SOT | Society of Toxicology

Creating a Safer and Healthier World by Advancing the Science of and Increasing the Impact of Toxicology

2015 Annual Meeting Abstract Supplement

Late-Breaking Abstract Submissions

All Late-Breaking Abstracts will be presented on Thursday, March 26, from 8:30 am–12:00 noon.

These abstracts will be available via the mobile event app, online planner, and a downloadable PDF from the SOT website.

54th Annual Meeting and ToxExpo™

San Diego, California

March 22–26, 2015

SOT 2015 San Diego

THURSDAY POSTER SESSION MAP

March 2015—8:30 AM to 12:00 Noon—Sails Pavilion

Poster Set Up—7:00 AM to 8:30 AM

260	259	258	257	256
251	252	253	254	255
250	249	248	247	246
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ENTRANCE

Photography in all poster sessions is prohibited without the consent of the poster presenter(s)/author(s). Please respect your colleagues' rights to privacy.

Thursday, March 26, Poster Session by Location

Session Title	Abstract #s	Poster Board #s
Late-Breaking Poster Session 1		
Alternatives to Mammalian Models and Animal Models	2469-2498	101-130
Chemical & Biological Weapons	2499-2507	131-139
Ecotoxicology, Food Safety, and Natural Products	2508-2525	140-157
Metals	2526-2537	158-169
Persistent Organic Pollutants (POPs)	2538	170
Late-Breaking Poster Session 2		
Cardiovascular Toxicology/Hemodynamics, Inhalants, Cardiopulmonary	2539-2557	201-219
Kidney and Liver	2558-2576	220-238
Cell Death/Apoptosis	2577-2582	239-244
Epigenetics	2583-2584	245-246
Oxidative Injury and Redox Biology, and Receptors	2585-2591	247-253
Carcinogenesis	2592-2595	254-257
Late-Breaking Poster Session 3		
Regulation/Policy	2596	301
Risk Assessment	2597-2615	302-320
Safety Assessment: Nonpharmaceutical	2616-2621	321-326
Safety Assessment: Drug Development and Discovery	2622-2635	327-340
Clinical and Translational Toxicology	2636-2638	341-343
Computational Toxicology and Bioinformatics	2639-2648	344-353
Mixtures	2649-2653	354-358
Education, Ethical, Legal, and Social Issues	2654	359
Late-Breaking Poster Session 4		
Children's Health/Juvenile Toxicity	2655-2661	401-407
Developmental Basis of Adult Disease and Developmental Toxicology	2662-2675	408-421
Endocrine Toxicology and Reproductive Toxicology	2676-2690	422-436
Nanotoxicology	2691-2704	437-450
Systems Biology and Toxicology	2705-2711	451-457
Late-Breaking Poster Session 5		
Neurotoxicity and Neurodegenerative Disease	2712-2747	501-536
Pesticides	2748-2749	537-538
Immunotoxicity and Autoimmunity/Hypersensitivity	2750-2756	539-545
Inflammation and Disease, Methods and Mechanisms	2757-2763	546-552
Gene Regulation/Signal Transduction/Genotoxicity and DNA Repair	2764-2774	553-563
Skin	2775-2778	564-567
Medical Devices	2779-2781	568-570
Late-Breaking Poster Session 6		
Biomarkers	2782-2801	601-620
Exposure Assessment/Biomonitoring	2802-2815	621-634
Biotransformation/Cytochrome P450	2816-2820	635-639
Disposition/Pharmacokinetics and Pharmacogenomics/Genetic Polymorphisms	2821-2825	640-644
Biological Modeling	2826-2829	645-648
Epidemiology	2830-2831	649-650
Stem Cell Biology and Toxicology	2832-2840	651-659

