalents/g at 8 hr, and in pup plasma 0.15 ug chlorpyrifos equivalents/ml at 8 hr. No chlorpyrifos was detected in milk, pups, or pup plasma. TCP was detected by HPLC to be the only identified metabolite in milk, pups, and pup plasma. The maximum concentration of TCP detected in maternal plasma was 3.2 ug/ml at 2 hr, in milk 0.38 ug/ml at 4 hr, in pups 1.2 ug/g at 8 hr, and in pup plasma 0.04 ug/ml at 8 hr. Pharmacokinetic analysis best described radioactivity and TCP as disappearing biexponentially from maternal plasma with terminal half-lives of 26 and 29 hr, respectively. Radioactivity and TCP disappeared mono-exponentially from milk, pups, and pup plasma with half-lives of 29 and 50 hr in milk, 15 and 15 hr in pups, and 25 and 15 hr in pup plasma, respectively. The results indicate that maternal metabolism of chlorpyrifos is rapid resulting mainly in TCP, and that distribution of TCP to pups and pup plasma is rapid, while elimination of TCP is prolonged.

1979 ENDOSULFAN-ALPHA INDUCES CYP2B6 AND CYP3A4 BY ACTIVATING THE PREGNANE X RECEPTOR (PXR).

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Endosulfan is a widely used organochlorine pesticide that has been shown to have endocrine disrupting activity in animal studies. Our previous work determined that endosulfan-alpha is metabolized by cytochrome P450 (CYP) 2B6 and 3A4. We postulated that endosulfan's endocrine disrupting properties may be due to its ability to activate PXR and/or the constitutive androstane receptor (CAR) to induce CYP2B6 and 3A4, which results in increased testosterone metabolism. CYP induction was explored by transient transfection assays in HepG2 cells using CYP2B6 or 3A4 reporter plasmids concomitant with human PXR (hPXR) or mouse CAR (mCAR) expression plasmids. Endosulfan-alpha at 10 μM induced CYP3A4 and CYP2B6 promoter activities by 11- and 16-fold, respectively, in the presence of hPXR. With mCAR, endosulfan-alpha induced the CYP2B6 promoter by only 3-fold over control. Human hepatocytes were treated with endosulfan-alpha and western blots of S9 fractions showed a dose-dependent induction of CYP2B6 protein, but induction of 3A4 protein was only observed at higher doses. CYP activity was measured by testosterone metabolism assay using the same S9 samples and results showed an increase in testosterone hydroxylation, although only at higher

doses of endosulfan-alpha. To investigate the ability of endosulfan to regulate CYP3A *in vivo*, sleep time studies were done using the anesthetic tribromoethanol (TBE) in PXR null and PXR humanized male mice. We found that endosulfan-alpha induces CYP3A metabolism of TBE via PXR. The ability of endosulfan-alpha to induce key CYP enzymes and enhance testosterone metabolism may be an important mechanism by which endosulfan affects endocrine homeostasis and causes reproductive toxicity.

1980 METABOLISM OF PYRETHROID PESTICIDES BY RAT AND HUMAN CYP450S AND SERUM.

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Pyrethroids are neurotoxic pesticides in which the parent chemical acts via the modulation of nerve axon ion channels. Both the pharmacokinetic and pharmacodynamic behavior of pyrethroids plays a role in their toxicity. The phase I biotransformation of pyrethroids occurs via cytochrome P450 mediated oxidation or esterase hydrolysis. Deltamethrin and esfenvalerate are type II pyrethroid pesticides, which are metabolized primarily by oxidative metabolism in rat liver microsomes. In human liver microsomes, esfenvalerate is also metabolized primarily by oxidative metabolism. In contrast, deltamethrin is metabolized almost entirely by hydrolysis in human liver microsomes. This research aims to determine which cytochrome P450 enzymes are responsible for the metabolism of deltamethrin and esfenvalerate in rat and human liver microsomes. In addition, this research examined the hydrolysis of deltamethrin and esfenvalerate in rat and human serum. Initial studies indicate that rat CYPs 1A1, 2C6, 2C11, and 3A2 metabolize both deltamethrin and esfenvalerate. Deltamethrin was metabolized most rapidly by 2C6 followed by 2C11>1A1> and 3A2. Esfenvalerate was metabolized most rapidly by 3A2 followed by 2C6>2C11> and 1A1. Esfenvalerate was also metabolized by human CYP2C9 while deltamethrin was not metabolized significantly by any human P450s. The hydrolysis of pyrethroids occurs primarily via carboxylesterase. Rat serum, which contains carboxylesterases, metabolized both deltamethrin (15.33 ± 3.24 pmoles/min/mL) and esfenvalerate (9.97 ± 2.94 pmoles/min/mL). Human serum however, which does not contain significant amounts of carboxylesterase, did not metabolize either deltamethrin or esfenvalerate. These species differences in the liver, P450, and serum metabolism of deltamethrin and esfenvalerate may influence extrapolation of exposure-dose relationships between species. (SJG is supported in part by NHEERL-DESE, EPA CT826513. This abstract does not represent EPA policy)

1981 EFFECT OF INDUCTION AND INHIBITION OF CYP3A4/7 ON ALACHLOR CYTOTOXICITY IN HEPG2 CELLS.

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Alachlor (2-chloro-2',6'-diethyl-N-(methoxymethyl)-acetanilide) is cytotoxic to human hepatoblastoma HepG2s, a cell line that expresses constitutive CYP3A7 and dexamethasone (DEX)-inducible CYP3A4 and CYP3A7. CYP3A4 catalyzes alachlor N-dealkylation to 2-chloro-N-(2,6-diethylphenyl)acetamide (CDEPA), precursor of 2,6-diethylbenzoquinoneimine, putative reactive metabolite for rat nasal carcinogenicity. We have observed time- and dose-dependent alachlor cytotoxicity in HepG2 cells (EC50 ~ 500 μM and 264 ± 17 μM at 6 and 24 h, respectively) as assessed by LDH leakage. We thus hypothesized that alachlor cytotoxicity was mediated by CYP3A4 and would be increased with dexamethasone (DEX), reported by others to induce CYP3A4 mRNA in HepG2 cells (Krusekopf et al. 2003. Eur. J. Pharmacol. 466:7-12). Although DEX pretreatment (25 μM, 48 h) did not alter CYP3A4 enzyme activity, it significantly increased CYP3A7-catalyzed luciferin 6' benzylether O-debenzylation. However, DEX had no effect on alachlor toxicity. Further, CYP3A4/7 inhibitor triacetyloleandomycin (100 μM) did not prevent, but rather potentiated, alachlor cytotoxicity. In agreement, the alachlor metabolite, CDEPA was less toxic than parent alachlor. Since HepG2 CYP3A4 activity was unaffected by DEX pretreatment, studies were done in DPX-2 cells, a HepG2 derivative engineered to overexpress pregnane-X receptor (Puracyp, Inc. Carlsbad, CA) that exhibits rifampicin (RIF) induction of endogenous CYP3A4. Alachlor cytotoxicity in these cells, measured as loss of ATP, occurred over a concentration range equivalent to that in HepG2 cells. CYP3A4 activity of DPX-2 cells treated with RIF (10 μM, 48 h) was twice that of untreated cells, but RIF did not increase alachlor toxicity. These results do not support metabolic activation of alachlor to a cytotoxic product by either CYP3A4 or CYP3A7. Support: Johnston Science Foundation and Louisiana Board of Regents.

1982 MECHANISM OF TRIFLURALIN INDUCED THYROID TUMOR IN RATS.

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Groups of 15 male F344 rats were exposed to control, trifluralin-fortified (6500 ppm) diet, or gavaged with phenobarbital (PB; 75 mg/kg/day) for 2 weeks. Ten rats/group were sacrificed, sera analyzed for T3, T4 and TSH and liver analyzed for selected T4-specific UGTs gene expression and enzyme level. Bile was collected from 5 rats/group for 2 h by terminal surgery and analyzed for free and conjugated T3 and T4. Serum T3 and T4 were decreased by 17 and 90% and TSH increased by 37% in trifluralin treated rats. The sera TSH level was increased by PB with little impact on T3 and T4. Both compounds induced mRNA expression of selected hepatic UGTs (up to 6.4-fold for trifluralin and 58-fold for PB) and total UGT enzyme activity (2-3 fold). The bile flow (g/h/kg) was 14 and 105% higher resulting in 20 and 86% increase in total bile collected in 2 h from PB and trifluralin-treated rats, respectively than the controls. Elimination of the total T4 in bile remained unchanged between control and PB-treated rats (42±11 and 41±5 ng) and more than doubled (86±19 ng) in trifluralin treated rats. Trifluralin treatment caused a 216% increase in biliary elimination of conjugated T4 and a 38% decrease in free T4 elimination. Biliary elimination of conjugated T3 in trifluralin-treated rats was 63% higher than in control, whereas remained unchanged in PB-treated rats. Decreases in serum T3 and T4 were due to enhanced peripheral metabolism and increased bile flow that resulted in a compensatory increase in TSH synthesis and secretion. These data suggest that high levels of TSH with chronic exposure to trifluralin would exert a continuous stimulation of thyroid gland leading to cellular hypertrophy and proliferation predisposing to the development of follicular cell tumors. Such TSH-mediated promotion of thyroid tumors has little relevance to humans since human thyroid follicular cells are less susceptible to TSH stimulation, in addition to the presence of high-affinity T4 binding protein (TBG) to modulate any dramatic decreases in T4 levels observed in F344 rats.

1983 90-DAY SUBCHRONIC ORAL TOXICITY OF SS-220I IN RATS.

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A 90-day subchronic toxicity study was conducted to determine the oral toxicity of the candidate insect repellent SS-220i (CAS# 77251-47-9). Entomological studies have demonstrated SS-220i to be at least as effective or better than N,N-diethyl-m-toluamide (DEET) in repelling various species of mosquitoes and biting flies. The entomological data supports continued development of SS-220i as a topical insect repellent. Information generated from this study, along with results from other laboratory animal toxicity studies, will be utilized to predict the potential risk to human health upon exposure to this compound. SS-220i was administered via the oral route to young adult Sprague-Dawley rats five days per week for ninety days at dose levels of 0, 100, 500, 750 or 1000 mg/kg/d. The subchronic 90-day oral study indicated that oral exposure to SS-220i produced systemic toxicity in rats at the 500, 750, and 1000 milligram per kilogram (mg/kg/d) dose groups, manifested as gastric changes, blood changes, morbidity, seizure activity, ataxia, lethargy and differences in body weights. These observations were not reported in all dose groups or sexes, but none of these observations were noted in the control and 100 mg/kg dose groups. The possible target organs identified from the 90-day oral study for SS-220i were the stomach, nervous system, blood (possible anemia), and liver. Female rats appeared to be more susceptible to the effects of SS-220i than males. This study shows that the No-Observed-Adverse-Effect-Level (NOAEL) for subchronic oral exposure to SS-220i for 90-days is 100 mg/kg/d. The views expressed in this paper are those of the authors and do not necessarily reflect the views and policies of the U.S. Army. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

1984 COMMUNICATING PESTICIDE RISKS TO AGRICULTURAL WORKERS.

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Abstract: The goals of the US EPA pesticide worker safety program are to protect human health and the environment by ensuring the competency of pesticide applicators, to minimize pesticide exposure to occupational pesticide users and agricultural field workers, to assure use of pesticides, and to engage health care providers in improving the recognition and management of pesticide poisonings. The worker safety program includes statutory requirements, regulations and label requirements.

To augment these components, US EPA uses education, outreach and training, guidance documents, partnerships, compliance assistance and capacity building, and oversight of implementing agencies.

A new hazard communication pilot study was undertaken to identify the best ways to communicate pesticide risk with agricultural workers. In many areas, most agricultural workers are native spanish speakers, and literacy rates can be low. Effectively communicating the risks of various pesticide exposures can be challenging. We developed and tested a variety of color-coded, icon-based tools for communicating PPE use, potential symptoms of over-exposure and actions workers could take to avoid pesticide exposures. The symbols were tested with agricultural workers in both Florida and California in the spring of 2006. A hazard training program was developed based upon the best-rated symbols, and again the training program was evaluated in focus groups in Florida and California agricultural workers during 2006. Through research, and stakeholder review, US EPA has identified gaps in the framework of the agricultural worker protection regulations. These gaps compromise US EPA's ability to ensure the safe use of pesticides and the protection of pesticide exposed agricultural workers. To reduce risk, ensure competency, promote consistency, and raise the standards of the federal program to those of many states, regulatory amendments are necessary. These studies identify risk communication tools that can be used to help fill these gaps.

1985 ORGANOCHLORINE EXPOSURE DURING FIRST TRIMESTER OF PREGNANCY AND ITS EFFECT ON ANOGENITAL DISTANCE IN MEXICAN CHILDREN. PRELIMINARY FINDINGS.

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DDE is considered an antiandrogenic compound. In rodents, prenatal DDE exposure has been shown to reduce anogenital distance in male progeny. In humans, DDE exposure has been linked to alterations in semen quality, pre-term birth and fetal loss. Our aim was to evaluate the impact of DDE, DDT, and HCH prenatal exposure on anogenital distance (AGD), a potential marker for androgenic fetal function. We conducted a cross-sectional study in a sample (n=76/382 births) pertaining to an ongoing cohort study evaluating the effects of organochlorine pesticides (OCP) on children neurodevelopment. Serum samples from the mothers were obtained during the first trimester of pregnancy to determine OCP by electron capture gas-liquid chromatography. We used the anal position index (API) as an indicator of AGD. We measured the distance between the center of the anus and the coccyx [anococcygeal distance (AC)] and the distance between the center of the anus and the posterior base of the scrotum for boys and the fourchette for girls [perineal distance (PA)]. We added AC and PA to calculate the coccyx-scrotum (CS) and coccyx-fourchette (CF) distances. The API was calculated as the PA to CS ratio for boys and PA to CF for girls (Reisner et al., 1984). Multivariate analysis showed that maternal DDE serum levels at first trimester of pregnancy were associated with a significant reduction of API ($\beta=-0.015$; $p=0.04$) in boys, suggesting the presence of antiandrogenic effects. No significant associations were observed in girls. Further studies in larger populations are needed to confirm this finding and to validate API as a marker of antiandrogenic effects. (Supported by: Fogarty International Center/NIH. Grant D43 TW00640). CONACyT grants 31034-M and 41708)

1986 PRELIMINARY ASSESSMENT OF BENZENE'S NEUROTOXIC POTENTIAL FROM CHRONIC OCCUPATIONAL EXPOSURE USING AUTOANTIBODIES.

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Benzene's acute neurotoxicity, carcinogenicity and hematotoxicity are documented. However, few studies have investigated the neurotoxic potential of chronic exposures, although some suggest central-peripheral neuropathy. Furthermore, reports suggest that benzene's potential neurocytotoxicity may be due to its metabolites. Recent reports indicate an increase in autoantibodies (AuAb) to several proteins as a result of occupational exposure to benzene. Previously, AuAb to nervous system (NS) proteins have been detected following exposures to heavy metals, pesticides and volatile organic compounds. In the present preliminary study, detection of serum AuAb, using ELISA, to neuronal proteins: the neurofilament triplet (NF) and glial proteins (glial fibrillary acidic protein: GFAP, and myelin basic protein: MBP) were used as indices of nervous system insult in workers occupationally-exposed (7±1 years) to benzene (BZ) in a refinery (n=40; 20 females and 20 males; 25

mg/m³±7) compared to demographically and socioeconomically-matched reference (R) workers in a food processing plant (n=60; 30 females and 30 males). Urinary phenol (UPh; µg/g creatinine), a marker of exposure, was significantly (p<0.01) higher in BZ than in R (65±2 and 8±1, respectively). More than 50% of BZ had IgG and IgM AuAb titers (ng/ml) to NF68, NF160 and NF200 compared to 13% of R (Z=3.2; p≤0.01). Mean titer levels were significantly (p≤0.01) higher in the BZ population. There was no statistical difference in either immunoglobulin to GFAP, although a higher percentage of BZ had titers. IgG to MBP were significantly (p≤0.01) higher in BZ than in R. A comparison based on gender also indicated a significant difference based on exposure. A comparison between exposed females and males indicated differences in only IgM to NF 160 and GFAP. These findings suggest that the neurotoxicity of chronic exposure to benzene warrants further study and that AuAb detection may be used in monitoring potential neurotoxic effects.

1987 EFFECTS OF VISUAL CONTRAST SENSITIVITY IN WORKERS EXPOSED TO STYRENE.

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Several studies have shown that styrene has ophthalmotoxic properties. Visual contrast sensitivity (CS) loss has been observed among workers exposed to organic solvent mixtures and styrene. Little attention has been paid to the relation between biological exposure index and CS loss. The present study aimed to (1) assess the relationship between urinary metabolites and CS loss and (2) examine visual function in relation to cumulative styrene exposure. CS was measured for 165 male workers exposed to styrene in four fiberglass and reinforced plastics factories (length of exposure>3 years) and 125 male non-exposed workers and college students using the Vistech MCT 8000 chart. All subjects were asked to complete questionnaire in which the items concerned their work history, occupational exposure or non-occupational solvent exposure, lifestyle habits, drug use and anamnesis. The personal styrene exposure in the air and end shift urinary mandelic acid (MA) and phenylglyoxylic acid (PGA) were measured, and the individual cumulative exposure index (CEI) was calculated. The results showed a significant reduction in CS at in styrene-exposed workers compared to the controls (p<0.001), after adjusting age, alcohol and smoking consumption. There was a significant effect of urinary MA+PGA level, with workers in high and medium concentration (>=200mg/g creatinine) group compared to low concentration group and controls at different spatial frequencies. With respect to CEI, there was a significant reduction in CS in 12 cycles per degree in high group workers than those in low group. The results suggest that urinary MA plus PGA less than 400 mg/g creatinine in long term exposure to styrene might lead to CS loss and CS loss might appear with increase of CEI.

1988 EXPOSURE-RESPONSE ANALYSIS FOR BERYLLIUM SENSITIZATION AND CHRONIC BERYLLIUM DISEASE AMONG WORKERS IN A BERYLLIUM METAL MACHINING PLANT.

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The current Occupational Exposure Limit (OEL) for beryllium has been in place for more than 50 years and was believed to be protective against chronic beryllium disease (CBD) until studies in the 1990s identified beryllium sensitization (BeS) and subclinical CBD in the absence of physical symptoms. Inconsistent sampling and exposure assessment methodologies have often prevented the characterization of a clear exposure-response relationship for BeS and CBD. Industrial hygiene (3,831 personal lapel and 616 general area samples) and health surveillance data from a beryllium machining facility provided an opportunity to reconstruct worker exposures prior to the ascertainment of BeS or the diagnosis of CBD. Airborne beryllium concentrations for different job titles were evaluated, historical trends of beryllium levels were compared for pre- and post-engineering control measures, and mean and upper bound exposure estimates were developed for workers identified as beryllium sensitized or diagnosed with subclinical or clinical CBD. Four methods were used to reconstruct historical exposures of each worker: industrial hygiene data were pooled by year, job title, era of engineering controls, and the complete work history (life-time weighted average) prior to diagnosis. Results showed that exposure metrics based on shorter averaging times (i.e., year versus complete work history) better represented the upper bound worker exposures which could have contributed to the development of BeS or CBD. It was observed that all beryllium sensitized and CBD workers were likely exposed to beryllium concentrations greater than 0.2 µg/m³ (95th percentile) and 90% were exposed to concentrations greater than 0.4 µg/m³ (95th percentile) within a given year of their work history.

Based on this analysis, it would appear that BeS and CBD generally occurred as a result of exposures greater than 0.4 µg/m³ and that maintaining exposures below 0.2 µg/m³ 95% of the time may prevent BeS and CBD in the workplace.

1989 PHARMACOKINETIC MODEL FOR ASSESSMENT OF LEUKEMIA RISK ASSOCIATED WITH DERMAL EXPOSURE TO CYCLOPHOSPHAMIDE AMONG NURSES.

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Cyclophosphamide (CP) is a human carcinogen. Oncology nurses are exposed to CP during daily work. This study aimed at assessing the leukemia risk of occupational exposure to CP in nurses.

Task-based dermal exposure intensities for oncology nurses were used to calculate dermal exposure levels to CP. Also, a worst-case exposure scenario was made using the maximum frequency for each task and the maximum intensity of exposure. Internal CP doses were estimated applying a PBPK model to assess the delivered dose for CP and its active metabolites, phosphoramidate mustard and acrolein, in bone marrow. This dose was related to epidemiological information from a longitudinal study with CP-treated patients using linear extrapolation from high to low dose to estimate the risk of leukemia for oncology nurses after 40 y occupational exposure to CP.

The cumulative phosphoramidate mustard and acrolein amounts in bone marrow were simulated to be 12.3 nmol for an average oncology nurse after 40 y occupational dermal exposure. The worst-case scenario with maximum task frequencies resulted in cumulative amounts of metabolites in bone marrow of 1.83 µmol.

Epidemiological data from a cohort of 111 ovarian cancer patients with a total CP dose of 46.35 grams over a 12 m period showed a cumulative risk for leukemia of 11.1% after a ten year period. This administered dose was inserted into the PBPK model as a monthly administration of 14.78 mmol CP into the blood compartment, which resulted in a cumulative dose of CP active metabolites in bone marrow of 5.02 mmol, after 12 m.

Based on this model the risk of developing leukemia for nurses exposed to CP via the skin of hands for 40 y, was estimated to be 0.3 per million oncology nurses. This risk estimate could potentially increase to a maximum of 40 per million oncology nurses for nurses with the maximum exposure frequency and intensity for all tasks (worst-case scenario).

1990 EPIDEMIOLOGICAL INVESTIGATION OF CANCER RATES WITHIN A TWO MILE RADIUS OF VERMICULITE ORE PROCESSING SITES IN UTAH.

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Vermiculite ore from Libby, Montana contained high levels of amphibole asbestos and asbestiform fibers and has been associated with asbestos related disease among Libby residents. Human exposure to the contaminated vermiculite has possibly occurred in communities throughout the United States located near facilities that received vermiculite ore from the Libby mine. A major source of exposure to asbestos is from exfoliation of the ore at exfoliation facilities. The U.S.E.P.A. identified two former exfoliation facilities in Salt Lake City, Utah, which operated from 1941-1987.

The Utah Department of Health investigated the incidence of asbestos-related cancers and asbestos related mortality in a two-mile radius around these sites for the following time periods: 1973-79, 1980-85, 1986-90, 1991-95, and 1996-2000. Cancer data were obtained from the Utah Cancer Registry. Mortality data were obtained from the Office of Vital Records for the same population group.

Cancer incidence and mortality rates in the two-mile radius study area for all asbestos-related cancers combined, cancer of the respiratory system and intrathoracic organs, and cancer of the lung and bronchus were significantly elevated during the time periods analyzed.

Rates for mesothelioma and asbestosis, diseases directly related to asbestos exposure, were not elevated in the study area. This suggests that exposure from the vermiculite processing facilities likely were not a significant contributing factor to the increased rates observed in other asbestos related cancers and diseases in the study area.

Factors that must be considered in the development and etiology of cancers, but that could not be evaluated in this investigation, include latency period, population migration, personal habits, diet, occupational exposures, and familial history of cancer. These issues limit the conclusions that can be drawn from this investigation. Additional research is needed to address the impact of these risk factors on the elevated rates of respiratory cancers in the study area.

1991 META-ANALYSIS OF ASBESTOS-RELATED DISEASE AMONG SKILLED CRAFTSMEN IN VARIOUS OCCUPATIONAL SETTINGS.

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A number of epidemiology studies have evaluated the risk of asbestos-related disease for different categories of workers who were potentially exposed to asbestos in non-manufacturing occupations over time. In this presentation, we provide an extensive review of the available literature on this topic, and present the methodology and key findings of a meta-analysis of asbestos-related disease among skilled craftsmen in various occupational settings. Our initial review of the literature included over 100 published and unpublished studies that were identified during various search efforts. Studies were included in a meta-analysis based on an evaluation of study and control populations, source of mortality data, diagnostic methods, exposure classifications, time period of exposure, latency period, and possible confounders. The different crafts evaluated were insulators, pipefitters and plumbers, boilermakers, carpenters, electricians, laborers, millwrights, sheet metal workers, and welders. Separate analyses were also performed for study setting (shipyard vs. non-shipyard) and disease outcome (mesothelioma vs. lung cancer). The results of each analysis yielded relative risk (RR) estimates and 95% confidence limits. Our findings indicated that, not surprisingly, the greatest risk of asbestos-related disease was historically observed among workers with the most potential for asbestos exposure, such as insulators or crafts employed in Naval shipyards, and workers with the longest duration of exposure. Elevated relative risks were also observed for some other crafts in non-manufacturing occupations, such as in the construction industry, where sprayed asbestos or insulation removal activities often occurred. Despite some limitations, the results of this analysis were generally consistent with prior estimates of asbestos exposure for various craftsmen in different occupational settings.

1992 CHARACTERIZATION OF RISKS TO WELDERS DUE TO EXPOSURES TO ASBESTOS IN WELDING ROD FLUX.

B. L. Finley and J. Pierce. *ChemRisk, San Francisco, CA.*

Due to its unique chemical properties, chrysotile asbestos was formerly incorporated into a wide variety of products, including the outer covering, or "flux" of certain classifications of general arc welding electrodes. The purpose of this analysis was to review the historical exposure and epidemiology information relevant to asbestos in welding rods and assess whether mild steel welders are at increased risk of developing asbestos-related diseases as a result of welding rod use. We found that studies that have attempted to measure airborne asbestos directly in welding rod fumes found no measurable fibers; this is likely due to the fact that the fibers degrade at the high temperatures present in the welding arc. In addition, "worst-case" use simulation studies, specifically intended to generate airborne flux particles, reported that airborne concentrations were undetectable or very low. The airborne concentrations generated were always below the current OSHA permissible exposure limit (0.1 f/cc TWA), and the lifetime doses associated with "worst-case" use were found to be far below plausible thresholds for mesothelioma and lung cancer. The epidemiology literature is complicated by the fact welders often experienced significant "bystander" exposure to amphibole-containing insulation. We have reviewed over 65 studies of asbestos-related disease that either specifically examined welders or included welding as a job category. Of these, roughly 40% concluded that welders are not at an increased risk of developing mesothelioma or lung cancer; approximately 60% concluded that there is an increased risk of one or both diseases. Of these latter studies, 1). there was almost always clear evidence of significant insulation exposure, and 2). none of the authors attributed the disease to exposures to asbestos in welding rod flux. We conclude that the weight of evidence indicates that welders were not historically at risk of developing asbestos-related diseases as a result of welding rod use.

1993 CATEGORIZATION OF ASSOCIATIONS BETWEEN EXPOSURE TO THE HERBICIDES USED IN VIETNAM OR THEIR CONTAMINANTS AND HEALTH OUTCOMES.

J. A. Cohen and M. B. Paxton. *The National Academies, Washington, DC.* Sponsor: *M. Catlin.*

Between 1962 and 1971, United States military forces sprayed herbicides over Vietnam. Because of ongoing uncertainty about the long-term health effects on Vietnam veterans of the herbicides sprayed, Congress passed the Agent Orange Act of 1991. That legislation directed the Secretary of Veterans Affairs to request the National Academy of Sciences, a non-profit organization that provides independent, objective advice on scientific issues, to perform a comprehensive review and evaluation of scientific and medical information regarding the health effects of ex-

posure to Agent Orange, other herbicides used in Vietnam, and the various chemical components of those herbicides, including dioxin. The first comprehensive review was published in 1994 and biennial updates have integrated all subsequent relevant peer-reviewed information. In assessing the evidence for an association of exposure to the herbicides or their components with a health outcome, the committee responsible for the report reviews the available literature and categorize the evidence as 1) sufficient for an association; 2) suggestive of an association; 3) inadequate or insufficient to determine whether an association exists; or 4) suggestive of NO association. In addition, conclusions regarding the biological plausibility of the health effects and the risk to Vietnam veterans are made. The latest of reports, Veterans and Agent Orange: Update 2006, will be released in March 2007. The assessments in the reports are used by the Department of Veterans Affairs to make policy decisions regarding compensation to veterans for service-related illnesses. The methods used by the committee, as well its most recent conclusions, will be discussed.

1994 CHILDHOOD CANCER AND PESTICIDES: POSSIBLE GENE-ENVIRONMENT INTERACTIONS.

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Introduction: Growing evidence supports the association between pesticide exposure and pediatric acute lymphoblastic leukemia (ALL). Studies of the association of pesticides with ALL have been limited by ecological study designs, reliance on self-reported exposures, and lack of biological measurements.

Study Question: Does the interaction between environmental factors (pesticides) and genetic polymorphisms lead to childhood cancer.

Methods: Genomic DNA was extracted and SNP analysis conducted for GPX1, COMT, PON1, GSTM1 and GSTT1. Urine specimens were analyzed for pesticide content using Tandem Mass Spectrometry.

Results: Metabolites of organophosphorus pesticides (OP) had elevated levels in cases compared to controls. Overall, for most compounds these correlations were not statistically significant. Genetic polymorphisms in several genes for xenobiotic metabolizing enzymes indicated that cases were more likely to be impaired in their ability to detoxify OP relative to controls. The odds ratios for the three PON1 genotypes increase with the increase in the number of copies of mutant gene. For mothers the OR for GG vs. AG is 2.08 [95% CI: (0.41, 11.79); p=0.51], while the OR for GG vs. AA is 4.58 [(0.81, 28.31); p=0.10], indicating a trend of increasing risk with increasing number of copies of the A allele. The trends in the OR for children were similar: OR for GG vs. AG is 1.41 [95% CI: (0.21, 11.08); p>0.99] while OR for GG vs. AA is 3.33 [(0.32, 37.60); p=0.48].

Conclusions: The results of this pilot study suggest that cancer cases were more likely to be impaired in their ability to detoxify OP relative to controls.

1995 FAMILIAL INTERSTITIAL PNEUMONIA (FIP) IS PHENOTYPICALLY AND GENETICALLY HETEROGENEOUS.

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The Idiopathic Interstitial Pneumonias (IIPs) are complex conditions, with limited treatment options and unknown etiology. A whole genome microsatellite screen for Familial Interstitial Pneumonia (FIP, the familial form of IIP), revealed multiple linkage peaks on chr 10 with a maximum LOD score of 2.1. To determine if either phenotypic classification or environmental exposures could better define the linkage peaks, ordered subset analysis (OSA) was conducted. In the presence of genetic heterogeneity, OSA uses family level covariate data to define a more homogeneous subset of families that maximize linkage. The 83 families were divided into 2 phenotypic classifications: 40 homogeneous families where all cases were diagnosed with the idiopathic pulmonary fibrosis type of IIP and 43 heterogeneous families where more than one type of IIP was observed within each family. Smoking (proportion of cases that ever smoked in a family) and age of onset (average age of diagnosis for cases in a family, and youngest age of diagnosis in a family) were also investigated as covariates using OSA. On chr 10, an early age of onset was found to increase evidence for linkage amongst all families (LOD 2.1 to 3.2) and the homogeneous families (LOD 1.9 to 3.7, p=0.01 at the more centromeric peak), while cigarette smoking was found to be suggestive of an increase in linkage amongst all

families (LOD 1.0 to 3.0) and heterogeneous families (LOD 1.8 to 2.1 at the more telemeric peak). Thus, it appears that different phenotypic classifications and environmental risk factors maximize linkage in distinct regions of chr 10. It is therefore likely that multiple genetic risk factors play a role in FIP, with both environmental and phenotypic classifications distinguishing more homogeneous sub-groups.

1996 BLOOD GROUP TYPE B IS ASSOCIATED WITH HIGH BLOOD LEAD LEVELS IN CHILDREN WITH CHRONIC EXPOSURE.

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Children chronically exposed to environmental lead are at risk of suffering severe adverse health effects. The aim of this study was to assess a possible association between blood group type (as a marker of susceptibility) and blood lead concentrations (PbB) above 10mg/dL, value considered as indicative of lead poisoning. The study was conducted in Torreón, Coahuila, Mexico, a city where lead contamination in soil, dust and air around a smelter site has resulted in increased lead body burden in scholar children, living in the vicinity of the major smelter complex of Latin America. We examined PbB in 233 children, 5-7 years old, and determined their blood group type. Blood group type O was the most common type (153 [66.2%] children) followed by types A (24.2%), B (8.2%) and AB (1.3%). Median PbB was 8.39 µg/dL in children with blood group type A, 9.6 µg/dL in children with blood group type O, 10.6 µg/dL in children with blood group type AB and 12.4 µg/dL in children with blood group type B. In univariate analysis higher risks of PbB above 10 µg/dL were observed in children with blood group type B (OR=3.33; P=0.03) and blood group type O (OR=1.87; P=0.05), in male children (Odds Ratio 1.58; P=0.08) and in children living closer to the smelter complex (OR=13.8; P=0.000). After controlling for gender and vicinity to the smelter complex, children with blood group type B had the highest risk of PbB >10 µg/dL (OR=4.48; P=0.02), followed by children with blood group type O (OR=3.12; P=0.006), when compared to blood group type A.

1997 GENETIC SUSCEPTIBILITY TO ORGANOPHOSPHATE TOXICITY IN A GROUP OF MIGRANT SEASONAL FARMWORKER WOMEN OF MEXICAN ORIGIN RESIDING IN TEXAS.

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Organophosphate pesticides (OPs) are currently the most widely used pesticides in the world. Studies have demonstrated that the effect of OPs toxicity can be modified by genetic polymorphisms. These variations can place individuals at a higher risk for the toxic effects of OPs. The serum paraoxonase (PON1) gene at position 192 codifies for the synthesis of the paraoxonase enzyme. This enzyme is important in the hydrolysis of OPs. A common polymorphism (Q192R) has been established, and is mostly responsible for PON1 activity. The frequency of the Q192R polymorphism varies between individuals. Studies in Hispanics are still lacking. The Hispanic population is of special interest to health researchers because of their increasing number in the U.S., and because the majority of individuals occupationally exposed to OPs are of Mexican origin. The overall goal of this study was to predict OP sensitivity in a population of migrant seasonal farmworker (MSF) women of Mexican origin residing in Texas. A total of 200 women were randomly selected from an ongoing study conducted by The Center for Research on Minority Health at UTMDACC. The Q192R PON1 polymorphism was genotyped by Real-Time PCR using the TaqMan System and the serum PON1 Activity was analyzed spectrophotometrically using paraoxon as a substrate. Genotype frequencies were QQ=0.26, QR=0.495 and RR=0.245. The mean PON1 activity among genotypes were QQ=479.7 U/L, QR=969.4 U/L and RR=1518.5 U/L, respectively (p=.001), with the lowest PON1 activity observed among women with the QQ genotype, which makes them particularly vulnerable to OP toxicity. In summary, this study can help determine (1) which Hispanic sub-populations are at a higher risk for OP toxicity and (2) establish future policies regarding OP exposure to help reduce health disparities in Hispanic populations.

1998 PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING OF PERSISTENT ORGANIC POLLUTANTS FOR LIFETIME EXPOSURE ASSESSMENT: A NEW TOOL FOR BREAST CANCER EPIDEMIOLOGICAL STUDIES.

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Persistent organic pollutants (POP) have been shown to promote breast cancer development in experimental models. Meta-analyses of epidemiological studies did not support an association between POP exposure and breast cancer incidence in humans. This may be due, in part, to difficulties in relevant exposure assessment and to the lack of tools to adequately estimate blood or tissue POP concentrations at critical time periods of the carcinogenic process. This study aimed to build a physiologically-based pharmacokinetic model (PBPK) which could be used as a tool for the estimation of past internal exposure to POP in epidemiological studies of breast cancer. The developed lifetime PBPK model simulates woman body physiological processes (e.g., growth, pregnancy, breastfeeding) and POP kinetics for given exposure scenarios. Using data on height, weight and age, the model estimates the values of physiological parameters (e.g., organ volume, blood flow and composition) throughout the entire life of a woman. The model allows to consider temporal variations in the daily intake from ingestion. The latter can be based on data obtained from a questionnaire on exposure or on theoretical average scenarios. Several model runs with different physiological profiles were simulated using the POP hexachlorobenzene. Simulations showed that women with a same blood concentration at 55 years of age may have completely different lifetime toxicokinetic profiles. Aside from exposure scenarios, factors that were shown to have the greatest impact on the lifetime toxicokinetic profile are the time and duration of lactation periods and weight history. This PBPK modeling tool will permit researchers conducting environmental epidemiology studies to reduce the uncertainty linked to past POP exposure estimation and to consider exposure time windows that are hypothesized to be mechanistically critical in carcinogenesis. (SH is recipient of a research scholarship from FRSQ)

1999 ESTIMATING HEALTH RISKS IN WORKERS ABOARD CRUDE OIL TANKERS DUE TO EXPOSURES TO N-HEXANE, TOLUENE, AND BENZENE.

J. Pierce, J. Warmerdam and B. L. Finley. ChemRisk, San Francisco, CA.

Most health studies of petroleum workers involve exposures at fixed facilities on land (e.g. refineries). There is a paucity of literature regarding the degree to which petroleum workers aboard ships (such as sea-going crude oil tankers) might represent an at-risk population, even though these workers are often aboard the ships in enclosed spaces for extended periods of time. We relied on historical on-board industrial hygiene data, employee work records and self-reported activities to estimate the magnitude of exposure to benzene, toluene, and n-hexane experienced by individuals aboard these vessels. A total of over 65 surveys performed on 15 ships spanning the years 1988 to 2003 were evaluated in this analysis. A Monte-Carlo model was developed that incorporated available concentration information during nine distinct work tasks that comprised a majority of work performed by crew members on these vessels during crude oil loading and while these vessels were underway. It was determined that airborne concentrations were location- and activity-dependent, with the highest levels experienced during work performed in partially enclosed spaces or near tank vents. Lifetime exposures to n-hexane, toluene, and benzene were calculated to be 9.6, 0.7, and 1.1 ppm-year at the 50th percentile and 28.4, 2.6, and 6.1 ppm-year at the 95th percentile. The estimated exposures are substantially below the levels suspected to cause clinical or subclinical neurological effects, and the estimated upper-bound cumulative benzene exposures are below the effects threshold for acute myelogenous leukemia reported in the well-studied "Pliofilm cohort" of rubber industry workers (200-400 ppm-year). Additionally, the estimated exposures are below the levels expected if the contemporaneous permissible exposure limit (PEL) concentrations were experienced over a working lifetime (20,000 ppm-year for n-hexane, 8000 ppm-year for toluene, and 40 ppm-year for benzene). These results are consistent with the available epidemiology literature on seamen aboard crude-oil tankers.

2000 A COMPREHENSIVE REVIEW OF OCCUPATIONAL EXPOSURE TO DIACETYL IN MICROWAVE POPCORN FACILITIES.

J. D. Roberts, D. A. Galbraith and B. L. Finley. ChemRisk, San Francisco, CA.

In May 2000, eight former employees of a microwave popcorn packaging plant were reportedly diagnosed with a pattern of respiratory symptoms "consistent with" respiratory obliterative bronchiolitis (OB), a severe and sometimes fatal lung disease. It was suggested that the symptoms were a result of exposure to diacetyl, a

chemical used in the popcorn butter flavorings. Since that time the National Institute of Occupational Safety and Health (NIOSH) has conducted cross-sectional industrial hygiene and medical investigations in four microwave popcorn facilities: Gilster-Mary Lee Popcorn Plant, Agrilink Popcorn Plant, American Popcorn Company, and Con Agra. The conclusions offered in the NIOSH reports appear to generally support the belief that respiratory disorders are elevated in certain workers and that diacetyl appears to be a likely candidate for the causative effects. This analysis summarizes the strengths and weaknesses of the existing information and identifies key data gaps. In addition to a detailed analysis of the NIOSH reports, we reviewed the PUBMED and MEDLINE databases for studies that reported animal toxicology and epidemiology results for diacetyl and artificial butter, known or suspected causes of OB, and diagnostic criteria for OB. There exists little toxicology or epidemiology literature for diacetyl, although cases of OB have been noted in agricultural workers and attributed to oxides of nitrogen. There are significant gaps in our knowledge of the effects of diacetyl and definitive conclusions regarding its toxicity to occupationally-exposed workers are difficult to offer.

2001 EXPOSURE OF CHILDREN TO VARIOUS CHEMICAL CONTAMINANTS THROUGH FISH CONSUMPTION.

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Exposure to arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb), polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs), polybrominated diphenylethers (PBDEs), polychlorinated diphenylethers (PCDEs), hexachlorobenzene (HCB), and polycyclic aromatic hydrocarbons (PAHs) through fish and seafood consumption by children of Catalonia, Spain, was assessed. In 2005, samples of the 14 most consumed edible marine species in Catalonia (sardine, tuna, anchovy, mackerel, swordfish, salmon, hake, red mullet, sole, cuttlefish, squid, clam, mussel, and shrimp) were randomly acquired in various cities of the country. Analysis of the above chemical contaminants was determined according to the appropriate analytical techniques. Intakes were subsequently estimated. The daily intakes for the analyzed contaminants were the following (boys/girls): total As, 126.5/110.6 µg; Cd, 0.48/0.61 µg; total Hg 7.45/5.60 µg; Pb, 1.55/1.27 µg; PCDD/Fs, 3.19/2.35 pg WHO-TEQ; PCBs (dioxin-like), 18.20/13.83 pg WHO-TEQ; PCNs, 0.97/0.52 ng; PBDEs, 12.17/8.21 ng; PCDEs, 21.08/17.58; HCB, 7.76/4.04 ng, and PAHs, 0.12/0.12 µg. For most pollutants, intake was higher in boys than in girls. Average exposure of children from Catalonia to metals through fish and seafood consumption did not exceed the respective tolerable daily intake of those pollutants for which it has been already established (metals, PCDD/Fs and dioxin-like PCBs, HCB, and PAHs). In relation to body weight, intake by children of most contaminants was higher than that found for other age groups of the general population of Catalonia.

2002 NON-LINEAR EXPOSURE-RESPONSE RELATIONSHIPS BETWEEN AMBIENT PM10 AND DAILY MORTALITY.

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PM time-series studies (e.g. NMAAPS, 2003; Zanobetti and Schwartz, 2005; Abrahamowicz et al., 2003) have suggested the existence of a non-linear exposure-response relationship between ambient particulate matter (PM10) and health endpoints such as non-accidental mortality, with steeper exposure-response slopes at lower PM10 levels compared to higher PM10 levels. Because this is diametrically opposed to what would be expected on the basis of toxicology, we explore the mathematical constraints placed on the shape of such exposure-response relationships when the environmental measure, or independent variable, is lognormally distributed (e.g. PM10) and the effect, or dependent variable, has a different distributional shape, for example, a Poisson or Gaussian distribution (e.g. daily mortality). For this analysis, we rely on published literature that reports correlations between daily fluctuations in PM10 (measured at central monitors) and daily fluctuations in population mortality (aggregated on the city or county basis) to establish the general magnitude of the relationship. We examine the shape of the exposure-response relationship that results from constraints imposed by the distributional shapes of the independent and dependent variables, considering as well the distributional properties of both confounders and error terms. Results of our analyses show that a non-linear slope is an expected outcome of correlations between data distributions where one is lognormally distributed and the other has a different distributional form. Comparisons with published data sets show that our analysis yields similar shapes of exposure-response curves and similar differences in slopes between low versus high PM10 levels. The analysis shows that caution should be exercised when assigning biological significance to non-linear exposure-response relationships in some instances, and that consistency of findings among numerous epidemiological

studies is an insufficient basis for concluding that the finding of steeper slopes at lower PM10 levels is of biological significance, as all studies share a common alternative explanation.

2003 EXPOSURE LEVELS OF ULTRAFINE PARTICLES DURING DIATHERMY SURGERY IN OPERATING THEATRES IN SWEDEN.

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Diathermy smoke contains complex hydrocarbons and organic material, and may also contain viable tumour cells or viral particles. These particles measure from 0.05 to more than 25 µm and exposure to such particles may have adverse effects on health. For that reason, the surgical operations personnel were concerned about the level of particles in the operating theatres. The objective of this study was to determine the exposure level of ultrafine particles for health care personnel working in an operating theatre.

Measurement of particles was performed during surgical operations using diathermal equipment. Personnel sampling equipments were carried by the physician using a TSI's P-TRAK® Ultrafine Particle Counter. The background levels in the operating theatre during surgery were measured with a TSI's P-TRAK® Ultrafine Particle Counter. Temperature, carbon dioxide (CO₂), and air humidity was also measured in the operating theatre.

The concentration of ultrafine particles in the operating theatres ranged from 73 to more than 300 000 particles/cm³. The background levels in the room during surgery were high with peaks levels of 160 000 particles/cm³. The temperature in the operating theatres was 19-26°C, the CO₂ level was 485-786 ppm and the air humidity was 16-38%.

The ultrafine particle levels in the operating theatres are extremely high compared to those of other work environments. For instance, the level in offices is 1000 – 2000 particles/cm³. The high levels of this study indicate that the local exhaust in the operating theater is not sufficient for particle removal.

2004 MEASUREMENT OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) ASSOCIATED WITH FINE PARTICULATE MATTER TO ESTIMATE STATEWIDE CUMULATIVE EXPOSURES IN NORTH CAROLINA.

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In the United States, there are over 1000 sites that routinely collect particulate matter less than 2.5 µm in aerodynamic diameter (PM_{2.5}) on Teflon filters for non-destructive gravimetric assay. By law, these filters are subsequently archived under refrigeration for at least 1 year. In the State of North Carolina, there are 32 sites that monitor PM_{2.5} every third day, but there are no routine measurements of organic species. Recently, analytical methods have been developed to exploit archived PM_{2.5} filters to retrospectively assess particle bound polycyclic aromatic hydrocarbons (PAHs) exposures without the need for special monitoring instrumentation. PAHs are of public health concern because they are ubiquitous in the environment (from all combustion sources) and have been linked to human carcinogenicity. We selected specific filters from the archives that corresponded to the 50 largest fire events from 2004 and 2005 and also chose spatial and temporal control filters. Each filter was analyzed for a suite of 9 PAHs ranging from 4- to 6-rings using solvent extraction followed by gas chromatography – mass spectrometry (GC-MS). From consideration of PM_{2.5} mass and measured PAHs content, we calculated the relative impact of forest fires to local environments. We also mapped overall PAHs across the State and interpreted the incremental risk posed by the State's 4000 wild fires documented each year with respect to the baseline risk.

2005 EXPOSURE TO ASBESTOS WITHIN THE AUTOMOTIVE REPAIR INDUSTRY BEYOND BRAKE COMPONENTS.

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Beyond those for brake friction material, limited exposure data are currently available to characterize the level of asbestos released during the maintenance work involving automotive asbestos-containing materials (ACMs). Two independent investigations were conducted to evaluate the potential release of asbestos during the

servicing of vehicles which incorporated ACMs. The first study was divided into 14 test sessions, in which seam sealant containing chrysotile fibers was removed from the wheel wells of vintage Ford Mustangs using either a hand scraper or a pneumatic chisel. A total of 70 area and 14 personal air samples were collected during this workplace simulation. In addition, asbestos exposure was assessed during clutch replacement. This investigation was divided into two sessions during which a total of 8 area and 4 personal samples were collected. The air samples collected during the individual exposure assessments were analyzed using NIOSH Methods 7400 Phase Contrast Microscopy (PCM) and 7402 Transmission Electron Microscopy (TEM). Among all air samples ($n = 84$) collected during the removal of seam sealant, 22 % ($n = 19$) of the samples were determined via TEM to contain chrysotile asbestos. The average PCM and Phase Contrast Microscopy Equivalent (PCME) airborne fiber concentrations were 0.025 and 0.005 fibers per cubic centimeter (f/cc), respectively. In comparison, 33% ($n = 4$) of all air samples ($n = 12$) collected during clutch replacement were identified via TEM to contain chrysotile fibers. The mean PCM fiber concentration was 0.041 f/cc, while the mean PCME fiber concentration was 0.002 f/cc for samples identified to contain asbestos during the clutch replacement study. The results of these current studies support the findings of previous exposure assessments that report asbestos concentrations well below the current occupational exposure limit for asbestos of 0.1 f/cc during automotive repairs.

2006 MTBE DETECTIONS IN GROUNDWATER OR DRINKING WATER SUPPLIES IN THE UNITED STATES.

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Detections of MTBE in ambient groundwater and some drinking supplies have raised concerns about the potential impact of MTBE on the nation's water quality. In this presentation, we provide a review and synthesis of the available water quality monitoring data that have been collected as part of various national, regional, statewide, and community-specific or focused assessments. Original data sets for each study were obtained and analyzed whenever possible. Based on analysis of more than 25 studies, we characterized the detection frequency and detected concentrations of MTBE for different geographic regions during the 1990s through early 2000s. The available data included a combination of water types, including drinking and non-drinking water, treated and untreated water, public and private water, and water served by groundwater and surface water. According to the most recent comprehensive national assessment, MTBE was found in about 9% of all community water supplies, but in only 5% of sources served by groundwater. MTBE was found in about 9% of all source waters sampled, but in only 5% of sources served by groundwater. Similar findings were observed in prior regional and national water quality assessments. A number of states or communities were found to have lower (<3%) or higher (>10%) detection frequencies for MTBE. In most cases, MTBE detections were associated with population density, use of reformulated gasoline, urban locations, or proximity to known leaking underground storage tanks. The median detected concentration of MTBE for most studies was less than 1 ppb, and few samples were found to exceed the U.S. EPA's organoleptic-based advisory levels for MTBE of 20-40 ppb. Comparisons between studies was sometimes limited due to differences in study purpose and design and data collection efforts. Despite these limitations, the available data indicate that although some drinking water supplies have been affected by MTBE, the majority of drinking water sources nationwide have either not been affected or have been found to contain MTBE at levels unlikely to pose an aesthetic or health risk.

2007 EUROPEAN CONSUMER EXPOSURE TO COSMETIC PRODUCTS A FRAMEWORK FOR CONDUCTING POPULATION EXPOSURE ASSESSMENTS.

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In order to correctly evaluate the toxicological safety of ingredients contained in cosmetic products, the risk assessor must have access to reliable data on consumer exposure. This study was carried out under the sponsorship of COLIPA with the aim to construct a probabilistic European population model of exposure. The study updates the current data on the daily quantities of body lotion, shampoo, antiperspirant/deodorant spray and non-spray, facial moisturizer, lipstick and toothpaste used by consumers. The data were collected using a combination of the market information databases and a controlled product use study. In total 44,100 households

and 18,057 habitual users (males and females) of the products in five European countries provided data using their own products, including use out of home. The raw data were analyzed using Monte Carlo simulation to construct a European Statistical Population Model of exposure. Distributions obtained largely confirm the exposure data currently used by the cosmetics industry. A significant finding in the study was an inverse correlation between the frequency of product use and the quantity used per application for four of the product types. Thus it is not appropriate to calculate daily exposure to these products by multiplying maximum values for both frequency of use and quantity per application. Design of this study could serve as a model for conducting population exposure assessments to chemicals.

2008 COMPARISON OF XRF AND FIXED BASE LABORATORY METHODS FOR ANALYSIS OF METALS.

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X-Ray Fluorescence (XRF) spectroscopy provides a tool for rapid and inexpensive field analytical measurements for metals. However, when XRF analysis is used for regulatory decision making, there is a concern that inaccuracy in the XRF results may lead to samples being misidentified as meeting regulatory requirements when, in fact, they exceed regulatory limits. Presumably these false negatives would in turn lead to cleanup decisions that might pose a risk to human health. To address this concern a subset of the samples analyzed by XRF in the field are also sent to a fixed-based laboratory. These paired data are then evaluated to assess the performance of the XRF field analysis. Traditional analysis has relied on correlation statistics to assess XRF performance. However, alternative statistical techniques may better address the performance associated with the ability of XRF to correctly identify a soil sample as above or below acceptable regulatory criteria. A dataset was assembled from multiple sites in southern California with analytical results from both XRF and a fixed-base laboratory. An analysis was conducted to compare the performance of different statistical techniques to evaluate the suitability of XRF results compared to the 'gold standard' fixed-based laboratory results. The analysis was conducted using several hypothetical regulatory criteria. Results suggest that correlation provides an estimate of the association between XRF and fixed-base laboratory results but may miss the true concern with XRF analysis, the predictive value for detecting exceedences in field use (i.e. the degree of false negatives). Based on our analysis, contingency table statistics may provide a better evaluation of XRF performance.

2009 COMPARATIVE DISTRIBUTION OF PERFLUOROOCTANOIC ACID IN MALE, FEMALE AND PREGNANT MICE FOLLOWING TREATMENT WITH 8-2 FLUOROTELOMER ALCOHOL (FTOH).

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The global occurrence of perfluorooctanoic acid (PFOA) in environmental and mammalian matrices has spurred regulatory interest in potential sources of this stable compound. 8-2 FTOH, a primary compound used in polymer synthesis, has been shown to undergo biotic and abiotic transformation into PFOA. An appropriate animal model is needed to fully elucidate the mechanisms of metabolism and distribution in mammals. The objective of this study was to compare the metabolism of 8-2 FTOH in male, female and pregnant mice. CD-1 mice received a gavage dose (30 mg 8-2 FTOH/kg BW) in a propylene glycol/water (1:1) vehicle on day 0 and timed-pregnant mice were dosed on gestational day (GD) 8. Animals were serially sacrificed and tissues excised for analysis via GC/MS. 8-2 FTOH was rapidly metabolized and no longer present by 24 hrs post-treatment (PT) and the estimated half-life ($t_{1/2}$) of 8-2 FTOH in serum of male mice was 6.2 hrs. In male mice, maximal PFOA concentrations in serum (992 ± 38 ng/mL) and liver (902 ± 27 ng/g) were at 12 hrs PT and temporally decreased up to 120 hrs. The maximum concentrations in serum and liver of female (768 ± 25 ng/mL and 699 ± 38 ng/g) and pregnant (789 ± 41 ng/mL and 673 ± 23 ng/g) mice also occurred by 24 hrs PT. Using linear regression, the calculated $t_{1/2}$ in male mice was 15.1 d and was comparable to female mice (14.9 d). However, during gestation (GD 8-18), the $t_{1/2}$ was 31.3 d, but values after parturition were similar to non-pregnant females (12.9 d). Estimated PFOA $t_{1/2}$ in liver was 8.2, 9.2 and 28.4 d for male, female and pregnant mice, respectively. In conclusion, 8-2 FTOH is rapidly metabolized to PFOA in male, female and pregnant mice. Sex-mediated elimination is absent, but pregnant mice retain PFOA approximately 2x longer in serum than non-pregnant females suggesting that physiologic changes during pregnancy increase PFOA retention.

2010 A COMPARISON OF THE METABOLISM AND DISPOSITION OF TWO BROMINATED FLAME RETARDANTS: TBBPA AND TBBPA-DBPE IN MALE F-344 RATS.

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Studies were conducted to characterize and compare the metabolic and dispositional fates of two brominated flame retardants, Tetrabromobisphenol A (TBBPA) and Tetrabromobisphenol A bis[2,3 dibromopropyl ether] (TBBPA-DBPE). Each compound was administered orally or intravenously (IV) to male Fischer-344 rats (20 mg/kg). Regardless of route of administration, [14C] TBBPA and [14C] TBBPA-DBPE were eliminated extensively in the feces (oral: >95%, IV: >70%). These results suggested that biliary excretion of [14C] equivalents of these compounds precedes fecal elimination. When bile was collected from rats dosed by oral gavage with [14C] TBBPA, 50% of the administered dose was found in the bile as metabolites within 2 h. However, by 24 h, only 1% of the dose of [14C] TBBPA-DBPE was detected in bile as metabolites following oral administration. These results suggest dramatic differences in absorption from the intestinal tract. Pharmacokinetic analysis of blood revealed that the systemic bioavailability of both compounds is low after oral administration (TBBPA: 1.6%, TBBPA-DBPE: 1.8%). Thus, although TBBPA is absorbed, it is extracted by the liver, extensively metabolized and excreted as glucuronide-conjugates (as determined LC-MS/MS analyses) into the bile, while TBBPA-DBPE is poorly absorbed from the GI tract and is excreted in the feces primarily as parent compound. Its slow fecal elimination following IV administration is due to retention in the liver. (This research was supported by NIEHS NTP Grant Number N01-ES-45529 and NIEHS-sponsored Southwest Environmental Health Science Center Grant Number P30-ES-06694.)

2011 ESTIMATION OF RELATIVE BIOAVAILABILITY OF DIOXINS AND FURANS IN SOIL IN RATS AND SWINE: IMPACT OF DIFFERENTIAL EROD INDUCTION ON BIOAVAILABILITY ESTIMATES.

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A subchronic pilot study of relative bioavailability for a dioxin- and a furan-contaminated soil was conducted. Rats and swine were administered soils mixed with feed (rats) or in gelatin tablets (swine) and matched to dioxin and furan dosages administered in corn oil (rats) or in gelatin tablets (swine). Relative bioavailability (RBA) estimates were higher in rats than swine with different estimates across congeners noted in rats. Higher hepatic EROD activity (a marker for induction of hepatic CYP1A1) was observed in rats (but not swine) dosed with the corn oil formulations compared to the soil/feed groups. The differential EROD induction might have enhanced clearance for some congeners in rats thereby affecting RBA estimates. Therefore, a follow-up study in rats with the furan-contaminated soil was conducted with a 20%, 50% and 80% reduction in the corn oil-administered doses relative to the soil dosage in order to control for EROD induction. When EROD induction was equalized, the apparent RBA for one furan congener, 2,3,7,8-tetrachlorodibenzofuran, was reduced significantly. Rats exhibited approximately 30% and 60% RBA for the dioxin- and furan-contaminated soils, respectively. In contrast, results from swine showed approximately 20 to 25% RBA for both soils (and no differential EROD induction). The bioavailability difference between rats and swine suggests important species differences for soils contaminated with chlorinated dioxins and furans. Species differences and experimental design issues, such as controlling for enzyme induction between treatment groups, are relevant significant factors where relative RBA inputs are important for theoretical exposure characterization.

2012 DIFFERENTIAL URINARY PROTEIN BINDING OF PBDES IN MICE.

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Polybrominated diphenyl ethers (PBDEs), commercial chemicals used as flame retardants in consumer goods, have become the focus of increasing scientific research due to their persistent detection in humans and the environment in conjunction with their potential for toxicity. PBDE congeners 47, 99, 100, 153, and 154, the primary congeners detected in human and wildlife samples, have different pharma-

cokinetic behaviors in rodents which appear to be species-, sex-, and congener-dependent. Many of these differences appear to be driven by urinary excretion. Therefore, this study investigated urinary excretion in C56BL/6 mice of PBDE congeners 47, 99, 100, and 153 in an effort to characterize mechanisms responsible for their excretion. Female mice were administered a single intravenous (iv) dose of PBDE 47, 99, 100, or 153 and urinary excretion was monitored for 5 days. Urine was analyzed for protein binding by Sephadex G-75 columns, gel electrophoresis and Western blotting. Analyses of 0-24h urine for PBDEs 47 and 99 dosed mice and pooled 0-120h urine (pooled due to limited amounts of urinary radioactivity) for PBDEs 100 and 153 dosed mice showed that the majority of PBDE in urine was protein-bound (>65%). All the PBDEs investigated were bound to three mouse Major Urinary Protein (mMUP) isoforms, and were readily excreted via urine. These results suggest that the physiological parameters associated with MUP, such as increased MUP secretion in male mice versus females, could therefore contribute to understanding of the different pharmacokinetic parameters observed in rodents. In conclusion, urinary binding proteins could affect the pharmacokinetics of PBDEs in rodents. (This abstract does not reflect EPA policy. Work partially funded by EPA DESE CT 826513.)

2013 DETECTION OF COCAINE AND METHADONE IN DIFFERENT POST-MORTEM FLUID AND TISSUE SAMPLES.

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The results of about 64 samples taken at postmortem examinations are presented and discussed. The samples analysed are blood (femoral and cardiac), urine, vitreous humour, liver, bile, muscle and stomach contents. Ethical requirements are fulfilled. Various analytical qualitative and quantitative techniques are used including Enzyme-Immuno-Assay (EIA), Gas-Chromatography/Mass Spectrometry (GC/MS) and Liquid-Chromatography/Mass Spectrometry (LC/MS). The mean drug ratio to femoral blood was studied. Cocaine mean ratio to femoral blood was as follow for cardiac blood 1.8 with range (1.6-2.0), Vitreous humour 2.7 with range (2.5-2.9), Stomach 57 with range (42.2-71.6), Bile 14.6 with range (4.3-24.8), Urine 37 with range (27.5-46.3). Liver 0.7 with range (0-2.2) and Muscle 0.37 with range (0-0.9). Statistical analysis of the results shows that concentrations of tissue cocaine, methadone and their metabolites tend to be proportional to those of the blood and that these drugs can be detected in the tissue including muscle and liver depending on the post-mortem interval and their volume of distribution. There are variations in the drug concentrations in both liver and muscle samples. Typically, liver samples tend to show higher drug concentrations than those of the muscle, however, in this study, some muscle samples have shown more drug concentrations than those of the liver within the same postmortem cases. Possible explanations of this observation are given and discussed. It is clear from this study that femoral blood is still the sample of choice for toxicological analysis of cocaine and methadone and their metabolites but muscle and liver samples can be good alternatives to blood if the latter is unavailable.

2014 BAYESIAN PBPK MODELING OF GENISTEIN IN RATS.

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Genistein is an estrogen-receptor-binding compound that occurs at high levels in soy beans and derived food products, with widespread human exposure. Numerous scientific publications describe its dosimetry, mechanisms of action, and health effects. To relate effects in rats to its internal, target-tissue dosimetry, we developed a PBPK model and calibrated it using pharmacokinetic (PK) data from adult male and female rats. Complexities of the model include enterohepatic circulation, binding of both genistein and its conjugates to plasma proteins, and use of multiple compartments to describe transport through the bile duct and GI tract. In other ways the model is simple, assuming perfusion-limited transport to the tissue groups and first-order rates of metabolism, uptake, and excretion. Initially, point estimates and approximate confidence intervals were obtained for the model parameters by standard optimization methods. But given the individual animal PK data available, we sought to apply Markov Chain Monte Carlo (MCMC) Bayesian analysis to quantify population variability and uncertainty in the fitted parameters. A set of 15 physiological parameters (tissue volumes, blood flows) and 8 partition coefficients were described using fixed distributions since the current data contain little information on them. Then 21 genistein-specific parameters were estimated by Bayesian

analysis. The sparse data set (typical for PK studies) and lack of prior information on the parameters caused significant computational challenges, however. Ultimately we had to impose bounds derived from point-estimate likelihood calculations to obtain convergence to biologically realistic values.

This research was supported in part by the American Chemistry Council, USA, and the Food Standards Agency, UK. Views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S.EPA.

2015 CLINICAL AND PHARMACOKINETIC CHARACTERISTICS OF AN ORALLY INGESTED TOBACCO PELLET.

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The tolerability and pharmacokinetic characteristics of a tobacco pellet intended to be entirely ingested was evaluated in 12 healthy adult Caucasian smokers (6 male; 6 female). The pellet prototype was comprised of powdered tobacco, fillers, binders, non-caloric sweeteners, flavors and pH modifiers with a finished weight, pH, and nicotine content of about 175 mg, 8.0, and 1.0 mg, respectively. Plasma nicotine and cotinine pharmacokinetic profiles from 4 randomized single-treatments compared smoking a cigarette to the oral ingestion of a pellet by 3 methods; dissolution in mouth, swallow whole and chew and immediately swallow. Profiles from 3 non-randomized multiple-treatments were also studied after oral ingestion of: 2 pellets; 1 pellet every 20 min for 1 h; and 1 pellet every 30 min for 6 h. Under all conditions the ingestion of pellets was well tolerated. Mean peak nicotine concentrations were 11.4 ng/ml after smoking and between 2.3-3.3 ng/ml after single-pellet ingestions. Times to these peak concentrations were 0.2 h after smoking and between 0.9-1.3 h after ingestion. Ingestion of 1 pellet every 30 min for 6 h resulted in a peak nicotine level of 15.7 ng/ml, a level within or below the range reported for regular tobacco users. Peak nicotine level increases were proportional when 1 or 2 pellets were ingested, but less than proportional when ingested over extended periods, attributed to nicotine's rapid terminal half-life (2.2-4.1 hr). Nicotine and cotinine bioavailability were proportional after multiple treatments. The lowest measures of nicotine bioavailability were after immediate swallowing, indicating rapid first-pass metabolism after GI tract absorption. Pharmacokinetic measures in this study demonstrated nicotine absorption rates from the pellet are relatively slower than achieved by cigarette smoking, and more similar to rates reported for other commercially available oral tobacco products.

2016 THE IMPORTANCE OF OXALATE RENAL CLEARANCE IN THE ENHANCED SENSITIVITY OF MALE WISTAR RATS TO ETHYLENE GLYCOL NEPHROTOXICITY.

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Ethylene glycol-induced nephrotoxicity is caused by the precipitation of the metabolite, oxalic acid, with calcium in renal tubule epithelium. Male Wistar rats are the most sensitive animal studied, with a NOEL of 150 mg/kg/day identified in a recent chronic study where ethylene glycol was administered via the diets at 0, 50, 150, 300 or 400 mg/kg/day for up to one year. Animals in the 400 mg/kg/day group were sacrificed early (day 203) due to increased morbidity and mortality. Kidneys from all animals were analyzed for total oxalates with no differences observed among control, 50 and 150 mg/kg/day groups. At 300 and 400 mg/kg/day, oxalate levels increased proportionally with the nephrotoxicity score (5-point scale ranging from no nephropathy to end-stage) with the most severely affected animals having oxalate levels up to 10.6% of the total tissue mass. The renal clearances of oxalic acid and inulin were evaluated in 5 animals/dose that survived one year with no treatment-related effects observed. Based upon nephrotoxicity scores and oxalate levels, it is conceivable that clearances would have been impacted in animals exposed to 400 mg/kg/day had they survived. In studies with naïve male Wistar and F344 rats (a less sensitive strain), a significant difference was observed in oxalate clearances between young rats (i.e. Wistar clearance < F344) but not in age-matched old rats. Regardless, the ratios of oxalate:inulin clearances in these two strains of rats, including those exposed to ethylene glycol, were all <1, indicating that a fraction of the filtered oxalate is reabsorbed. Other species, including humans, typically have ratios >1 and are more effective at clearing oxalic acid. Thus, the lower renal clearance and kidney accumulation of oxalates in male Wistar rats enhances their sensitivity, which will be a factor in human risk assessments. *Funded by ACC EO/EG Panel.*

2017 PHARMACOKINETIC DATABASE OF PHOSPHORODIAMIDATE MORPHOLINO OLIGOMERS (PMOS).

P. L. Iversen and A. Amantana. *Toxicology, AVIBioPharma, Inc., Corvallis, OR.*

Phosphorodiamidate morpholino oligomers (PMOs) bind to their target mRNA sequences and suppress gene expression by blocking translation or interfering with pre-mRNA splicing. A pharmacokinetic database was established for various PMOs administered at different dosing levels via different routes in various species including rats, non-human primates (monkeys) and human. The PMOs ranged from two to thirty on the basis of the number of subunits. The database was designed to determine the relationship between molecular weight of PMO and pharmacokinetics so as to serve as a predictive model. The database revealed a strong positive correlation between molecular size and volume of distribution as well as the elimination half-life. The relationship between certain motifs in the base sequence and PMO pharmacokinetic profile is yet to be established. In summary, the database shows a strong influence of molecular size on tissue biodistribution of various PMOs.

2018 PHARMACOKINETIC PROFILES OF DIMER AND TRIMER PHOSPHORODIAMIDATE MORPHOLINO OLIGOMERS (PMOS) IN RATS.

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Phosphorodiamidate morpholino oligomers (PMOs) are neutral antisense molecules that bind to complementary RNA sequences and suppress gene expression by blocking translation or interfering with pre-mRNA splicing. The objective of this study was to examine the pharmacokinetic profiles of pyrimidine dimer (TT, T=Thymidine) and pyrimidine-inosine trimer (TTI) PMOs and their distribution in the central nervous system. The compounds were administered at 5mg/kg by intravenous injection and plasma concentrations were monitored at different time points by fluorescence for pharmacokinetic evaluation. The plasma concentration versus time curve of the trimer but not the dimer PMO revealed enterohepatic circulation. The trimer and dimer PMOs showed elimination half-lives of 20.12 h and 5.07 h respectively. The trimer PMO showed urinary excretion of less than 1% of the dose administered compared to 95% of the dimer. Where as urinary excretion peaked at 12 h postdose in the case of the trimer PMO, the dimer PMO showed maximum urinary excretion at 4 h postdose. Both the dimer and trimer PMOs demonstrated broad tissue distribution following administration with the kidney being the primary site of accumulation. Also, measurable concentrations within the brain were observed at 1 h to 72 h postdose. The emerging data shows that a single base in a given PMO sequence can alter the pharmacokinetic profile of the compound.

2019 INFLUENCES OF INJECTABLE SEDATIVES ON INTRAVENOUS GLUCOSE TOLERANCE TEST (IVGTT) IN CYNOMOLGUS MONKEYS.

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Type 2 diabetes mellitus is a progressive and complex disorder that is difficult to treat and diagnose effectively. Glucose Tolerance Test (GTT) has been used in diagnosis of diabetes as a complicating factor in cardiovascular disease and has also been used as a disease model to study efficacy of glycemia-regulating therapeutics. Since the spontaneous occurrence of diabetes in cynomolgus monkeys is rare, GTT may be an important disease model in the development of therapeutics for hyperglycemia. Given that the effects of stress (from procedural handling) on glucose levels is well known, the influence of commonly used injectable sedatives on intravenous (IV) GTT in 3 female cynomolgus monkeys was studied. The following sedatives were administered intramuscularly ~ 10 min prior to IVGTT: ketamine (10 mg/kg) alone or with xylazine (2 mg/kg), acepromazine (1 mg/kg) and Telazol (5 mg/kg) and compared to results from IVGTT in the same 3 animals awake for the dosing and blood collection procedures. Food was withheld from the monkeys for 13 to 16 hours prior to each IVGTT. Blood samples were obtained pre-dose (twice, at least 5 min prior to each glucose injection) and at 2, 5, 10, 20, 30, 40, and 60 minutes after administration of glucose (0.6 g/kg, IV). Glucose and insulin levels were compared and analyzed using WinNonlin software to determine clearance and disposition. Glucose (0.6 g/kg) administered IV produced hyperglycemia within 2 minutes that was reduced to normoglycemic levels 30 to 60 minutes post-injection. Sedation did reduce the variability in IVGTT results and the choice of sedative for performance of IVGTT does influence the resultant glucose clearance in cynomolgus monkeys.

2020 DISPOSITION OF ORAL AND INTRAVENOUS DOSES OF [¹⁴C]CUMENE IN MALE F344 RATS.

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Cumene, a petrochemical, is used in the manufacture of chemicals, as a thinner for paints, lacquers, and enamels, and in a variety of petroleum products. The oral LD₅₀ of cumene in rats is 1.4 g/kg. We have investigated the disposition of oral (1.4, 14, and 140 mg/kg) and intravenous (IV) (1.4 mg/kg) doses of [¹⁴C]cumene in male F344 rats. Vehicles were corn oil for oral doses and 1:1:8 ethanol:Alkamuls:water for the IV dose, and the radiochemical purity of [¹⁴C]cumene in the dose formulations was ~98%. Urine, feces, and volatile organics (VOCs) were collected through 24 h after dosing. Recovery of radioactivity (% dose) was determined in excreta and in tissues at 24 h. Excretion of radioactivity in urine accounted for ~70-80% of the oral dose, with ~1% recovered in feces by 24 h. Recovery of radioactivity as volatile organics (VOCs) increased from ~1% at the 1.4 mg/kg oral dose to ~9% at the 140 mg/kg oral dose, which suggests saturation of one or more processes involved in cumene disposition at the high oral dose. After the 1.4 mg/kg IV dose, 90% was recovered in urine, ~1% in feces, and ~5% as VOCs. Urinary excretion of radioactivity was more rapid after the IV compared with oral doses; by 12 h ~60% was excreted after the IV dose versus 30-40% after oral doses. At 24 h after dosing 17-25% of the dose was present in tissues, primarily in the small intestine and cecum, regardless of dose or route of administration. The high degree of recovery in the gastrointestinal (GI) tract is consistent with incomplete absorption after the oral dose. However, the extent of recovery in the GI tract after the IV dose suggests significant biliary excretion of parent and/or metabolites. Based on these results we will conduct studies of [¹⁴C]cumene in bile-duct cannulated rats to further understand the metabolism and disposition of cumene. [Supported by NIEHS Contract No. N01-ES-25483]

2021 DISPOSITION AND METABOLISM OF N-BUTYL GLYCIDYL ETHER IN F344 RATS AND B6C3F1 MICE.

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The disposition and metabolism of ¹⁴C-labeled n-butyl glycidyl ether (BGE) was investigated in F344 rats and B6C3F1 mice. BGE is an industrial diluent of epoxy resins and is being studied by the Nation Toxicology Program (NTP). Animals were dosed by gavage in corn oil at 2, 20, and 200 mg/kg. The percentage of the radiolabeled BGE dosage recovered in the urine and feces of rats and mice ranged from 68-92% and 2.6-7.7%, respectively, within 24 hours. A higher percentage of the administered dose was recovered as ¹⁴CO₂ in respired air from mice (10-18%) than rats (1.5%). Trace amounts (0.1-0.4%) of non-¹⁴CO₂ volatiles were recovered in respired air. BGE did not accumulate in tissues, 1.5-4.4% of the dose remained after 24 hours. The highest concentrations were in the stomach, the site of dosing. There was no dose effect on the disposition from 2 to 200 mg/kg doses. Male rats were dosed at 20 mg/kg daily for 10 days. The concentration of BGE 24h after the last of the multiple doses was lower in all tissues than 24h after a single 200 mg/kg dose. A trace amount of BGE was covalently bound in the liver. BGE underwent extensive metabolism in both rats and mice; the parent compound was not present in urine. A species difference in BGE metabolism was observed. A total of fourteen metabolites were identified by NMR and MS analysis. Three of these metabolites are derived from a glutathione (GSH) conjugate of BGE. The remaining metabolites are from addition of water to BGE to form a diol followed by further metabolism. In vitro studies confirmed that BGE conjugated with GSH with or without the addition of glutathione S-transferase (GST). This addition is regiospecific to the C-1 position of the epoxy ring.

2022 DIFFERENTIAL SYSTEM MODELS [PROSTATE, COLORECTAL AND CNS] OF POLYCYCLIC AROMATIC HYDROCARBON TOXICITY.

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Polycyclic aromatic hydrocarbons are persistent environmental contaminants that exhibit a myriad of toxic symptoms in mammals. Because it is suspected that there is both a genetic and environmental contribution to the etiology of B(a)P-induced 1) prostate and colon cancer and 2) developmental CNS dysfunction, it has been hypothesized that exposure to B(a)P will shift the dose-response curve to the left in the 12t-7f transgenic LADY mouse model. Initial results from prostate specific cell lines are in support of this hypothesis in that these prostate specific cell lines are ca-

pable of metabolizing B(a)P. Congruent with this hypothesis is that B(a)P exposure will also accelerate the progression of colon cancer using the Apc Min transgenic mouse model. Results from B(a)P exposed microsomes from liver, small intestine and colon samples demonstrate a concentration-dependent increase in total B(a)P metabolite levels with the highest metabolite concentrations found in liver>jejunum>colon. B(a)P exposure was also hypothesized to generate reactive oxygen species which potentiates toxicity to cause deficits in motor function. This hypothesis was tested in C57BL mice and motor activity was significantly impaired suggesting B(a)P exposure-induced insult to dopaminergic mechanisms. Finally, the hypothesis that gestational exposure to B(a)P dysregulates glutamate receptor subunit composition development was tested in C57BL mice. The results from "ex vivo" primary cortical neuronal cultures from exposed offspring support this hypothesis and demonstrate significant downregulation of NR2B and GluR1 developmental expression. Collectively, our results using B(a)P exposure as a model environmental contaminant will contribute to understanding the etiology of B(a)P-induced neurological dysfunction and cancer. [Supported by NS041071 and 1S11ES014156].

2023 DIETARY FAT-MODULATED FLUORANTHENE METABOLISM AND DNA ADDUCT FORMATION IN F-344 RATS.

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Harmful health effects such as cancer and toxicity to humans may be attributed to consumption of fatty foods contaminated with environmental toxicants. The objective of this study was to characterize the effect of dietary fat on disposition; metabolism and DNA adduct formation of fluoranthene (FLA), a polycyclic aromatic hydrocarbon compound. Fluoranthene was administered to 10-week old F-344 male rats in unsaturated (olive oil) and saturated (palm kernel oil) fats at doses of 50 and 100µg/kg via oral gavage. Blood, small intestine, liver, lung, testis, adipose tissue, urine and feces were collected at various time points post-FLA exposure. Samples were subjected to liquid-liquid extraction with methanol, chloroform and water. The extracts were concentrated under nitrogen, reconstituted in methanol and analyzed by reverse-phase high-performance liquid chromatograph equipped with a fluorescence detector for detection and quantitation of FLA parent compound and metabolites. DNA was isolated from the tissues of FLA-exposed rats, subjected to ³²P-post labeling and resolved by thin layer chromatography to measure FLA-DNA adducts. The concentrations of unchanged FLA (FLA parent compound) and its metabolites showed an increase for the saturated fat treatment group compared with the unsaturated fat group. The FLA-DNA adduct concentrations were high in tissues of rats that received FLA through saturated fat. The concentration of FLA metabolites and FLA-DNA adducts showed a dose-dependent increase, and this increase was statistically significant (P < 0.05) for saturated fat. These findings demonstrate that the high residence time of FLA parent compound in saturated fat contributes reactive metabolites of FLA that bind with DNA and cause damage to target organs (supported by NIH grants ES012168, GM08037, G12 RR03032, and 1S11ES014156-01A1).

2024 PRELIMINARY BIOLOGICAL SAMPLE ANALYSIS OF (R)-(+)-PULEGONE IN FEMALE AND MALE F344N RAT PLASMA SAMPLES FROM A PTK STUDY USING IV DOSING.

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Pulegone, a monoterpene ketone, is the major constituent of pennyroyal and other essential oils derived from the *Labiatae* plant family. Because it is primarily used as a mint fragrance or flavoring for foods, beverages and perfumes, pennyroyal oil is under toxicological evaluation by the NIEHS. In this work, an un-optimized gas chromatographic method with flame ionization detection (GC/FID) was developed to quantitate (R)-(+)-pulegone in female and male F344N rat plasma samples from a preliminary, single IV dose, toxicokinetic study (PTKS). A twelve-point (R)-(+)-pulegone spiked plasma curve defined the concentration range from ~0.02 to ~100 µg/mL. For sample preparation, proteins were precipitated using trichloroacetic acid (TCA) followed by extraction with 400 µL of hexane containing the internal standard, hexadecane. The experimental limit of quantitation (ELOQ) was estimated to be ~0.02 µg/mL of (R)-(+)-pulegone in rat plasma. The preliminary toxicokinetic study was conducted in 10 female and 10 male rats administered a single intravenous injection of (R)-(+)-pulegone at 10 mg/kg, with plasma samples drawn at 6, 15, 30, and 60 minutes and 2, 4, 6, 8, 12, and 24 hours post-dose. The PTKS determined (R)-(+)-pulegone concentrations ranged from 0.120 µg/mL to 0.417 µg/mL for the male rat plasma, while the female rat plasma

concentrations ranged from 0.021 µg/mL to 0.297 µg/mL. Using noncompartmental analysis for an IV bolus administration model with linear trapezoidal interpolation, the toxicokinetic parameters included: area under the concentration curve (AUC), maximum concentration (C_{max}), time to maximum concentration (T_{max}), and plasma half-life (t_{1/2}). These values for the male rats were: AUC = 103.8462 min µg/mL; C_{max} = 0.4170 µg/mL; T_{max} = 30 min; and t_{1/2} = 223.8356 min; and for female rats: AUC = 81.2049 min µg/mL; C_{max} = 0.2970 µg/mL; T_{max} = 120 min; and t_{1/2} = 166.3321 min.

2025 EFFECT OF DRINKING WATER PRECONDITIONING ON DICHOROACETIC ACID (DCA) AND DIBROMOACETIC ACID (DBA) TOXICOKINETICS (TK) IN F344 RATS AND B6C3F1 MICE.

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Dihaloacetic acids (DHAs) have been detected in drinking water as a result of chlorine disinfection. Due to the potential for toxicity, NIEHS selected DCA and DBA for toxicity testing and, in support of these studies, TK studies were conducted to evaluate whether repeated exposure via the drinking water (preconditioning) affects the TK of DCA and DBA. Male or female F344 rats and B6C3F1 mice were used. Single gavage administration dosages (mg/kg) were 40 (DCA) and 40 and 100 (DBA) for the rats and 100 or 200 (DCA and DBA) for the mice. Preconditioned animals were given DCA or DBA in the drinking water for 14 days (ad libitum), untreated drinking water (overnight on Day 14), and a single gavage administration on Day 15. The drinking water and preconditioned gavage target dosages (mg/kg) were 42.6 (DCA) and 34.3 and 68.6 (DBA) for the rats and 74.0 and 148 (DCA) and 100 and 200 (DBA) for the mice. Rat and mouse dosing volumes were 5 and 10 mL/kg (gavage). Plasma samples were analyzed for DCA and DBA concentration using a validated capillary GC-ECD method. A one-compartment model with first order (or equal) absorption and elimination was used to describe the gavage profiles. For DCA, elimination was substantially decreased following preconditioning (k₁₀, 0.0342 vs 0.000842 min⁻¹; AUC, 762 vs 85700 µg/mL.min) for the rats, whereas a modest decrease was observed for the mice (e.g., 200 vs 148 mg/kg; k₁₀, 0.117 vs 0.0579 min⁻¹; AUC, 1380 vs 4840 µg/mL.min). For DBA, elimination was only mildly affected (approximate 2- to 3-fold decrease) by preconditioning for rats and mice. The results of this study indicate that the kinetics of DCA and DBA are affected by repeated exposure, which produced a large decrease in the elimination of DCA and only modest decreases in the elimination of DBA. [Study supported by NIEHS Contract No. N01-ES-55551.]

2026 DIBROMOACETIC ACID TOXICOKINETIC STUDY IN F344 RATS AND B6C3F1 MICE.

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Dihaloacetic acids (DHAs) have been detected in drinking water as a result of chlorine disinfection. Due to the potential for toxicity, three DHAs were selected by NIEHS for toxicity testing – dichloroacetic acid (DCA), bromochloroacetic acid (BCA) and dibromoacetic acid (DBA). In support of the toxicity studies, toxicokinetic (TK) studies were conducted and the results of the DBA TK study are presented here. Female F344 rats and male B6C3F1 mice were given a single intravenous (IV) administration of DBA in normal saline at 10 (rats) and 100 (mice) mg/kg or a single gavage administration of DBA in Milli-Q water at 10, 40, or 100 mg/kg (rats) and 100, 200, or 400 mg/kg (mice). Rat and mouse dosing volumes were 2 and 4 mL/kg (IV) and 5 and 10 mL/kg (gavage). Plasma samples were analyzed for DBA concentration using a validated capillary GC-ECD method (LOQ = 0.0934 µg/mL). A two- (rats) and one- (mice) compartment model with first order elimination were used to describe the IV profiles. For the rat, the C_{2min} (µg/mL), volume of distribution (mL/kg), k_e (min⁻¹), and AUC_{inf} (µg/mL.min) were 29, 172, 0.226, and 258 and, for the mice, were 147, 617, 0.0773, and 2110, respectively. A one-compartment model with equal first order absorption and elimination was used to describe the gavage profiles. For rats, the C_{max} (µg/mL), T_{max} (min), k_a or k_e (min⁻¹), and AUC_{inf} (µg/mL.min) values were 2.6, 20, 0.0267, and 202 at 10 mg/kg; 37, 20, 0.0320, and 2260 at 40 mg/kg; and 104, 40, 0.0183, and 15000 at 100 mg/kg. For mice (same TK parameters and units) were 42, 20, 0.0510, 1730 at 100 mg/kg; 133, 20, 0.0471, and 5770 at 200 mg/kg; and 247, 45, 0.0338, and 20400 at 400 mg/kg. In addition to providing TK parameters for DBA (IV), this study showed that in both species a larger than proportional increase in DBA plasma concentrations occurred following a single gavage administration over the dosage range tested. [Study supported by NIEHS Contract No. N01-ES-55551.]

2027 PHARMACOKINETICS OF A SINGLE DERMAL DOSE OF DEET (N, N-DIETHYL-M-TOLUAMIDE), ALONE OR IN COMBINATION WITH PERMETHRIN, AND/OR PYRIDOSTIGMINE BROMIDE (PB) IN MALE RATS.

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We examined pharmacokinetic interactions of a single dermal dose of the insect repellent, DEET (400 mg/kg), when administered with a single dermal dose of the insecticide permethrin [3-(2,2-dichloroethyl)-2,2 dimethylcyclopropanecarboxylic acid (3-phenoxyphenyl)methyl ester] (1.3 mg/kg), and/or a single oral dose of the nerve agent prophylaxes, PB (13 mg/kg), in male rats. Blood, tissues, and urine samples were collected at 0.5, 1, 2, 4, 8, 24, 48 and 72 hr after dosing. Plasma, liver, kidneys, brain, testes, stomach and urine samples were analyzed for the parent compounds and selected metabolites. The application site of the skin retained approximately 0.1% of DEET following administration, 72 hr after dosing, either alone or in combination with PB, or permethrin. DEET and its metabolites m-toluamide and m-toluic acid were identified in rat plasma, tissues and urine samples. Concurrent application of DEET, PB and/or permethrin significantly increased bioavailability of DEET and decreased the urinary excretion of its metabolites compared to its application alone. The data show that the pharmacokinetic of DEET was altered in the presence of the other test compounds, alone or in combination. These results demonstrate that a profound interaction was observed between DEET, permethrin and/or PB indicating possible competition for metabolism enzymes/distribution processes in the rat. Alterations of the pharmacokinetic profiles in the presence of the other compounds could explain previous reports of increased toxicity following combined exposure of test compounds that may have contributed to the Gulf War Veterans, illnesses. Supported in part by the U.S. Army Medical Research and Materiel Command under the contract number: DAMD 17-99-1-9020.

2028 COMPARATIVE TOXICOKINETIC STUDY OF VICRIVIROC IN CYNOMOLGUS MONKEYS OF DIFFERENT ORIGINS.

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The cynomolgus monkey (*Macaca fascicularis*) is one of the non-rodent species of choice for pre-clinical safety evaluation studies conducted in support of drug registration. These monkeys originate from various geographical regions, including mainland China (Chinese), as well as the Indonesian and Philippine islands (Island). Baseline hematological parameters, such as reticulocyte, erythrocyte and white blood cell counts, as well as various serum biochemistry parameters, have been shown to differ in cynomolgus monkeys of different origins. These differences have the potential to alter the pre-clinical toxicity profile secondary to alterations in the pharmacokinetics and systemic exposure. This study was undertaken to examine whether the toxicokinetics (TK) of vicriviroc (VCV), a CCR-5 antagonist in clinical development for the treatment of HIV, could be altered when administered to cynomolgus monkeys of different origins. Chinese and Island monkeys received VCV by nasogastric gavage at a daily dose of 15 or 40 mg/kg, doses that encompassed the range in prior TK studies. On Days 1 and 7, blood samples were collected from VCV-dosed monkeys at 1, 2, 4, 8 and 24 hr post dose. Plasma concentrations of VCV and its carboxylic acid metabolite were determined using a liquid chromatographic with tandem mass spectrometric (LC MS/MS) method. TK parameters, including C_{max}, T_{max}, AUC and metabolite:parent ratios, were determined using non-compartmental analysis. Additional blood samples were collected from control monkeys for the determination of VCV plasma:red blood cell partition coefficients (PC) using equilibrium dialysis. Certain differences in the hematological parameters between Chinese and Island monkeys that could have altered the TK profile of VCV would have been reflected by differences in the PCs. Our findings demonstrated that the systemic exposure to VCV and its metabolite, and the VCV plasma:red blood cell partition coefficients were similar between Chinese and Island monkeys.

2029 COMPUTATIONAL MODELING OF THE IMPACT OF PLASMA LIPID LEVELS ON CHOLINESTERASE INHIBITION AND DOSIMETRY OF CHLORPYRIFOS IN THE DAM AND FETUS.

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Developing fetuses are a unique subpopulation with potentially altered susceptibility to the effects of chemical exposures. Chlorpyrifos pharmacokinetics (PK) and pharmacodynamics (PD) are altered in the pregnant rat and fetus and

cholinesterase (ChE) inhibition is generally less in fetal than maternal tissues. During gestation, changes in physiology and metabolic capacity will impact fetal and maternal PK/PD. Placenta and mammary compartments and a fetal sub-model were added to a previously published chlorpyrifos physiologically based pharmacokinetic (PBPK) and PD model. Altered lipid levels are one of the many physiological changes observed during pregnancy, where lipid levels increase up to 5-fold. The effect of increasing total plasma lipid fraction on predicted partitioning, PK, and ChE inhibition in the dam and GD18-20 fetus was assessed. The fetal model included brain, liver, and fat compartments with esterase inhibition in the brain, liver, and plasma, and transfer to the fetus was based on blood flow. Bioactivation and detoxification were included in the fetal liver as a fraction of the rates in adult liver. This uncomplicated late gestational PBPK model either predicted, or slightly under-predicted literature-reported fetal plasma chlorpyrifos levels and plasma and brain ChE inhibition following repeated gavage exposures to the dams. An algorithm was used to predict changes in blood partitioning of chlorpyrifos when total plasma lipid levels increase 2-3.5x. Increased solubility in the blood resulted in an improved model fit by predicting increases in plasma chlorpyrifos and in plasma ChE inhibition in the dam and GD18-20 fetus. Predicted brain inhibition was also predicted to be increased in dams, but was not appreciably altered in the fetus. These results show the potential impact of lipid changes during pregnancy and the importance to predict PK/PD differences of a lipophilic compound like chlorpyrifos during pregnancy and gestation.

2030 DISPOSITION OF PYRITHIONE IN THE RAT AFTER ORAL, DERMAL AND INTRAVENOUS ROUTES OF ADMINISTRATION.

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Zinc pyriethione (ZPT) is used in commercial and consumer products as a fungicide. In the present study, radiolabeled and unlabeled pyriethione (PT) were measured in female, Sprague-Dawley rats at select time points after single administration. Rats were prepared with an indwelling jugular cannula for serial blood samples and allowed at least 1-day to recover. Cannula patency was maintained with daily flushings of heparinized saline. Blood was collected at baseline and 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 48 and 72 hr following gavage, dietary and dermal routes of exposure. For intravenous administration, blood was collected at baseline and 5, 10, 30 min, 1, 2, 4, 24, 48, and 72 hr after injection. Approximately 250 μ L of blood was withdrawn at each time point. Radioactivity was determined in blood at each time point and in urine and feces at 8, 24, 48 and 72 hr after dosing. The concentration of ¹⁴C-PT was measured in select tissues 72 hr after dosing. Plasma concentration of PT was determined using LC/MS/MS. Urine excretion was the primary route of elimination for all routes of administration. The concentration of total radioactivity in blood was higher and more persistent than plasma PT. This is consistent with studies in which PT metabolites, including 2-methylsulfonylpyridine, were found as the main constituents in the blood. There was no apparent bioaccumulation of radiolabel PT in any of the tissues evaluated in this study. The highest amounts of radioactivity were in the liver, kidney and blood for all routes of administration. Approximately 70-80% of the gavage or dietary ¹⁴C-ZPT was bioavailable compared to <10% for dermal application. There was an 8-12 hr lag time for ¹⁴C-PT or PT to appear in the blood/plasma following dermal exposure. From this study, it is concluded that urinary excretion is the principle route of elimination of ZPT, systemic availability of ZPT following topical administration is limited and concentrations of blood ¹⁴C-PT over predicts the plasma PT.

2031 TOXICOKINETIC AND TISSUE DISTRIBUTION STUDY OF DELTAMETHRIN IN ADULT SPRAGUE-DAWLEY RATS.

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Deltamethrin (DLM) is a widely-used type II pyrethroid insecticide and a relatively potent neurotoxicant. The objective of this project was to evaluate the toxicokinetic behavior and tissue distribution of DLM in male, adult Sprague-Dawley (S-D) rats. For the toxicokinetic study, 10 mg DLM/kg was administered by gavage and 2 mg/kg iv in glycerol formal to cannulated rats. Serial plasma samples were collected over 96 hr and analyzed for DLM content by HPLC. For the dose-dependent tissue distribution study, male S-D rats received 0.4, 2, or 10 mg/kg by gavage. Serial plasma, whole brain, fat, liver, and skeletal muscle were collected for up to 3 weeks. The absolute bioavailability of DLM was 15%, and the peak concentration in plasma was 0.84 ± 0.24 μ g/ml. The area under the curve (AUC_0^∞) was 5.49 ± 0.22

μ g $\cdot$ hr/ml. In the tissue distribution study, DLM concentrations vs time curves showed dose-dependent kinetics in plasma and tissues. After a single oral dose, DLM was rapidly absorbed (T_{max} = 1, 1 and 2 hr for 0.4, 2 and 10 mg/kg, respectively). The maximum plasma concentrations of DLM were 0.5 and 1.2 μ g/ml for 2 and 10 mg/kg, respectively. Fat showed the highest AUCs (143 and 514 μ g $\cdot$ hr/ml for 2 and 10 mg/kg, respectively) and long elimination half-lives (168 and 200 hr for 2 and 10 mg/kg, respectively). Muscle also showed long elimination half-lives (191 and 190 hr for 2 and 10 mg/kg, respectively). These long elimination half-lives of DLM in fat and muscle suggest that DLM may accumulate in these tissues. The AUC for DLM in the brain, the target organ, was much less (13%) than that in plasma at 10 mg/kg. This is, to our knowledge, is the most complete internal dosimetry data set available for a pyrethroid. It provides a basis for beginning to understand the internal disposition of pyrethroid insecticides, as well as data for development of a PBPK model. (Supported by EPA STAR Grant R830800)

2032 BLOOD AND BRAIN CONCENTRATIONS OF BIFENTHRIN CORRELATES WITH DECREASED MOTOR ACTIVITY INDEPENDENT OF TIME OF EXPOSURE.

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Pyrethroids are neurotoxic insecticides used in a variety of agricultural and household activities. Due to the phase-out of organophosphate pesticides, the use of pyrethroids has increased. The potential for human exposure to pyrethroids has prompted pharmacodynamic and pharmacokinetic research to better characterize exposure and risk. This work tested the hypothesis that blood and brain pyrethroid concentrations are predictive of neurotoxic effects. Adult male rats received acute oral bifenthrin in corn oil vehicle. Exposures for the 4 and 7 hr time points were 0, 0.1, 1, 2, 4, 6, 8, 12, 16 mg/kg and 0, 0.05, 0.5, 1, 3, 4.5, 6, 9 mg/kg, respectively (n=4 or 8/group). Motor activity was measured in figure 8 mazes for 1 hr at 4- and 7-hr post exposure. Whole blood and brain tissue were collected immediately following behavioral testing. Tissues were vortex extracted in hexane, eluted through silica solid phase extraction cartridges, and analyzed using LC/MS/MS. Limits of quantitation were 100 pg/ml and 150 pg/g for blood and brain. Bifenthrin exposure decreased motor activity in a dose dependent manner. Doses of 3 mg/kg or higher decreased motor activity 20% to 70% of vehicle control levels in both the 4- and 7-hr groups. Combining the 4- and 7-hour groups, the relationship between whole blood or brain bifenthrin concentrations and motor activity were described using a Hill model. Model fits to the blood data ($R^2=0.68$, $p<0.0001$) estimated ED_{50} of 323 ng/ml tissue with a slope factor of 1.4. Model fits to the brain concentration data ($R^2=0.69$, $p<0.0001$) estimated the ED_{50} of 219 ng/g with a slope factor of 2.3. These data suggest that blood or brain concentrations can be used as a dose metric to estimate effects on motor function, and that this relationship is independent of time of exposure. (This abstract does not represent EPA policy).

2033 DISPOSITION AND MUSCLE WEAKNESS IN THE RAT AFTER REPEAT DOSE ADMINISTRATION OF ZINC PYRITHIONE.

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In the present study, ZPT-derived radioactivity (¹⁴C) and parent, i.e., pyriethione (PT), were determined in female, Sprague-Dawley rats at select time points after single and repeat dose (10-day) administration of ¹⁴C-ZPT. Muscle weakness and tone were measured in the same group of rats to determine the relationship between PT plasma/blood concentrations and toxicity. Rats were prepared with indwelling jugular cannula for serial blood samples. ZPT (1.25, 3 and 10 mg/kg, gavage; 10, 30 and 100 mg/kg, dermal; 250 ppm, diet) was administered for 10 days. Blood or plasma concentrations of ¹⁴C-PT or PT, respectively, were determined at select time points following day 1 and day 10 of dosing. Comparison of the pharmacokinetic parameters across routes of exposure shows that the time to reach maximum concentrations of PT was much greater with dermal exposure than either oral route. This is consistent with the long dermal exposure (6 hr/day) and the lag time required for ZPT-derived compounds to penetrate the skin. Area-under-the-curve (AUC) for rats exposed to ZPT via diet or gavage was similar. In contrast, the AUC values adjusted for dose for rats treated topically with ZPT were much lower than the corresponding values for animals administered ZPT orally. These differences in bioavailability of ZPT following 10-days of dosing were supported by analysis of urinary excretion which provided the same relationship. There was no evidence of

accumulation of ¹⁴C-PT following any route of exposure, which is consistent with a log P value for ZPT of 0.97. The day 10 plasma AUC(0-∞) for PT was positively correlated with combined measures of muscle weakness and tone. The concentration of plasma PT after 10 days of administration determines the degree of muscle weakness independent of the route of administration. These data are the first to show repeated administration of ZPT does not accumulate in the rat and that the concentration in plasma is predictive of the degree of muscle weakness in this species.

2034 PHARMACOKINETICS AND TISSUE DISPOSITION OF ATRAZINE IN MALE C57BL/6 MICE.

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Atrazine (ATR) is a widely used herbicide and exposure to it has been associated with a number of toxicological effects on reproductive, endocrine, nervous, and immune systems. As is the case with many other environmental contaminants, a growing number of in vivo toxicity studies with ATR are conducted in mice. Yet, at present, there are no data describing ATR's pharmacokinetics and tissue dosimetry in the mouse. Hence, the objective of this study was to address this data gap. Male C57BL/6 mice were orally exposed to a dose range (5-250 mg/kg) of ATR. Plasma, tissues (liver, spleen, thymus, kidneys, and brain), urine, and fecal material were collected at various time points up to 96 h post dosing. Using our newly developed LC-MS method, levels of ATR and its metabolites didealkyl ATR (DACT), desisopropyl ATR (DIP), desethyl ATR (DE), ATR-glutathione conjugate (ATR-GSH), and ATR-mercapturate (ATR-MER) were determined in the various matrices. ATR was rapidly metabolized in mice and DACT was the most abundant metabolite detected in the urine and plasma samples, as well as in the various tissues. For example, in the 0-24 h urine samples, DACT levels in mice exposed to 250 mg/kg ATR were more than 150-fold greater than ATR and more than 60-fold greater than the levels of DE and DIP. There was a good correlation between plasma and tissue levels of ATR and its metabolites, with the highest levels observed within the first 4 h after exposure. In addition to determining the levels of ATR and its metabolites in the liver and kidneys, we also readily detected them in potential target organs such as the brain, thymus and spleen. Therefore, in addition to accurately describing the pharmacokinetics and tissue disposition of ATR in the mouse, this study also provides the basis for the development of a mouse-specific PBPK model, as well as for the calibration of models that are being developed by others in the rat. [Supported by NIH P20RR017661].

2035 INHIBITION OF HUMAN GLUTATHIONE S-TRANSFERASE OMEGA BY TOCOPHEROL SUCCINATE.

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Glutathione S-transferases (GSTs) are a family of multifunctional enzymes that are present in all living organisms. Their main function is the detoxification of electrophilic compounds. Glutathione conjugation is the major detoxification pathway available to the organism to trap toxic substances. Based on their substrate specificity, sequence structure, catalytic activity, immunogenicity and sensitivity to inhibitors, the mammalian

GSTs form seven distinct classes termed alpha, mu, pi, sigma, theta, zeta, and new class of human GSTs designated omega. Human GST omega 1-1 (hGSTO1-1) is identical to human monomethylarsenic acid (MMAV), the rate-limiting enzyme for biotransformation of inorganic arsenic. It is expressed in a wide range of human tissues, including brain. Several studies have indicated a role for an Omega-class GST gene in the early onset of both Alzheimer's and Parkinson's diseases, and it is possible that hGSTO1-1 may be involved in the modulation of the activity of interleukin-1 (IL-1) which play a major role in a wide range of inflammatory disease. Compounds that target IL-1 production are being investigated. We found that (+)-α-tocopherol succinate inhibited the reduction monomethylarsenate (MMAV) and dimethylarsenate (DMAV) in a concentration-dependent manner with an IC₅₀ of 4 and 3 μM, respectively. The kinetics indicated an uncompetitive inhibition of the MMA(V) and DMA(V) reducing activity of hGSTO1-1.

2036 IMPACT OF ANIMAL HANDLING ON THE TOXICOKINETICS OF BIS(2-CHLOROETHOXY)METHANE IN MALE F344 RATS.

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Conventional methods for conducting toxicokinetic (TK) studies with rodents require not only large numbers of animals, but also significant handling of the animal for dosing and sample collection. Advent of automated instruments for dosing and

blood sample collection allows TK studies to be conducted using fewer animals that are freely-moving and non-handled for dosing and sampling. This work compared the TK profile of bis(2-chloroethoxy)methane (CEM), a potential cardiotoxin, from the manual to an automated method. F344 male rats were given a single intravenous (IV) administration of CEM at 20 mg/kg by the automated dosing instrument or manually via an indwelling jugular vein catheter. Plasma samples were collected at specified time points by the automated sample collection instrument via a surgically implanted femoral vein catheter or manually from the retro-orbital plexus. CEM plasma samples were analyzed by a validated GC/MS method. The plasma CEM concentration-time profiles were characterized by a two-compartment kinetic model with bolus input and first-order elimination. The TK parameter estimates for the initial decay rate (alpha), elimination from the central compartment (K₁₀), distribution from the central to the peripheral compartment (K₁₂), AUC, and systemic clearance were not statistically different between the manual and automated methods. There were statistically significant differences (p<0.05) for the terminal decay phase (beta), volume of distribution, and rate of distribution from the peripheral to the central compartment (K₂₁) between the manual and automated methods. However, the differences in these TK parameters were considered marginal, thereby suggesting they were biologically insignificant. These results demonstrated that the automated method for dosing and sample collection resulted in similar estimates for CEM TK parameters when compared to the manual method. [Supported by NIH-ES-05-03]

2037 DETERMINATION OF THE PHARMACOKINETICS FOR MODIFIED RELEASE RECOMBINANT HUMAN THYROID STIMULATING HORMONE (MR-RHTSH) IN RATS AND DOGS.

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Purpose: Pharmacokinetics (PK) studies were executed in Sprague Dawley rats and Beagle dogs to assess and compare the pharmacokinetics of recombinant human thyroid stimulating hormone (rhTSH) prepared in sodium carboxymethylcellulose (mr-rhTSH) and rhTSH prepared in sterile water for injection (rhTSH). More specifically, studies were conducted to evaluate the effect of concentration, viscosity, and injection volume on the PK of mr-rhTSH.

Methods: Test articles were intramuscularly (IM) administered to rats and dogs at doses ranging from 0.05 to 1.0 mg/kg with various viscosities of mr-rhTSH ranging from 18-149 cps. In addition, dogs received IM administration of mr-rhTSH at dose volumes of 0.07 or 0.15 ml/kg. PK blood samples were collected at various post dosage timepoints via jugular vein cannulas and evaluated by ELISA.

Results: Pharmacokinetic analysis demonstrated a statistically significant decrease in C_{max} and an increase in T_{max} for mr-rhTSH compared to rhTSH without eliciting significant differences in exposure (area under the curve, AUC). Further analysis indicated that altering the viscosity range in between 18 and 149 had no significant impact on mr-rhTSH pharmacokinetics. Injection volume studies in beagle dogs demonstrated a statistically significant shift in C_{max} and T_{max} for mr-rhTSH relative to rhTSH, regardless of whether the injection volume was 0.07 or 0.15 ml/kg. Significant changes in C_{max}, T_{max}, and t_{1/2} were also observed with mr-rhTSH dosed at either, 0.1, 0.2, or 0.05 mg/kg compared to rhTSH.

Conclusions: These results indicate that following intramuscular administration, maximal concentrations are reduced and the time to C_{max} is prolonged with mr-rhTSH relative to rhTSH. Such changes occur in the absence of differences in exposure and occur independent of mr-rhTSH viscosity, concentration and injection volume.

2038 TISSUE DISPOSITION OF GOLD NANOPARTICLE FOLLOWING IV INJECTION IN SWINE.

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Gold nanoparticles have many potential uses in biology and medicine, including molecular level imaging and treatment of diseases such as cancer. The ability to image morphological changes associated with disease at the molecular level will lead to earlier disease detection and treatment and increase the possibility of a successful outcome. Preliminary experiments have been conducted with the objective of determining the disposition of hybrid gold nanoparticles (AuNPs) in blood and tissues following the I.V. administration of a gum-arabic preparation of AuNPs to juvenile swine. In these studies juvenile male swine were anesthetized with isoflurane, and venous catheters were placed in the cranial vena cava. While anesthetized, the gold nanoparticles were administered via the catheter at dosages ranging from 1.88 to 4.00 mg/kg BW. Serial blood samples were collected at 5, 15, 30, 60, 120, and

240 minutes post-dosing. A final blood sample was then collected 24 hours post-dosing and pigs were humanely sacrificed and tissues were collected for gold analysis using atomic absorption spectrophotometry. In these experiments gum-arabic AuNPs were distributed according to a two-compartment model, with a distribution phase lasting approximately 4 hours and an elimination phase that continues through 24-hours. There were measurable levels of gold in several tissues, however, the lung and liver had the greatest concentrations. Preliminary results indicate that these hybrid gold nanoparticles are distributed rapidly to many tissues in the body and the lung and liver are sites of accumulation. These hybrid gold nanoparticles could potentially be used to increase the sensitivity of CT or ultrasound imaging of these organs or in targeted treatment and radiotherapy of cancer and other disease in these organs. Work supported by the National Institutes of Health/National Cancer Institute, Cancer Nanotechnology Platform program (grant number: 1R01CA119412-01).

2039 NASAL AND PULMONARY UPTAKE AND METABOLISM OF INHALED SULFURYL FLUORIDE IN ADULT RATS.

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Sulfuryl fluoride (SF), a structural and post-harvest fumigant, is a key alternative to methyl bromide, a stratospheric ozone depleting substance scheduled for phaseout by the Montreal Protocol. Extensive toxicological studies suggest that fluoride (F) ion may play a role in the mode of action of SF in insects and mammals. This study was designed to determine contribution of the upper (URT; nasal) and lower (LRT; pulmonary) respiratory tract to the absorption and metabolism of inhaled SF and to provide data necessary to develop a PBPK model to describe tissue dosimetry of SF, and the hydrolysis products fluorosulfate (FS), and F in the adult rat. Male F344/DuCrI rats (n=6/group), anesthetized with urethane, were exposed to 0 or 300 ppm SF for 4 h. One half of the rats were surgically-modified to separate the URT from the LRT before exposure. In surgically-modified rats humidified breathing air (0 ppm SF) was supplied directly to the lungs via a tracheal cannula, while the test atmosphere was pulled through the URT at a rate of 150 ml/min. The fractional uptake of SF by the URT was estimated from the difference in the SF concentration, determined by GC/FID, before and after passage through the URT. After exposure, the concentration of SF in blood and lavage fluids and the concentration of FS and F in serum, lavage fluids, and tissue homogenates of nasal mucosa, lung, cerebrum, olfactory bulb and cerebrum were determined by GC/MS or ion chromatography, respectively. Based on the difference in SF concentration before and after passage through the URT, the average percent of SF absorbed by the URT was 5%. No SF was detected in any sample from either surgically-modified or unmodified rats, however, elevated levels of FS and F were detected in serum, lavage and tissue samples of SF-exposed rats. These data indicate that even at high exposure concentrations, absorbed SF is rapidly and completely hydrolyzed to FS and F, and is, therefore, not available for systemic exposure of tissues beyond the point of contact (URT and LRT).

2040 PULMONARY ABSORPTION AND LUNG DOSIMETRY; THE ISOLATED AND PERFUSED RAT LUNG EXPOSED TO DRY POWDER AEROSOLS OF BUDESONIDE, FORMOTEROL AND TERBUTALINE.

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When rats are exposed nose-only to aerosols of solutes, disposition in the respiratory tract proceeds along several routes; absorption through the air/blood barrier, mucociliary clearance with swallowing, macrophage engulfment of slow dissolving particles and various systemic effects. These overlaying processes tend to obscure the key process of solute absorption through the air/blood barrier. Our aim was to create a model for measuring the pulmonary absorption of inhaled solutes in great detail. Hence, one important task is to link the systemic concentration profile with the dosimetry of the exposed tissue; primarily the air/blood barrier of the peripheral lung. The isolated and perfused rat lung (IPL) has been widely used to study deposition, absorption and metabolism of inhaled drugs and toxicants. However few options have been available for exposing the IPL to dry powder aerosols in a controlled way. The recently developed DustGun aerosol technology can reproducibly expose the IPL to a respirable dry powder aerosol. We studied the pulmonary absorption of three generic drugs by exposing distal regions of the IPL to respirable aerosols of budesonide, formoterol and terbutaline. The IPL was perfused in a single pass mode and repeated samples of the perfusate were taken up to 80 min post exposure. The concentration of drug in perfusate and in lung extracts was measured using LC-MS/MS. An overall mass balance was established by summing up cumu-

lative clearance with the tissue dose at the end of the perfusion period. To describe absorption and retention of the substances in the lungs, independent of the concentrations, the retention of total deposited dose as a function of time was calculated. The fractional retention in the lung at 80 min post exposure for budesonide, formoterol and terbutaline was 0.2, 0.18 and 0.04 respectively.

2041 BIOAVAILABILITY OF PBDES IN MALE RATS FROM ORALLY ADMINISTERED HOUSEHOLD DUST.

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Recently, household dust has been implicated as a major source of polybrominated diphenyl ether (PBDE) exposure in humans. This finding has very important implications especially for young children, who are thought to ingest more dust than adults, and may be more susceptible to some of the putative developmental effects of PBDEs. No absorption parameters of PBDEs from ingested dust have ever been determined, therefore, the objectives of this study were to determine the absorption, distribution, and excretion of PBDEs in male Sprague-Dawley rats fed either dust (NIST Standard Reference Material 2585) or oil contaminated with ppb levels of 20 PBDE congeners for 21 days in the diet. Following cleanup by an automated Fluid Management System utilizing triphasic silica and basic alumina cartridges, the tissue residues were quantitated by HR-GC/HR-MS analysis. Results from adipose tissue and livers showed that uptake of individual PBDE congeners did not differ between comparable doses of either vehicle. Bioconcentration factors of PBDEs in adipose were the same for all dosage groups, i.e. 10-20 for tri- to hexa-BDEs, 1-4 for hepta- to nona-BDEs, and <1 for deca-BDE. The liver-to-adipose ratios on a lipid-weight basis (L:A) for tri- to hexa-BDEs were <0.5, while the L:A was >1.0 for hepta- to deca-BDEs. The present results show that PBDEs in dust are at least as bioavailable as those dissolved into an oil vehicle, and that higher brominated congeners are less bioavailable than lower brominated congeners. These results indicate that dust can be a facile source of PBDEs in mammals, and that the familiar PBDE fingerprints in biota is a result, at least in part, of bioavailability of individual congeners. (This abstract does not reflect Agency policy.)

2042 OLD PRINCIPLES IN A NEW FIELD: PARTICOKINETICS AFFECTS SIZE AND TIME DEPENDENT TOXICITY OF NANO SILICA IN MACROPHAGES.

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The rapid growth in the use of in vitro methods for nanoparticle toxicity assessment has proceeded with limited consideration of the unique kinetics of these materials in solution. Cellular dose is a function of several systemic and particle factors: media density and viscosity, particle size, shape and density. Collectively, these processes determine the rate of transport of nanoparticles to cells in culture. Here we use a computational model of particle kinetics and dosimetry in vitro to study the influence of size and agglomeration dependent nanoparticle delivery rates on the pharmacodynamics of the macrophage inflammatory response to amorphous silica. RAW267 macrophages were exposed to 10, 150, 500 and 1000 nm amorphous silica and the expression of TNF α mRNA was determined 2, 4 or 8 hours after exposure. Two sets of doses were selected, equal nominal media surface area concentrations, and equal delivered particle surface area determined with the computational dosimetry model. The agglomeration state of these particles was determined by scanning electron microscopy and used as input into the dosimetry model. We show that the kinetics of delivery significantly impact the apparent dynamics of the inflammatory response and of the relative potency of particles of different sizes. Additional experiments demonstrate that media volume in vitro can affect dose to the cell and the dynamics of the measured inflammatory response, particularly for dense particles. Incorporating particokinetics and principles of dosimetry are critical for nanomaterial toxicity assessment. Funded through U.S. Department of Energy under Contract DE-AC05-76RL01830.

2043 PERCUTANEOUS ABSORPTION OF POLYCYCLIC AROMATIC COMPOUNDS (PAC) FROM PAVING- ASPHALT FUME CONDENSATES.

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Four asphalt fume condensates (AFCs) were obtained from the head-space vapors of storage tanks containing straight-run paving asphalts at 147 $\pm$ 2.7 deg.C, for measuring percutaneous absorption through human skin. The Asphalt Institute

(Lexington, KY) provided the AFCs, which were analyzed by the Fraunhofer Institute (Hannover, Ger.) for specific polycyclic aromatic hydrocarbons (PAH). The Heritage Research Group provided analyses of total 3-6 ring PAC. One inch circular pieces of human back skin from a single donor (250-350 μ m thickness) were mounted in Franz diffusion cells. Tissue integrity was assured by measuring electrical resistance. Neat condensate was applied at "infinite dose" (200 μ l/tissue) to the stratum corneum; the dermis was in contact with the receptor fluid, which was an aqueous solution of polyoxyethylene 20 oleyl ether. Receptor fluids were sampled at intervals over 48 hr and analyzed by HPLC using fluorescence detection; the mobile phase was 60-100% acetonitrile/water gradient. Flux rates of anthracene, fluoranthene, pyrene, and total 3-6 ring PAC were measured. The average ($n=4$) neat AFC concentration (ppm) was: anthracene, 50.6 $\pm$ 14; fluoranthene/pyrene, 66.5 $\pm$ 47; and 3-6 ring PAC, 833 $\pm$ 302. Flux rates (ng/cm²/hr) were: anthracene, 4.4 $\pm$ 1.2; fluoranthene/pyrene, 3.8 $\pm$ 2.8; PAC, 34.3 $\pm$ 14.4. The apparent permeability coefficient (Kp) for the four samples, as determined by results for anthracene was 1.0 $\pm$ 0.10 $\times 10^{-4}$ cm/hr. Results show that skin penetration of PAH, at concentrations naturally present in fume condensates are measurable by *in vitro* methods. Rate and extent of PAH and PAC skin penetration can be a useful parameter in the risk assessment of potential toxicity from asphalt fume exposure. This research was supported by the Asphalt Paving Environmental Council.

2044 IN VITRO ABSORPTION OF ¹⁴C-DECAMETHYLTETRASILOXANE (¹⁴C-L4) IN HUMAN SKIN.

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The absorption of ¹⁴C-L4 was evaluated when applied to human skin *in vitro* using flow-through diffusion cells. Human epidermis with a top layer of dermis (300-500 μ m) was prepared from intact abdominal skin by dermatoming. Skin discs from four donors were assessed in triplicate. A physiological receptor fluid was pumped underneath the skin samples for 24 hr and collected at predetermined time points using a fraction collector. Barrier integrity of the skin was initially screened with ³H-H₂O. Each skin sample was dosed with neat ¹⁴C-L4 at a targeted dose of 10 mg/cm². Immediately after dosing, charcoal baskets were placed above the exposure site and secured into a custom designed holder to capture volatilized material. At the end of 24 hr, the charcoal baskets were removed and extracted, skin was washed, tape stripped and solubilized, and receptor fluid was collected. The radioactivity content in each sample was measured by liquid scintillation counting. The percent dose absorbed was determined as the amount of radioactivity in the receptor fluid combined with the amount remaining in the skin after washing and tape stripping relative to the applied dose. Almost all (99.9%) of the recovered ¹⁴C-L4 was captured in the charcoal baskets placed above the exposure site. The percent of applied ¹⁴C-L4 recovered from all analyzed samples was 104.7%. Only a small amount of applied dose (0.06%) was found on the skin surface after a 24 hr exposure or remained in the skin after washing (0.03%). Little, if any (0.001%) of the applied dose penetrated through the skin into the receptor fluid with the mean cumulative penetration ($\pm$ SEM) over the 24 hr being only 0.07 $\pm$ 0.007 μ g equivalents L4/cm² of exposed skin. The total percent dose absorbed was estimated to be 0.03% of applied dose with virtually all of the absorbed ¹⁴C-L4 retained in the skin. (Supported by the Silicones Environmental, Health and Safety Council of North America)

2045 DISPOSITION AND ELIMINATION OF TUNGSTEN IN RODENTS AFTER REPEAT GAVAGE AND DRINKING WATER EXPOSURES.

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Tungsten (W) has been nominated for study to the National Toxicology Program (NTP) because of reported associations between concentrations of W in drinking water and childhood leukemia. As part of the NTP program, the chemical disposition of W (administered as sodium tungstate dihydrate in water) in plasma, liver, kidney, uterus, femur, and intestine of rodents (Sprague-Dawley Rats and C57Bl/6N mice) have been characterized after repeat exposure by gavage (10 mg/kg) or drinking water (560 mg/L) for 14 consecutive days. Separately, the same species were exposed to drinking water (560 mg/L) during gestation for 9 (mice) or 10 (rats) days. For the rodents exposed during gestation, all the aforementioned tissues were analyzed in addition to pups to assess transplacental disposition. Animals were euthanized 24 hr after the last day of dosing. At sacrifice, plasma, intestine, liver, kidney, femur, uterus, and fetus were collected for analysis by

Inductively Coupled Plasma Mass Spectrometry. W was detectable in plasma and all tissues and in pups of both rats and mice. In many tissues, W accumulation was readily apparent with concentrations in intestine, kidney, and femur significantly above baseline levels. Explanations for enrichment/retention in these tissues include intestine being the primary route of entry for oral exposures, kidney being the primary route of elimination (>95% eliminated through the urine as shown in previous studies), and the ability of W to displace P in bone and become retained in femur. Funding from NIEHS Contract # N01-ES-25483

2046 NON-COMPARTMENTAL PHARMACOKINETIC AND PHARMACODYNAMIC ANALYSIS OF A RECOMBINANT IFN- β AND NEOPTERIN FOLLOWING A SINGLE SUBCUTANEOUS INJECTION IN CYNOMOLGUS MONKEYS.

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Noncompartmental pharmacokinetic (PK) and pharmacodynamic (PD) analyses are an integral part of Investigative New Drug (IND) submissions. Here, WinNonlin (v. 5.0.1) was used to determine the PK and PD profile of a recombinant human IFN- β and Neopterin, an established biomarker of IFN- β activity, in the plasma of cynomolgus monkeys following a single subcutaneous injection. Molecular quantifications were performed using validated ELISAs. Separate groups of animals (3-5/sex/group) were dosed at 0, 3.5, 11, or 35 μ g/kg IFN- β , and bled at ten time points for the purpose of PK/PD analyses on Day 1 of a 13-week repeat dose safety study. Animals were also bled on Day 89, but neither IFN- β nor Neopterin were detected. Maximum IFN- β plasma concentrations (C_{max}) were reached between 4 to 8 hours (T_{max}) following administration, with the fastest absorption occurring at the high dose level. Both the C_{max} and the area under the curve (AUC(0- ∞)) increased beyond dose proportional rates. The goodness-of-fit statistic (R²) applied to the data indicated that estimations based on the terminal elimination rate constant (K_{el}) were reliable. The terminal half-life (T_{1/2}) of IFN- β was estimated to be between 5 and 6 hours. Neopterin plasma concentrations linearly increased beyond baseline values at approximately the IFN- β T_{max} for each group, with a dose dependent slope increase until the maximum effect (R_{max}) was observed at the final time point (T_{max} = 24 hrs). However, Neopterin R_{max} and AUC(>B) increases across dose levels fell short of dose proportionality. Interestingly, C_{max} and AUC(0- ∞) for IFN- β in this experiment were substantially higher than those found in the published data for a commercially available IFN- β at the same dose levels in the same species, despite a similar T_{max} and T_{1/2}, suggesting improved bioavailability.

2047 ALTERATION OF TRICHLOROACETIC ACID (TCA) RENAL ELIMINATION BY PROBENECID IN SPRAGUE-DAWLEY (S-D) RATS.

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Probenecid (PRB), a nonspecific inhibitor of the organic anion transporter/multidrug resistance-related protein (OAT/MRP), members of ATP-binding cassette transporter superfamilies, was used to investigate the role of active transport in the renal clearance of TCA in young S-D rats. Previous studies from our group showed that the induction of CYP2E1 by pyridazine enhanced trichloroethylene (TCE) metabolism and increased the renal clearance of TCA in animals dosed with TCE or TCA. The roles of the transporters for the small organic anions and their conjugates are well established, but there is apparently no information on the involvement of these transporters with TCA.

Male S-D rats (~ 175 g bw) were cannulated (carotid artery and/or jugular vein), housed in metabolism cages individually, allowed to recover from surgery for 24 hr, given PRB (500 mg/kg in 5% emulsion) ip (controls were given saline), followed by the administration of TCA (50 mg/kg, iv). Serial blood samples were taken for 72 hr via the indwelling catheter, and urine collected and stored at -20 $^{\circ}$ C until analysis. TCA in blood and in urine were derivatized and analyzed using the headspace gas chromatography. Basic pharmacokinetic parameters (AUC, t_{1/2}, CL, CLR and VD) for TCE and TCA were estimated using WinNonLin. The excretion of TCA in urine was significantly reduced from that of controls as expected. The modulation of TCA clearance may have important implications in cancer risks thought to be posed by TCE to certain subpopulations (e.g., diabetics, alcoholics, obese individuals and persons taking CYP2E1-inducing medications). This work was supported by DOE Cooperative Agreement DE-FC09-02CH11109.

2048 DIFFERENTIAL GENE EXPRESSION IN THE UTERUS AND THE CERVIX: IMPLICATIONS FOR CHARACTERIZING THE EFFECTS OF ESTROGEN AND SERMS.

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When analyzing gene expression data, it is critical to minimize variation within dose-groups in order to enhance the ability to identify mode-of-action pathways. One contribution to heterogeneity within dose-groups can occur when tissues have regional variations of cell-types, such as the female reproductive tract. We describe a study where uterine gene expression data were generated on Affymetrix RAE230V2.0 GeneChips using ovariectomized (OVX) Sprague-Dawley rats. The rats were dosed daily with vehicle, 17 α -ethinylestradiol (EE) or tamoxifen (tam) for 28 days and at necropsy, the uterus was collected, weighed, and a portion flash frozen in liquid nitrogen for RNA isolation. The remaining portion was fixed in formalin and paraffin embedded. Global data analysis utilizing Principal Component Analysis (PCA), Multiple Dimensional Scaling (MDS) and Hierarchical Clustering (HCA) revealed that both the EE and tam dose-groups contained two samples that looked very different from the remaining three samples in each dose-group and that these four samples appeared to be more similar to each other than to their respective dose-groups. Detailed analysis of the gene changes in the outlier samples suggested the presence of a stratified squamous epithelium consistent with that found in cervix. Further investigation, including histological examination and molecular analysis of the formalin fixed and paraffin embedded sections, confirmed that cervical contamination of the uterus resulted in a dramatically different expression profile for the animals dosed with EE or Tam. In a separate confirmatory study in OVX rats with EE and tam, the uterine horns were dissected from the cervix and the two tissues were profiled separately on Affymetrix arrays. Analysis of these expression profiles revealed that the cervix and uterus have distinct profiles with some overlap of EE and tam induced genes. This work demonstrates the importance of removing cervical tissue as it confounded pathway analysis in the uterus.

2049 INTERACTIONS BETWEEN CYTOCHROME P450 2E1 AND MICROSOMAL EPOXIDE HYDROLASE IN EFFECTS OF 4-VINYLCYCLOHEXENE DIEPOXIDE IN THE MOUSE OVARY.

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4-Vinylcyclohexene diepoxide (VCD) is an occupational chemical that destroys ovarian small pre-antral follicles in rodents. VCD is detoxified to an inactive tetrol metabolite, 4-[1,2-dihydroxy] ethyl-1,2-dihydroxycyclohexane by microsomal epoxide hydrolase (mEH). *In vivo* dosing with VCD (80mg/kg, i.p.) showed no difference ($p>0.05$) in follicle loss between CYP2E1 +/+ and CYP2E1 deficient (-/-) mice (28-day old). A post-natal day 4 (PND4) mouse whole ovary culture system (enriched in target small pre-antral follicles) has been used to investigate ovotoxic effects of VCD as well as another ovarian toxicant, 7, 12-dimethylbenz[a]anthracene (DMBA), *in vitro* and it was shown that CYP2E1 deficient (-/-) mouse ovaries are less susceptible to VCD-induced follicle loss than their wild-type counterparts (129S1/SvImJ). Conversely, when incubated with DMBA, which, unlike VCD is bio-activated by mEH, there was more follicle loss in cultured ovaries from CYP2E1 -/- compared with CYP2E1 +/+ mice. Thus, these studies suggest a possible interaction between ovarian CYP2E1 and mEH activity. Lack of correlation between *in vivo* and *in vitro* studies may be explained by differences in age and heterogeneity of the older ovary. The present study was designed to evaluate expression of mEH in CYP2E1 +/+ and -/- PND4 mouse ovaries exposed to VCD. Ovaries were cultured in VCD (5 μ M) or control medium for 15d and expression of mRNA encoding mEH was measured by Real-Time PCR. Basal expression levels of mEH mRNA in CYP2E1 -/- ovaries was lower than that in +/+ ovaries, however, exposure to 5 μ M VCD caused a 3.5-fold increase in expression of mEH in -/- ovaries, whereas there was no change in +/+ ovaries. These findings suggest that CYP2E1 may play a role in regulation of mEH expression in the mouse ovary. (ES09246 and Center grant 06694)

2050 OVARIAN MICROSOMAL EPOXIDE HYDROLASE (MEH) IS INVOLVED IN 7, 12-DIMETHYLBENZ[A]ANTHRACENE (DMBA) INDUCED FOLLICLE LOSS IN AN IN VITRO OVARIAN CULTURE SYSTEM.

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DMBA causes ovarian follicle disruption in mice. Disruption of follicles by DMBA is attributed to the bioactivation of DMBA to a 3,4-diol-1,2-epoxide intermediate. DMBA is bioactivated in hepatic tissue by CYP 1B1 to a 3,4-epoxide which is then

hydrolyzed to form a 3,4-diol by mEH enzyme. This compound further undergoes epoxidation at the 1,2 position by CYP 1A1 or 1B1 to form the ultimate ovotoxicant DMBA-3,4-diol-1,2-epoxide. The mouse ovary expresses CYP 1A1, CYP 1B1 and mEH, and mRNA for these enzymes is inducible. These findings suggest that the ovary is capable of bioactivating DMBA to the 3,4-diol-1,2-epoxide. Previous studies have shown that DMBA induces significant ($p<0.05$) follicle loss by 6h in an ovarian culture system. The present study was designed to evaluate whether this loss of follicles in culture depends on ovarian mEH enzyme activity. Ovaries from post natal day 4 (PND4) B6C3F1 mice were incubated with vehicle medium (1% DMSO), mEH enzyme blocker cyclohexene oxide (2mM; CHO), 1 μ M DMBA, or 1 μ M DMBA and 2mM CHO for 6h. Following incubation, ovaries were collected and prepared for histological evaluation by classification and counting of follicles. Following 6h incubation, follicle numbers in control medium (DMSO) were: primordial, 386 $\pm$ 19.9 follicles counted/ovary; and primary, 311 $\pm$ 26.4 follicles counted/ovary. There was no effect of CHO on follicle numbers. DMBA incubation significantly ($p<0.05$) decreased follicle numbers (primordial, 204 $\pm$ 22.3 follicles counted/ovary; and primary, 203.5 $\pm$ 10.6 follicles counted/ovary). Whereas, addition of CHO to ovaries incubated with DMBA prevented ($p<0.05$) follicle loss (primordial, 357 $\pm$ 18 follicles counted/ovary; and primary, 297 $\pm$ 36.9 follicles counted/ovary). These observations support the hypothesis that PND4 mouse ovary is capable of bioactivating DMBA to its ovotoxic form 3,4-diol-1,2-epoxide, and ovarian mEH enzyme activity is likely involved (ES09246 and Center Grant 06694).

2051 TOXICITY OF A CLINICALLY RELEVANT DOSE OF HYDROXYUREA (HU) ON EMBRYO DEVELOPMENT.

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Sponsor: A. Ramesh.

The purpose of this study was to investigate the risks associated with a dose of HU used for the treatment of sickle cell disease (SCD) on ovarian physiology and embryo development. In Expt 1, 21 day-old female C57BL/6J mice were randomly assigned to a treatment or a control group (N=20/gp). Treatment consisted of oral HU (30 mg/kg) for 30 days; while control mice received saline (HU carrier). Four days to the cessation of HU dosing, all mice were subjected to folliculogenesis induction with PMSG (2.5 IU; IP) followed 48 hours later by ovulation induction with hCG (2.5 IU; IP). Ten mice/gp were anesthetized at 48 hours post PMSG to facilitate blood collection via cardiac puncture for E2 measurement by RIA. 16 hours post hCG, the remaining mice were sacrificed, ovaries excised and weighed and embryos harvested and cultured in Whitten's medium supplemented with CZBt (WCZBt). In Expts 2 and 3, folliculogenesis and ovulation were induced in untreated mice as described above, followed by embryo recovery. Embryos were either cultured continuously (Expt 2) or intermittently (Expt 3) in the presence or absence of bioavailable HU (18 μ g HU/ml of WCZBt). Data on ovarian wt, ovulation rate and serum E2 were compared with unpaired "t"-test, while those on embryo development were analyzed by Chi-square. Treated mice sustained decreased ovarian wt, ovulation rate and circulating E2 compared with controls ($P<0.05$). Fewer embryos retrieved from HU treated mice developed to blastocyst stage (32%) compared with those from controls (60%; $P<0.05$). Furthermore, continuous or intermittent *in vitro* exposures of embryos to HU also resulted in reduced development to blastocyst stage ($P<0.05$), with continuous *in vitro* exposure to HU fairsing worse compared with controls. Though HU is beneficial for the alleviation of the clinical manifestations of SCD, it compromises ovarian physiology and the ability of generated embryos to develop and consequently, women on HU should not attempt pregnancy (Supported by NIH grants G12 2RR003032, U-54 HD044315 & RO1 HD020419-19S1).

2052 AN INNOVATIVE STUDY OF OVARIAN AND REPRODUCTIVE EFFECTS OF LANSOPRAZOLE ADMINISTERED TO JUVENILE RATS.

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In earlier studies, ovarian weights and corpora lutea numbers were reduced in neonatal rats (7 days old) dosed with the proton pump inhibitor lansoprazole for 28 days at 50 mg/kg/day. In contrast, weanling rats (21 days old) dosed for 40 days at 150 mg/kg/day had no ovarian effects. The day of vaginal opening was not adequately assessed in either study. We designed a study to reconcile the different outcomes and determine any lasting effects on ovaries or reproductive capacity. Neonatal female rats were given vehicle, 1.5 or 50 mg/kg/day lansoprazole by gavage from 7 days old. Weanling female rats were given vehicle or 150 mg/kg/day from 21 days old. Both age cohorts were treated daily until 8 days after vaginal opening for all animals in the age cohort. Ten female pups (2/litter) from each of these groups were then necropsied (Interim) and an additional 2 female pups/litter were held until 70 days of age and then bred to untreated males (Reproductive). In

the Reproductive phase, estrous cycling was followed prior to mating and continued until mating was verified. Mated females were allowed to litter. Their parturition and offspring were assessed. Reproductive phase dams were necropsied 4 or 5 days after parturition. Ovaries from all Interim and Reproductive phase rats were weighed and examined for numbers of corpora lutea. The uteruses of Reproductive phase dams were examined for implantation sites. The day of vaginal opening was not affected by lansoprazole administration except in weanlings dosed at 150 mg/kg/day, where the mean day of opening was significantly increased (Day 34) compared to their controls (Day 31). Individual values were all within the historical range. This delay was attributed to a nonspecific slower growth rate in this group, rather than a specific effect on the reproductive system. Vaginal opening occurred at approximately 110 grams of body weight for the 150 mg/kg/day group and their controls. Lansoprazole had no effects upon ovarian weights, numbers of corpora lutea or reproductive function in this study.

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THE INHIBITION OF PROGESTERONE (P) FORMATION OF CULTURED OVARIAN THECA-INTERSTITIAL (TI) CELLS BY THE METHOXYCHLOR (MC) METABOLITE, HPTE, IS NOT MEDIATED THROUGH THE ESTROGEN RECEPTOR (ER) OR ANDROGEN RECEPTOR (AR).

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The pesticide MC is used on agricultural crops and livestock. MC exposure in rodents is linked to impaired steroid production, ovarian atrophy and reduced fertility. Following in vivo administration, it is rapidly converted to HPTE, reported active metabolite, by the liver. Both MC and HPTE have weak estrogenic and antiandrogenic activities, and these effects are thought to be mediated through the ER and ARs, respectively. Previous in vivo studies in female animals demonstrated decreased P production but no change in serum estrogen levels following exposure to MC. We showed a decrease in androgen and P production of follicular cells by HPTE in-vitro. The current studies examined the mechanism of actions of HPTE on P production by cultured ovarian TI cells from immature rats. To isolate TI cells, 24-25-day-old female Sprague-Dawley rats were injected (s.c.) with 20 I.U. of pregnant mare serum gonadotropin. Approximately 48 h following treatment, ovarian tissue was digested with collagenase and dispersed. TI cells were cultured in DMEM / F12 medium and exposed to HPTE for 24 h on the day of plating. Exposure to HPTE (0, 10, 50, 100 nM) alone inhibited P formation in a dose-dependent manner with a significant decline to ~12 % of control at 100 nM. In contrast, estrogens (estradiol, bisphenol-A, and 4-tert-octylphenol), pure anti-estrogen ICI 182,780 (ICI), and anti-androgens (4-OH-flutamide or the vinclozolin metabolite M-2) had no effect on P production even at 1000 nM. Co-treatment of HPTE with ICI did not block the effect of HPTE on P formation. These studies revealed that HPTE inhibition of P production by cultured TI cells is unique, and does not appear to be mediated through the ER or AR.

The findings and conclusions in this abstract have not been formally disseminated by NIOSH and should not be construed to represent any agency determination or policy.

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IN VIVO METHYL-PARATHION EXPOSURE IMPAIRS MALE FERTILIZING ABILITY IN MICE.

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Evidence supports that some environmental agents can interact with sperm DNA causing genetic damage that may affect fertility. Organophosphorous pesticides (OP) are among the most used agrochemicals and have been associated with male reproductive effects. We previously showed that methyl-parathion (Me-Pa), an OP, alters sperm quality and induces genotoxic effects in sperm that were related to oxidative damage. This study was conducted to determine the ability of mice spermatozoa to fertilize oocytes after Me-Pa exposure (20 mg/kg bw/ip), evaluating two stages of spermatogenesis in spermatozoa collected from cauda epididymis-vas deferens at 7 days post-treatment (dpt; cells at epididymal maturation at the time of exposure) and at 28 dpt (cells at meiosis at the time of exposure). Sperm quality, mitochondrial function using JC-1 staining, acrosome reaction induced by GABA (induced-AR), fertilization rate at 8 h after IVF, and lipid peroxidation (LPO) as malondialdehyde (MDA) production, were evaluated. Acute exposure to Me-Pa altered sperm quality (viability and motility) at both times, and a decrease in mitochondrial function was observed that was more pronounced at 7 than at 28 dpt

(19% and 77% of the control). The fertilization rate (pronuclear formation) was decreased and was also more severe at 7 dpt than at 28 dpt (21% and 55%) compared with the control group (73%). This suggests that cells at both spermatogenic stages are target of Me-Pa toxicity. An increase (3.2-fold) in MDA production was observed only at 7 dpt and was negatively correlated with mitochondrial activity ($r = -0.886$) and induced-AR ($r = -0.880$). These correlations observed between LPO and mitochondrial activity and AR suggests that the induction of oxidative stress by Me-Pa exposure may be involved in the alteration of events preceding fertilization, therefore, compromising the ability of spermatozoa to fertilize. This study was supported by CONACyT-Mexico (Grant #44643 given to BQV).

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PROTECTIVE ROLE OF α -TOCOPHEROL ON THE OXIDATIVE AND GENETIC DAMAGE CAUSED BY METHYL-PARATHION EXPOSURE IN MICE SPERMATOZOA.

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Organophosphate pesticides (OP) are widely used in Mexico and OP exposure has been associated with fertility problems in agricultural workers. OP induces the generation of reactive oxygen species which alter DNA integrity. We previously showed that methyl parathion (Me-Pa), an OP, alters sperm DNA that was associated with oxidative damage. Since oxidative stress can be reversed by antioxidant treatment, we evaluated the protective effect of α -tocopherol on the oxidative and genetic damage caused in spermatozoa by Me-Pa treatment. Adult ICR male mice were exposed to Me-Pa (3, 6 and 12 mg/kg, bw/i.p/5 days) or co-exposed with the highest dose of Me-Pa (12 mg/kg, bw/i.p/5 days) and α -tocopherol (50 mg/kg/i.g/5 days). Blood and sperm cells from cauda epididymis-vas deferens were extracted 24 h after the last administration, and erythrocyte acetylcholinesterase (AChE), sperm quality, lipid-peroxidation (LPO) by malondialdehyde (MDA) production and the chromatin structure by SCSA (DFI% parameter) were evaluated. Me-Pa exposure caused a dose-response decrease in AChE (52% to 71% inhibition). An increase in MDA at 6 (1.9-fold) and 12 mg/kg (2.5-fold) was observed, as well as an increase in DFI% (fragmented DNA), showing higher values of 6- and 22-fold at doses of 6 and 12 mg/kg, respectively, compared to the controls. Sperm viability and motility also showed dose-response decreases after Me-Pa exposure. On the other hand, animals co-exposed with Me-Pa and the antioxidant α -tocopherol showed values of LPO, DNA damage and sperm quality similar to the controls, compared with the exposed group; while inhibition of AChE was similar to the Me-Pa exposed group. These results strongly suggested that oxidative damage caused by Me-Pa is prevented by the antioxidant treatment, and that oxidative stress is involved in the sperm chromatin structure damage observed after OP exposure, but not in the AChE inhibition. This study was supported by CONACyT-Mexico (Grant #44643 given to BQV).

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TIME-DEPENDENT EFFECTS OF DI(N-BUTYL) PHTHALATE ON THE GENE EXPRESSION PATTERNS IN THE TESTIS OF SPRAGUE-DAWLEY RATS.

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Time-dependent effects of di(n-butyl) phthalate (DBP) on expression patterns of testicular gene were studied in Sprague-Dawley for different periods of exposure (1, 7, 14, and 28 days). A control group receiving corn oil was also monitored for comparative purposes. Gene expression patterns were measured using the reverse transcription-polymerase chain reaction (RT-PCR). Serum concentrations of DBP were increased with the time of exposure and were significantly higher in treated rats when compared to controls. Similar to significant decrease in testes weights, for 14 and 28 days, epididymis weight was also markedly reduced after 14 and 28 days exposure. DBP caused significant induction of steroidogenic acute regulatory (StAR) and SR-B1 mRNA at 14 and 28 days exposure in the group receiving 750 mg/kg/day. P450_{sc} mRNA was decreased in the group treated with 750 mg/kg/day at day 7; thereafter, an apparent increase in P450_{sc} mRNA was observed after 14 and 28 days exposure. High dose of DBP significantly increased the CYP17 mRNA level after 28 days exposure. At 7 days, significant reduction of CYP19 mRNA was observed only in the group exposed to 750 mg/kg/day of DBP. In addition, DBP significantly decreased mRNA levels of Spag4 and LDHA after 7 days exposure. Data indicate that acute and chronic exposure to DBP showed mechanistically distinct effects on steroidogenesis pathway in the testes of Sprague-Dawley rats. These results indicate that the steroidogenesis-related genes investigated in this study may provide insights into the molecular mechanisms underlying DBP induced testicular dysgenesis in humans.

2057 REVERSIBILITY OF THE ADVERSE EFFECTS OF 1-BROMOPROPANE EXPOSURE IN RATS.

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Previous experiments have revealed that 1-Bromopropane (BP), an alternative to chlorofluorocarbons, is toxic to the nervous and reproductive system. Here we investigated the reversibility of its toxic effects in rats. 72 male Wistar rats were divided into 3 groups of 24 each and exposed to 0, 400 and 1000 ppm of 1-BP via inhalation for 6 wks (8h/d, 7d/w). 8 rats from each group were sacrificed at the end of 6 wks of exposure. 4 and 14 wks postexposure, 8 rats from each group were again sacrificed to see the recovery changes. Sperm count, motility, morphology and testicular histopathology were studied. Blood pressure, skin temperature (for autonomic nervous system assessment) and hind limb muscle strength were also measured every 2 wks. For 1000 ppm the testicular and epididymal weights, sperm count and motility all remained significantly reduced for 14 wks postexposure along with an increase in abnormal sperm morphology. Testicular histopathology at 1000 ppm showed marked atrophy with no signs of recovery even until 14 wks postexposure. Retained spermatids were increased at 400 ppm at the end of 6 wk exposure. Also at 1000 ppm skin temperature got reduced following 6 wk exposure, blood pressure remained elevated until 4 wk postexposure and hind limb muscle strength remained reduced even until 14 wks postexposure. Brain Gamma enolase was also measured but not significant between the different groups. In terms of reversibility, 1-BP like 2-BP caused testicular atrophy with spermatogonial damage and remained irreversible until 14 wks postexposure. In the nervous system blood pressure and skin temperature effects were reversible but the muscle strength effects lasted longer until the end of the experiment. Differently from the previous study showing less toxic effect of 1-BP to spermatogonia at 800 ppm or below, 1-BP at a higher dose may cause depletion of spermatogenic cells and a longer lasting adverse effect on the nervous system.

2058 DISRUPTION OF TESTOSTERONE HOMEOSTASIS AS A MODE OF ACTION FOR THE REPRODUCTIVE TOXICITY OF TRIAZOLE FUNGICIDES IN THE MALE RAT.

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Triazoles associated with a range of reported reproductive effects in experimental animals were selected to assess potential toxic modes of action. Wistar Han rats were fed myclobutanil (M: 100, 500, or 2000 ppm), propiconazole (P: 100, 500, or 2500 ppm), or triadimefon (T: 100, 500, or 1800 ppm) from gestation day 6 to postnatal day (PND) 120. One male per litter was necropsied on PND1, 22, 50, or 92. Measurements included anogenital distance (AGD) at PND0, body/organ weights, serum hormones, age at preputial separation (PPS), sperm morphology and motility, and fertility and fecundity. AGD increased by the high dose of all 3 triazoles. T1800 delayed PPS. Relative liver weights increased at PND1, 50, and 92 by all 3 triazoles. Hepatocellular hypertrophy was present (PND50: P2500 and T500; PND92: M2000, P2500, and T1800). Relative pituitary weights decreased (PND92: M500 and M2000). Absolute testis weights increased (PND1: M100 and M500; PND22: M500; PND50: P500, T100, and T500). Relative ventral prostate weights increased (PND92: M500, T100, and T500). Serum testosterone increased (PND50: T500 and T1800; PND92/99: M2000, P500, P2500, T500 and T1800). Insemination and fertility were impaired (M2000 and T1800). In addition to the reproductive system effects, total serum thyroxine levels decreased (PND92: T1800). The reproductive effects are consistent with disruption of testosterone homeostasis as a key event in the mode of action for triazole-induced reproductive toxicity. [AKG and CRB were funded by U.S. EPA Cooperative Research Agreement No. CT826512010 with NCSU. This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy.]

2059 EFFECT OF MOTORCYCLE EXHAUST INHALATION EXPOSURE ON THE REPRODUCTIVE SYSTEM IN MALE RATS.

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Motorcycle exhaust (ME) is a major source of air pollution in the urban areas where motorcycles are a popular means of transportation. ME contains carcinogenic benzene and benzo(a)pyrene and poses a potential health hazard. The major objective

of the present study was to determine the male reproductive effects of ME using rats exposed to diluted ME by inhalation one hour each in the morning and afternoon, Monday through Friday, for 4 weeks. The ME inhalation exposure resulted in decreases of organ weights and sperm numbers in testis and epididymis. ME exposure produced testicular histological changes including seminiferous tubule atrophy and germ cell necrosis. The exposure induced epididymal pathological effects such as empty of sperm cells and formation of clusters of pyknotic and necrotic sperm cells. ME inhalation exposure decreased cytosolic superoxide dismutase and microsomal 7-ethoxycoumarin O-deethylase activities in the reproductive organs. ME increased the formation of thiobarbituric acid reactive substances by epididymis homogenate in lipid peroxidation assay. The results of RT-PCR analysis indicated that ME increased proinflammatory cytokine interleukin-6 and cell death regulators bax and cyclooxygenase-2 mRNA expression in testis and epididymis. These present results suggest that inhalation exposure to ME has the ability to induce reproductive toxicity in male rats and oxidative stress is a possible mechanism of toxicity.

2060 RECOVERY FROM ETHYLENE GLYCOL MONOETHYL ETHER-INDUCED DAMAGE IN THE REPRODUCTIVE SYSTEM OF MALE RATS.

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Ethylene glycol monoethyl ether (EGEE) can cause damage in the testes of several animals. In the present study, we examined the dose-dependent effects of chronic inhalation of the solvent on reproduction and sperm motion in male rats, and the recovery from the damage after termination of the exposure. Male Sprague-Dawley rats at 8 weeks old were exposed to EGEE at 0, 80, 250 and 700 ppm by inhalation, 8 hr/day, 6 days/week, for 9 weeks. The ratios of testes, epididymides and prostate to body weight were decreased by about 20% and significantly lower in the highest EGEE group than in the control. The percentages of motile sperm in cauda epididymis were dose-dependently decreased in the three EGEE groups, with significant difference between each of the EGEE groups and the control. The progressive sperm was also decreased in a similar manner to that of motile sperm. Velocity parameters (curvilinear velocity, VCL, average path velocity, VAP, and straight line velocity, VSL), parameter showing the swimming pattern (amplitude of lateral head displacement, ALH, and beat cross frequency, BCF), parameters such as straightness (STR) and linearity (LIN) were also affected in the medium and highest EGEE groups. With the sperm from spermatids, similar results were obtained as in the cauda epididymides. Some animals were allowed to live for 10 weeks after exposure, and the recovery was estimated. Although the ratios of reproductive organs to body weight were still lower in the highest EGEE group, the motion of sperm was normal, and there was not statistically significance between the control and any of EGEE groups. Part of the animals was mated with female rats after the termination of exposure, and the effect of EGEE on the reproduction of male rats was evaluated.

2061 TESTICULAR EFFECTS OF DI(2-ETHYLHEXYL)PHTHALATE (DEHP) FED IN A LOW PHYTOESTROGEN DIET TO SPRAGUE-DAWLEY RATS.

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DEHP, a major plasticizer in polyvinylchloride plastics, shows toxicity in multiple rat organs. Testes are the most sensitive target of DEHP, with developing animals more sensitive than adults. Most studies have utilized standard chow diets, and we have examined the effects of DEHP administered in a low phytoestrogen (soy- and alfalfa-free) diet. Weanling Sprague-Dawley rats were placed on Purina 5K96 diet and mated at PND 75. Pregnant dams were given feed containing 0, 2.5, 25, 250, 2500, and 10000 ppm DEHP from gestation day 7. Pups were continued on the same treatment as their dams after weaning and were terminated on postnatal day (PND) 14, 21, or 70. Absolute testes weights were significantly less than controls for all ages at 10000 ppm. Histopathological examination of testes revealed severe depletion of germ cells in all PND 70 males treated with 10000 ppm DEHP, but not in other dose or age groups. Mild Leydig cell hyperplasia was observed on PND 14 at 2500 and 10000 ppm, and on PND 70 at 10000 ppm. Serum and testicular testosterone levels were significantly lower than controls at 10000 ppm on PND 70, but serum inhibin B levels were not altered. However, relative to controls, testicular inhibin B, Sertoli cell counts (determined by sox-9 immunostaining), and serum FSH were significantly higher at 10000 ppm on PND 70. mRNAs for markers of Sertoli cells (inhibins, anti-müllerian hormone, androgen-binding protein) and Leydig cells (3 β -hydroxysteroid dehydrogenase) were significantly induced by DEHP treatment in PND 70 males. These results are consistent with studies utilizing standard chow diets in that high dose DEHP, approximately 1200 mg/kg/day, decreased germ cells and testosterone in adult F₁ males. In addition, dietary exposure of DEHP under the conditions of this study induced gene expression of markers for Sertoli and Leydig cells, increased Sertoli cell numbers, and increased serum FSH in F₁ adult male rats.

2062 AN ORAL TWO-GENERATION REPRODUCTIVE TOXICITY STUDY OF S-111-S-WB IN RATS.

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S-111-s-WB, a mixture of perfluoro fatty acid ammonium salts with varying carbon chain lengths (C7-C13), was administered via oral gavage to 30 animals/sex/group at dosage levels of 0.025, 0.125 and 0.6 mg/kg/day over two generations in Crl:CD(SD) rats. This study was conducted to evaluate potential adverse effects on reproductive outcome and on neonatal survival, growth and development. One litter per dam was produced in each generation. In addition, blood levels of the major components were determined one week prior to mating, and during gestation and lactation for dams and their offspring. No test article-related morbidity or deaths occurred except for one F0 male that was euthanized in extremis following 14 weeks of dose administration. Parental reproductive performance, reproductive organ weights, mean number of F1 or F2 pups born, survival, general physical condition and pre-weaning weights, and developmental landmarks were unaffected at any dose. Lower mean body weight gains and/or body weight losses were observed in F0 and F1 males in the 0.6 mg/kg group after 7-8 weeks of dosing and continuing throughout the remainder of each generation. Higher liver weights were noted for F0 and F1 parental males in the 0.125 and 0.6 mg/kg groups, F0 and F1 parental females in the 0.6 mg/kg group, F1 male and female pups in the 0.125 and 0.6 mg/kg groups and F2 male and female pups in the 0.6 mg/kg group. Hepatocellular hypertrophy was noted for the majority of F0 and F1 males in all test article-treated groups and several F0 females in the 0.6 mg/kg group. Higher kidney weights were observed for F0 and F1 parental males and females in the 0.125 and 0.6 mg/kg groups and correlated to renal tubule hypertrophy, noted for the majority of F0 males, all F1 males and several F0 females in the high dosage group. Serum concentrations in dams and pups were generally considered to be proportional to the dosage.

2063 EVALUATION OF HEXAMETHYLDISILOXANE (HMDS) IN A 2-GENERATION REPRODUCTIVE TOXICITY STUDY IN RATS.

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Hexamethyldisiloxane is an intermediate for the manufacture of other silicone materials. It is also used as a solvent in the electronics industry and as an ingredient in a variety of personal care products. Groups of male and female Sprague-Dawley rats (30/sex/group, ~7 weeks old) were exposed via whole-body vapor inhalation to a concentration of 0, 100, 400, 1600 or 5000 ppm for 6 hr/day. Exposure for the F0 and F1 generations started at least 70 days prior to mating and lasted through weaning of the respective pups on postnatal day (PND) 21. Female exposures were interrupted from gestation day 21 through PND 4, resumed on PND 5 and continued until weaning on PND 21. Clinical signs, body weights and food consumption were recorded at appropriate intervals. Unselected F1 and F2 weanlings were necropsied on PND 21 or 28. There were no test article-related clinical signs in any generation. Mean body weights for both sexes in the 5000 ppm group were reduced throughout the majority of both generations. Increased kidney weights were noted in the 1600 and 5000 ppm group males of both generations with corresponding microscopic findings at 5000 ppm of hyaline droplets (consistent with alpha-2 urinary globulin) and basophilic renal tubules. Reproductive performance, offspring physical condition, postnatal survival and attainment of landmarks of sexual development were unaffected by HMDS exposure. Estrous cyclicity, time between pairing and mating, mating and fertility indices, duration of gestation and parturition in F0 and F1 generations were unaffected by HMDS exposure. HMDS-related decreased offspring body weight gains were observed for F1 and F2 pups in the 5000 ppm group from PND 4-21 and PND 4-14, respectively. In this study, the NOEL for parental reproductive toxicity was 5000 ppm HMDS and was 1600 ppm for neonatal toxicity due to decreased offspring weight at 5000 ppm. (Supported in part by the Silicones Environmental, Health and Safety Council of North America).

2064 EVALUATION OF THE REPRODUCTIVE AND DEVELOPMENTAL TOXICITY OF SODIUM TUNGSTATE IN RAT MODEL.

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The Green Armament Technology (GAT) program, a U.S. Army pollution prevention initiative recommended substitution of lead (Pb) with tungsten (W) in military munitions. Accordingly, this led to increased W utilization in several military

applications with potential rise in occupational exposure of military personnel to W. The toxicological profile of W is sketchy and incomplete and recent events such as high solubility of W in drinking water have generated new concern. Consequently, the purpose of this study is to investigate one-generation reproductive effects of soluble W using USEPA OPPTS Guideline 870.3800 with minor modification. Adults Sprague-Dawley rats (P0 generation) were dosed daily by oral gavage with 125, 62.5 or 5 mg/kg/day of sodium tungstate (NaW) or dH2O (n=40/sex group) for 70 consecutive days. Mating occurred after 14 days and dosing continued through pregnancy. Evaluated effects include weight gain, gestation and offspring viability. Significant tissue retention of W (p<0.001; n≥3) was detected in the spleen, ovaries and femur of P0 and F1 (offspring) generation exposed to NaW when compared to the controls. Gestation lengths (22.081±0.089) in days for 125 mg/kg/day group were significantly different (p<0.05; n≥37) from controls (21.548 ± 0.097). Weight gain in P0 (5 mg/kg/day) males was 16.4% (p<0.001; n≥30) more than the controls. Weight gain in F1 generation (62.5mg/kg/day) on PND 20 when weaning occurred and on PND 70 was 5.2% to 33.3 % (p<0.05; n≥25) more than the controls for both sexes. Histopathology report in several P0 males (125 mg/kg/day) includes histological lesions, lymphohistiocytic inflammation with cardiomyocyte degeneration and necrosis within the heart. Hematology, serum chemistry and immunology effects are ongoing. These results suggest that oral exposure to high levels of soluble W may cause some signs of reproductive, developmental or systemic toxicity.

2065 28-DAY ORAL AND REPRODUCTIVE/DEVELOPMENTAL SCREENING TOXICITY STUDIES OF VINYL-TRIS(2-METHOXYETHOXY)SILANE.

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Vinyl-tris(2-methoxyethoxy)silane (VTMES) is used as a cross-linker in ethylene propylene diene monomer rubber and for moisture-curable thermoplastic polyolefin applications. The potential toxicity of VTMES, a High Production Volume Chemical (HPV), was evaluated in a combined repeated dose toxicity with a reproductive/developmental toxicity screening test. The study design utilized the protocol in accordance with the OECD and EPA test guidelines Nos. 422 and 870.3650, respectively. The test article was administered in dehydrated corn oil by gavage to 10 rats/sex/group, seven days a week, at 0, 25, 75 and 250 mg/kg/day. There were no test article-related effects on body weight, body weight gains, Functional Observation Battery or locomotor activity evaluation except for a reduction in body weight gain in the high dose males. Reductions in red blood cells, reticulocytes, platelets, eosinophils and basophils in both sexes in the high dose group correlated microscopically with bone marrow hypocellularity. An overall decrease in the erythroid:myeloid ratio was considered a disproportionate mild suppression of both myeloid and erythroid elements. Reduced thymic weights in both sexes in the high dose group correlated microscopically with lymphoid depletion. Reductions in testicular weights in the 250 mg/kg group correlated microscopically with seminiferous tubule degeneration. Decreased prostate weights in the 75 and 250 mg/kg groups males correlated with decreased secretion and/or atrophy. Reduced fertility, increased mean number of days between pairing and coitus and gestation length were observed in the 250 mg/kg group. Only one female in the high dose group delivered, but all pups were dead on PND 0. Live litter size and postnatal survival were also reduced in the 75 mg/kg group. The NOELs were 25 and 75 mg/kg for male and female systemic toxicity, respectively, and 25 mg/kg for reproductive and developmental toxicity. (Sponsored by the Silicones Environmental, Health and Safety Council).

2066 TWO-GENERATION REPRODUCTION STUDY OF BISPHENOL A (BPA) IN CD-1® SWISS MICE.

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A 2-generation study (OECD Test Guideline 416), incorporating estrogen sensitive endpoints, was conducted to evaluate the potential reproductive/developmental effects of BPA in mice. The following groups (28 parental mice/sex/group) were 6 BPA groups with doses from ~0.003 to 600 mg/kg/day (mkd; 0.018-3500 ppm); 17-β-estradiol (E2) positive control (0.08 mkd; 0.5 ppm); 2 negative control groups; 1 unbred F1 male/litter/group exposed concurrently with the F1 parental males. The F0 generation was exposed through weaning (pnd 21) of the F1 pups when selected F1 pups were similarly exposed to study termination at F2 weaning.

Body weights (wt), feed consumption, clinical observations, F0/F1 estrous cyclicity, F1/F2 anogenital distance and acquisition of F1 puberty (vaginal patency and preputial separation [PPS]) were recorded. Organ wt and histopathology were recorded for F0/F1 adults and F1/F2 weanlings, and andrology was assessed for F0/F1 males. E2 resulted in systemic, reproductive and developmental effects consistent with a potent estrogen and comparable to effects observed in a previous E2 reproduction study. At 3500 ppm BPA (~600 mkd), systemic effects included reduced body wt (F1 only), increased kidney and liver wt, centrilobular hepatocyte hypertrophy, and kidney nephropathy in F0/F1 adults; reduction in pup body wt (F1 only), reduced spleen wt (both sexes). Reduced testes wt, with seminiferous tubule hypoplasia (F1/F2 weanlings), and delayed PPS (F1) were indicative of delayed development. At 300 ppm BPA (~55 mkd), the only treatment-related effect was an increased incidence of centrilobular hepatocyte hypertrophy in adults (both sexes). The NOEL was 30 ppm BPA (~5.5 mkd) for systemic toxicity and 300 ppm BPA for developmental toxicity. There were no reproductive effects at any BPA dose.

2067 AN UPDATED WEIGHT OF THE EVIDENCE EVALUATION OF REPRODUCTIVE AND DEVELOPMENTAL EFFECTS OF LOW DOSES OF BISPHENOL A.

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Recent public concern has focused on potential reproductive and developmental human health effects from exposure to low levels of Bisphenol A (BPA). An expert panel convened by Harvard's Center for Risk Analysis published a weight-of-evidence evaluation of reproductive/developmental toxicity from BPA exposure ≤5 mg/kg-day (Gray et al., 2004), and we updated this to include >50 studies published through February 2006 (Goodman et al., 2006). Since then, several additional relevant low-dose studies have been conducted, including a two-generation study in CD-1 Swiss mice. Results from these studies are consistent with those reviewed by the Harvard Panel and by us, including two multi-generation rat studies. Overall, although some statistically significant effects of BPA have been reported in rats and mice, positive findings for most endpoints are countered by more numerous studies showing no effects. Thus far, positive findings have not been replicated, do not exhibit coherence and plausibility, and do not show consistency across species, doses and time points. These unconfirmed positive findings include morphological changes in testes and sperm in oral mouse studies. In addition, some mouse studies report morphological changes in male and female reproductive organs after non-oral exposures to BPA. Owing to the lack of first-pass metabolism, results from non-oral studies are of limited relevance to oral human exposure. Human biomonitoring indicates exposures significantly lower than the "low" doses in the reviewed animal studies, and reports of human health impacts are very limited and inconsistent. Taken together, the weight of evidence does not support the hypothesis that low oral doses of BPA adversely affect human reproductive and developmental health.

2068 BENZO(A)PYRENE DECREASES BRAIN AND OVARIAN AROMATASE mRNA EXPRESSION.

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Aromatase catalyzes the conversion of androgens to estrogens. In fish, there are two isoforms of aromatase (CYP19): CYP19A1, a gonad form and CYP19A2, a brain form. Estrogen affects the dimorphic, anatomical, functional and behavioral aspects of development of both sexes. CYP19 expression was measured following BaP exposure (0, 10, 100 µg/L waterborne for 10 or 15 days) in *Fundulus* adults, juveniles and embryos by *in situ* hybridization. Constitutively, CYP19A1 was expressed mainly in the ooplasm of stage I and II oocytes (primary growth stage) as strong and evenly distributed signals, but in the BaP treated group, the CYP19A1 expression was significant decreased in the 3 month old *Fundulus*. Expression of CYP19A2 was detected in the brain including the hypothalamus in 4, 6, 8, 10, and 14 dpf embryos. In adult fish brain, the highest expression was detected in the hypothalamus and hypophysis of both sexes. At 14 dpf, BaP exposed embryos had significantly decreased (3.31-fold) CYP19A2 compared to controls. Similarly, in adults, CYP19A2 expression was significantly decreased particularly in the brain hypophysis and hypothalamus regions (85% and 81% of controls, respectively). These studies provide a greater molecular understanding of the mechanisms of action of BaP and its potential to impact reproduction or development. Supported by NIEHS R01ES012710.

2069 ASSESSING THE TOXICITY AND ESTROGENICITY OF 4-PENTYLANILINE USING MEDAKA (*ORYZIAS LATIPES*).

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A variety of natural and synthetic chemicals have been shown to be estrogenic and capable of altering reproduction in aquatic organisms. *In vitro* methods using rainbow trout (RBT) for determining estrogen receptor (ER) binding and liver slice vitellogenin mRNA (VTG) induction are being used to develop QSARs for predicting the estrogenic activity of chemicals in lower vertebrates. For risk assessments, it is important to establish the relationship of *in vitro* screening methods to higher level effects such as growth, survival and reproduction. The goal of this study was to use reproduction assessments in medaka to determine the relationship of higher level effects to *in vitro* endpoints. These comparisons were performed with 4-pentylaniline (PA, CAS 33228443) a chemical shown to be estrogenic using RBT *in vitro* assays. Thirty-six medaka breeding pairs were distributed (unbalanced) into 2 L glass aquaria and exposed to PA at 0.06, 0.28, 0.69, 2.45, and 8.76 µM. The fecundity and fertility of these pairs were assessed for 21 days. Spawns from days 15-16 were incubated to measure hatching success. A kinetic uptake study indicated that the BCF of PA in medaka livers was ~ 65. At the LOEC of 2.47 µM PA, the following effects were observed; VTG induction (100-fold) in males, reductions in fecundity, male mortality (2 of 4) and decreased hatching success. At higher PA levels, female mortality (4 of 4) and 375-fold VTG induction in males were observed. Using the BCF of 65, the medaka liver PA level at the LOEC was 160 µM, a level similar to the RBT *in vitro* EC50 for VTG induction. The *in vivo* LOEC for fecundity and male VTG induction were similar to the *in vitro* EC50 for VTG induction. These data suggest, with further *in vivo* testing, that QSARs based on *in vitro* assays for weak estrogens are useful for prioritizing environmental chemicals most likely to impact reproduction.

2070 REPRODUCTIVE TOXICITY TESTING AND CONTROL BACKGROUND DATA IN THE CYNOMOLGUS MONKEY.

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To assess the effects of test substances on reproductive functions and embryo-fetal development in cynomolgus monkeys (*Macaca fascicularis*), menstrual cycle and fetal evaluation control data are particularly important. Menstrual cyclicity in cynomolgus monkeys (146 female monkeys for a total of 907 cycles), pregnancy ratio, control background data from teratology studies (fetuses evaluated at gestation day [GD] 100) and peripheral blood lymphocyte subsets data (flow cytometry) were evaluated. The menstrual cycles were variable within and between females (average of all individual cycles: 30.5 ± 7.7 days, average of mean value of each female: 30.9 ± 4.5 days). In teratology studies (control animal data), the mean abortion/embryo-fetal death incidence was 7.7%, and 62% of the abortions occurred during the first half (GD20-GD35) of the organogenesis period (GD20-GD50). The mean fetal weight at GD 100 was 114 g. There were no external fetal abnormalities, a low incidence of visceral abnormalities (absence of left thyroid in 1 out of 98 fetuses, 1.0%), and a 2.6% incidence of skeletal abnormalities. The most common variation was lumbar rib (10.4%). In a teratology validation study using thalidomide as the proximate teratogen (15 to 20 mg/kg/day; dams dosed on GDs 26, 27, and 28), abnormalities extensively published such as micromelia/amelia, paw/foot hyperflexion, ectrodactyly, polydactyly, syndactyly or brachydactyly were observed. The continued accumulation of normal background data accompanied by the low incidence of abortions and fetal abnormalities/variations establishes the female cynomolgus monkey as a more suitable nonhuman primate animal model for reproductive and developmental toxicity testing.

2071 FEASIBILITY OF GROUP HOUSING OF SEXUALLY MATURE CYNOMOLGUS MONKEYS FOR REPRODUCTIVE TOXICITY STUDIES.

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Among nonhuman primates, cynomolgus monkeys (*Macaca fascicularis*) are the species most commonly used for reproductive and developmental toxicity evaluation and animals are housed singly in order to avoid interferences imposed by social rank and associated behaviour. However, European guidelines emphasize social housing for nonhuman primates in order to provide improved social interactions

and enriched environments. We, therefore investigated the effects of single vs same sex group housing of mature monkeys on reproductive parameters (semen analysis, testis size, ovarian cycle length, endocrine analysis). Initially, a newly formed group of 7 males was studied over 26 weeks and a group of 8 females over 15 cycles (3 pre-dose, 12 following group formation). Social rank was determined by food challenge test. First analysis revealed (1) marked albeit transient alterations of testicular size without consistent changes of semen parameters (ejaculate weight, sperm motility and number) in subordinate animals and females transiently exhibited irregular ovarian cycles. Among females these effects correlated with absent/decreased pre-ovulatory estradiol peak and luteal progesterone increase. In contrast, no effects on testosterone, inhibin B and FSH could be found in males. Importantly, reproductive parameters were less affected when smaller groups (n= 4) and animals sharing the room prior to group transfer were used (n=10). In conclusion, group housing of sexually mature animals markedly affected certain reproductive parameters. Small group size and appropriate animal selection might provide a feasible avenue towards using group-housed mature cynomolgus monkeys in reproductive toxicity studies. Further work is needed to confirm and extend the latter aspect.

1. Niehoff et al. (2005) Zitat aus Toxicol. Sci (abstracts von SOT 2005)..

2072 MARMOSETS (CALLITHRIX JACCHUS) AS MODEL FOR DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) EVALUATION.

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Generally, for nonhuman primate DART studies, macaque monkeys are the model of choice. However, due to development of therapeutic antibodies with limited cross-reactivity or test items with specific metabolic properties, DART studies in the marmoset model became a need. In general, reproductive physiology of the marmoset is different from that of human and Old World Monkeys (1) with the clinical relevance of this animal model being unclear (2). However, the sensitivity of marmosets to teratogens such as thalidomide derivatives is proven (3). This evaluation deals with the feasibility of DART studies in the marmoset model. Since marmosets lack menstrual bleedings, a standardized approach based upon progesterone (not estradiol) determination and experimental induction of luteolysis using 0.8 µg cloprostenol was established for the monitoring of ovarian cyclicity and ovulation: Cycle duration was 28.7 ± 6.3 days for 110 individual cycles. This approach permits female fertility evaluation and was used to develop an embryofetal development study regimen. Controlled cycling females were housed with males (1:1), pregnancy was detected by ultrasonography (mating success of >70% in >130 matings) and dosing lasted from day 25 to 85 post-ovulation or longer. Cesarean sections were performed on day 110 ± 1 yielding 1-4 live fetuses/female. Fetal evaluation was feasible using standard macaque protocols. A panel of male fertility parameters was also established comprising testosterone, testicular volumes (by caliper), semen evaluation (sperm number, motility and morphology), testicular flowcytometry and spermatogenesis staging. However, unlike in macaques, reliable assays for inhibins and FSH are not available for marmosets. In conclusion, (limited) DART evaluation is feasible in marmosets if macaque models cannot be used. 1. Abbott et al 2003 Comp Med 53:339; 2. Zuehlke & Weinbauer 2003 Toxicol Pathol 31:123; 3. Merker et al 1988 Arch Toxicol 61:165;

2073 COMPARISON OF VAGINAL CYTOLOGY CONTROL DATA GENERATED AT SEVEN LABORATORIES IN FISCHER 344 RATS AND B6C3F1 MICE.

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To understand variations in vaginal cytology analysis, the control animal data from 51 studies were compiled for comparison. The slides and necropsy data came from seven different NTP contract laboratories performing 90-day toxicology studies for NIEHS.

Twelve-day vaginal cytology samples were collected by seven NTP laboratories following the same sampling procedures. Slides were fixed, stained and subsequently submitted to Gene Logic, Inc. for evaluation. Estrous stages were determined and the data were evaluated for cycling or non-cycling, cycle numbers and cycle length. Data from the control groups of 51 studies were summarized, and discrepancies between studies and laboratories on these parameters are exemplified and compared. In conclusion, there were no obvious discrepancies noted in the percent cycling, percent regular cycle, cycle numbers or cycle length among studies or laboratories, in either F344 rat or B6C3F1 mouse studies. The percent of the cycle spent in the various stages was also comparable.

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2074 AN ASSESSMENT OF THE EFFECT OF THE ROUTE OF DOSE ADMINISTRATION ON ESTROUS CYCLES OF CDOIGS RATS FOR FEMALE FERTILITY (ICH-1) STUDIES.

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During the course of female fertility and early embryonic development (ICH-1) studies estrous cycles are monitored. Various methods of dose administration are employed during the course such studies e.g., oral gavage, subcutaneous injection, nose-only inhalation exposure and continuous intravenous. Data was obtained from rats (CD-IGS) that were 10 to 12 weeks of age at the start of treatment. Stages of the estrous cycles were determined daily by examination of wet smears prepared from a vaginal lavage. Cyclicity data were collected during the 2-week acclimation period prior to the start of dosing and only females with normal estrous cycles were assigned to the studies. Estrous cycle data was then collected during the 2-week pre-mating treatment period and during the mating period until identification of mating. For inhalation studies, the females were acclimated to the restraint tubes for increasing periods of time for a minimum of 3 consecutive days prior to the initiation of treatment, of up to 1-hour duration. The females dosed by continuous infusion had catheters surgically implanted under general anesthesia in the vena cava, via the femoral vein, at least 1 week before the start of the pre-mating dosing period and two weeks prior to the start of treatment. Comparison of data for the pre-study period shows no effect of pre-study procedures for either the inhalation or intravenous infusion groups. During the dosing period values for cycle length, number of days in estrous and number of cycles were comparable for the various treatments. The various methods of administration showed no adverse effect on the estrous cycle parameters of female CDO IGS rats. It was concluded that estrous cyclicity data collected during the pre-mating dosing period for fertility studies with CDO IGS rats were unaffected by either routine, nose-only inhalation exposure or continuous infusion treatment procedures.

2075 COMPARISON OF CONTROL MALE REPRODUCTIVE DATA FROM SEVEN LABORATORIES.

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To understand variations between strains and laboratories in sperm motility, sperm density, spermatid head counts and male reproductive organ weights, the control animal data obtained from studies of NTP chemicals conducted at 7 different contract laboratories are compiled for comparison.

Data on sperm motility (manual evaluation), sperm density, organ weight (right testis, right epididymis, and right cauda epididymis), and terminal body weights were collected by NTP laboratories performing 90-day toxicology studies (55 studies). The spermatid head counts were collected by Gene Logic from frozen testes harvested by the NTP laboratories. Fischer 344 rats and B6C3F1 mice were the species and the strains used predominantly; a few studies used Long Evans rats or AM3-C57B1/6, BALB/c, TgAC, or FVB mice.

In general, all measured parameters are comparable between Fischer 344 and Long Evans rats. Variations among the 7 laboratories are considered minimal in all tested endpoints. When compared to B6C3F1 mice, sperm density (per mg cauda epididymis and per cauda epididymis) was lower in TgAC and FVB; the spermatid concentration (per testis) was lower in TgAC, FVB, and BALB/c mice; the reproductive organ-to-body weight ratios were lower in TgAC, FVB, and AM3-C57B1/6 mice; and the terminal body weight and reproductive organ-to-body weight ratios were lower in BALB/c mice.

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2076 EVALUATIONS IN CD-1 MICE INCLUDING REFLEX AND PHYSICAL DEVELOPMENT, PASSIVE AVOIDANCE AND SPERM ANALYSES IN FERTILITY, PERI-POSTNATAL, PEDIATRIC AND/OR DEVELOPMENTAL NEUROTOXICOLOGY EVALUATIONS.

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Although rats are more frequently used than mice in reproductive/developmental, pediatric and developmental neurotoxicology studies, because of a smaller size and/or specific sensitivity to a test material there is often a need to evaluate mice. The current presentation provides historical control data for reproductive performance, peri-postnatal development and sperm evaluation. Data are from studies performed under GLP conditions using Crl:CD1(ICR) mice. In 21 studies, mean estrous stages/14 days = 3.0 pretreatment and 3.5 with treatment. Mean time in

cohabitation was 4.2 days (87% mated by the male 1 and 13% mated by male 2), with 96% fertility. Daily evaluations of surface righting showed that 23.8% of the mice were able to right on postpartum day (PPD) 1, and that by PPD 10, 100% attained criterion. Pinna unfolding was first evident PPD 3 and present for all mice PPD 7. Eye opening was first present PPD 13 and present for all mice PPD 17. The acoustic startle reflex was present in 57.9% of the pups on PDD 13 and for all pups on PPD 19. In 4 studies, passive avoidance learning for both male and female mice required 4.1 (3.6 to 4.6) trials to criterion on day 1 of training. No mouse failed to learn. Passive avoidance retention was evident for both male and female mice, with 2.6 (2.3 to 2.9) trials to criterion on day 2 of training. Females had slightly lower latencies than males [mean latency of 50.2 sec. (41.6 to 59.4) versus 47.3 sec. (36.8 to 58.9) on day 2, although the variability precludes a sex-related difference. Sperm analyses (Hamilton Thorne IVOS) in 11 studies (269 male mice) gave a vas deferens sperm count mean of 416.7 motile (348.2 to 498.1) and 90.8% motile (87.8% to 93.8%) and an epididymal sperm count of 75.9 (55.6 to 103.1) and sperm density of 2097.40 (1510.10 to 2858.84). Based on these data, mice can be studied for these parameters, and the data are no more variable than for rats.

2077 CAESAREAN DERIVATION AND FOSTERING OF RAT PUPS IN ICH PRE- AND POST-NATAL DEVELOPMENTAL TOXICITY STUDIES.

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The ICH pre- and post-natal study design involves treatment during gestation and lactation up to weaning. Such studies are almost exclusively performed in the rat, with no feasible non-rodent species. This limitation poses a severe problem for drugs, such as non-steroidal anti-inflammatory agents, that block the onset of parturition in rodents by interference with prostaglandin synthesis (an effect that is not pertinent to non-rodent species). For short-acting agents, it is usually considered acceptable to suspend treatment at the end of gestation for two or three days while parturition takes place, but this is not applicable for long-acting drugs. A possible solution is to treat rats throughout gestation, remove the pups by caesarean on the presumed day of parturition and have the pups raised by a treated foster mother. Having developed the technique in a small investigative trial, a larger experiment was performed to generate reference data for use in regulatory studies. 30 female SD rats were treated with 5 mL/kg of 2 % cremophor in isotonic saline by gavage from days 6 to 20 of gestation. The females were submitted to a caesarean section on day 21 or 22 of gestation. The live pups were removed and given to a foster dam that had littered within the last 24 hours. The foster dams were then treated at the same dose volume from days 1 to 20 of lactation. 24 litters were successfully fostered. One further dam failed to adopt 2 successive litters. In total, 31 out of 286 pups (11%) adopted by the foster dams died before litter standardisation on day 4 of lactation. This compares with a historical average of 4% or less for non-fostered pups born to untreated rats in our laboratory. There were no pup deaths after day 4. The pup weights on day 1 were lower than usual (6.6g on average, compared with 7.6g), but the difference was recovered by the time of weaning. In conclusion, the caesarean derivation and fostering technique is considered feasible for use in regulatory developmental toxicity studies for drugs that block the onset of parturition in rodents.

2078 OCCURRENCE OF HEADLESS SPERMS IN ADOLESCENCE RAT URINE.

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Increased incidence of headless sperms (HS) in urine was spontaneously observed in adolescence naive male Crj:CD(SD) rats. To clarify the factors contributing to this event, the incidence of HS in urine and the epididymis was periodically examined in conjunction with the measurement of testis and epididymis weights, motility of sperms, testicular testosterone and transferrin or serum FSH levels. The urinary HS incidence was 61%, 69%, 44%, 30%, 14%, 9% and 7% in 100 sperms counted at 8, 9, 10, 11, 12, 13 and 14 weeks old, respectively; namely, peaked at 9 weeks old, gradually decreased from 10 weeks old, and became almost a plateau from 12 weeks old onwards. The epididymal HS incidence, 14.7%, 3.6%, 0.4% and 0.4% at 8, 10, 12 and 14 weeks old, which was extremely less than that in urine, decreased from 10 weeks old with increased sperm motility, and became mostly zero from 12 weeks old. In scanning electron microscopy of HS in the epididymis, a narrow gap between the head and neck of sperm was clearly seen along with the posterior ring. Testicular transferrin concentrations, a marker for Sertoli cell maturation, increased from 10 weeks old, and testicular testosterone increased from 12 weeks old. In contrast, no change in serum FSH concentration was seen

throughout the study period. These results demonstrate that an increase in urinary and epididymal HS incidence in adolescence naive rats aged 8 to 9 weeks old would be a physiological phenomenon seen in the process of the Sertoli cell maturation.

2079 MATERNAL MORTALITY ASSOCIATED WITH A TWO-GENERATION REPRODUCTION TOXICITY STUDY WITH HFC 245FA IN SPRAGUE DAWLEY RATS.

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The study was conducted following the OECD Guideline 416 "Two-Generation Reproduction Toxicity Study". The exposure levels of 0; 2,000; 10,000 and 50,000 ppm were based on results from a 13-week study.

Unexpected mortality of lactating dams in the medium and high dose group was observed in both generations beginning with day 10 of lactation. Histopathology revealed that the brain, particularly the cerebellum and to a minor degree the heart, turned out to be the toxicological target tissues of the substance.

Statistically significant histopathological alterations were observed in the cerebellum of females of the high dose group of both generations. No changes were found in the brain in males or non-pregnant females for all exposure groups.

The neuronal necrosis and degeneration mainly in the cerebellar cortex was the most severe finding. Spongiosis in diverse severities was found in 7 females of the F0-generation and in 9 females of the F1-generation of the high dose group. The vacuolation of the brain tissue appeared more pronounced in the cases of the F1-generation. The vacuolation was most severe in the Purkinje cell layer due to accumulation of fluid in the astrocytes.

The lesions found in the cerebrum were restricted to the F0-generation and less pronounced than in the cerebellum. The main findings were glial scars in the corpus callosum of two female rats of the high dose groups.

The increased incidence of myocardial fibrosis and mononuclear cell infiltration in the males, indicating a cardiomyopathy, was only seen in the F0-generation. Only the females of the F1-generation showed a statistically significant increase of minimal myocardial fibrosis. Serum enzyme measurements showed no evidence for acute myocardial damage.

The reason for the maternal mortality is possibly an indirect interaction of the compound and the stress associated with lactation.

2080 THE EFFECT OF INHIBITORS ON JET FUEL ALIPHATIC HYDROCARBON INDUCED TOXICITY IN HUMAN EPIDERMAL KERATINOCYTES.

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Human epidermal keratinocytes (HEK) were exposed to JP-8, pentadecane (pent), tetradecane (tetra), tridecane (tri), undecane (un), or control for 5 min. The hydrocarbons (HC) were removed, cells rinsed and incubated for 0, 1, 4, 8, and 24 h. Media from each set of treatments was pooled and stored at -80°C until assayed for cytokines. Cell viability was determined by neutral red. Additional studies were conducted to determine the ability of inhibitors to block HC-mediated inflammation. Studies were identical, with a 1 h preincubation of HEK with parthenolide (P), isohelenin (I), SB 203580 (SB), substance P (SP), or recombinant human IL-10 (rHIL-10). HEK were incubated in media containing the inhibitor after HC exposure. At 24 h, JP-8 and un caused the highest HEK mortality, followed by tri, tetra, and penta. While the addition of the inhibitors did not change this trend, each inhibitor alone significantly (p<0.05) decreased HEK viability within each treatment. DMSO, the vehicle for P, I, and SB, had minimal effect on viability. Overall, terminal IL-8 was suppressed by each inhibitor. P was the most effective inhibitor of IL-8; un, tri, tetra, and penta caused significantly less IL-8, while JP-8 caused significantly higher release than controls. Normalizing by the control indicates which inhibitors suppress IL-8 to control levels. In addition to P, SP suppressed HC effects; I suppressed un and penta, while SB and rHIL-10 had little effect. No inhibitor suppressed JP-8 to control levels. P, I, and SB suppressed IL-6 values below the controls. HEK viability, IL-8, and, to a lesser extent, IL-6 are reliable biomarkers to assess the effectiveness of these inhibitors. JP-8, a mixture of over 200 HC, had the greatest toxic effect on HEK viability and inflammation, followed by un. No difference was noted between tri, tetra, and penta. P, SP, and I, inhibitors of the NF-κB pathway, are effective in suppressing HC-induced inflammation in HEK. (Supported by AFOSR FA 9550-04-1-0376.)

2081 A COMPARISON OF TRENDS IN THE PARTITIONING OF 32 ALIPHATIC AND AROMATIC JET FUEL CONSTITUENTS IN VARIOUS SPME/SOLVENT MATRIXES.

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Recently publications have highlighted the difference in accepted octanol/water (Ko/w) values that exist for many analytes. In addition to the range of values available, a decision must be made whether to use calculated or experimentally derived values. For many researchers the choice of Ko/w value to use can be arbitrary, habitual, or based upon the values that give the best fit their distinct data. Using 3 commercially available SPME matrixes, Polydimethylsiloxane (PDMS), Polyacrylate (PA), and Carbowax/divinylbenzene (CAR), to represent varying degrees of hydrophobicity (from hydrophobic to hydrophilic respectively) we are exploring the partitioning trends of 32 aliphatic and aromatic jet fuel constituents from a 50% aqueous/50% organic (water/ethanol) or 4% BSA solutions. Once the mixture reaches equilibrium the experimental K value, determined from the membrane coated fiber technique (K_{mcf}), should more accurately describe the partitioning in skin than available Ko/w values for the same analytes of interest. Calculated values were obtained from well-known sources such as SPARC's, ClogP, ADME, and Syracuse Research Corporation (SRC), and experimental values from SRC and ChemDraw. In initial comparisons there was a 3-25% variance of Ko/w's between found values. Comparisons of K_{mcf} values and experimental databases resulted in R² of 0.60-0.83, while calculated comparisons varied between 0.33-0.84. In making a decision of what value to compare our results against we could have chosen several options; the most complete data set (from one source), the one set that gave the best fit for the majority of comparisons, the dataset that fit the best based on each individual scenario (SPME/solvent matrix), or even selecting the individual value that best fit from all available sources. These initial results further support creation of an in vitro system, such as K_{mcf}, to predict trends of skin partitioning in untested analytes of interest. Supported by USAFOSR FA9550-04-1-0376.

2082 PROPOSED MECHANISM OF JP-8-INDUCED MOLECULAR RESPONSES IN THE SKIN.

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The jet fuel, JP-8, has been shown to cause an inflammatory response in the skin, which is characterized histologically by erythema, edema and hyperplasia. Studies in laboratory animal skin and cultured keratinocytes have identified a variety of changes in protein levels related to inflammation, oxidative damage, apoptosis and cellular growth. Most of these studies have focused on prolonged exposures and subsequent effects. In an attempt to understand the earliest responses of the skin to JP-8, we have investigated changes in gene expression in the epidermis for up to 8 hrs after a 1-hr cutaneous exposure in rats. After exposure, we separated the epidermis from the rest of the skin with a cryotome and isolated total mRNA. Gene expression was studied with microarray techniques and changes from sham-treatments were analyzed and characterized. We found consistent two-fold increases in gene expression of 27 transcripts at 1, 4 and 8 hrs after the beginning of the 1-hr exposure that were related primarily to structural proteins, cell signaling, inflammatory mediators, growth factors and enzymes. Canonical signaling pathways from Ingenuity's Pathway Analysis (IPA) were used to identify pathways that were activated by JP-8 at the various time points. Analysis of pathways changed showed that several signaling pathways (for example, IL-6, ERK/MAPK, and chemokine) were increased at one hour and that the most significant changes at eight hours were related to metabolic pathways, many of which were downregulated. These results confirm and expand many of the previous molecular studies with JP-8. Based on the one-hour changes in gene expression, we hypothesize that the "trigger" of the JP-8-induced, epidermal, stress response is a "physical" disruption of osmotic, oxidative and membrane stability which activates gene expression in the signaling pathways and results in the inflammatory, apoptotic and growth responses that have been previously identified. (Sponsored by the Air Force Office of Scientific Research, AFOSR/NL)

2083 INVESTIGATION OF DERMAL IRRITATION POTENTIAL OF SELECTED JET FUEL CHEMICALS (NONANE, DODECANE AND TETRADECANE) BY IN VIVO MICRODIALYSIS.

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Purpose: To quantify the release of inflammatory markers in the skin using dermal microdialysis after topical application of selected aliphatic hydrocarbons (nonane, dodecane and tetradecane) in hairless rats. Methods: Linear microdialysis probes,

10 mm, were inserted in the dermis of hairless rats under urethane anesthesia. Dermal exposures were carried out by occlusive application of chemicals (230 µl for 2 h using Hill top Chambers®). The expression of neuropeptides [substance P (SP)] and prostaglandin E₂ (PGE₂) in the skin as a result of dermal exposures was measured for 7 h in dialysate by enzyme immunoassay. The in vitro recovery of SP from the microdialysis probes was determined at a flow rate of 2 µl / min. At the end of the study, plasma and exposed skin samples were subjected to quantitative analysis for IL-1α and TNF-α using enzyme immunoassay (EIA) kits. Results: The mean concentrations of SP after insertion of the probe (20.73 ± 1.58 pg/ml), application of nonane and dodecane (70.441 ± 4.69 and 49.71 ± 6.66 pg/ml) and 5 h after their removal demonstrated significant (P < 0.001) SP release following 2 h dermal exposure. Tetradecane did not show significant influence on SP release. Similarly, the mean concentrations of PGE₂ after insertion of the probe (230.51 ± 25.39 pg/ml), application of nonane (396.80 ± 7.35) and 5 h after its removal demonstrated significant (P < 0.05) nonane induced PGE₂ release. Dodecane and tetradecane showed no significant effect on PGE₂ release. Furthermore, nonane significantly (P < 0.001) increased while dodecane and tetradecane did not show any increase in the expression of IL-1α and TNF-α in the blood and skin as compared to untreated animals. Thus, microdialysis (SP and PGE₂) and cytokine data demonstrated irritant potential of the chemicals in the order of nonane > dodecane > tetradecane. Conclusion: Dermal microdialysis data correlates well with biomarker expressions after dermal exposures with irritant chemicals and thus can be used to quantify dermal irritation.

2084 TATTOO PIGMENTS SKIN: TATTOOING AND QUANTITATIVE EXTRACTION OF PIGMENT RED 22.

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In the western world millions of people have a tattoo to decorate the skin. Frequently tattoo colorants are mixtures of organic pigments containing also precursors and byproducts of pigment synthesis. Exposure to UV light or laser removal is proven to result in potentially toxic or carcinogenic decomposition products. To assess the health risk of tattooing and the light interaction with the respective pigments, it is important to determine the concentration of pigments in tissue. Surprisingly, this concentration is unknown. Thus, we developed an extraction method and quantified the amount of pigment that is punctured in the skin. Pig skin is tattooed using a standard tattooing machine and the original tattoo pigment Pigment Red 22 (purity of 80%), as well as the highly pure synthesised pigment with the concentration of 10% and 25% (w/vol). After tattooing, the skin specimen is punched and gently dissolved using a tissue lysis buffer. The pigment is extracted via liquid extraction with isopropanol/ dichloromethane and concentrated in a keeper by heating and nitrogen stream. The samples are dissolved in methylene chloride and analysed by HPLC. Preliminary studies have yielded recovery rates of the extraction method with > 95%. The amount of pure pigment punctured into skin is ranging from 140 – 500 mg per 100 cm² of tattooed skin. Our results show that the concentration of pigments in the skin depends critically on the size of the pigments and the concentration of the pigment applied to the skin surface.

In light of these high concentration punctured into the skin we should remind the fact that the application of such pigments have no established history for safe use. Moreover, after puncturing into the skin a part of the pigments is transported away into the human body and can be found in other organs like the lymph nodes. The work is supported by a grant of the 'Deutsche Forschungsgemeinschaft' (DFG), grant number BA1741/3-1.

2085 MEASUREMENT OF SKIN THICKNESS IN CONTROL RATS (SD) AND MICE (CD1) FROM TWO SUBCUTANEOUS CARCINOGENICITY STUDIES.

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Epidermal, dermal and hypodermal thickness of control male and female Sprague-Dawley rats and CD1 mice from two subcutaneous carcinogenicity studies was measured in an attempt to understand the implications of variable skin thickness on the incidence and distribution of the neoplastic and vascular injection site lesions observed during these studies. To our knowledge, this report represents the first comprehensive assessment of skin thickness in aged rodents. Skin sections from 40 rats and 40 mice were evaluated morphometrically at the following injection sites: upper dorsal thoracic (rats only), left and right scapular, left and right dorsal thoracic and left and right lumbar. The thickness of each site was averaged

and each average was submitted to two-sided statistical analysis. Differences in the thickness of the epidermis, dermis and hypodermis (the skin layer in which the compound was deposited) were observed craniocaudally in rats of both sexes, while differences in mice were limited to the dermis in males and the hypodermis in both sexes. In male rats, the epidermis and dermis were thinnest and the hypodermis was thickest at the scapular region. In female rats, greater epidermal thickness at each region compared to the lumbar region was deemed biologically insignificant due to its small magnitude (<2 microns). As was found in males, the dermis was thinnest and the hypodermis thickest at the scapular region. In mice, similar differences were observed – the dermis was thinnest at the scapular region in male mice and the hypodermis was thickest at this region in mice of both sexes. In conclusion, sites used for dorsal subcutaneous injection of xenobiotics in rodents are not equivalent. In particular, the thickness of the hypodermis varies significantly. This, along with the volume and formulation of the compound injected, may influence the occurrence of injection site lesions and make their interpretation difficult regarding relevance for human safety.

2086 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN
INDUCED MATRIX METALLOPROTEINASE-1
EXPRESSION IN A2058 MELANOMA CELLS REQUIRES
THE AHR AND ERK PATHWAYS.

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There has been a 34% increase in melanoma-related mortality in the United States from 1973 to 1992. Although excessive UV exposure is considered the main etiological factor in melanoma initiation, epidemiological and experimental evidence suggests that exposure to environmental carcinogens contributes to melanoma. Our data show that activation of the aryl hydrocarbon receptor (AhR) pathway activates expression and activity of the matrix metalloproteinases (MMPs). Matrix metalloproteinases are a family of zinc- and calcium-dependent proteinases classified by extracellular matrix substrate specificity and are essential for tissue remodeling events and cancer progression. We examined the effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the expression of MMP-1, a proteolytic enzyme that degrades type I collagen, in A2058 melanoma cells. We have shown that TCDD increased MMP-1 expression is AhR dependent and that it requires sequences in the distal portion of the promoter region. Recently it has been discovered that approximately 60% of all melanomas contain an activating mutation, V599EBRAF, in the Ras/Raf MAP kinase pathway. To investigate the contribution of the Ras/Raf signaling pathway on TCDD/AhR-induced MMP expression, we used specific chemical inhibitors for several signaling pathways and examined the effect of TCDD and AhR-activation on phosphorylation events in these cascades. TCDD treatment caused alterations in ERK phosphorylation, and co-treatment with the MEK inhibitor, U0126, abolished phosphorylation of ERK. Further, a minimal MMP-1 promoter construct is induced 4 fold by TCDD, but is not inducible upon co-treatment with U0126. These data demonstrate that TCDD-induced expression of MMP-1 in A2058 cells requires Ras/Raf signaling as well as the AhR pathway.

2087 GLUTATHIONE-MEDIATED PROTECTION AGAINST
CHROMIUM-INDUCED DERMAL TOXICITY.

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Occupational exposure to compounds containing chromium has been identified as a risk factor for developing irritant and contact dermatitis among workers. Induction of oxidative stress has been implicated as one of the major mechanisms responsible for the toxicity and the adverse health effects due to exposure to chromium. Presently, we have studied the role of glutathione in the toxicity of chromium following its dermal exposure in mice. The tissue glutathione levels of C57BL/6 mice were depleted by providing them with drinking water containing 20 mM buthionine sulfoximine (BSO). Hexavalent chromium (K₂Cr₂O₇) was applied on the skin of control and BSO-fed mice on alternate days at doses of 0, 1, 5 and 10 mg/kg b.w. either for 2 or 4 weeks. The chromium-induced lesions noticed on the skin of the mice correlated with the dose and duration of the chemical application. In general, the animals with depleted tissue levels of glutathione exhibited more severe skin lesions compared with the control animals indicating the enhanced susceptibility of the GSH-depleted mice to chromium-induced dermal toxicity. Similarly, the amounts of 8-hydroxydeoxy guanosine (8-OHdG) and malondialdehyde (MDA) – indicators of oxidative damage to tissue DNA and lipids, respectively, were higher in the skin of the GSH-depleted mice treated with chromium compared with the controls. The enhanced dermal toxicity of chromium noticed among the GSH-depleted mice compared with the corresponding controls was further supported by the results of gene expression and histopathology studies. In conclusion, these results suggest that the tissue level of glutathione is an important determining factor in the response of mice to the dermal toxicity induced by hexavalent chromium.

2088 DEVELOPMENT OF A METHOD FOR
PHARMACOLOGICAL MANIPULATION OF EPIDERMIS
USING INTRADERMAL INJECTIONS IN THE RAT.

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Our goal is to deliver signaling molecules or their antagonists directly to epidermal cells of intact rat skin in an attempt to understand chemical-induced changes in signaling pathways. The Stratum corneum limits penetration of most molecules including signaling proteins or their antagonists. We used an intradermal injection technique that avoids disrupting the skin at the site of experimental chemical exposures, by penetrating the skin with a needle about 20 mm away from the exposure site and tunneling subcutaneously to beneath the future site of exposure. In an attempt to determine if injected molecules would diffuse through the basal layer of the epidermis from below, we injected 50 µL of a 0.27 mg/mL solution of recombinant Enhanced Green Fluorescent Protein (EGFP) (27 KDa) in phosphate buffered saline. After 1.5 hours, injected skin was sectioned and studied under a fluorescent microscope. A high concentration of EGFP was observed in the injected region of the dermis. In addition, we were able to visualize some EGFP that moved from the injection site into the epidermal region. To investigate the efficacy of delivering signaling molecule antagonists, we also intradermally injected 100 µL of interleukin 1 receptor antagonist (Il-1ra) (17 KDa) or phosphate buffered saline as a control. We used antibody conjugated with Cy3 to detect Il-1ra, and a FITC-conjugated antibody to keratin 14 to locate the position of the basal membrane of epidermis. Preliminary results from the fluorescent microscopic study show the presence of Il-1ra in the epidermal region. The epidermis was stretched compared to control skin. Although the successful intradermal injection caused a protrusion on the surface like a blister, there was no separation of dermal layers in the region of the skin where the bleb is formed. We conclude that this intradermal injection technique could deliver signaling molecules and their antagonists, which are smaller than 27 KDa, to the rat epidermis. (Supported by the AFOSR)

2089 HUMAN SKIN IN ORGAN CULTURE AND HUMAN
SKIN CELLS (KERATINOCYTES AND FIBROBLASTS) IN
MONOLAYER CULTURE FOR ASSESSMENT OF
CHEMICALLY-INDUCED SKIN DAMAGE.

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Human skin cells (epidermal keratinocytes and dermal fibroblasts) in monolayer culture and human skin in organ culture were exposed to agents that are known to produce irritation (redness, dryness, edema and scaly crusts) when applied topically to skin. Among the agents used were three well-accepted contact irritants (i.e., all-trans retinoic acid [RA], sodium lauryl sulfate [SLS] and benzalkonium chloride) and five contact allergens (oxazolone, nickel sulfate, eugenol, isoeugenol and ethylene glycol dimethacrylate [EGDM]). The corrosive organic mercury compound, aminophenyl mercuric acetate (APMA), was used as an addition test agent. Based on dose-dependent differences in i) cytotoxicity in a 4-hour assay with keratinocytes and fibroblasts, ii) growth suppression in a 2-day assay with the same cells, iii) histological changes (necrosis, altered proliferation and differentiation) in organ-cultured skin, and iv) the profile of secreted molecules from organ-cultured skin, the corrosive agent could be distinguished from the other contact irritants, which, in turn, could be distinguished from the agents with allergenic potential. The use of organ-cultured skin in conjunction with cells derived from the skin in monolayer culture may provide an initial approach to screening agents for deleterious changes in skin.

2090 SUSPECTED INFLUENCE OF P-GLYCOPROTEIN ON THE
PENETRATION OF IVERMECTIN IN PORCINE SKIN.

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The purpose of this study was to determine the contribution of P-glycoprotein (P-gp), a transmembrane energy-dependent drug efflux pump, on the dermal absorption of ivermectin, as a possible P-gp substrate. An 8 hour flow-through diffusion cell experiment was performed on porcine skin with perfusate samples collected hourly. The skin was dosed with Ivomec Pour-On for cattle (Ivomec®) spiked with BODIPY-ivermectin (Molecular Probes, Eugene, OR). Verapamil was used as a P-gp inhibitor, while DMSO was used with the BODIPY-ivermectin as a positive control. The skin samples were frozen and viewed on a Nikon C-1 confocal system

to visualize the localization of the fluorescently labeled ivermectin using a combination of confocal-differential interference contrast (DIC) microscopy and confocal fluorescent microscopy. The samples treated with Ivermec® and BODIPY-ivermectin exhibited fluorescence in the outermost layers of the stratum corneum. The samples that had verapamil added showed more fluorescence, and penetration through the stratum corneum and into the stratum granulosum. In the DMSO controls, fluorescence was present throughout the entire cross section of porcine skin with a higher concentration in the stratum corneum. The presence of ivermectin in the stratum corneum is consistent with what was expected because the highly lipophilic ivermectin would have an affinity for the lipid matrix of the stratum corneum. The co-administration of verapamil increased the absorption of ivermectin, presumably by inhibiting the action of P-glycoprotein. It is important to note that topical ivermectin is usually applied monthly, and an 8 hour experiment is a preliminary step in understanding its transport process.

2091 IN-VITRO HUMAN SKIN PENETRATION OF THE FRAGRANCE MATERIAL HMPCC.

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An in vitro human skin absorption study was conducted on the fragrance material HPMCC (4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxyaldehyde), under both unoccluded and occluded conditions. Skin permeation and distribution of HPMCC was determined using epidermal membranes from cosmetic surgery donors. Skin membranes were mounted into Franz-type diffusion cells with the stratum corneum facing the donor chamber. The average area available for diffusion was 1.2 cm². HMPCC was applied, at the maximum in-use concentration of 1.5% in 70/30 (v/v) ethanol/water to the skin surface at a target dose of 5 µl/cm². The donor chamber of the occluded cells was covered with a greased, glass coverslip immediately after dosing. Permeation was measured over 24 h at 12 time points, using PBS as receptor. At 24 h, 5.54 ± 0.6 µg/cm² (unoccluded) and 18.6 ± 1.9 µg/cm² (occluded) (mean ± standard error, SE) of HMPCC had permeated, corresponding to 7.37 ± 0.86% (unoccluded) and 24.8 ± 2.5% (occluded) of the applied dose of 75.2 µg/cm². The 24 hour surface wipes contained 43.4 ± 2.8% (unoccluded) and 31.2 ± 2.2% (occluded) HMPCC and donor chamber wash/wipes contained 20.7 ± 1.5 (unoccluded) and 16.8 ± 0.8% (occluded) of the applied dose. The stratum corneum contained 7.75 ± 0.62% (unoccluded) and 6.20 ± 0.61% (occluded) of the applied HMPCC. The epidermis plus any remaining stratum corneum after tape stripping contained 6.06 ± 0.60% (unoccluded) and 8.69 ± 0.57% (occluded). Levels of HMPCC in the epidermis (plus any remaining stratum corneum after tape stripping), filter paper membrane support and receptor fluid were combined (as per SCCNFP guidelines) to result in a total absorbed dose value of 14.3 ± 0.9% (unoccluded) and 36.4 ± 2.6% (occluded). Overall recovery of HPMCC at 24 h was 86.2 ± 2.0% (unoccluded) and 90.5 ± 0.7% (occluded). The evaporative loss of HMPCC was low when estimated by measuring the loss from PTFE (polytetrafluoroethylene) sheets.

2092 PREDICTING CUTANEOUS PERMEABILITY OF BIOCIDES USING LSER APPROACH.

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The permeation of a chemical through skin is influenced by its physico-chemical characteristics and the biological characteristics of the skin. In predicting dermal absorption, scientists have proposed different mathematical models to correlate physico-chemical (especially solvatochromatic parameters) and biological interactions occurring in the process of permeation. Previous studies have shown that hydrophobicity, polarity, molecular volume and hydrogen bonding capability of chemicals are among the most important characteristics to be used for predicting permeability. The aim of this study is to predict dermal absorption of different biocides (with wide range of Kow) using modified Linear Solvation Energy Relationship (LSER) approach, with a calibrated flow through diffusion system. Infinite doses of biocides belonging to five different classes; phenols, morpholines, oxazolindines, isothiazolines and triazines in aqueous solutions were applied to porcine skin flow through diffusion cells. These perfusates were analyzed by GC-MS and HPLC to determine skin permeability. The system coefficients (r, s, a, b, v) of a calibrated set of solutes of the system were determined from multiple linear regression analysis and plugged into the LSER equation to predict permeability values for the several biocides. Our preliminary data with 2 phenols; o-phenylphenol and p-chloro-o-cresol, have shown that the predicted permeability values were very close to observed values, demonstrating that our calibrated system can efficiently predict permeability of biocides. In further studies, the permeability of biocides will be determined in various solvents and commercially available metalworking fluids,

so to assess the behavior of biocides in mixtures and to determine the solvatochromatic parameter that has the most prominent effect in predicting permeability. NIH OH003669-06 supported this research.

2093 METABOLIC TRANSFORMATIONS OBSERVED DURING IN VITRO DERMAL PENETRATION STUDIES.

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Since the adoption of the European Commissions chemical strategy (REACH) and the Ground Water Directive (COM(2003)550) the environmental fate of a large number of chemicals, including pharmaceuticals, is becoming of increased public and regulatory concern. Under the proposed legislation risk assessments of a large number of chemicals, including dermal exposure, will be required.

The dermal penetration of [14C]-labelled parabens (octyl and propyl) was investigated in static Franz cells using isolated skin preparations obtained from both man and Sprague-Dawley rats. The rates of percutaneous absorption were compared to those obtained using standard reference compounds, [14C]-benzoic acid, [14C]-caffeine and [14C]-testosterone.

Since the skin possesses significant enzyme activities, some topically applied substances are metabolised as they pass through the skin. In the present study, the effect of metabolism on the percutaneous penetration of topically applied substances was investigated. Both octyl and propyl paraben were metabolised to p-hydroxy benzoic acid on passage through the skin, although the permeation rates were notably different. Testosterone was also metabolised, to androstenedione, on passage through the skin.

2094 HUMAN IN VITRO MODELS OF DERMAL METABOLISM.

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Dermal metabolism is recognized as an important consideration in evaluating the topical exposure of chemicals present in topically applied pharmaceutical and cosmetic products. Intact skin placed in flow-through diffusion cells, skin homogenates, and isolated keratinocytes are some of the models that have been used to study the metabolism of specific chemicals. The objective of this research was to establish human in vitro models for studying the dermal metabolism of chemicals. The metabolic capacity of freshly excised full thickness human skin and human keratinocytes was evaluated using probe substrates for known xenobiotic metabolizing cytochrome P450 (CYP) enzymes, transferases (N-acetyl, glucuronosyl, and sulfonyl) and esterases. Measurable CYP enzyme activities were observed for all transferases and esterases as well as for CYP1A2, CYP2B6, CYP2E1, and CYP3A4. Both freshly excised full thickness human skin and keratinocytes exhibited a similar pattern of enzymes with measurable activities, which correlated with enzyme activities previously reported in the literature. The effects of storage conditions, (1) storage medium, (ice vs Belzer UW solution) and (2) length of storage (up to 48 hours), on the metabolic capacity of freshly excised skin were also evaluated. The observed metabolic activities were well maintained by storage of the skin on ice for up to 48 hours. In conclusion, freshly excised human full thickness skin and human keratinocytes are suitable models for evaluating potential dermal metabolism of chemicals.

2095 IN VIVO AND IN VITRO DIBUTYL PHTHALATE SKIN ABSORPTION IN HAIRLESS GUINEA PIG SKIN.

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The in vitro assessment of skin absorption of lipophilic chemicals can be problematic when conducting in vitro diffusion cell skin absorption studies, because the chemicals may not freely partition from skin into an aqueous receptor fluid. In order to evaluate the in vitro-in vivo agreement in absorption of lipophilic chemicals, the cosmetic ingredient, dibutyl phthalate (DBP), was chosen as a model lipophilic compound due to reported toxicological concerns. Studies were done to measure DBP dermal absorption in vitro and in vivo using hairless guinea pig skin. Skin absorption was determined in these studies using an emulsion dosing vehicle containing 14C-DBP. In vitro absorption was measured for 24 or 72 hr by using flow-through diffusion cells (0.64 cm²) with a receptor fluid consisting of HHBSS (pH 7.4). In vivo absorption of DBP 24 hr after dermal application resulted in systemic absorption of 62.0 ± 2.0 (mean ± SEM) of the applied dose absorbed (%ADA) with 2.2 %ADA remaining in the skin. The majority of systemically ab-

sorbed DBP *in vivo* was found excreted in urine. *In vitro* DBP absorption over 24 hr found 28.9 ± 2.1 %ADA in receptor fluid with approximately 21.8 % remaining in skin. Extended absorption studies for 72 hr found receptor fluid levels of 55.9 ± 3.9 %ADA with 17.3 %ADA remaining in skin. Charcoal impregnated filter paper attached (for 1 hr) to the top of the diffusion cell or to the top of the *in vivo* dosing chamber improved recovery by trapping the volatile DBP. These studies indicate there is considerable DBP absorption in skin. In conclusion, DBP tends to form a skin reservoir *in vitro* as shown by the increased receptor fluid levels found after 72 hr when compared to 24 hr. The 72 hr *in vitro* absorption values correlated most closely with the 24 hr *in vivo* results. These studies help determine the importance of including DBP remaining in the skin at the end of an *in vitro* study as potentially available for systemic absorption.

2096 ASSESSMENT OF DERMAL ABSORPTION BY THERMAL GRAVIMETRIC ANALYSIS – DEVELOPMENT OF A 2-COMPARTMENT DIFFUSION MODEL.

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Recent work in our laboratory shows that it is possible to register the dermal uptake of chemicals *in vitro* by thermal gravimetric analysis (TGA), i.e. by exposing a piece of skin to chemical vapour of known concentration while recording its weight in a high-precision scale during carefully controlled temperature and humidity conditions.

We have previously studied several organic solvents with the TGA technique using trypsinated porcine skin. The data indicate that the shape of the weight curve is related to the skin:air partition (P) and diffusion (D) coefficients of the chemical. To define the relations more precisely, a solution to Fick's second law of diffusion was derived, for the special case of recording total weight over time in the TGA setup. A good fit to observed data was obtained using a 2-compartment diffusion model. The parameters P and D were obtained by best fit to observed weight curves of butyl acetate, methanol, 2-propanol and toluene using the Berkeley Madonna software.

The same chemicals were also studied using conventional Franz' diffusion cells and dermatomed porcine skin. A good correlation was obtained between the diffusion coefficients derived from the Franz' cell experiments and those derived from the faster of the two compartments in the TGA diffusion model.

In conclusion, experiments with a limited number of chemicals indicate that skin:air partition and diffusion coefficients can be obtained by fitting a TGA-specific solution to Fick's second law to TGA weight curves and that the numerical values correlate well to the those obtained in conventional diffusion cell experiments. Further studies on several different types of chemicals are needed to confirm the usefulness of the method.

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2097 MARKOV CHAIN MODEL FOR QUANTITATING DERMAL ABSORPTION FROM COMPLEX MIXTURES.

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Exposure to pesticides dosed in complex chemical mixtures is a common environmental and occupational health concern. Risk assessors need to know the rate at which a pesticide is absorbed in to the skin in order to predict the toxicity of exposure to the pesticide. However, experimentally obtaining the absorption rates for even a small number of possible pesticide mixtures are expensive and time consuming. The objective of this study is to create a Markov chain model to predict pesticide absorption rates through the skin. The model parameters were fit to absorption data collected in the isolated perfused porcine skin flap (IPPSF) for a mixture of ethyl-parathion in ethanol. The model was investigated both analytically and through simulations using Gillespie's algorithm and was fit to the data using least squares methods. A model with three internal states, two absorbing states plus a delay appears to describe these data adequately. The structure of this model was compared to classical modeling approaches commonly used in dermal absorption studies (e.g. compartmental models and their reduction, PBPK models). Future work will expand the number of pesticide mixtures analyzed as well as search for correlations between absorption parameters and the physical and chemical properties of the pesticides and the mixture components into which they are exposed. These correlations may enable risk assessors to predict absorption profiles after chemical exposures in complex mixtures or formulations based on mixture component physical and chemical properties. (Supported by NIOSH R01 OH 07555).

2098 INCORPORATION OF COMPLEX CHEMICAL MIXTURE EFFECTS INTO A QSAR MODEL OF DERMAL ABSORPTION.

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Significant progress has been made on predicting dermal absorption/penetration of topically applied compounds by developing quantitative structure activity relationship (QSAR) models based on linear free energy relations (LFER). However, all of these efforts have employed compounds applied to the skin in aqueous or single solvent systems, a dosing scenario that does not mimic occupational, environmental nor pharmaceutical exposure. We have explored using hybrid QSAR equations describing individual compound penetration based on the molecular descriptors for the compound modified by a mixture factor (MF) which accounts for the physicochemical properties of the vehicle/mixture components. The MF is calculated based on percentage composition of the vehicle/mixture components and physical chemical properties selected using principal components analysis. This model has previously been applied to 12 different compounds in 24 mixtures for a total of 288 treatment combinations obtained from flow-through porcine skin diffusion cells (Toxicol. Appl. Pharmacol. 208:99-110, 2005). The data reported here includes using three fundamentally different LFER models (Abraham, Potts and Guy, Hostynek and Magee) on this diffusion cell dataset as well as applying them to a new experimental dataset in the *ex vivo* isolated perfused porcine skin flap model consisting of 10 of the same compounds in 5 mixtures for a total of 50 treatment combinations. The use of the MF in combination with classic LFERs based on penetrant properties significantly improved the ability to predict dermal absorption of compounds dosed in complex chemical mixtures across three different LFER models and two biological systems. (Supported by NIOSH R01 OH 07555).

2099 CUSTOM MADE PANI FIBER TO ASSESS DERMAL PARTITIONING AND ABSORPTION OF BIOCIDES.

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A custom-made polyaniline (PANI) coated metal alloy fiber was evaluated for its use in assessing dermal partitioning and absorption of certain biocides. This PANI coating provides a unique functionality compared to three commercially available fibers polyacrylate (PA), polydimethylsiloxane (PDMS), and Carbowax/templated resin. The production of this custom-made fiber is a relatively simple process that can be controlled by utilizing cyclic voltammetry (CV). The CV program for coating the metal alloy fiber consisted of one initial scan from -0.2V to +1.1V followed by several sets of scans from -0.2V to +0.8V with a 15 second equilibration time at -0.2V between scan sets and scan rate of 50 mV/sec. The electrolyte solution was 0.1M aniline in 1.0M sulfuric acid. The bare metal alloy fiber SPME assembly, commercially donated, served as the working electrode. In our experiments, cyclic voltammetry appeared to give more consistent results and control in coating the metal alloy fiber with PANI as opposed to using a constant deposition potential. To assess the PANI fiber, solid-phase microextraction (SPME) was performed by direct immersion into water solutions containing either a single component or a mixture of biocides at 37°C and stir rate of 400 R.P.M. The PANI fiber was desorbed directly in the GC-MS injector port for 5 minutes at 200°C following immersion. This PANI coated fiber exhibited good reproducibility and durability. The RSD's averaged 6 to 9% over a period of three days for the first 46 injections of a single component (5ppm 2-phenylphenol) in water with only three outliers. Time profile experiments revealed that both p-chloro-o-cresol and o-phenylphenol reached equilibrium before 10 minutes while the equilibrium time for o-benzyl-p-chlorophenol was greater than 90 minutes. This research was supported by NIH OH003669-06.

2100 PREDICTING SKIN PERMEABILITY OF MOLECULES FROM CHEMICAL MIXTURES USING AN INERT MEMBRANE-COATED FIBER ARRAY.

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Predicting skin permeability of compounds from chemical mixtures is required for transdermal drug delivery, safety evaluation of cosmetics and risk assessment of chemicals, where most exposure occurs in complicated chemical mixtures, not individual chemicals. We have developed a membrane-coated fiber technique that can be used to measure the partition coefficients of molecules from chemical mixtures with high precision and throughput. This novel approach predicts skin permeability based on its correlation with the partition coefficients from chemical mixtures determined in a fiber array. Each of the membrane-coated fibers represents a pattern of molecular interactions: polydimethylsiloxane (PDMS, lipophilic), polyacrylate (PA, polarizable) and CarboWax (Wax, polar). Molecular interactions occurring during percutaneous absorption can be simulated by the multiple fibers:

$\log(kp) = c + a \log K_{MCF1} + b \log K_{MCF2} + \dots + \log K_{MCFn}$. The feasibility of this approach was demonstrated with 25 diverse chemicals; their skin permeability [$\log(kp)$] from chemical mixtures were measured by in vitro flow through diffusion experiments and partition coefficients of the chemicals ($\log K_{MCFn}$) were determined by the membrane-coated fiber technique. Multiple regression analysis was used to define equations linking $\log(kp)$ to $\log K_{MCFn}$ for the three MCFs. A significant correlation equation was defined: $\log(kp) = -2.73 - 1.31 \log K_{wax} - 0.31 \log K_{pdm} + 2.34 \log K_{pa}$ with a R^2 of 0.92. The correlation coefficients in the equation reflected the mixture-skin interactions in different vehicles. These data clearly demonstrate that dermal absorption of diverse chemicals can be estimated from data collected in the inert fiber array which reflect the physicochemical diversity of interactions seen when chemicals partition into the stratum corneum. (Supported by NIOSH OH-07555 and USAFOSR FA 9550-04-1-0376).

2101 ENVIRONMENTAL TOBACCO SMOKE (ETS) EXACERBATES ATHEROSCLEROSIS PROGRESSION IN APOE^{-/-} MICE USING NON-INVASIVE REAL-TIME ULTRASOUND BIOMICROSCOPY (UBM) IMAGING.

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Environmental tobacco smoke (ETS) has been listed by American Heart Association as a significant risk factor for cardiovascular diseases. Atherosclerosis is a major underlying cause of angina, heart attacks, strokes, and peripheral vascular disease. In this study, using non-invasive in vivo ultrasound biomicroscopy (UBM), we developed a novel imaging protocol to follow lesion progression in atherogenic mice. We also employed this protocol to assess the effects of long-term, low-concentration ETS exposure on plaque acceleration in atherosclerosis susceptible mice. Tobacco and Health Research Institute's 1R4F cigarettes were used to generate a side-stream cigarette smoke (SS, a surrogate for ETS) atmosphere. ApoE^{-/-} mice (n=24/group) fed with either high-fat chow (HFC), or normal chow (NC), were exposed to a low dose of SS (480 ug/m³) or filtered air (FA) for 6 hr/d, 5 days/wk, for up to 6 months. Before exposure and at 2, 4, 6 months during exposure, non-invasive UBM (Vevo660, Visualsonics, Canada) was performed on each mouse to assess plaque progression in aorta, brachiocephalic and left carotid arteries. The plaque volume was then measured by ImageJ. In addition, after each UBM assessment, 8 mice per group were sacrificed and histological analysis was performed for comparison with the results obtained using UBM. At 4 and 6 months, using UBM, SS exposure produced the greatest change in plaque volume in the left common carotid artery of HFC mice as compared to FA controls or NC controls, but not in brachiocephalic artery. Furthermore, UBM showed significant increases of plaque volume in the brachiocephalic artery in all four groups every other month. Our results show that this novel UBM imaging protocol is a useful non-invasive tool to follow plaque progression in atherogenic mice. The results also suggest that ETS exposure exacerbates plaque development in atherosclerosis susceptible mice.

2102 ESTROGEN REPLACEMENT THERAPY AND DEVELOPMENT OF VASCULAR LESIONS.

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Estrogen is known to increase vascular endothelial cell growth which may contribute to the increase in risk of coronary heart disease in women exposed to unopposed estrogen replacement therapy (ERT). Although the mechanism is not clear, estrogen may put women at risk by its promotion of abnormal proliferative vascular lesions and subsequent thickening of the vasculature. How estrogen may support or promote vascular lesions is not clear. In this study, we have examined whether estrogen stimulated endothelial tube formation is dependent on cellular redox state. Methods: Vascular lesion formation was determined in a 3-dimensional Matrigel assay. Prior to estrogen treatment, MnSOD and catalase were overexpressed in human umbilical vein endothelial cells (HUVEC) via adenoviral vectors. Endothelial tube formation was measured by AngioQuant software, an automated image analysis tool for quantification of angiogenesis. Results: Physiological concentrations of E2 found in human serum were tested. Estrogen (100pg/ml) treatment for 6 h stimulated a 2-fold increase in endothelial tubes. Endothelial tube formation was significantly inhibited to the level of control by overexpression of both MnSOD and catalase. In addition, co-treatment with the antioxidant ebbsen showed a significant reduction in tube formation. Conclusions: We have shown that overexpression of MnSOD and catalase significantly inhibits endothelial tube formation. It appears that early estrogen signaling does not require estrogen receptor genomic signaling because we can inhibit tubule formation by antioxidants. Findings of this study highlight the potential damaging consequences of unopposed ERT in vascular disorders dealing with oxidative stress conditions.

2103 THE DIETARY FLAVONOID QUERCETIN BLOCKS PCB77 INDUCED PRO-INFLAMMATORY RESPONSES IN VASCULAR ENDOTHELIAL CELLS.

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Polychlorinated biphenyls (PCBs) are widespread environmental contaminants that can cause a wide variety of toxic effects in exposed organisms. Co-planar PCBs can induce oxidative stress and activation of pro-inflammatory signaling cascades which are associated with atherosclerosis. Vascular endothelial cells have been shown to be sensitive to chemical insult and cellular dysfunction after co-planar PCB exposure. The majority of the toxicological effects elicited by co-planar PCB exposure are associated to activation of the aryl hydrocarbon receptor (AHR) and subsequent induction of responsive genes. Quercetin, a dietary flavonoid, has been demonstrated to possess antioxidant and anti-inflammatory properties in various in-vivo and in-vitro models. Previous studies from our group have shown that flavonoids can significantly reduce PCB77 induction of oxidative stress and expression of the AHR responsive gene cytochrome P450 1A1 (CYP1A1). To determine if quercetin can block PCB77 induced expression of pro-inflammatory genes associated with atherosclerosis, porcine endothelial cells were exposed to PCB77 in combination with quercetin, and expression of pro-inflammatory proteins was analyzed by western blot. Upon confluence, cells were serum deprived for 8 h, then treated with PCB77 (1 µM), quercetin (10 µM), or PCB77 plus quercetin for a period of 16 h. Quercetin co-treatment significantly blocked PCB77 induction of the pro-oxidative and inflammatory proteins: CYP1A1 and vascular cell adhesion molecule 1 (VCAM1). These results suggest that quercetin intervention is protective against PCB77 induced endothelial cell dysfunction. (Supported by grants from NIEHS, NIH (P42ES07380) and the University of Kentucky AES).

2104 HIV ANTIRETROVIRAL DRUG COMBINATIONS INDUCE ENDOTHELIAL MITOCHONDRIAL DYSFUNCTION AND ROS PRODUCTION, BUT NOT APOPTOSIS.

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Numerous studies report that HIV patients administered antiretroviral therapy are at a greater risk for cardiovascular diseases. Endothelial dysfunction is an early event and a sensitive marker for cardiovascular diseases, such as atherosclerosis. We previously reported that HIV antiretroviral therapy induces direct endothelial dysfunction in vivo, and induces only a slight increase in cell death, as indicated by the LDH release assay. We also observed that endothelial mitochondrial function was compromised following antiretroviral treatment. To determine whether antiretroviral-induced endothelial dysfunction is via mitochondrial dysfunction and whether mitochondrial dysfunction culminates in endothelial cell apoptosis, we treated HUVEC with micromolar amounts of AZT, didanosine, or AZT plus didanosine. These treatments induced a decrease in cellular O₂ consumption, a collapse in the mitochondrial membrane potential, and an increase in the production of reactive oxygen species (ROS) in endothelial cells. An MnSOD mimetic, MnTBAP, abolished the increased ROS production, suggesting that the ROS were generated by mitochondria. Transfection of the endothelial cells with a mitochondrially-targeted catalase decreased antiretroviral-induced ROS and 8-isoprostane production, confirming the mitochondrial localization of the ROS produced. Finally, to determine whether antiretroviral-mediated mitochondrial dysfunction culminates in endothelial cell apoptosis, we assessed levels of apoptosis using the TUNEL, the DAPI staining, the caspase activation, and the DNA fragmentation assays, but none of these assays indicated a marked increase in apoptotic cells following antiretroviral treatment. Our studies thus suggest that antiretrovirals induce mitochondrial dysfunction and increase mitochondrial ROS in endothelial cells, but the decreased mitochondrial function does not culminate in apoptosis.

2105 PCB 77 ACTIVATES ENOS VIA PI3K AND AKT PHOSPHORYLATION IN ENDOTHELIAL CELLS BY A CAVEOLIN-1 DEPENDENT MECHANISM.

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Polychlorinated biphenyls (PCBs) are persistent environmental contaminants that can contribute to the pathology of atherosclerosis by activating inflammatory responses in vascular endothelial cells. We hypothesize that plasma membrane mi-

crodomains called caveolae are critical in endothelial activation and toxicity mediated by PCBs. Caveolae are particularly abundant in endothelial cells and play a major role in endothelial trafficking and signaling pathways associated with the pathology of vascular diseases. eNOS is co-localized with caveolae and is a critical regulator of vascular homeostasis. Dysfunctional eNOS signaling may be important in the early pathology of atherosclerosis and the activation of eNOS can lead to excessive NO formation. The current study was designed to test the hypothesis that PCBs can activate endothelial cells via eNOS signaling. Human endothelial cells (EA.hy926) were treated with PCB77 (2.5 μ M) for 5 and 10 minutes to explore protein activation. Exposure to PCB77 rapidly induced caveolin-1 and eNOS phosphorylation as well as activation of PI3K and Akt. Caveolin-1 silencing abolished the PCB-stimulated eNOS phosphorylation. Furthermore, PI3K inhibitors (Wortmanin, Ly294002) blocked eNOS and caveolin-1 phosphorylation. Cellular exposure to PCB77 also increased nitrotyrosine levels, which may have implications in oxidative-stress mediated inflammatory mechanisms. These findings suggest that PCB77 induces eNOS phosphorylation in endothelial cells through an Akt-dependent mechanism, events regulated by caveolin-1. Our data provide evidence that caveolae may play a critical role in regulating vascular endothelial cell activations and toxicity induced by persistent environmental pollutants such as coplanar PCBs.

2106 INSIDIOUS VASCULAR INFLAMMATORY RESPONSE AND IDENTIFICATION OF EARLY PREDICTIVE BIOMARKERS IN RAT FOLLOWING ORAL ADMINISTRATION OF THE PDE IV INHIBITOR SCH 351591.

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We previously reported that the PDE IV inhibitor SCH 351591 induced mesenteric arterial hemorrhage and necrosis in rats. The objective of the current study was to characterize the SCH 351591-induced vascular injury that precedes arterial hemorrhage and necrosis. Male Sprague-Dawley rats were administered orally a single dose of 20 mg/kg SCH 351591. Groups of animals (n=6) were then sacrificed at 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min after dosing. Microscopic evaluation of the mesentery at the 15 min time point revealed an increase in the number of mast cells, apparent activation of mast cells and vasodilatation in the microvasculature. This was paralleled by a transient increase in circulating lymphocytes at 15 minutes. Serum beta-hexosaminidase showed a similar but non-significant increase. Responses observed at the 30-45 min time point included a significant increase in the number of mast cells, recruitment of eosinophils, marked vasodilatation, endothelial cell activation, and perivascular inflammatory reaction in small veins. Recruitment of neutrophils, eosinophils, lymphocytes, and mononuclear cells with progressively worsening perivascular inflammatory reactions in the arteries and veins was observed from 60-240 min after dosing. Adhesion of leukocytes to activated endothelial cells and transendothelial migration of circulating leukocytes were also observed at these later time points. Circulating granulocytes significantly increased by 240 minutes as had the circulating levels of GRO/CINC-1. Arterial hemorrhage and necrosis had not developed by the 240 min time point. Confirming previous pilot studies, IL-6 was not elevated at any time point studied. Mast cell degranulation was very rarely observed at any time point. Pathological, hematological and biomarker changes were seen in the very early stage of drug-induced mesenteric vascular injury, well before medial hemorrhage and necrosis.

2107 PLASMA CYTOKINE LEVELS ARE INCREASED IN OLDER APOLIPOPROTEIN E DEFICIENT MICE FOLLOWING EXPOSURE TO DIESEL EXHAUST.

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The cardiovascular (CV) system is exquisitely sensitive to adverse effects following increases in ambient particulate matter (PM), as exemplified by increases in morbidity and mortality following periods of greater air pollution. Diesel accounts for a large percentage of PM in an urban setting. Inflammation is associated with coronary events such as atherosclerotic plaque rupture, thrombogenesis and embolization. Plasma cytokines play a critical role as both mediators and markers of the inflammatory response to PM. We hypothesize a change in plasma cytokine levels following exposure to diesel exhaust (DE) in a model of CV disease, the apolipoprotein E null (apoE^{-/-}) mouse, that will suggest the mechanisms involved in PM mediated CV events. To test this, male apoE^{-/-} mice 25-35 weeks old (N = 15/group) were exposed to 200 μ g/m³ DE or filtered air (FA) for 1 day, 1 week or 8 weeks. At sacrifice, plasma was analyzed for interleukin (IL)-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, granulocyte-macrophage colony stimulating factor (GM-CSF), interferon (IFN)- γ and tumor necrosis factor (TNF)- α using the BioRad BioPlex cytokine kit. All of the cytokines had the greatest increases following 1 day of DE

exposure with a return to FA exposed levels with longer DE exposures. IL-4, IL-10, and IL-12, were significantly increased following exposure to DE compared to FA. With the cytokines IL-2, IFN- γ , GM-CSF and TNF- α , the FA exposed groups were below the limits of detection, as plasma concentrations are usually low, while the DE exposed groups were measurable. As both T_H1 and T_H2 cytokines were increased in the circulation following DE exposure, we can infer both a cell mediated and antibody mediated response to DE. This change in circulating cytokines may directly affect the vasculature, cardiac muscle, or atherogenesis to produce an adverse effect. We conclude that the complex inflammatory response that may signal CV effects to DE exposure is mediated by cytokines.

2108 DIESEL EXHAUST ENHANCES VENOUS CONGESTION IN A MODEL OF HEART FAILURE.

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Air pollution has been associated with hospital admissions and death due to heart failure. However, the exact mechanism(s) and causal components remain to be clarified. Recent studies suggest that air pollution enhances vasoconstriction systemically. Thus, we hypothesized that exposure to air pollutants induce vasoconstriction that, in the presence of a failing heart, leads to venous and pulmonary congestion. To study this, we used 1) isolated arteries and veins exposed to organic components common to many combustion sources, and 2) a hamster model of cardiomyopathy exposed to a common ambient air pollutant, diesel exhaust via inhalation. Previous work in our laboratory has demonstrated that the gaseous components of diesel exhaust increased the vasoconstrictive effects of ET-1 in isolated ApoE^{-/-} septal coronary arteries. Current work in our laboratory is focused on mesenteric arteries and veins from C57BL/6. Isolated mouse mesenteric veins and arteries showed similar proconstrictive effects when exposed to combined acetaldehyde, formaldehyde, acetone, hexadecane and octadecane at concentrations found in particle filtered diesel exhaust exposed saline; however, the individual compounds did not appear to replicate the effects. In a related study, cardiomyopathic hamsters (HCM) were obtained late in life exhibiting cardiac impairment (fractional shortening 0.15-0.20, normal ~0.60). HCM were exposed to 300 mg/m³ of whole diesel exhaust for 1 hr/day for 2 days and venous pressures (Pven) were taken via radiotelemetry through catheterization of the thoracic vena cava. Exposure to whole diesel exhaust significantly increased Pven to ~13 mmHg compared to control animals (Pven ~5 mmHg). Furthermore, aortas taken from the diesel exposed animals exhibited significantly more oxidant generation than control as measured by lucigenin-enhanced chemiluminescence, indicating elevated vascular oxidative stress. These data suggest that air pollutants can induce congestion and vasoconstriction, which may play a role in exacerbation of cardiopulmonary symptoms related to congestive heart failure.

2109 SUB-CHRONIC EXPOSURE TO CONCENTRATED AMBIENT PARTICULATE AIR POLLUTION AND CARDIOPULMONARY RESPONSES IN RATS.

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Ambient particulate matter (PM) has been linked with intensification of respiratory and cardiovascular diseases. The central hypothesis tested in this *in vivo* study was that long-term exposure to PM will result in adverse pulmonary and systemic effects. Specifically, we expected a diminished endothelial function (as monitored by von Willibrand Factor and endothelin-1), reduced fibrinolysis (as monitored by tissue plasminogen activator and plasminogen activator inhibitor-1) and increased blood coagulation (as monitored by thrombin generation tests). To test this hypothesis the effect of concentrated ambient particles (CAPs) was studied in rats that had a mild pulmonary inflammation at the onset of CAPs exposures induced by ozone.

Using an Ambient Fine Particle Concentrator (AFPC) in total 20 male Fischer F334 rats were exposed to CAPs (0.15-2.5 μ m). 15 animals were exposed for 4 weeks, 5 days/week for 6 hours a day and 5 animals, equipped with radiotelemetry for recording ECG, were prolonged exposed for 13 weeks. On average CAPs concentrations of 285 and 227 μ g/m³ were reached. Cardiopulmonary responses were investigated 24 hours after the last exposure in both groups. The effect on inflammation (protein, lactate dehydrogenase and several cytokines in broncho alveolar lavage fluid), pathological and haematological indicators (see above) were examined. Vascular function was assessed in arteries using the myograph system. First results suggest that neither CAPs exposure for 4 weeks nor for 13 weeks induced significant changes of inflammation in the lung, in haematological indicators, ECG measurement or vascular function.

Contrary to our expectations no major effects were associated with CAPs exposure for 4 weeks up to 13 weeks.

2110 ROLE OF THE NITRIC OXIDE PATHWAY IN FENOLDOPAM-INDUCED VASCULAR INJURY.

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Fenoldopam, a dopaminergic DA1 agonist, causes mesenteric vascular injury in the rat but also increases mesenteric and renal arterial blood flow. This vasodilation is mediated by nitric oxide (NO). Nitric Oxide synthase (NOS) is regulated by the shear-stress mediating cell surface protein caveolin-1 (cav-1). Profound vasodilation and accompanied decreased shear stress are early events that could initiate vascular injury. Therefore, determining the role of cav-1 and the NO pathway in fenoldopam-induced vascular injury would be of interest. At specific sites of mesenteric arterial injury induced by fenoldopam, there was loss of cav-1 and apoptosis determined immunohistochemically in endothelial and smooth muscle cells (SMC). Additionally, evidence of gap and tight junction loss localized to injury sites was seen in response to fenoldopam-induced vascular damage. In order to determine the sequence of events, rat SMC were cultured with pharmacologically and structurally diverse in vivo vascular toxicants and the cells analyzed temporally for activated caspase 3, annexin V and cav-1 using cellomics, flow cytometry and western blot analysis. The results showed a dose and time dependent cav-1 loss 6 hours post-treatment that preceded activation of caspase 3 and expression of annexin V as markers of apoptosis. Because functional loss of cav-1 is associated with increased NOS activity and vasodilation via NO, in vivo studies were conducted to show that continuous intravenous infusion of a NO donor produced vascular lesions morphologically similar to those induced by fenoldopam. Moreover, the incidence and severity of vascular injury were reduced when a nitric oxide synthase inhibitor (L-NAME) or a scavenger of NO-generated free radicals were co-administered with fenoldopam. Collectively, these data suggest that the NO pathway may play an important role in vasodilatory drug-induced vascular injury.

2111 THE EFFECT OF DIESEL PARTICLES ON CAPILLARY ENDOTHELIAL TUBES GROWN IN VITRO.

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To determine whether inhaled diesel particles have deleterious effects on the lung vasculature, we used an in vitro culture model system. 125,000 human umbilical vein endothelial cells were plated in the wells of 6-well dishes. Some wells were evenly coated with 2 mg Matrigel (Becton Dickinson). On a plastic substratum, cells proliferated, doubled every 24 hr, and formed a confluent monolayer by 48 hr. On Matrigel, cell proliferation was limited and capillary tubes began forming within 3 hr. Microscopy revealed that capillary tube formation was complete by 12 hr and that every cell was a component of a tube. With daily changes of medium, capillary tubes were stable for a minimum of 8 days. Next we tested how different concentrations of diesel particles affected the survival of endothelial cells and tubes. The "zero" time point for addition of diesel particles was defined as 12 hr after plating cells on plastic or Matrigel. Automobile diesel particles from Japan (courtesy of Dr. Sagai, Tokyo) were sonicated in PBS for 5 min, then added at 1 ug/ml, 10 ug/ml, and 100 ug/ml medium to monolayers or capillary tubes. Within 4 hr, microscopy showed that the highest concentration of particles caused monolayer cells to extend processes, and caused capillary tubes to appear swollen or inflated. To understand this effect, monolayers were examined by a quantitative assay [the Cell Titer 96 Aqueous One Solution Assay (Promega)] to detect viable cells. This colorimetric method is capable of evaluating either proliferation or cytotoxicity, and demonstrated that 100 ug/ml particles caused cell death: Within 24 hr, 50% of the cells died. By 48 hr 80% died. However, 1 ug/ml and 10 ug/ml diesel particle concentrations had no effect on cell survival. Cell doublings occurred every 24 hr, equivalent to the rate of proliferation of non-treated cells.

2112 SHORT-TERM EXPOSURE TO PARTICULATE MATTER POTENTIATES VASOMOTOR TONE CHANGE.

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Recent studies have implicated air-borne particulate matter <2.5 µm (PM_{2.5}) in vasoconstriction and potentiation of hypertension in humans. The precise mechanisms remain to be characterized. We investigated the effects of short-term exposure to PM_{2.5} in an angiotensin II (A II) rat model.

Sprague-Dawley rats were exposed to concentrated ambient PM_{2.5} or filtered air (FA) in a whole-body exposure chamber for 10 weeks. Blood pressure (BP) was monitored by radiotelemetry. At week 9, an osmotic minipump containing A II was implanted subcutaneously. Rats were sacrificed 1 week after A II intervention, and the aorta excised and subjected to evaluation.

Baseline BP after 9-week of PM_{2.5} exposure was similar between groups. Mean BP (MBP) was higher in A II-PM_{2.5} starting at day 3 (ΔMBP = 57±3 vs. 20±4 mmHg at day 5, p<0.05 vs. A II-FA). Vasoconstrictor response to phenylephrine was exaggerated in the PM_{2.5} group (107.1±6.9 vs. 78.3±5.5%, p<0.05 vs. FA) while dilator response to acetylcholine was potentiated in precontracted aortic segments (-74.5±2.7 vs. 95.3±3.2%, p<0.05 vs. FA). Superoxide production in aorta was increased by PM_{2.5} (3378±159 vs. 1528±151 pixels/mg dry weight in A II-PM_{2.5} and A II-FA groups, respectively) by an NAD(P)H oxidase mediated mechanism, as evidenced by inhibition with apocyanin and upregulation of the NAD(P)H oxidase subunits, p22phox and p47phox (2.4 and 2.6 fold increase in A II-PM_{2.5} vs. A II-FA by real time RT-PCR, p<0.05) in the aortic vessel wall. There were no differences in expression of p67phox, gp91phox, nox1, and nox4. RhoA protein and mRNA were similar; while Rho associated kinase-1 (ROCK-1) mRNA was increased (2.1 fold increase in A II-PM_{2.5} vs. A II-FA).

Short-term air pollution exposure may exaggerate A II-induced hypertension through NAD(P)H oxidase and Rho-kinase dependent pathways.

2113 FINE AMBIENT AIR PARTICULATE MATTER EXPOSURE INDUCES MOLECULAR ALTERATIONS INDICATIVE OF CARDIOVASCULAR DISEASE PROGRESSION IN ATHEROSCLEROTIC SUSCEPTIBLE MICE.

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Epidemiological, clinical, and toxicological studies have demonstrated that exposure to ambient air particulate matter (PM) can alter cardiovascular function and may influence cardiovascular disease (CVD). It has been shown that exposure to concentrated ambient air particles (CAPs) results in an increase in plaque area in mice that are susceptible to CVD development. Although the effects of CAPs on plaques have been well characterized phenotypically, alterations at the gene expression level have not yet been examined. The present study utilizes laser capture microdissection to recover atherosclerotic plaques from ApoE^{-/-} male mice exposed daily for 6 months to either air or CAPs (Inhal Tox 17:217-224, 2005). Total RNA extracted from the microdissected plaques was amplified and analyzed for alterations in gene expression using the Affymetrix platform and mouse genome 430 2.0 array. Differential gene expression analysis was conducted at a Bayesian p-value estimate of 0.05 and 1.2 fold change. Approximately 2000 genes were altered: 500 genes down regulated and 1500 genes up regulated. Ontological analysis ranked alterations to groups of genes, in order of significance, involved in serotonin receptor, phospholipase C activating pathway, neutrophil chemotaxis, cardiac inotropy, inflammatory response, regulation of muscle contraction, cell proliferation, oxidative stress, and heart development. These data are consistent with previous studies demonstrating an increase in lesion area, cellularity, and oxidative stress following CAPs exposure. These technologies will aid in the identification of specific molecular changes which influence CVD progression following ambient PM exposure as well as provide insight into the contribution that environmental air pollution has on chronic health effects. (Funding: USEPA-NCSSU Cooperative Training Agreement CT 833235-01-0. This abstract does not necessary reflect EPA Policy)

2114 SHORT-TERM AIR POLLUTION EXPOSURE POTENTIATES INSULIN RESISTANCE.

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Background: Although there are no direct links implicating air pollution with metabolic syndrome (MS), there is a correlation between the latter and urbanization. We hypothesized that short-term exposure to fine particulate matter <2.5 µm (PM_{2.5}) induces insulin resistance, and potentiates vasoconstriction.

Methods: C57BL/6 mice (n=32, male) fed with high fat chow (HFC) for 5 months, were exposed to concentrated ambient PM_{2.5} or filtered air (FA) in a whole-body exposure chamber for 10 weeks (6 h/d, 5 d/wk).

Results: Insulin/glucose ratios, glucose tolerance test, and weights were identical prior to exposure; PM_{2.5} exposure worsened fasting hyperglycemia (168±6 vs. 126±7 mg/dl p<0.01 vs. FA-HFC), and after glucose loading (370±35 vs. 315±25 mg/dl at 30 minutes, both p<0.01 vs. FA-HFC). Basal nitric oxide (NO) release

assessed by the degree of constriction to NG-monomethyl-L-arginine (L-NMMA, 10^{-4} M) was reduced in PM_{2.5} vs FA-HFC group. Stimulated NO release in the aorta assessed by response to acetylcholine (10^{-5} M) and insulin (500 mU) was attenuated in the PM_{2.5}-HFC group vs. FA-HFC ($51 \pm 6\%$ vs. $75 \pm 4\%$; $17 \pm 6\%$ vs. $29 \pm 9\%$, $p < 0.01$ vs. FA-HFC respectively). Response to phenylephrine was potentiated (136 ± 6 vs. $100 \pm 6\%$, PM_{2.5}-HFC vs. FA-HFC, $p < 0.01$) in denuded aortic segments, with the exaggerated response inhibitable by LY294002 and Wortmannin, inhibitors of phosphatidylinositol 3-kinase. Phosphorylation levels of Akt and eNOS were significantly down-regulated in the PM_{2.5} group compared with FA group ($49 \pm 11\%$ vs. $100 \pm 19\%$; $51 \pm 3\%$ vs. $100 \pm 16\%$, respectively).

Conclusion: PM_{2.5} aggravates insulin resistance, attenuates NO release and potentiates vasoconstriction at the level of the vasculature through PI3K/Akt pathways. These findings suggest an important new link between air pollution and MS.

2115 MECHANISM OF DISRUPTION OF ENDOTHELIAL MONOLAYER INTEGRITY BY SODIUM ARSENITE.

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Arsenic contamination of ground water is a global problem and drinking water is a significant source of arsenic exposure to humans. Epidemiological studies have shown an association between elevated arsenic levels in drinking water and cardiovascular disease. Arsenic exposure has been shown to exacerbate atherosclerosis, beginning with activation of the endothelium that lines the vessel wall. Endothelial barrier integrity is maintained by proteins of the adherens junction such as vascular endothelial cadherin (VE-cadherin) and β -catenin and their association with the actin cytoskeleton. In the present study, human aortic endothelial cells were exposed to 1, 5 and 10 μ M sodium arsenite [As(III)] for 1, 6, 12 and 24 h, and the effects on endothelial barrier integrity were determined. Immunofluorescence studies revealed formation of actin stress fibers and non-uniform VE-cadherin and β -catenin staining at cell-cell junctions that were concentration and time-dependent. Intercellular gaps were observed with a measured increase in endothelial permeability. In addition, concentration-dependent increases in tyrosine phosphorylation (PY) of β -catenin and activation of protein kinase C γ (PKC γ) were observed. Inhibition of PKC γ restored VE-cadherin and β -catenin staining at cell-cell junctions and abolished the As(III) induced formation of actin stress fibers and intercellular gaps. Endothelial permeability and PY of β -catenin were also reduced to basal levels. These results demonstrate that As(III) induces activation of PKC γ , which leads to increased PY of β -catenin downstream of PKC γ activation. Phosphorylation of β -catenin plausibly severs the association of VE-cadherin and β -catenin, which along with formation of actin stress fibers, results in intercellular gap formation and increased endothelial permeability. The intercellular gaps and increased permeability induced by arsenic could potentially accelerate/enhance the process of atherosclerosis. (Supported by NIH grant P20 RR17670)

2116 SMOOTH MUSCLE DYSFUNCTION INDUCED BY MONOMETHYLARSONOUS ACID (MMAIII): A CONTRIBUTING FACTOR TO ARSENIC-ASSOCIATED CARDIOVASCULAR DISEASES.

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Arsenic in drinking water is reported to be associated with cardiovascular diseases and cancers in humans. Recent studies suggested that methylated trivalent arsenicals, the metabolites of inorganic arsenic, could be responsible for the arsenic toxicity. To study the role of monomethylarsonous acid (MMAIII), a major methylated trivalent arsenicals, in arsenic-induced cardiovascular diseases, we investigated the effect of MMAIII on blood vessels using organ bath systems. In isolated rat thoracic aortic rings, pre-treatment of MMAIII suppressed agonist-induced vasoconstriction irreversibly in a dose-dependent manner, regardless of agonist type, such as phenylephrine (PE), serotonin or endothelin-1. Similar effects were also observed in aortic rings without endothelium, suggesting that vascular smooth muscle function was directly affected by MMAIII. In contrast to the pre-treatment, a post-treatment of MMAIII after induction of vasoconstriction by PE has no inhibitory effect, showing that MMAIII affected on the initiation process of vasoconstriction, not on the maintenance of vasoconstriction. The effect of MMAIII was mediated by inhibition of PE-induced Ca²⁺ increase as found in aortic strips and rodent smooth muscle cells, A7r5 using confocal microscopy. Extracellular influx and release from intra-cellular store were blocked concomitantly by MMAIII treatment. In calcium-permeabilized aortic strips, MMAIII did not affect vasoconstriction stimulated by Ca²⁺ addition, confirming the specific effect on Ca²⁺ mobilization, not through effect on other contractile machinery. Consistent with the in vitro results, pre-treatment of MMAIII intravenously attenuated PE-induced blood pressure increase in in vivo rat models. In conclusion, MMAIII induces smooth muscle dysfunction mediated through inhibition of Ca²⁺ increase, which results in impaired vasoconstriction and blood pressure change. This study will provide a new insight into the role of trivalent methylated arsenicals in arsenic-associated cardiovascular diseases.

2117 ARSENITE ENHANCES PLATELET ADHESION TO ENDOTHELIAL CELLS THROUGH MODULATING ADHESION MOLECULE EXPRESSION ON PLATELET SURFACE.

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Arsenic, an important contaminant in drinking water, is known to be a risk factor to cardiovascular diseases (CVD) in human, but the exact mechanism underlying remains unclear. In this study, we investigated whether arsenic could affect adhesion of platelet to vascular tissue that is, endothelial cell, which can accelerate the progression of CVDs ultimately. To examine the effect of arsenite on the platelet adhesion molecules, expression level of glycoprotein (GP) IIb/IIIa, GP Ib/IX/V, or P-selectin on platelet was measured using flow cytometric analysis after arsenite treatment. As a result, arsenite did not affect the expression of these adhesion molecules in human platelets independently. However, in the presence of sub-threshold level of thrombin, arsenic potentiated increases of GP IIb/IIIa and P-selectin expression, important adhesion molecules that serve as a receptor for adhesive proteins, in a concentration-dependent manner. In contrast, GP Ib/IX/V, which is decreased in activated platelets on the platelet surface, was found to disappear from surface in arsenite-exposed platelets. In accordance with the adhesion molecule expression, arsenite increased the adhesion of platelets to human aortic endothelial cells. In conclusion, arsenic-exposed platelets become more active in adhesion molecule expression and it leads to enhanced adhesiveness to endothelial cells. These results suggest that arsenic enhances the sensitivity of platelet adhesion to endothelium, and potentiated platelet adhesion could be an important aggravating factor to CVDs in arsenic-exposed human populations.

2118 A NEW METHOD FOR STUDYING PLATELETS, BASED UPON THE LOW-ANGLE LIGHT SCATTERING TECHNIQUE, AND ITS APPLICATION IN EXPERIMENTAL TOXICOLOGY.

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Platelets, being highly-labile structures, can be activated or inhibited under small influences. Despite understanding of the role of platelets in hemostasis, and a variety of methods for investigating their functionality, there is a need for simple quantitative analysis of their sensitivity to the main physiological agonists: ADP, thrombin, PAF, etc. A new method is presented for simultaneous monitoring of changes in shape and aggregation of platelets. The signal of light scattering alterations at angles below 6 degrees was shown to be determined by platelet aggregation dynamics. Over a range of larger angles (6-15 degrees), cell shape changes also contributed to the signal: (I) rounding, and (II) pseudopodia formation. The EC50 value for ADP-induced rounding was 40 nmol l⁻¹. Aggregation kinetics showed 2nd order kinetics in relation to initial cell concentration. The rate constant depended on stirring conditions and on [Ca²⁺] in the medium. The method was used as a tool in experimental low-level intoxication by organophosphates and for studying delayed effects. Platelets of rats exposed to Russian VX differ from the control ones by their pronounced instability, which was sometimes expressed by the development of spontaneous activation and aggregation without adding ADP into the medium. Kinetic parameters of aggregation were significantly increased with regards to both maximal velocity of aggregation U_{max} and effective concentration EC50. Toxicological experiments with fluoroacetate (FA), an inhibitor of TCA cycle, showed a hypersensitivity followed by a refractory (insensitive) state of rat platelets. The work was supported by the BioIndustry Initiative (BII) of the U.S. Department of State (Grant# BII-ISTC-2629).

2119 COOXIDATION MEDIATED XENOBIOTIC ACTIVATION AND CYTOTOXICITY BY 12-LIPOXYGENASE IN INTACT PLATELETS.

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Peroxidase mediated cooxidation process has been suggested as a major alternative pathway for xenobiotic activation other than hepatic cytochrome P450 monooxygenase system. Despite the wealth of reports on the potential involvement of cooxidation in the activation of various compounds, manifestation of cooxidation mediated xenobiotic activation in intact cell system without extra sources of peroxidase system has been very difficult to demonstrate, mainly due to the natural scarcity of peroxidase enzymatic activities. In the present study, arachidonic acid dependent

activation of the representative phenolic compound, α -naphthol was observed in intact platelet system as shown by covalent binding increases in arachidonic acid and α -naphthol concentration dependent manner. It suggests that prostaglandin H synthase (PHS) or 12-lipoxygenase (12-LPO), two major arachidonic acid dependent peroxidase systems in platelets, can mediate bioactivation of α -naphthol. Furthermore, this cooxidation process has resulted in enhanced cytotoxicities of α -naphthol against platelets. Interestingly, baicalein and CDC, inhibitors of 12-LPO attenuated the bioactivation of α -naphthol while inhibitors of PHS, indomethacin or aspirin was not effective, implying dominant role of 12-LPO in arachidonic acid initiated cooxidation in intact platelets. In addition, anti-oxidants prevented bioactivation and manifestation of α -naphthol cytotoxicity effectively, supporting the generation of radical or reactive oxygen species in the course of α -naphthol cooxidation. With these results, we believe that an important in vitro evidence of peroxidase-mediated xenobiotic activation was provided for understanding toxicological significances of peroxidase-mediated cooxidation in the xenobiotic bioactivation.

2120 DIFFERENTIAL ROLE OF NRF2 IN REGULATING THE INDUCIBILITY OF ANTIOXIDATIVE/ANTI-INFLAMMATORY DEFENSES BY 3H-1, 2-DITHIOLE-3-THIONE AND SIMVASTATIN IN MACROPHAGES.

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Macrophages release reactive intermediates, including oxyradicals, which mediate inflammatory responses. On the other hand, macrophages are also targets of oxidative stress. These cells play a critical role in the pathogenesis of various chronic inflammatory disorders, such as atherosclerosis. Thus, understanding the signaling mechanisms controlling the antioxidative/anti-inflammatory defenses in macrophages is of importance for developing strategies for intervention of chronic inflammatory disorders. Accordingly, this study was undertaken to investigate the role of Nrf2 signaling in regulating both constitutive and inducible expression of antioxidative/anti-inflammatory defenses in mouse macrophages. The basal expression of a series of antioxidative/anti-inflammatory defenses was significantly lower in Nrf2^{-/-} macrophages than in wild-type (Nrf2^{+/+}) cells. These included catalase, glutathione (GSH), GSH peroxidase, glutathione reductase, GSH S-transferase, NAD(P)H:quinone oxidoreductase 1, and heme oxygenase-1. Incubation with 3H-1,2-dithiole-3-thione (D3T), a chemoprotectant, resulted in significant induction of the above antioxidative/anti-inflammatory defenses in Nrf2^{+/+} macrophages, but not in Nrf2^{-/-} cells. Notably, incubation of macrophages with the lipid-lowering drug, simvastatin at 1-10 μ M also led to significant induction of the above antioxidative/anti-inflammatory defenses. Surprisingly, induction of the above cellular defenses by simvastatin was independent of Nrf2 signaling. Regardless of the differential role of Nrf2 signaling in D3T- and simvastatin-mediated upregulation of cellular defenses, treatment of macrophages with either D3T or simvastatin afforded significant protection against oxidative and electrophilic injury. Studies are currently underway to investigate if upregulation of the above cellular defenses also attenuates the formation of macrophage proinflammatory cytokines.

2121 INHIBITION OF CHOLESTERYL ESTER HYDROLASE IN THP-1 CELLS BY ORGANOPHOSPHORUS OXONS.

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Cholesteryl ester hydrolase (CEH) is an enzyme found in human monocytes and macrophages that catalyzes the hydrolysis of cholesteryl esters to yield free cholesterol. This step is believed to play an important regulatory role in macrophage reverse cholesterol transport, the process in which cholesterol in atherosclerotic plaques is transported to the liver via high density lipoprotein particles for secretion in bile. Human CEH differs from hepatic carboxylesterase 1 (CES1) by only 5 amino acids. CES1 is inhibited stoichiometrically by organophosphorus (OP) oxons, the active metabolites of OP pesticides. We hypothesize that exposure to OP pesticides may adversely affect macrophage reverse cholesterol transport thus increasing the risk of atherosclerosis. To test this hypothesis, we have measured the ability of several OP oxons to inhibit CEH/CES1 activity. CEH/CES1 activity was measured using para-nitrophenyl valerate as the substrate. Chlorpyrifos oxon, paraoxon, and methyl paraoxon all inhibited CEH/CES1 activity in THP-1 cell lysates. IC50 values are in the nanomolar range with chlorpyrifos oxon being the most potent and methyl paraoxon the least potent. We also treated intact cells in culture with the three oxons and found that CEH/CES1 was inhibited by each of the three oxons with paraoxon being the most potent. Chlorpyrifos oxon was found to be less stable in the culture media than paraoxon possibly accounting for the difference in relative potency in cell lysates versus whole cells. THP-1 cell viability was not affected by exposure to the oxons. Our results also suggest that treatment of THP-1 cells inhibits CEH/CES1 in the intact cell. We conclude that exposure to OP oxons does have the potential to slow macrophage reverse cholesterol transport by inhibiting CEH/CES1. (Supported by NIH-NCRR RR070710 and NIH-P20 RR017661)

2122 IMPACT OF RNA DEGRADATION ON AFFYMETRIX GENE EXPRESSION PROFILES.

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RNA quality appears to be one of the most important factors influencing the success of in vivo gene expression experiments. RNA degradation can occur within minutes after the animal sacrifice due to the presence of RNases in tissues, or can be induced by the treatment with toxicants that produce tissue necrosis. The present study was undertaken to evaluate the impact of RNA degradation on reproducibility and comparability of gene expression profiles. Rat liver total RNA was extracted from frozen tissue using a combined Trizol/RNeasy® method. RNA was then partially degraded by incubation with different RNase A concentrations. RNA quality was assessed on the Agilent 2100 bioanalyzer and expressed as the RNA Integrity Number (RIN), a new metric developed by Agilent® based on the overall electropherogram profile of the RNA sample. Four groups of three RNA samples were prepared with decreasing qualities, corresponding to RIN values of 9, 8, 7 and 5, respectively. RNA were then analyzed on pangenomic Affymetrix GeneChip® Rat Genome 230 2.0 arrays. Comparisons were performed between lower and high quality RNAs (taking the highest RIN RNA as the baseline). Transcripts with more than a 1.5-fold modulation factor and a p-value lower than 0.05 were selected. Our results show that RNA degradation produced artefactual changes of transcriptomes and that the number of modulated transcripts increased with RNA degradation: 33 transcripts were found to be modulated between RIN 9 and 8 groups, 281 between RIN 9 and 7 groups and 878 between RIN 9 and 5 groups. In addition, we found that the more pronounced the degradation of the RNA, the greater the apparent modulation of the transcripts. Sample clustering analysis showed a clear separation between RNA with a RIN of 5 and the other samples. We have shown in this experiment that RNA degradation can lead to biases which lead to erroneous conclusions on Affymetrix gene expression profiling experiments. These findings show the pertinence of the RIN as a RNA quality parameter and its potential value in the definition of RNA quality thresholds.

2123 DETECTION OF HIGH MW PROTEINS IN MALDI-TISSUE IMAGING.

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BTraditional matrix assisted laser desorption/ionization (MALDI) mass spectroscopy tissue imaging provides excellent coverage of protein profiles within the 2-25 kDa range. We used the traditional method applying matrix with a robotic spotter to the surface of a tumor bearing kidney section from an Eker rat treated with a renal carcinogen 2,3,5-tris-(glutathion-S-yl) hydroquinone. Tumor specific proteins with MW at 10944 (annexin II light chain), 11636 (β -2-microglobulin), histone H4 (11234 Da), 13901 Da (acetylated histone H2B), and 14005 Da (methylated histone H2A.3) were detected. In order to detect proteins with higher MW and resolution to determine posttranslational modifications, we developed a method that offers sensitive coverage of proteins of 25-100 kDa. The new method is derived from the MALDI sandwich method where a frozen tissue section is placed on top of a drop of matrix dissolved in a fixation solution. More matrix is deposited on top of the tissue in a manner to limit protein delocalization and to enhance the signal. Triton X-100 doubled the number of separate MALDI peaks to >1000 peaks. A series of quality controls was developed. Protein peaks of 41841 and 41873 Da, corresponding to the α -cardiac and α -skeletal muscle isoforms of actin, were detected in the heart. Evidence for phosphorylation of cardiac myosin light chain (MLC) was region specific. MALDI images of a contracting rat colon and stomach were obtained. Similar to the heart, it was possible to distinguish between isoforms of actin differing by 20-40 Da. Signals of the appropriate M/z to be regulatory smooth muscle MLC and MLC-P were also observed. Evidence from MALDI and immunohistochemistry is presented for the presence of a phosphoprotein upstream of MLC-P. In summary, the ethanol Triton X-100 sandwich method allows the detection of large MW proteins in tissue images. The strength of the signal will allow hypothesis testing, in conjunction with other techniques, rather than simple profiling. (GM39338, ES06694)

2124 MICROARRAY ANALYSIS OF THE GLOBAL GENE EXPRESSION RESPONSE TO N-NITROSODIMETHYLAMINE EXPOSURE UNDER DIABETIC CONDITIONS.

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The incidence of diabetes has risen dramatically in the United States during the past decade. Recent evidence implicates diabetes as a risk factor for development of chronic non-alcoholic liver disease and hepatocellular carcinoma (HCC). The ex-

pression of CYP2E1 is increased under low insulin (diabetic) conditions, and CYP2E1 catalyzes the metabolism and bioactivation of numerous xenobiotics, including nitrosamines. To examine the hypothesis that diabetic conditions result in increased risk of hepatic disease and HCC associated with increased xenobiotic metabolism, global gene expression profiles resulting from N-nitrosodimethylamine (NDMA) treatment were obtained using high density microarrays. Primary cultured rat hepatocytes, maintained under low insulin were treated with 1, 10, and 100 μ M NDMA for 24 hours. Gene expression changes in the NDMA-treated samples were compared with untreated controls using Agilent Rat whole genome oligonucleotide microarrays. Stringent data filtering by statistical analysis, minimum intensity criteria, and fold change cutoffs identified over 100 genes that fall into two primary clusters, which are up- and down-regulated in a dose-dependent manner. Principal components analysis reveals subtle changes in expression between untreated cells and those treated at the two lower concentrations, while the 100 μ M treatment results in a significant change in expression for many genes. Pathway analysis of the differentially expressed genes indicates that the phosphatidylinositol signaling, calcium signaling, and MAPK pathways are altered in response to NDMA treatment. Bioinformatics analysis of the upstream regions of co-clustered genes provides evidence of common transcription factors, including Sp1, Egr, and RREB. This research has been supported by National Institute of Environmental Health Sciences grants ES03656, ES06639 and Agilent Technologies.

2125 ENVIRONMENTAL INFLUENCES ON THE FETAL EPIGENOME.

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A growing body of evidence suggests that epigenetic mechanisms of gene regulation, such as DNA methylation and histone modifications, are influenced by the environment, and play an important role in the early origins of adult disease. Using the viable yellow Agouti (Avy) mouse, we investigated environmental determinants of DNA methylation following maternal exposures. Methylation of cytosine-guanine (CpG) dinucleotides is stochastic among isogenic Avy mice, causing variation in coat color from yellow (unmethylated) to pseudoagouti (methylated). Unmethylated mice also experience higher rates of obesity, diabetes, and tumorigenesis due to constitutive ectopic Agouti expression. Since the epigenome is particularly susceptible to dysregulation during embryogenesis, we investigated the effect of dietary genistein supplementation of mice during gestation, at levels comparable with humans consuming high-soy diets. Maternal genistein shifted the coat color distribution of Avy offspring toward pseudoagouti and increased methylation of six CpG sites in the Avy retrotransposon. We also investigated the effect of low-level dietary bisphenol A (BPA) exposure of mice during gestation. Maternal BPA shifted the coat color distribution of Avy offspring toward yellow and decreased methylation of six CpG sites in the Avy retrotransposon. These studies provide the first evidence that *in utero* dietary exposure to genistein or BPA affects gene expression and alters susceptibility to disease by permanently modifying the epigenome. Interestingly, maternal co-exposure to BPA and genistein or BPA and methyl donors such as folic acid, choline, and betaine restores normal stochastic variation in offspring coat color. Similar to rescuing the adverse phenotype of a genetic knockout, these results indicate that environmental co-exposures may result in a cumulative effect that restores normal stochastic variability at Avy and other metastable epialleles, providing interesting implications for public health interventions for environmental exposures affecting the epigenome.

2126 EFFECT OF BISPHENOL A ON SELECTED DIFFERENTIATION MARKER GENES IN EMBRYOID BODIES DERIVED FROM CYNOMOLGUS MONKEY EMBRYONIC STEM CELLS.

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Embryonic stem (ES) cells are useful model systems to test drugs and chemicals and a potential to predict or anticipate toxicity in humans. Experiments using murine ES cells to determine embryotoxicity have been reported, but there is little information available concerning embryotoxicity tests using primate ES cells. In this study, we examined the effect of 0.1 μ M and 10 μ M bisphenol A (BPA) on differentiation of the CMK-6 Cynomolgus monkey ES cell line to determine embry-

otoxicity. Embryoid bodies (EB) were cultured for 7, 14, and 21 days, and a real time RT-PCR was performed to determine gene expression of Oct-3/4, a stem cell marker gene, and several other differentiation markers. Treatment with 0.1 μ M BPA slightly stimulated the expression of Oct-3/4, indicating an inhibition of EB differentiation. BPA also slightly stimulated the expression of α -fetoprotein, an endoderm differentiation marker, suggesting BPA can affect the differentiation of ES cells to various cell types. These results indicate that this system may work as a model for determining embryotoxicity.

2127 TAMOXIFEN AND ETHYNYLESTRADIOL CO-TREATMENT IN C57BL/6 MOUSE UTERUS: GROSS, MORPHOMETRIC AND CDNA MICROARRAY GENE EXPRESSION ANALYSIS.

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Tamoxifen (TAM), the treatment of choice for estrogen receptor positive (ER) breast cancer, has been associated with an increased incidence of endometrial cancer in post-, but not pre-menopausal women. Compared to ethynyl estradiol (EE), a potent agonist, TAM elicits a partial ER-mediated uterotrophic response in immature rodents. Moreover, cotreatment with 1000 μ g/kg TAM antagonizes the uterotrophic effect induced by 30 μ g/kg EE. To further investigate the molecular basis of the antiestrogenicity of TAM in the uterus, a time course analysis was conducted in immature, ovariectomized C57BL/6 mice treated with EE, TAM, EE with TAM or vehicle (sesame oil) following a single dose at 2, 4, 8, 12, 18, 24 hrs or after three daily treatments (3x24 hrs). Uterine wet weight increased by 18 hrs for all treatments. However, at 3x24 hrs, EE-induced uterotrophy is hindered upon cotreatment with TAM. This inhibitory effect is reflected by the absence of an EE-mediated increase in luminal circumference. However, TAM did not antagonize the hallmark estrogenic increase in luminal epithelial cell height. cDNA microarray analysis at 3x24 hrs, using a 2x2 factorial study design, identified 127 EE-induced genes that were further induced in the presence of TAM. These differentially expressed, TAM-augmented genes represented pathways involved in vascularization (i.e. Pgf, Smad5) and ion homeostasis (i.e. App, Commd1). In addition, the expression of 107 EE-induced and 16 EE-repressed genes were antagonized by TAM cotreatment. These antagonized genes were associated with cell cycle regulation (i.e. Cdc25c, Rb) and DNA replication (i.e. Helb, Nusap1), and, in general, consistent with morphometric results. These changes indicate that TAM selectively alters EE-elicited gene expression, consistent with its ability to antagonize EE induction of uterine wet weight.

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2128 THE ISOTHIOCYANATE MOIETY IS REQUIRED FOR SULFORAPHANE (SFN)-MEDIATED INHIBITION OF LIGAND ACTIVATION OF THE HUMAN STEROID AND XENOBIOTIC RECEPTOR (SXR).

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SFN [1-isothiocyanato-4-(methylsulfonyl)butane] is one of several naturally occurring isothiocyanates (ITC) found in broccoli. SFN has chemoprotective properties, demonstrated both in vitro and in a number of animal models. Chemoprevention is presumed to occur via activation of the NRF2/Keap1/Antioxidant Response pathway. We previously demonstrated that SFN is an effective antagonist of the human SXR (also called hPXR). SXR is involved in the regulation of expression of many genes integral to drug metabolism, including CYP3A4 and MDR1. HEPG2 cells were transiently transfected with human SXR and a luciferase reporter coupled to the CYP3A4 promoter. We then evaluated the ability of the ITCs Cheirolin [1-isothiocyanato-3-(methylsulfonyl)propane], Iberin [1-isothiocyanato-3-(methylsulfonyl)propane], Erucin [1-isothiocyanato-4-(methylthio)butane] and phenylethyl isothiocyanate (PEITC), and SFN-nitrile (5-(methylsulfonyl)pentanenitrile, which lacks the ITC moiety) to inhibit rifampicin activation of SXR. SFN-nitrile had no inhibitory activity towards SXR, but Iberin and Cheirolin showed antagonistic activity nearly equipotent to SFN. The results also demonstrated that the alkyl sulfide moiety must be at least partially oxidized to maintain strong SXR antagonism, as Erucin, the analog with a fully reduced alkyl sulfide moiety, showed significantly less antagonism than the other analogs. Molecular modeling identified several cysteine and lysine residues in the ligand binding domain that might be targets for covalent interaction with ITCs. Future studies will evaluate the functionality of these sites via site directed mutagenesis. (Supported by a pilot project from P30 CA15704).

2129 EVALUATION OF THE EFFECTS OF XENETIX® AND DOTAREM® ON HEPATIC AND RENAL GENE EXPRESSION IN RATS.

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Xenetix® and Dotarem® are leading contrast agents for X-ray and magnetic resonance imaging, respectively. The present study was undertaken to evaluate the effects of these compounds on hepatic and renal gene expression in rats. Male Wistar Han rats were treated by a single intravenous injection of Dotarem® at 2.5, 5 or 10 mmol/kg or Xenetix® at 2, 4 and 10 g iodine/kg. Liver and kidneys were sampled 4 and 48 hours after treatment. Blood biochemistry investigations were performed on blood samples taken at necropsy. A microscopic examination was carried out for liver and kidney samples preserved in buffered formalin. RNA was extracted from the tissues and transcriptomes were studied with Affymetrix pangenomic rat arrays. Transcripts with at least a statistically significant two-fold modulation factor between treated and control groups were retained at each time point. Concerning the in-vivo part of the study, effects of the compounds were low in intensity, consistent with those described in the literature and included increased blood urea levels in high dose groups on day 1 and liver and kidney vacuolations at microscopic examination on both days. In animals given Dotarem®, 109 genes (85/31 on day 1/3) were modulated in the kidney and 86 (62/26 on day 1/3) in the liver. Some of these modulated genes were implicated in oxydoreductase activity. After treatment with Xenetix®, gene expression profiling showed that 201 genes (169/22 on day 1/3) were differentially expressed in the kidney and 97 (66/34 on day 1/3) in the liver. The principal ontologies involved were symporter activity, cell migration in the kidney and cation homeostasis in the liver. The effect of treatment with Dotarem® or Xenetix® on the whole kidney and liver transcriptomes appeared to be immediate, with more pronounced effects in the kidney. However, these changes were rapidly reversible, with a return to basal levels on day 3. These results are consistent with the low toxicity in non-clinical studies and with the good tolerance profile of these products in clinical use.

2130 DOWN-REGULATION OF THE UVA-INDUCED COLLAGENASES EXPRESSION BY THE SAPONINS ISOLATED FROM ROOTS OF PLATYCODON GRANDIFLORUM IN HUMAN FIBROBLASTS.

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Exposure of ultraviolet (UV) light, a harmful environmental stress, on the skin often induces chronic skin diseases such as skin cancer as well as photoaging, and the mechanisms of these skin damages are closely associated with up-regulation of collagenases activities. Moreover, UV-induced collagenases cause connective tissue damage and the skin to become wrinkled and aged. In this study we investigated the effect of saponins isolated from Roots of Platycodon grandiflorum (CKS) on UVA-induced collagenase-1 and collagenase-3 expression in human fibroblasts. Treatment with CKS significantly decreased the reactive oxygen species (ROS) formation under UVA irradiation. To investigate the significant cis-acting regions which collagenases promoters, transient transfection experiments were carried out using reporter vectors harboring deleted collagenases promoters. The transcriptional factor binding sites for AP-1 could be important for the induction of collagenase-1 and -3 by CKS. These results suggested that CKS can inhibit UVA-induced collagenase-1 and -3 expressions might be mediated through the AP-1 inactivation and inhibited ROS formation in human fibroblasts.

2131 CCAAT/ENHANCER BINDING PROTEIN β AND STEROIDOGENIC FACTOR-1 BINDING REGIONS IN THE PROMOTER OF TESTOSTERONE BIOSYNTHESIS ENZYMES ARE DIFFERENTIALLY BOUND FOLLOWING *IN UTERO* DIBUTYL PHTHALATE EXPOSURE IN RATS.

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It has been repeatedly demonstrated that prolonged *in utero* exposure of fetal male rats to dibutyl phthalate (DBP) can result in feminized phenotype characterized by malformed epididymides, hypospadias, cryptorchism, and retained thoracic nipples among others. These symptoms likely result from decreased expression of steroidogenic enzymes, and therefore reduced testosterone biosynthesis. However, the molecular mechanisms involved in changes in gene expression profiles are unknown. To understand these mechanisms in rats, an *in vivo* DNase footprinting protocol

was developed to provide a quantitative map of changes in DNA-protein interactions in the promoter region of steroidogenic genes, including StAR, SR-B1, CYP11A1, and CYP17, that are down-regulated following an *in utero* DBP exposure. This map was compared to vehicle control and a non-toxic phthalate diethyl phthalate (DEP). Regions with altered DNase protection were coordinated with a specific DNA binding protein by electrophoretic mobility shift assay, and binding activity confirmed with chromatin immunoprecipitation. Results demonstrated altered DNase protection at multiple putative transcription factor binding sites in each gene. These results were observed along with diminished testis testosterone and mRNA levels of the corresponding steroidogenic gene. Of these DNA binding proteins, only regions mapping to CCAAT/enhancer binding protein beta (c/ebp β) and steroidogenic factor-1 (SF-1) were altered in all genes examined. ChIP confirmed declines in DNA-protein interactions of c/ebp β in DBP treated animals, while SF-1 was observed to be reduced in both DEP and DBP treatments. These results suggest that while decreased SF-1 activity is involved, interference of c/ebp β binding is critical in the observed inhibition of transcription. These observations provide a snapshot of changes in the structure of transcriptional machinery and suggest a possible mechanism of action resulting from DBP exposure.

2132 CHARACTERIZATION OF GENOMIC EXPRESSION IN THE ZUCKER DIABETIC FATTY RAT MODEL SYSTEM VERSUS ZUCKER RATS.

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Three groups of 10 males (resulting in 2 pools of n=5) representing 3 Zucker strains were included in the study. Two strains of Zucker diabetic fatty (ZDF) rats (an accepted rodent model for Type 2 diabetes) and normal Zucker rats were subjected to weekly oral glucose tolerance (OGT) tests at 10 to 13 weeks of age to assess the onset of diabetic symptoms. ZDF animals exhibited associated symptoms starting at approximately 12 weeks of age.

Liver was isolated from test animals and total RNA was analyzed for global gene expression using Affymetrix Rat Genome 230 2.0 arrays. Discriminant analysis was able to distinguish male Zucker Diabetic Fatty rats (Obese and Hyperinsulinemic) from male Zucker rats (Normal). Previous analyses of both male and female 18 week old strains and of younger Diabetic and/or Hyperglycemic animals identified 19 relevant probe sets. Of these common sets, 18 distinguished the normal strain from the two Obese/Hyperinsulinemic strains. Only one probe set distinguished each diseased strain from the other.

The results indicate that gene expression changes in the ZDF model system are highly consistent between diseased strains and differ significantly from normal strains.

2133 COMPENSATORY MECHANISMS IN BILIARY EXCRETION AFTER 2/3-PARTIAL HEPATECTOMY (2/3-PH) IN MICE.

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Bile formation is one of the most important functions of liver and maintenance of biliary excretory function is crucial in various pathological conditions. Bile flow and composition are good indicators of liver function. During the last decade, mice have become a very important model in biomedical research, because of the availability of genetically modified mice. However, neither the flow nor composition of bile is well characterized in mice. Therefore, we examined the effects of an important model of liver regeneration, 2/3-PH, on bile formation and composition of main bile constituents; and on gene expression of important transporters in liver. Bile and liver samples were collected from sham and 2/3-PH mice 24 and 48 hrs after surgery. Bile flow in 2/3-PH mice was similar to that of the sham-operated control mice, calculated per body weight, and about 55% higher in 2/3-PH mice when calculated per liver weight. Bile flow depends mainly on bile acid and glutathione excretion. After 2/3-PH, the biliary excretion of glutathione decreased, whereas bile acid excretion increased per gram liver weight. Therefore, the compensatory increase in bile flow was due to a 2.4-fold increase in bile-acid excretion per gram of liver. The mRNA expression of neither the bile acid uptake transporter, Ntcp, nor the canalicular efflux transporter, Bsep, was altered by 2/3-PH, indicating that these bile acid transporters may have functional reserve capacity. The biliary excretion of cholesterol did not change after 2/3-PH, but phospholipid excretion into bile was markedly increased. The increased expression of the phospholipid transporter, Mdr2, may be responsible for this latter phenomenon. In conclusion, the compensatory maintenance of bile flow in mice after 2/3-PH is largely due to an increased excretion of bile acid, and the increased expression of Mdr2 markedly enhances the excretion of phospholipids, that is known to protect the biliary tree against the harmful effect of enhanced bile acid excretion. (Supported by NIH-grant ES-09716)

2134 CHEMICAL GENETIC APPROACHES TO UNRAVEL THE MOLECULAR PATHWAYS CONTROLLING REGENERATION.

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Zebrafish have the remarkable ability to regenerate a number of tissues following amputation or injury. Previously, we have demonstrated that TCDD exposure impairs caudal fin regeneration and we have used TCDD as a small molecule probe to disrupt the normal regenerative process to define the interactions between AHR and other signal transduction pathways. Our work with TCDD suggested that a small molecule screening approach may be a powerful way to identify molecular pathways that choreograph tissue regeneration. As a proof of this principle, we have developed a rapid throughput in vivo regeneration assay to identify chemicals that disrupt tissue regeneration. Two days post fertilization larvae were amputated and continuously exposed to individual chemicals from a 2000 member structurally diverse small molecule library. Regenerative progression was assessed microscopically at three day post amputation. A "hit" was defined as a small molecule that led to aberrant regeneration and approximately 2.6% of the chemicals were scored as "hits" in the primary screen. The hits were grouped into chemical classes and the largest chemical cluster consisted of 12 glucocorticoids. Consistent with the known actions of glucocorticoids as ligands for glucocorticoid receptors (GR), a number of known GR-target genes such as GILZ, FKBP5 and SOX9b were induced in glucocorticoid exposed larvae. To further reveal that small molecule targets can be defined in this model, we demonstrate that the GR is necessary and sufficient for the inhibitory response. We further characterized that glucocorticoids specifically target early stages of regeneration such as wound healing and blastema formation. Collectively, our studies indicate the feasibility of exploiting chemical genetics to unravel molecular signaling networks in vivo. Supported by NIEHS grants ES10820, ES00210, and #ES03850

2135 LOSS OF C/EBP ALPHA RESULTS IN DIMINISHED DNA DAMAGE INDUCED G1 CHECKPOINT.

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The ability of cells to respond to DNA damage is essential to ensure the integrity of the genome. DNA damage can initiate the activation of cell cycle checkpoints in G1, S and G2 phases preventing the replication of damaged DNA and allowing time for DNA repair. The basic leucine zipper transcription factor, CCAAT/enhancer binding protein α (C/EBP α) is involved in the regulation of mitotic growth arrest and differentiation in some cells. C/EBP α is induced by DNA damage through a p53-dependent pathway in keratinocytes. C/EBP α knockdown by siRNA resulted in a diminished G1 checkpoint after UVB-induced DNA damage suggesting a role for C/EBP α in the G1 checkpoint. Recently, we have shown that C/EBP α is induced by DNA damaging agents in cell types other than keratinocytes such as dermal fibroblasts. The objectives of this study are to determine the pathway of C/EBP α induction in fibroblasts and the physiological consequences of C/EBP α induction. We report here that C/EBP α induction by UVB in fibroblasts does not require p53, however, caffeine, an inhibitor of ataxia-telangiectasia-mutated kinase and ataxia-telangiectasia-mutated and Rad3-related kinase (ATM/ATR), blocks C/EBP α induction by UVB. Inhibitors of glycogen synthase kinase 3 beta (GSK-3 β) also block C/EBP α induction by UVB. To determine whether C/EBP α has a role in cell cycle regulation, fibroblasts were isolated from C/EBP α -null and wild type mice, placed in culture and synchronized by serum deprivation. After release into the cell cycle, fibroblasts from the two genotypes progressed similarly into S-phase and no differences were observed between the two genotypes. When cells were treated with UVB in early G1 phase, entry of cells into S-phase was increased in C/EBP α -null fibroblasts compared to wild type. These results provide genetic evidence that C/EBP α has a role in the G1 checkpoint. Our results also suggest that C/EBP α induction by DNA damage in fibroblasts involves ATM/ATR and GSK-3 β but does not require p53.

2136 PHARMACOKINETIC AND PHARMACODYNAMIC EFFECTS OF 2'-MOE MODIFIED PHOSPHOROTHIOATE ANTISENSE OLIGONUCLEOTIDES ADMINISTERED DIRECTLY TO LUNG AND CEREBROSPINAL FLUID.

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Objective: To determine the PK/PD relationships for proprietary modified antisense oligonucleotides (ASO) following either intrapulmonary delivery utilizing aerosols targeting IL-4R α mRNA in the mouse lung or direct intracerebroventricular

lar (ICV) or intrathecal (IT) injection targeting SOD-1 mRNA in the rat central nervous system (CNS). Methods: Animal studies were conducted to characterize the tolerability and pharmacokinetics. ASO concentrations in target tissues were measured using either hybridization ELISA or capillary gel electrophoresis (CGE). IL-4R α protein in lung was measured by flow cytometry and target mRNA measurements by qRT-PCR. Results: Aerosol doses up to 1 mg/kg IL-4R α ASO were well-tolerated in mice. Significant reduction in IL-4R α protein expressed in pulmonary epithelial cells and inflammatory cells resulted in significant amelioration of asthma symptoms in mice during induced acute inflammation. Minimum effective doses were observed at 1 to 10 μ g/kg and lung tissue concentrations < 50 ng/gram. The ASO half-life in the lung was estimated to be 2 to 4 days, and once weekly dosing sustained the IL-4R α antisense effect and reduced AHR and airway inflammation in mice. In a brain study, ICV administration in rats was well tolerated up to 100 μ g/day for one month of continuous infusion. At this dose, the brain and spinal cord SOD-1 ASO concentrations were 55 $\pm$ 15 μ g/g and 20 $\pm$ 12 μ g/g, respectively. The SOD-1 mRNA reduction was 40-60% of saline treated animals in brain and approximately 20% in spinal cord. The ASO half-life in the brain ranged from 14 to 30 days. Comparison of IT and ICV administration on kinetics and antisense effects will also be presented. Conclusions: Direct topical administration to lung and brain of 2'-MOE modified PS ASOs was well tolerated, exhibited potent dose and concentration-dependent antisense activity, and was well-correlated with the ASO pharmacokinetics.

2137 REGIONS OF ALTERED METHYLATION (RAMS) IN LUNG/BRONCHI OF RATS EXPOSED TO MAINSTREAM TOBACCO SMOKE (MTS).

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DNA methylation, i.e., 5-methylcytosine, is an epigenetic modification regulating gene expression. Aberrant methylation plays a key role in carcinogenesis and we hypothesize that it also contributes to other toxicities. We evaluated the effect of MTS on the methylation status of rat lung/bronchiole DNA. Male and female Sprague Dawley rats were exposed to air only, sham or low, medium, or high (0.06, 0.20, or 0.80 mg wet total particulate matter/L) doses of MTS for 1 hr/day, 5 days/wk for 13 wk. After treatment, a procedure involving methylation-sensitive restriction digestion, arbitrarily primed PCR, and capillary electrophoretic separation of the PCR products was utilized to identify regions of altered DNA methylation (RAMs). In males, a dose-response relationship was evident based on the total number of RAMs that formed in each treatment group, and a trend towards increasing RAMs was observed for the females. In the males, total RAMs detected in the low, medium and high dose groups were 26, 32 and 41, respectively. In the females, total RAMs discerned in the low, medium and high dose groups were 29, 31 and 33, respectively. Of the 41 total RAMs in the males at the high dose, 3 were also seen in the low/medium dose groups, while 38 were unique to the high dose rats. The females displayed 33 RAMs at the high dose, 4 of which were also observed in the low/medium dose groups while 29 were unique to the high dose. While new methylations predominated, complete hypomethylations exhibited a dose-response relationship. Histopathological analysis revealed minor changes, an increase in the number of macrophages in both sexes in all dose groups, and an increase in goblet cells at the high dose in the male rats only. Importantly, this group exhibited the most RAMs. In conclusion, MTS treatment of both male and female rats alters DNA methylation in lung/bronchi, and it is possible that this epigenetic change may serve as a biomarker of exposure.



2138 AGING AS A MODULATOR OF CHEMICAL TOXICITY.

C. Corton¹ and H. M. Mehendale². ¹NHEERL, U.S.-EPA, Research Triangle Park, NC and ²Department of Toxicology, University of Louisiana, Monroe, LA.

One of the greatest achievements of the last 8 decades is better health care, which has led to a burgeoning aging population. By 2030, the number of individuals older than 65 will more than double to 72 million, and one of every five Americans will be older than 65. The rapid growth in the number of older Americans has many implications for public health, including the need to better understand the risks posed to older adults by environmental exposures to chemicals. The capacity to appropriately respond to toxicant exposure declines with normal aging and may be exacerbated in individuals with pre-existing health conditions. This decline can result in compromised pharmacokinetic and pharmacodynamic responses to environmental exposures encountered in daily activities. The objective of this symposium is to highlight recent examples of altered sensitivities to xenobiotic exposure by aging in a number of tissues and to bring forward substantial new information on what is

known about their mechanisms. This symposium will include an overview of molecular pathways that are altered in aging including those involved in xenobiotic metabolism and will include important examples of how aging alters chemical sensitivity in the liver, kidney and brain.

2139 GENETIC PATHWAYS THAT REGULATE AGEING IN MODEL ORGANISMS.

V. Longo. *University of Southern California, Los Angeles, CA.* Sponsor: C. Corton.

Mutations in genes affecting metabolism, stress responses, endocrine signaling, and telomeres can increase the life spans of a number of model organisms. These mutations revealed evolutionarily conserved pathways for aging that extend life span in response to sensory cues, caloric restriction, or stress. This talk will focus on how mutations affecting longevity pathways delay age-related disease, and how the molecular analysis of these pathways is leading to a mechanistic understanding of linkage of aging and chemically-induced disease susceptibility.

2140 IDENTIFICATION OF CHANGES IN XENOBIOTIC METABOLISM ENZYME EXPRESSION DURING AGING USING COMPREHENSIVE TRANSCRIPT PROFILING.

C. Corton. *NHEERL, U.S.-EPA, Research Triangle Park, NC.*

Aging changes the expression of enzymes and transporters important in the metabolism and fate of xenobiotics in the liver, kidney and intestine. Most notable are the changes in a number of CYP and xenobiotic transporter genes regulated by the nuclear receptors PXR, CAR and PPARalpha. In order to gain a comprehensive understanding of the effects of aging on xenobiotic metabolism, we are using DNA microarray analysis to study the changes that occur in a number of tissues relevant to metabolism of xenobiotic chemicals at different life stages in mice and rats. We show remarkable changes in phase I, II and III gene expression in the liver that are both species-dependent and -independent. We are using this information to create a biologically realistic pharmacokinetic model that predicts altered metabolism of xenobiotics in aged populations.

2141 INCREASED SUSCEPTIBILITY OF AGING KIDNEY TO ISCHEMIC INJURY: ROLE OF INCREASED MMP EXPRESSION.

A. Parrish. *College of Medicine, Texas A&M, College Station, TX.* Sponsor: C. Corton.

Aging is associated with an increased incidence and severity of acute renal failure; however, the molecular mechanisms underlying the increased susceptibility remain undefined. These experiments were designed to investigate the influence of age on the response of the kidney to ischemia. Renal slices were prepared from young (5 month), aged ad libitum (24 month) and aged caloric restricted (24 month) male Fischer 344 rats and subjected to ischemic stress (anoxia, 100% N₂) for 0-60 min or to the nephrotoxicant cisplatin (2 mM, 4 hr). As assessed by intracellular ATP and GSH content, and LDH and alpha-GST leakage into the culture media, slices from aged ad libitum rats were more susceptible to injury than young counterparts. Importantly, caloric restriction attenuated this increased susceptibility to injury. In an attempt to identify molecular pathway(s) underlying this response, microarray analysis was performed on tissue harvested from the same animals used for the functional analysis. Several age-related changes in the expression of MMPs were identified via microarray. Therefore, we examined the impact of aging, and caloric restriction, on the expression of MMPs and TIMPs in the rat kidney using quantitative RT-PCR. Aging was associated with increased expression of MMP-7, -9, -13 and -14 (MT1-MMP) and TIMP-1; interestingly these changes were attenuated by caloric restriction. The increased expression of these MMPs was associated with increased gelatinase, collagenase and matrix type MMP activity. Ablation of MMP-14 expression in NRK cells using shRNA attenuated several changes associated with ischemia, suggesting that MMPs may mediate ischemic injury in the kidney. In summary, overexpression of MMPs may be associated with the increased susceptibility of aging kidney to ischemic insult.

2142 AGING PROTECTS AGAINST CHLORDECONE AMPLIFIED PROGRESSION OF HALOALKANE HEPATOTOXICITY.

H. M. Mehendale. *Department of Toxicology, University of Louisiana, Monroe, LA.*

The prevailing general perception is that aging increases drug- and chemical-induced hepatotoxicity. Although considerable age-dependent adverse drug reactions and hepatotoxicity are known, the reasons for this variation remain to be fully ex-

plored. Among the different models used to study hepatotoxicity, chlordecone (CD) + CCl₄ interactive model is the most potent and well known for its advantages: toxicity at individually nontoxic levels, extensive liver damage leading to death due to hepatic failure, and mechanisms studied at length, etc. In adult rats (3 months) this interactive toxicity results in 67-fold amplification of hepatotoxicity and lethality. However, older F344 (14 months) and SD and F344 rats (24 months) are completely resilient to the interactive toxicity of CD+CCl₄. The toxic effects of this interaction in adult rats (3 months) include extensive hepatotoxicity characterized by elevated ALT and AST enzyme levels, hepatic necrosis, bankruptcy in cellular energy, failed tissue repair leading to progression of injury, hepatic failure, and death. In contrast, in aged rats, only transient hepatic damage was observed even though the initial bioactivation-mediated liver injury of CCl₄ in CD pre-exposed rats is the same as adults. Molecular changes in genes involved in the cell cycle may be the basis for this protection. The mechanisms underlying this remarkable resiliency in advanced age towards highly amplified toxicity of CCl₄ are of significant interest, particularly because these findings suggest that it may be possible to stimulate liver tissue repair in advanced age.

2143 AGING AND SENSITIVITY TO ORGANOPHOSPHORUS INSECTICIDE: TOXICOKINETIC AND TOXICODYNAMIC FACTORS.

C. Pope. *Oklahoma State University, Stillwater, OK.*

Organophosphorus insecticides inhibit acetylcholinesterase to elicit neurotoxicity. Aged rats exhibit higher acute sensitivity than adults to the prototype organophosphorus insecticide parathion. We studied possible toxicokinetic and toxicodynamic processes that may contribute to the differential sensitivity. While detoxification potential (paraoxonase, carboxylesterase activities) in liver and lung was generally similar between age groups, plasma carboxylesterase activity was significantly lower in aged rats and was highly correlated with acute sensitivity to parathion across multiple age groups. As carboxylesterase is particularly important in detoxification of parathion (by stoichiometric binding to its active metabolite, paraoxon), age-related differences in plasma carboxylesterase activity could contribute to the differential sensitivity. The neurochemical consequence of extensive acetylcholinesterase inhibition is accumulation of synaptic acetylcholine, which in turn causes overstimulation of cholinergic receptors and a variety of cholinergic signs of toxicity. We thus compared striatal acetylcholine release/accumulation in adult and aged rats both in vitro using brain slices and in vivo using microdialysis. Using superfused striatal slices, the muscarinic autoreceptor agonist carbachol was less potent at inhibiting acetylcholine release in aged rats compared to adults, suggesting potentially lesser presynaptic adaptation to elevated acetylcholine levels following anticholinesterase exposure in aged rats. In contrast, using in vivo microdialysis, markedly less striatal acetylcholine accumulation was noted in aged rats compared to adults after treatment with equitoxic dosages of parathion (9 vs 27 mg/kg, sc). These results suggest that presynaptic inhibition of acetylcholine release may be less effective with aging, but that cholinergic toxicity may be elicited by lesser brain acetylcholine accumulation in aged animals. Both toxicokinetic and toxicodynamic factors may therefore play a role in the differential sensitivity to anticholinesterases in aging.

2144 GENE EXPRESSION AND IMMUNE SYSTEM SUSCEPTIBILITY.

D. Germolec¹ and R. Luebke². ¹*Integrative Toxicology Group, NIEHS, Research Triangle Park, NC* and ²*Immunotoxicology Branch, U.S. EPA, Research Triangle Park, NC.*

Differences in gene expression, resulting from an individual's genetic makeup or induced by environmental exposures, may have a significant impact on an organism's response to pathogens, neoplastic cells and/or exogenous antigens. Immune system homeostasis and responses to pathogens or neoplastic cells requires a tightly regulated and progressive cascade of gene expression, protein synthesis and effector function. Exposure to drugs or chemicals may alter expression of genes culminating in suppression of function or inappropriate stimulation of normally protective responses. Inherited polymorphisms may also predispose the host to express greater sensitivity or susceptibility to exogenous agents, either as a direct result of xenobiotic-gene product interaction or indirectly by increasing effects of xenobiotics on gene expression. The use of microarray technologies as discovery tools to detect altered immune pathways and/or function by examining genome-wide changes in expression patterns following exposure to environmental agents is increasing. This technology can be used to evaluate human and laboratory animal samples for similarities in expression profiles following accidental or voluntary exposure to a variety of potential toxic compounds. The symposium will address two separate but related uses of genomics techniques: detection of modified gene expression in ostensibly normal individuals following exposure to xenobiotics, and characteriza-

tion of gene expression profiles that predispose an individual to greater adverse effects of xenobiotics. Presenters will address gene expression as a means to discover mechanisms of toxicity and/or targeted pathways that would not have been evident if standard functional assays were used in isolation, and how gene expression can be used to screen chemicals for skin sensitization potential and explain differential susceptibility to dermal sensitization or demonstrate systemic immune effects following exposure to inhaled metals. The influences of host genotype on inflammatory responses to environmental factors such as ozone and particulate materials will also be discussed.

2145 USE OF MICROARRAYS TO INVESTIGATE MECHANISMS OF IMMUNOTOXICITY.

S. B. Pruett. *Cellular Biology & Anatomy, LSU Health Sciences Center, Shreveport, LA.*

Mechanistic risk assessment for cancer has proved to be very useful and has allowed more realistic evaluation of risks for compounds that cause cancer in rodents by mechanisms that are not operative in humans. However, mechanistic risk assessment has not been actively pursued with regard to immunotoxicology end points. As genomic data are considered along with functional parameters used to screen for immunotoxicity, we will obtain information on both their predictive value as screening tools and their ability to identify potential mechanisms of action. Several practical issues must be considered in conducting such studies, including but not limited to: selection of a microarray platform, use of a purified cell type vs. whole organ homogenates, use of animals or cells that are or are not challenged to induce an immune response prior to analysis, the number of replicates needed, whether samples should be pooled or not, and whether confirmation by other methods is required. A study investigating the mechanisms by which ethanol inhibits innate immunity to bacteria in mice provides an example indicating that microarrays can provide mechanistic clues that would be difficult to obtain in any other way. Preliminary data suggest that following exposure to ethanol, similar changes in gene expression are observed in both stimulated and non-stimulated macrophages. Finally, the use of microarrays to develop a systems biology approach to immunotoxicology would seem to now be feasible, and this may allow generalizations and predictions of immunological effects not possible at the present time. This work was supported by grants from NIEHS and NIAAA.

2146 IDENTIFICATION AND PRIORITIZATION OF GENE EXPRESSION CHANGES IN DENDRITIC CELLS AS A SCREEN FOR CONTACT ALLERGY.

C. Ryan¹, L. Gildea¹, L. Foertsch¹, J. Kennedy¹, R. Dearman², I. Kimber² and E. Gerberick¹. ¹Procter & Gamble Company, Cincinnati, OH and ²Syngenta Central Toxicology Laboratory, Macclesfield, United Kingdom.

Allergic contact dermatitis (ACD) resulting from skin exposure to sensitizing chemicals is a common occupational and environmental health problem. A critical step in the induction of ACD is the uptake and processing of protein-bound allergens by Langerhans cells (LC), resident immature dendritic cells of the skin. Following an encounter with a chemical allergen, LC become activated and subsequently migrate from the skin to the draining lymph nodes, undergoing a maturation process during the journey. Many changes have been reported to occur in LC as a result of hapten exposure including the induction of signal transduction pathways and the modulation of cell surface markers and cytokine expression. To explore further the concept of chemical allergen induced LC activation and maturation, the process has been modeled in vitro using human peripheral blood-derived dendritic cells (DC) as surrogates for LC. Microarray analysis provided a list of genes which were significantly changed, at the transcriptional level, following exposure of the DC to a model contact allergen. Subsequent analysis of a sub-set of those genes identified specific genes which appear to demonstrate good sensitivity, specificity, dynamic range, and reproducibility and thus may prove useful in the development of screening methods for skin sensitization and may also provide mechanistic information on the immune recognition phases of ACD.

2147 CYTOKINE GENE POLYMORPHISMS AND SUSCEPTIBILITY TO CHRONIC INFLAMMATORY DISEASES IN OCCUPATIONAL SETTINGS.

B. Yucesoy and M. I. Luster. *HELD/TMBB, CDC/NIOSH, Morgantown, WV.*

There is a wide range of inter-individual variation in susceptibility to occupational and environmental related diseases. Mechanisms underlying susceptibility are largely unknown, but genetic predisposition is suspected to be a major factor. Recent evidence suggests that common polymorphisms in immune/inflammatory and antioxidant genes contribute to the pathogenesis of many complex human dis-

eases. In light of this, functional variants of these genes have been examined for evidence of association with pulmonary fibrosis in a group of ex-coal miners, accelerated decline in lung function in firefighters and irritant contact dermatitis in healthcare workers. Several significant associations appeared between cytokine gene variants and disease progress and/or severity. The tumor necrosis factor- α (TNF α) -308, -238 and interleukin-1 receptor antagonist (IL-1RA) +2018 variants were found associated with the development and severity of silicosis. Also, associations between accelerated decline in lung function and genetic variations in several genes which regulate inflammation were found in firefighters that can not be explained by smoking history. Specifically, the presence of IL-1 RA +2018, TNF α -238 and -308 variants were associated with the decline rate of lung function as measured by FEV₁. Individual differences in response to common irritants (sodium dodecyl sulfate, benzalkonium chloride and sodium hydroxide) were investigated in a group of healthcare workers. Initial results confirmed interindividual variability in irritant susceptibility and provided base for investigating the role of genetic variations in response to irritants. In conclusion, the data suggest that genetic variations play a role in individual variability to certain occupational diseases. Such information can be used to help identify the most susceptible populations and apply relevant information to the risk assessment process by determining safe exposure levels for the most susceptible groups of workers.

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2148 A PROSPECTIVE STUDY OF GLOBAL EXPRESSION PROFILING IN WHOLE BLOOD SAMPLES FROM INDIVIDUALS EXPOSED TO METAL FUMES.

D. Christiani^{1,5}, Z. Wang¹, D. Neuberg², L. Su¹, J. Kim³ and J. Chen⁴. ¹Harvard School of Public Health, Boston, MA, ²Harvard Medical School, Boston, MA, ³U.S. EPA, Research Triangle Park, NC, ⁴UNC School of Public Health, Chapel Hill, NC and ⁵Massachusetts General Hospital, Boston, MA. Sponsor: R. Luebbe.

Although inhalation of metal particulates is a documented cause of pulmonary and airway inflammation, an exploratory study of microarray analyses in whole blood total RNA sampled before and after occupational welding-fume exposure supported the existence of systemic responses following inhalation of metal particulate. To evaluate the duration of particulate-induced gene expression changes, we conducted a follow-up study using a similar population one year after the original study. We recruited 15 individuals with welding fume exposure and 7 non-exposed individuals. Twelve of the 22 individuals (8 in exposed group and 4 in non-exposed group) also were monitored in our previous study. Whole blood total RNA corresponding to three time points, including baseline, immediately after ~6 h of exposure, and 24 h after baseline, was analyzed using cDNA microarray technology. Similar to our previous observations, exposure-induced expression alterations in non-smoking individuals were found in genes clustered in several Gene Ontology Biological Processes, related to Immune Response and Programmed Cell Death. The small number of genes with changed expression 24 hours after baseline compared to baseline suggest that acute particulate-induced effects were transient. Comparison of paired *t* statistic distributions indicate more global changes of gene expression in the previous study, which had significantly higher level of exposure. Although smoking was a confounding factor, a dose-response pattern of global expression was evident in smokers with higher level exposure in the previous study, in contrast to almost no changes in smokers with lower level of exposure in this study. Taken together, our results demonstrated the importance of applying a paired-sample study design in population studies that generate high dimensional data and an expected high degree of inter-personal variations.

2149 ENVIRONMENTAL EXPOSURES, IMMUNE FUNCTION, AND HOST VULNERABILITY.

J. Hollingsworth. *Duke University, Durham, NC.* Sponsor: D. Germolec.

The severity of airways disease can result from a complex interaction between host genetic vulnerability and environmental exposures. Studies in humans and mice indicate that both the biologic response to inhaled toxins and immunologic function are dependent on genetic background. This presentation will focus on the mechanisms through which common environmental exposures, such as ambient ozone (O₃), can modify both innate and adaptive immune function, and the role of host genotype in determining the severity of effects. The biological response to inhaled O₃ includes: recruitment of inflammatory cells, enhanced airway hyperreactivity, perturbations in alveolar macrophage function, and alterations in the production of pro-inflammatory mediators. Exposure to ambient O₃ can enhance or suppress allergic inflammation depending on the dose and timing of exposure, indicating that inhaled O₃ can modify adaptive immune function. We recently observed that innate immunity was modified in mice exposed to O₃ and subsequently challenged

with an aerosol of LPS. Pre-exposure to O₃ dramatically reduced total cells, alveolar macrophages, and neutrophils in the lung following inhalation of LPS. However, mice that were pre-exposed to O₃ had higher concentrations of total protein in lavage fluid, suggesting enhanced lung injury. Moreover, O₃ pre-exposure resulted in elevated levels of proinflammatory cytokines in the lavage fluid and serum following LPS challenge. Studies using genetically-engineered animals demonstrated that the biologic response to O₃, inhaled endotoxin, and allergic response to antigen are dependent on the expression of innate immune genes. We have demonstrated the role of toll-like receptor 4 in airway response to O₃, inhaled endotoxin, and allergic asthma. Current studies are focused on characterization of novel innate immune genes, which can regulate the severity of airways disease to a variety of inhaled challenges. Cumulatively, these studies support a complex interaction between a commonly inhaled toxin, innate immune function, and host genetic vulnerability. Supported by NIEHS (ES12717, ES101945, and ES11961) and NIAID (AI058161).



2150 IMPACT OF THE QUALIFICATION OF SAFETY BIOMARKERS ON DRUG DEVELOPMENT AND REGULATORY REVIEW: ROLE OF THE PREDICTIVE SAFETY TESTING CONSORTIUM.

L. Y. Rosenblum¹ and D. Robinson-Gravatt². ¹*International Drug Development, Kyowa Pharmaceutical Inc., Princeton, NJ* and ²*Pfizer Global Research and Development, Groton, CT*.

Progress in the application of biomarkers of drug-induced toxicity in both drug development and regulatory decision-making processes depends upon two important achievements: (1) scientific consensus on the identification and qualification of biomarkers of toxicity that are animal-model specific and able to bridge preclinical and clinical applications; and (2) regulatory acceptance of newly qualified biomarkers. Both achievements require a formal venue for sharing data, assays and samples across multiple pharmaceutical companies; a process for the review of qualification study protocols and data at the FDA; and entry points for application of qualified biomarkers in regulatory decision-making. The Predictive Safety Testing Consortium (PSTC) has been organized through the C-Path Institute as a venue for sharing and critically evaluating qualification data from studies using model as well as proprietary compounds. In collaboration with the FDA, the PSTC will share data to support the development of a process for review and acceptance of biomarkers, as well as to define potential regulatory applications of accepted biomarkers. The PSTC has initially organized itself around four biomarker areas: nephrotoxicity, hepatotoxicity, vascular injury, and genotoxic and non-genotoxic carcinogenicity. This Symposium will highlight the collaborative work of the PSTC and discuss current and future regulatory applications at the FDA.



2151 PROCESS MAP FOR QUALIFICATION OF GENOMIC BIOMARKERS OF DRUG SAFETY.

F. M. Goodsaid. *Genomics/OCP/OTS/CDER/FDA/HHS, Silver Spring, MD*.
Sponsor: L. Rosenblum.

A key deliverable of the Critical Path at the FDA are biomarkers for drug safety evaluation that can improve drug safety for new drugs and the efficiency of drug development. A draft process map for biomarker qualification was developed as part of a CRADA and other collaborations. This process map has been discussed internally at the FDA and used in the design of enabling regulatory mechanisms for the evaluation of qualification data submissions. The results of the collaborations we have had thus far help in the discussion of these mechanisms. Our collaborations show that comprehensive biomarker qualifications must be defined in the context of the application of each biomarker. These qualifications are expensive and will require the knowledge, experience, compounds and tissue banks from more than one company to qualify for known valid biomarker classification. Our experience also shows a clear synergy between the willingness of the industry to generate qualification data and of the FDA to have an efficient and transparent qualification process in place to evaluate this data.



2152 OVERVIEW OF THE PREDICTIVE DRUG SAFETY TESTING CONSORTIUM.

W. B. Matres¹ and J. Vonderscher². ¹*The Critical Path Institute, Tucson, AZ* and ²*Genomics Institute of the Novartis Research Foundation, San Diego, CA*.

In keeping with the Critical Path Initiative, a collaborative qualification of preclinical biomarkers of drug safety benefits the drug-development process, and a clear definition on the application of qualified biomarkers in regulatory decision-making is a powerful driver for participating in such a collaboration. The Predictive Safety

Testing Consortium (PSTC) is the result of an effort by the pharmaceutical industry, the FDA and the C-Path Institute to assemble a team of scientists from the pharmaceutical industry and from health authorities to share data from studies on multiple compounds leading to a consensus both on the data as well as the regulatory process for the qualification of these biomarkers. Key to the successful founding of the PSTC was the creation of a novel legal framework to provide benefits to both those who discover the exploratory markers as well as those who participate in their qualification. In addition to addressing anti-trust issues, the legal agreement among participating companies and C-Path specifically addresses intellectual property (IP) considerations, including IP that companies may bring into the consortium, as well as that which may be developed during the efforts of the consortium, and describes data sharing processes and obligations. One of the challenges facing the PSTC is the development of a data management resource for biomarker qualification. These data include not only the discrete new biomarker measurements, but also the standard toxicological and histopathological parameters collected during biomarker studies. These data must be collected and stored in a standard format (e.g. SEND/CDISC) that 1) supports the range of analyses and correlation testing necessary for biomarker qualification, and 2) anticipates clinical as well as preclinical data. This session will review the organizational structure, plans, schedule, and data management tasks of this Consortium, as well as the impact of the PSTC on discussions of regulatory policy at the FDA.



2153 IMPACT ON DRUG DEVELOPMENT AND REGULATORY REVIEW OF THE QUALIFICATION OF NOVEL BIOMARKERS OF NEPHROTOXICITY.

F. D. Sistare¹ and J. Vonderscher². ¹*Safety Assessment, Merck and Co., Inc., West Point, PA* and ²*Genomics Institute of the Novartis Research Foundation, San Diego, CA*.

The need for improved biomarkers of nephrotoxicity in drug development is exemplified by experiences where routine measures of BUN and serum creatinine fail to report the low grade kidney histopathological lesions that can be seen in animal toxicology studies. The C-Path Predictive Safety Testing Consortium's Kidney Toxicity Working Group has collected and pooled comparative performance data from rat studies using 17 different kidney toxicants and 3 kidney non-toxicants, that are anchored in traditional histopathology and measurements of BUN, serum creatinine, and/or urinary albumin. Measurements were made among samples from these studies using several of 16 candidate accessible urinary biomarkers including lipocalin-2, uromodulin, trefoil factor-3, osteopontin, GST-alpha, GST-mu, clusterin. Initial data are demonstrating that some candidate biomarkers can add significant value over BUN and serum creatinine, but keen awareness of important limitations, especially regarding the kinetics of the biomarker response duration, regional specificity of the injury, and biological relationship to the underlying histopathologic process will define their potential clinical utility. Samples remaining from these studies, along with additional independent sample sets identified from rat and non-human primate studies of the member companies with 9 additional kidney toxicants and 2 kidney nontoxicants are targeted for expanded assessments of these endpoints to establish the most promising panel to bridge into a clinical qualification strategy.



2154 IMPACT ON DRUG DEVELOPMENT AND REGULATORY REVIEW OF THE QUALIFICATION OF NOVEL BIOMARKERS OF HEPATOTOXICITY.

P. Wier. *Safety Assessment, GlaxoSmithKline Pharmaceuticals, King of Prussia, PA*.

Hepatotoxicity is probably the most prevalent preclinical and clinical adverse drug effect. Traditional biomarkers of liver injury include serum enzyme activities and levels of bile constituents that variably reflect cholestatic injury or cytotoxic hepatocellular injury, serum markers of hepatic synthetic functions, and certain acute phase proteins. These markers have proven, practical utility but improvements in sensitivity and specificity of biomarkers for hepatotoxicity are still needed. Within the Predictive Safety Testing Consortium, the Liver Working Group (LWG) is primarily focused on the progression to validation and regulatory acceptance of novel, exploratory liver toxicity biomarkers with added value over current markers. This presents a unique opportunity to collectively define the largest gaps in the current arsenal of biomarkers for hepatotoxicity, to harvest toxico-/proteo-/metabolic biomarker-discovery research, generate cross-validation data, and foster a common understanding of validated uses for new biomarkers. The "omics" era of biomarker research has yielded novel candidate markers that may increase sensitivity or specificity for cholestatic and cytotoxic hepatocellular injury, candidate markers for manifestations of liver toxicity (e.g., steatosis) not well-covered traditionally, and candidate markers to suggest modes of hepatotoxicity. These candidate markers include non-invasive (e.g., blood or urine based) markers for use in concert with established Clinical Pathology makers as well as those for use in conjunction with histopathology, such as mRNA signatures. The LWG has embarked on a collective

process to identify the most promising of these novel markers, collect the breadth of data necessary to define their uses and limitations, and achieve consensus on their validation.



2155 IMPACT ON DRUG DEVELOPMENT AND REGULATORY REVIEW OF THE QUALIFICATION OF NOVEL BIOMARKERS OF VASCULAR INJURY.

M. P. Lawton¹ and R. Snyder². ¹Drug Safety Research and Development, Pfizer Inc., Groton, CT and ²Schering-Plough Research Institute, Lafayette, NJ.

There are no sensitive or specific biomarkers for drug-induced vascular damage. Moreover, extrapolation from preclinical findings of vascular injury to human risk is poorly understood. Restrictions in available knowledge and tools limit accurate risk management of vascular injury. These weaknesses can be addressed through the development and clinical translation of predictive biomarkers, mechanistic and clinical investigations to increase understanding of species differences, and advances in regulatory practices and policy based on emerging science and validation of new biomarkers. The Vascular Injury Working Group has exchanged data, samples, and reagents that members have generated and has used this information to help identify candidate exploratory and probable valid biomarkers for which comprehensive prospective qualification studies across Consortium members can be designed. Some of the candidate biomarkers resulted from the application of Systems Toxicology, in which the overlap of information across genomic, proteomic and metabolomic platforms has synergistically defined exploratory biomarker sets.



2156 AIR POLLUTION, OXIDATIVE STRESS AND NEUROTOXICITY: AN OVERVIEW.

B. Veronesi. NHEERL/NTD/CTMB, U.S. EPA, Research Triangle Park, NC.

Increases in neurodegenerative diseases (classic and variant patterns suggest a multifactorial etiology, that combines environmental exposures with susceptibility factors. The numerous biological and chemical contaminants carried on particulate matter (PM) can damage target cells and tissues through oxidative stress pathways. It is also known that inhaled particles exit the lungs and enter the circulation where they distribute to numerous organ systems, including the brain. Since the brain is highly vulnerable to oxidative stress damage, the possibility that it is also targeted by PM is of great concern. This workshop will present experimental evidences that identifies PM also damages the brain and defines oxidative stress as an important factor to that damage.

Dr. Veronesi (US EPA) will begin by describing the neuropathic response of transgenic (ApoE^{-/-}) mice chronically exposed to concentrated ambient PM (CAPs). Cultures of immortalized microglia, later exposed to the same CAPs responded with cellular and genomic evidence of oxidative stress-stimulated pathways and an up-regulation of the innate immunity, Toll-like receptor. Dr. Campbell (Western University of Health Sciences) will report on the response of ovalbumin sensitized BALB/c mice exposed to PM sampled from several California sites. Her data suggest that PM may induce CNS inflammation through innate immunity pathways. Dr. MohanKumar (Michigan State University) will present recently published data which indicate that PM can affect parts of the brain that are involved in neuroendocrine functions, such as stress. Finally, Dr. Block (NIEHS) will describe the microglia-mediated, degeneration of dopaminergic neurons in mouse mesencephalic cultures, exposed to diesel emission particles. She will also present new data indicating that the MAC1 pattern recognition receptor mediates the diesel induced, DA neurotoxicity. These presentations confirm that the brain is affected in many ways by PM exposure. It is hoped that a rigorous exchange of comments and suggestions for future research results from this workshop.



2157 AIR POLLUTION, OXIDATIVE STRESS AND NEUROTOXICITY: INTRODUCTION.

L. Chen¹ and B. Veronesi². ¹Environmental Medicine, New York University, Sterling Forest, NY and ²NHEERL/NTD/CTMB, U.S. EPA, Research Triangle Park, NC.

Long-term exposure to fine particulate air pollution (PM_{2.5}) is associated with an increased risk of death from cardiopulmonary diseases. In addition to cardiopulmonary effects, ambient PM can also produce neuropathic effects such as histopathological changes in the nasal tissues and in the olfactory and respiratory nasal mucosa, olfactory bulb, cortical and subcortical structures, alterations of the blood-brain barrier, damage to cortical neurons, apoptotic glial cells and neurofibrillary tangles. These changes are plausibly related to the oxidative stress- produced by inhalation of ambient air pollutants. It has been shown that inhaled particles quickly enter the systemic circulation and distribute to numerous organ systems. Through this mechanism, the brain could be targeted by ambient PM. Another

mechanism that PM could affect CNS is the transport of ultrafine particles through the olfactory tubercles to gain entry to various CNS targets. PM may also affect the CNS, indirectly, through oxidative stress-cytokine/chemokine pathways. Ambient PM may also perturb the normal variations in metabolism, energy utilization, and organ function to produce neurodegenerated effects. Furthermore, ambient PM could also enhance the recruitment and maturation of monocytes to produce cytokines and chemokines and promote local inflammatory response. Acting independently or in combination, these pathways could produce systemic effects leading to morbidity and mortality in subjects chronically exposed to elevated levels of ambient PM.

In this workshop, experimental evidence of PM's multiple effects on the brain will be presented. Such evidence indicates that PM exposure causes neuropathological, neuroimmunological, and neurochemical changes to the mammalian CNS and identifies the brain as an additional and critical target of air pollutants.



2158 PARTICULATE MATTER, OXIDATIVE STRESS AND NEURODEGENERATION.

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Particulate matter (PM) damages target tissues through oxidative stress (OS). Several reports indicate that inhaled ultrafine particles quickly exit the lungs, enter the blood stream and distribute to various organs, including the brain. To determine if there was a causal relationship between PM and neurodegeneration and if OS played a role in this susceptibility, the follow experiment was done. OS-prone, transgenic mice (Apo E^{-/-}) were chronically (4-6 mo) exposed by inhalation to concentrated ambient air pollution (CAPs) sampled from Tuxedo, NY. Morphometric analysis of serial brain sections showed a 29% reduction of immunohistochemically stained dopaminergic neurons sampled in the brain's substantia nigra. There was no significant loss of neurons in the brains of the CAPs-exposed background strain C57 mice, implicating the condition of OS as a predisposing factor to the susceptibility.

Since microglia mediate neuronal degeneration, their response to the above CAPs was examined in culture. Immortalized, mouse microglia (BV2), exposed to CAPs (>12 µg/ml) exhibited mitochondrial depolarization after 15 min exposure and significant increases in TNFα and IL-1β after 6 and 24 hr, respectively. The endogenous scavengers, glutathione and non-protein sulfhydryl, were significantly depressed after 2 hr exposure to CAPs (>25 µg/ml). Finally, cultures of BV2 microglia were exposed (4 hr) to CAPs (75 µg/ml) or to LPS (10ng), an endotoxin known to stimulate OS in microglia. RNA isolated from these cells, examined in Affymetrix microarrays, showed significant increases in genes coding for apoptotic pathways, superoxide demutase, TNFα and Toll receptors; and cell adhesion molecules. Together, these data suggest that the brain of OS-compromised animals can be damaged by exposure to CAPs and that microglia respond to CAPs with measurable expressions of OS.



2159 PARTICULATE MATTER PRESENT IN URBAN AIR POLLUTION ENHANCES INNATE IMMUNE RESPONSES IN THE MOUSE BRAIN.

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While epidemiological studies demonstrate that exposure to particulate matter (PM) is associated with increased morbidity and mortality related to pulmonary and cardiovascular dysfunction, few reports have addressed adverse CNS effects. Since the etiology of most cases of neurodegenerative disorders is unknown, gene-environment interactions are likely to play a role in initiation and progression of such disorders. Studies were conducted to determine if PM, derived primarily from vehicle emissions, can up-regulate innate immune responses in mouse brains. Two different size fractions of PM were evaluated. Ultrafine (UF) fractions have an aerodynamic diameter < 0.15 µm and fine plus ultrafine particles (F+UF) have an aerodynamic diameter < 2.5 µm. In the first study, exposure to particulate matter (4 hr/day, 5 days/wk, for 2 weeks) from a site in Los Angeles, CA, increased NF-κB activation and levels of proinflammatory cytokines in the brain of ovalbumin sensitized BALB/c mice. In the second study, the same animal model and exposure paradigm was used but at a site in Riverside, CA. There was an upward trend in the CNS levels of NF-κB activation, the innate immune receptor Toll-like receptor 2 (TLR2), and nitrotyrosine content. However, the levels did not reach statistical significance. In the third study, ApoE^{-/-} animals were exposed (5 hrs/day, 3 days/wk for 5 weeks) at a Site at Los Angeles, CA. There was a significant increase in NF-κB activation and the levels of TLR2. Evaluation of the PM from both Los Angeles and Riverside shows that there are differences in the composition. This suggests that the

chemical makeup and duration of exposure significantly determines the extent of CNS responses. Since inflammatory events contribute to the pathogenesis of neurodegenerative diseases, the potential of PM to accelerate progression of such disorders requires further examination.

2160 EXPOSURE TO CONCENTRATED AMBIENT PARTICLES INCREASES STRESS AXIS ACTIVITY BY AFFECTING BRAIN STEM NORADRENERGIC NEURONS.

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Exposure to concentrated ambient particles (CAPs) has been shown to increase stress axis activity. However, the underlying mechanism(s) for this effect is unknown. The paraventricular nucleus (PVN) of the hypothalamus contains corticotrophin releasing hormone neurons that are under the stimulatory control of noradrenergic neurons located in the brain stem. We hypothesized that CAPs exposure may lead to stimulation of noradrenergic neurons in the brain stem leading to increases in stress axis activity. Preexisting allergic airway disease may aggravate this phenomenon. In the present experiment, adult male Brown Norway rats were exposed to CAPs exposure (8 hr) with or without ovalbumin (OVA)-induced allergic airway disease. Rats were sacrificed 24 h after exposure and the brains were removed, frozen and sectioned. The PVN and brain stem noradrenergic neurons were microdissected. Serum was collected for corticosterone radioimmunoassay. Norepinephrine (NE) levels were measured in the PVN and the levels of tyrosine hydroxylase (TH) the rate-limiting enzyme in NE biosynthesis was measured in the brain stem. CAPs exposure significantly increased NE levels in the PVN, similar to that seen in OVA pretreatment. Corticosterone levels were elevated paralleling NE levels in the PVN. Site-specific changes were observed in TH levels with some noradrenergic nuclei showing a more pronounced increase than others. These results indicate that exposure to CAPs acts on the neuroendocrine system to stimulate the stress axis. The ability of CAPs exposure to affect brain stem neurons could have implications in autonomic regulation. The potential involvement of inflammatory mediators in this phenomenon is currently under investigation. Supported by NSF IBN 0236385 and NIH AG-027697.

2161 MICROGLIA MEDIATE DIESEL EXHAUST PARTICLES-INDUCE DOPAMINERGIC NEUROTOXICITY: THE ROLE OF MAC1.

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Inflammation and microglial activation are associated with Parkinson's (PD, a neurodegenerative disease that has been linked to environmental chemical exposure. Experimental reports indicate that exposure to air pollution can cause neuroinflammation and neuropathology. However, the underlying mechanisms are largely unknown. In this presentation, the response of microglia to nanometer-sized diesel exhaust particles (DEP) (< 0.22 µM) is examined. Mesencephalic neuron-glia cultures treated with DEP (5-50 µg/ml) responded with a dose dependent reduction of dopaminergic (DA) neurons, as determined by DA-uptake assay and tyrosine-hydroxylase immunocytochemistry (ICC). The selective toxicity of DEP for DA neurons was demonstrated by the lack of DEP effect on both GABA uptake and Neu-N immunoreactive cell number. The critical role of microglia was shown by the failure of neuron-enriched cultures to show DEP-induced DA neurotoxicity, where DEP-induced DA neuron death was reinstated with the addition of microglia to neuron-enriched cultures. OX-42 ICC staining of DEP treated neuron-glia cultures indicated microglia activation. Intracellular reactive oxygen species and superoxide were produced from enriched-microglia cultures in response to DEP. Neuron-glia cultures from NADPH oxidase deficient (PHOX^{-/-}) mice were insensitive to DEP neurotoxicity when compared to control mice (PHOX^{+/+}), emphasizing the importance of extracellular superoxide in DEP-induced neurotoxicity. Neuron-glia cultures from MAC1 knockout mice (MAC1^{-/-}) revealed that DEP (50 µg/ml) morphologically activated microglia in MAC1^{+/+} cultures, but not in MAC1^{-/-} cultures. Further, DEP failed to exert DA neurotoxicity in MAC1^{-/-} cultures, as determined by DA uptake and TH-positive cell counts. We conclude that DEP is selectively toxic to DA neurons through activation of the MAC1 receptor and the consequent production of extracellular superoxide.

2162 MOLDS, MYCOTOXINS AND DAMP BUILDING-RELATED ILLNESSES.

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Health risks arising from mold contamination in water-damaged residential and commercial structures remain a major concern to the U.S. public. The black mold *Stachybotrys* and its toxins have been suggested to cause a spectrum of illnesses in exposed building occupants ranging from respiratory to neurological problems. Although this mold represents a small component of the indoor air mycoflora, it readily propagates on cellulose-based building materials subjected to water damage from condensation, leakage and flooding. Lawsuit and remediation costs to the insurance and building industries due to *Stachybotrys* have reached into the billions of dollars. However, significant questions remain relative to (1) specific hazards posed by the fungus, (2) whether it has unique mechanisms compared to other airborne fungi, and (3) the doses of the fungus or its toxins required for putative adverse effects. Furthermore, there has been a lack of validated methods to measure contamination by this organism and estimate actual human exposure. These problems have exacerbated by extensive litigation and media attention which sometimes interfere with scientific judgment and prevent reasoned dialogue. Recognizing these concerns, the 2004 Institute of Medicine (IOM) report "Damp Indoor Spaces and Health" stated that although "in vitro and in vivo research on *Stachybotrys chartarum* suggests that effects on humans may be biologically plausible; these observations require validation from more extensive research before conclusions can be drawn". This symposium will focus on new advances in our understanding of *Stachybotrys* and its toxins since the IOM report relative to: (1) detection and quantitation in indoor air environments, (2) capacity to target specific tissues and cause illness in animal models, (3) novel mechanisms of action and (4) identification and use of unique biomarkers of exposure.

2163 INDOOR MOLD AND ILLNESS IN TEXAS HOMES WITH VISIBLE WATER DAMAGE AND/OR MOLD.

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Since the mid to late 1990s there has been increasing public attention on the presence of *Stachybotrys*, dubbed "black mold" by the lay media, in indoor air. An extremely wide range of symptoms and illnesses has been attributed to *Stachybotrys* by individuals in buildings where the mold has been found. This has led to a wave of litigation based largely on public perception of risks in the absence of sufficient scientific evidence to conclude that the presence of *Stachybotrys* in buildings is more likely to cause health effects than any other mold. Some molds, such as *Stachybotrys* and *Aspergillus* produce mycotoxins which have been shown to cause toxicity when ingested in foods highly contaminated with mold spores, but there is no evidence that inhalation of mycotoxin containing spores at concentrations found in indoor environments is sufficient to cause toxic effects. Current gaps in knowledge include an understanding of acute and/or chronic toxic effects of mycotoxins produced by indoor molds, assessment of likely exposure pathways in indoor environments, and the dose response relationship of mold spore inhalation and toxicity. In order to evaluate reported health effects after exposure to indoor mold, the indoor air mold spore level from 215 Texas homes with visible water and/or mold damage was compared to symptoms reported in building occupants. The most commonly reported health complaints by building occupants were allergies, allergy-like symptoms, sinus problems, respiratory symptoms, headache, and asthma. Twenty-two percent of the investigated homes had detectable airborne *Stachybotrys* spores ranging from 13 - 88,400 spores/m³. The mean *Stachybotrys* air concentration in homes was 1,082 spores/m³ (CI = 1,309). *Stachybotrys* spores contributed a median of 3% of the total spore count in indoor air. There was no statistical difference in the indoor airborne *Stachybotrys* spores in homes with occupants who reported health complaints and those that did not. In summary, the most commonly reported symptoms in homes with health complaints were those typically associated with exposure to many types of mold.

2164 ALLERGIC POTENTIAL OF INDOOR MOLDS.

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Many fungi have been associated with allergic lung disease, but few are well studied and even fewer allergens of fungal origin are well characterized. Exposure to damp moldy environments has been associated with the exacerbation of asthma, but the role of molds in the induction of allergic asthma is less clear. Genetically predisposed individuals who inhabit water-damaged buildings may be susceptible to the development or exacerbation of respiratory allergy. Five molds (*Scopulariopsis brevi-*

caulis, *Trichoderma viride*, *Penicillium crustosum* Group, *Stachybotrys chartarum*, *Wallersteina sebi*) found in water-damaged homes in the Cleveland area have been linked to asthmatics. The moderately severe asthmatic children from homes that underwent mold remediation subsequently had a lower rate of asthma exacerbations compared to control asthmatics (non-remediated homes). Our hypothesis is that certain mold species may be more potent than others in asthma induction. Our mold/allergy mouse model exhibits many characteristics of human allergic asthma and has been used to demonstrate cause and effect, dose response, has compared the potency of 3 molds including *S. chartarum* to house dust mite (HDM), and has identified specific allergens. Studies have found that *S. chartarum* causes sensory irritation in immunized and non-immunized mice and elevated IgE with repeated exposures, in addition to anecdotal and published reports associating it with asthma. Studies from our laboratory have found that *S. chartarum* extracts induces both antigen non-specific and specific allergic asthma-like responses and that it is half as potent as HDM in the induction of allergic responses (rat leukemia basophil assay). Currently, we are evaluating the allergic potential of the other 4 molds in the Cleveland area homes that have been linked to asthma in our mouse model. There are still questions, both qualitative and quantitative, that need to be answered before the risks associated with indoor exposure to molds can be adequately assessed.

(This abstract has been reviewed by the NHEERL, US EPA and does not reflect the EPA policies.)

2165 TRICHOCECENE-INDUCED INFLAMMATION AND APOPTOSIS: CELLULAR TARGETS AND MOLECULAR MECHANISMS.

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Trichothecenes are a family of over 180 sesquiterpenoid mycotoxins that induce immediate early gene responses and apoptosis via the ribotoxic stress response and activation of mitogen-activated protein kinases. These include the satratoxins and the macrocyclic trichothecenes produced by *Stachybotrys* and *Myrothecium*. Double-stranded RNA associated protein kinase (PKR), Src family kinases and other kinases transduce this response, possibly through direct interaction with the ribosome. These pathways are operable in both in vitro leukocyte and neuronal models. The macrocyclic ring plays a key role in the molecular toxic effects of satratoxin which can bind and modify eukaryotic ribosomes. Downstream pathotoxic sequelae of the trichothecene-induced ribotoxic stress response are demonstrable in several in vivo models which are shedding light on potential human health hazards.

2166 ESTIMATION OF HUMAN EXPOSURE TO STACHYBOTRYS CHARTARUM AND ITS MYCOTOXINS.

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The spores of *Stachybotrys chartarum* (SC), which are about 7 to 10 microns in diameter, are rarely found in the air of buildings. The mycotoxins (trichothecenes) produced by SC, aka satratoxins, are found in the greatest abundance on the spores of SC. People inhabiting SC contaminated buildings sometimes demonstrate symptoms associated with trichothecene poisoning. This was difficult to explain because it was thought that the only way in which the satratoxins could get into the human body was by inhalation of SC spores. The presence of airborne satratoxins on particles smaller than spores was investigated using graded filters. A competitive enzyme linked immunosorbent assay (ELISA) was used to analyze the filter extracts. The results showed that extracts from spore free filters demonstrated statistically significant ($p < 0.05$) antibody binding that increased with sampling time. These data showed that satratoxins can become airborne free of SC spores. We next examined the presence of airborne macrocyclic trichothecene mycotoxins (MTM) in indoor environments (8 test buildings with known SC contamination and 4 control buildings). We used a high volume liquid impaction bioaerosol sampler under static or disturbed conditions and the above described ELISA. For test buildings, the results showed that detectable trichothecene levels increased with sampling time and short periods of air disturbance. Trichothecene levels ranged from < 10 to $> 1,300$ pg/cubic meter of air. Our data showed that airborne MTM can exist in SC contaminated buildings. Finally, we wanted to see if we could detect MTM in the tissues of people who were exposed to SC in contaminated buildings. Sera from occupants of contaminated buildings (test samples, $n = 45$) and uncontaminated buildings (controls, $n = 27$) were analyzed using the above described ELISA. Twenty three samples were significantly different ($P < 0.05$) from normal human serum, whereas only one of the control samples tested positive. These data suggest that trichothecene mycotoxins can be demonstrated (by ELISA) in the tissues of certain individuals exposed to SC in contaminated buildings.

2167 PULMONARY TOXICITY OF STACHYBOTRYS AND THE IDENTIFICATION OF A NOVEL BIOMARKER.

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The lung as a target for *Stachybotrys* and its toxins will be discussed relative to animal models and human epidemiologic data. Acute pulmonary hemorrhage in young human infants continues to occur in the Cleveland area. The initial epidemiological suggestion of the involvement of *Stachybotrys* is substantiated by the continued observation that 90% of these infants (now 40 cases) come from *Stachybotrys* contaminated homes. Quantitative PCR has documented *Stachybotrys* elements in three of the infants' tracheal secretions. An infant animal model of acute instillation of *Stachybotrys* spores in the lungs of rat pups implicates macrocyclic trichothecenes (e.g. satratoxin G) in lung injury, although spores from isolates that produce only the simple trichothecenes ($> 100\times$ less toxic) still produce extensive injury. The interplay of several mechanisms of pathogenesis will be presented. We have documented the formation of covalent adducts of purified human serum albumin by the macrocyclic trichothecene, satratoxin G (Yike et al., *Environ Health Perspect*, 114:1222 (2006)). These adducts have also been found in the serum of patients who were exposed to *Stachybotrys* in their homes including the most recent infant with pulmonary hemorrhage and both of his parents. Infant rats exposed intra-tracheally to *Stachybotrys* spores also have the satratoxin-albumin adducts in their serum. These adducts are the basis of a novel quantitative biomarker assay currently being developed.

2168 NASAL TOXICITY OF STACHYBOTRYS TOXINS.

J. R. Harkema, Z. Islam, K. N. Corps, C. J. Amuzie and J. J. Pestka. *Michigan State University, East Lansing, MI.*

Nasal airways are likely deposition sites for inhaled fungal spores and potential sites for toxicity caused by associated mycotoxins. Satratoxin G (SG) is a macrocyclic trichothecene mycotoxin produced by *Stachybotrys chartarum*, the black mold suggested to contribute to illnesses associated with water-damaged buildings. This presentation will 1) review comparative nasal anatomy and physiology, 2) describe nasal and neurotoxicity of SG and other related mycotoxins in mice, and 3) discuss the implications of these murine studies for assessing the risk to human health. In a recent study, mice were exposed to a single intranasal instillation of SG in saline or saline alone (controls) and killed 6h, 24h, 3days, 7days, or 28 days post instillation (PI). Nasal and brain tissues were examined for SG-induced microscopic and molecular alterations. At 24h PI, SG induced an acute rhinitis and elevated mRNA expressions of pro-inflammatory cytokines in both nasal ethmoid turbinates and in the adjacent olfactory bulb (OB) of the brain. SG also induced apoptosis of olfactory sensory neurons (OSN) in olfactory epithelium (OE). Apoptosis of OSN was identified with immunohistochemistry for caspase-3, electron microscopy for ultrastructural cell morphology, and RT-PCR for elevated expression of pro-apoptotic genes. Dose-response analysis revealed that the no effect and lowest effect levels at 24 h PI were 5 and 25 μg SG/kg bw, respectively, with severity increasing with dose. Time course studies with a single instillation of SG (500 μg /kg bw) indicated maximum atrophy of OE at 3 days PI. Atrophy of the olfactory nerve and glomerular layers of the OB was detectable by 7 days PI along with mild encephalitis. OE atrophy and OSN loss were still evident at 28 days PI. These results suggest that neurotoxicity and inflammation in the nose and brain are potential adverse health effects of exposure to satratoxins and *Stachybotrys* in water-damaged buildings.

2169 SAFETY OF DIETARY SUPPLEMENTS USED FOR WEIGHT REDUCTION: LESSONS LEARNED.

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Recent results from the Center for Disease Control Survey indicate that an estimated 66% of U.S. adults are either overweight or obese. Use of dietary supplements to control body weight is rapidly increasing. A number of supplements that claim to help burn fat, block fat absorption, modulate carbohydrate metabolism, boost energy expenditure, suppress appetite, flush out excess water weight and control food cravings — all good outcomes for an eager dieter are readily available. A recent survey suggests that 7% of adults and 28% young obese women use weight loss supplements. Commercially, over 50 individual and 125 combination products of dietary supplements are available for weight reduction. Understanding the evidence of safety, efficacy, and quality for dietary supplements is critical when considering the potential public health impact of consuming these products. For example, although evidence of modest weight loss from ingestion of ephedra exists, potential

adverse effects have led the U.S. FDA to ban the sale of ephedra containing products. Evidence regarding the safety and efficacy of chromium, hydroxycitric acid, guar gum, chitosan, conjugated linoleic acid, ginseng, glucomannan, green tea, l-carnitine, psyllium, pyruvate, and St. John's Wort in weight loss has also been claimed by the manufacturers. The amount of well-designed research to substantiate the safety of many supplements for weight loss is sparse. In a recent article, Consumer Reports magazine published a list of the "dirty dozen" — 12 supplements, including some used for weight reduction, that have been linked either to serious adverse effects or to a strong theoretical risk. Critical safety assessment is the most logical way of understanding this issue. This session will take a critical look at previous safety assessments of dietary supplements used in weight loss and where and how the consideration of safety should or could be improved.



2170 ASSESSING THE HEALTH EFFECTS OF DIETARY SUPPLEMENTS.

P. M. Coates. *Office of Dietary Supplements, National Institutes of Health, Bethesda, MD.* Sponsor: M. Soni.

There is an increasing need for documentation of the health effects (both positive and negative) of dietary supplements used for weight management and weight reduction. Health effects identified in ecological or epidemiological studies have not always been replicated when ingredients are subjected to randomized controlled trials (RCTs). This is important, since there can be many confounding factors in assessing weight. Likewise, historical use of an ingredient (e.g., as part of an indigenous healing system) is no guarantee of safety or efficacy when it is provided as a dietary supplement for a 21st century application. There is a debate between accepting historical use without further testing and conducting the best RCTs whose applicability under different circumstances may be questioned. All of these issues must be considered when mounting a research agenda on the challenging topic of dietary supplements for weight management.



2171 EVIDENTIARY BASES FOR SAFETY AND EFFICACY CLAIMS IN SUPPLEMENTS FOR WEIGHT LOSS.

D. B. Allison. *Biostatistics, The University of Alabama at Birmingham, Birmingham, AL.* Sponsor: M. Soni.

The evidentiary bases for safety, efficacy, or toxicity claims in supplements for weight loss are sometimes difficult to discern. Different 'players' in the field (e.g., regulatory bodies, health care practitioners, consumer advocates, and the civil courts) often hold different views as to what constitutes adequate evidence. Indeed, the different evidentiary standards in such diverse settings are often quite appropriate and reflect fundamentally different goals of these groups. However, the critical questions are: What constitutes adequate evidence and from whose point of view? How studies should be designed and analyzed to offer such evidence? So, there is a need to review studies that are currently being designed and analyzed. The available data suggest that there is a large gap between a sound evidentiary basis and the type and amount of evidence that is actually collected in the weight loss supplement arena by virtually any standard. Finally, to make greater progress in this field, there is a need to highlight and discuss methodologic questions.



2172 SAFETY AND EFFICACY OF TRIVALENT CHROMIUM IN WEIGHT LOSS.

R. A. Anderson. *Beltsville Human Nutrition Research Center, Beltsville, MD.* Sponsor: M. Soni.

Trivalent chromium is an essential nutrient required for normal sugar and fat metabolism. Initial studies of supplemental Cr on lean body mass reported highly significant effects of Cr, 200 ug/d, as Cr picolinate within 6 weeks. The results appeared to be greater than would be expected and have been difficult to reproduce. However, others studies of longer duration, more than 12 weeks, and higher levels of Cr, at least 400 ug/d, have also reported significant effects of Cr on body weight and composition. In contrast, there have also been a number of well controlled studies that have not observed improvements in body weight and composition.

Several studies have also documented the safety of Cr but recent studies have questioned the potential toxicity of trivalent Cr. We have shown that the incorporation of Cr into tissues of rats for Cr chloride and Cr picolinate is linear in the range of intake from 5 to 100 ug of Cr per gram of diet. On a per kg body weight basis, this would be roughly a daily intake of 5000 ug/kg compared with less than 15 ug/kg for a person consuming 1000 ug per day of supplemental Cr. Recent studies in mice suggest that at intakes of roughly 25 ug of Cr per gram of diet by pregnant mice leads to signs of toxicity in the offspring for Cr picolinate but not for Cr chloride. There are also reports that Cr in the form of Cr picolinate can lead to free radical DNA damage in the presence of oxidants. We subjected human cultured HaCaT keratinocytes to cytotoxic levels of Cr chloride and Cr histidinate (the highest soluble levels of Cr picolinate did not cause cell toxicity) for 24 hours and then submitted cells to hydrogen peroxide-induced oxidative stress and did not observe DNA damage. In contrast, there were protective effects on DNA monitored by a decrease in % tail moment in the Comet assay. In summary, the apparent safety of Cr varies with the form of Cr and the test system used. While there are individual reports of possible Cr toxicity in people taking supplemental Cr, there are no confirmed cases of toxicity of supplemental trivalent chromium at intakes ranging from 50 to 1000 ug daily.



2173 MISLEADING TOXICITY REPORTS ON SAFETY OF WEIGHT REDUCING DIETARY SUPPLEMENTS: EXAMPLE OF *GARCINIA CAMBOGIA* EXTRACT.

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Garcinia cambogia extract is frequently used in dietary supplements for weight loss. (-)-Hydroxycitric acid, the principal acid of the extract, has been shown to inhibit ATP citrate lyase thus limiting the availability of acetyl-CoA units required for fatty acid synthesis and lipogenesis during a lipogenic diet. Recent reports on *Garcinia cambogia* extract indicate that high doses are effective in suppressing fat accumulation but highly toxic to the testis. A critical review of such information raises several questions such as (1) different isomers/forms of the active ingredient hydroxycitric acid in the extract, responsible for weight control and toxicity, (2) animal models, experimental study design used in safety assessment, (3) use of uncertainty factor for agents acting at physiological levels. These questions need logical consideration for safety assessment of dietary supplement used in weight reduction. In these evaluations, consideration such as use of lactone (which appears to be more toxic) form of hydroxycitric acid, inclusion of pair fed group, use of obese animal model (with higher lipogenic properties), etc. deserves important consideration in safety assessment.



2174 SAFETY ASSESSMENT OF DIETARY SUPPLEMENTS MARKETING FOR WEIGHT LOSS: FDA PERSPECTIVES.

V. H. Frankos. *Division of Dietary Supplements, Food and Drug Administration, College Park, MD.* Sponsor: M. Soni.

In evaluating dietary supplements, FDA looks at scientific information from a range of sources, including published research, evidence-based reports, adverse event reports, and data about the pharmacology or toxicology of a compound. The agency has particular interest in gathering safety data about certain weight loss dietary supplements suspected to cause cardiovascular stress such as the biogenic amines synephrine, octopamine and tyramine present in *Citrus aurantium*, also known as bitter orange. Concern has also been raised about usnic acid derived from *Usnea barbata*, marketed for weight loss and linked to human liver damage. It appears that supplements marketed for weight loss need custom studies to establish their safety. This presentation will discuss FDA's perspective on safety assessment of dietary supplements for weight reduction.

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Ahmad, M H	1656	Ancian, P	2122*, 2129	Arnold, L L	753, 761, 762*
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		Barber, D S	640, 1056*, 1394	Bedrick, E J	512
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Betts, C J	1152*, 1362	Blumenthal, G M	1978	Boverhof, D R	741, 1210, 1219, 1418*
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