Thursday, March 26, Poster Session by Topic

TOPIC	ABSTRACT #s	POSTER BOARD #s	Session Title
Alternatives to Mammalian Models and Animal Models	2469-2498	101-130	Late-Breaking Poster Session 1
Biological Modeling	2826-2829	645-648	Late-Breaking Poster Session 6
Biomarkers	2782-2801	601-620	Late-Breaking Poster Session 6
Biotransformation/Cytochrome P450	2816-2820	635-639	Late-Breaking Poster Session 6
Carcinogenesis	2592-2595	254-257	Late-Breaking Poster Session 2
Cardiovascular Toxicology/Hemodynamics, Inhalants, Cardiopulmonary	2539-2557	201-219	Late-Breaking Poster Session 2
Cell Death/Apoptosis	2577-2582	239-244	Late-Breaking Poster Session 2
Chemical & Biological Weapons	2499-2507	131-139	Late-Breaking Poster Session 1
Children's Health/Juvenile Toxicity	2655-2661	401-407	Late-Breaking Poster Session 4
Clinical and Translational Toxicology	2636-2638	341-343	Late-Breaking Poster Session 3
Computational Toxicology and Bioinformatics	2639-2648	344-353	Late-Breaking Poster Session 3
Developmental Basis of Adult Disease and Developmental Toxicology	2662-2675	408-421	Late-Breaking Poster Session 4
Disposition/Pharmacokinetics and Pharmacogenomics/Genetic Polymorphisms	2821-2825	640-644	Late-Breaking Poster Session 6
Ecotoxicology, Food Safety, and Natural Products	2508-2525	140-157	Late-Breaking Poster Session 1
Education, Ethical, Legal, and Social Issues	2654	359	Late-Breaking Poster Session 3
Endocrine Toxicology and Reproductive Toxicology	2676-2690	422-436	Late-Breaking Poster Session 4
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Epigenetics	2583-2584	245-246	Late-Breaking Poster Session 2
Exposure Assessment/Biomonitoring	2802-2815	621-634	Late-Breaking Poster Session 6
Gene Regulation/Signal Transduction/Genotoxicity and DNA Repair	2764-2774	553-563	Late-Breaking Poster Session 5
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Late-Breaking Abstracts

ABSTRACT FINAL ID: 2469 Poster Board -101
TITLE: Human Microvascular 3-D Spheroid Model for Toxicity Testing

AUTHORS (FIRST INITIAL, LAST NAME): J. K. Das[1], Q. H. Felty[1]
INSTITUTIONS (ALL): 1. Environmental & Occupational Health, Florida International University, Miami, FL, United States.
KEYWORDS: Human Vascular Spheroids, Proliferative Lesions, ID3

ABSTRACT BODY: Proliferative microvascular lesions that result from a focal budding of endothelial cells are an aggressive endothelial phenotype that may be modified by exposure to persistent environmental pollutants. The goal of this study was to establish a "Vascular Sphere in Dish model" for toxicity screening based on human vascular cells for the purpose of creating a biologically relevant *in vitro* model. We developed a method for preparing a 3-dimensional (3-D) vascular spheroid model that consisted of combining human microvascular endothelial cells, human vascular smooth muscle cells, and human fibroblasts. Our procedure cultured the combination of cells in specified ratios in an ultra-low attachment culture dish in B27 media to form the vascular spheres. Next, these spheres were transferred into a dish and cultured for up to 28 days without B27 supplement in a defined fibrin matrix seeded with fibroblasts and maintained in a culture medium comprised of DMEM/F12 and growth supplements. Our data showed that human vascular spheres imitate proliferation and tube formation *in vitro*. The ability of microvascular endothelial cells to sprout and form stable vascular networks in fibrin matrices was also investigated as a function of vascular stemness. We have previously studied the inhibitor of DNA binding and differentiation protein 3 (Id3). Under defined cell culture conditions, endothelial spheroids that overexpressed ID3 showed an increase in stem cell markers CD34 and VEGFR3; and formed a 3-D vascular tissue that morphologically resembled glomeruloid microvascular lesions. In summary, our findings show that vascular spheroids consisting of a combination of cell types while grown in a unique 3-D microenvironment and culture medium ingredients supported the growth of vascular spheroids. The application of this *in vitro* 3-D microvasculature sphere model may be used to study the effect of environmental pollutants in a more biologically relevant model compared to animal experiments and will allow for faster and less expensive screening.

ABSTRACT FINAL ID: 2470 Poster Board -102
TITLE: An *In Vitro* Model for the Evaluation of Chemical-Induced Multiple Organ Toxicity

AUTHORS (FIRST INITIAL, LAST NAME): M. Dong[1], J. Choi[1], D. Na[1]
INSTITUTIONS (ALL): 1. School Life Sciences and Biotechnology, Korea University, Seoul, Republic of Korea.
KEYWORDS: *In Vitro* Multiorgan Toxicity Assay, CYP Overexpression, IdMOC Plate

ABSTRACT BODY: A major weak point of an *in vitro* system is the lack of multiple organ interactions as observed in a whole organism. In this study, we developed *in vitro* assay system for the evaluation of chemical induced multiple organ toxicity using the integrated discrete multiple organ cell culture (IdMOC) plates. We plated human cell lines, HepG2, HK-2 and HUVEC, to observe the organ toxicity of the liver, kidney and blood vessel, respectively, on the IdMOC plate. For the metabolic activation of chemical, HepG2/CYPs5-13 cells which overexpress four human cytochrome P450 (CYP) isoforms, CYP3A4, 1A2, 2D6 and 2B6, were established and evaluated the CYP expression by enzyme activity and RT-PCR. mRNA levels of 3A4, 1A2, and 2D6 in HepG2/CYPs5-13 cells were 12, 560, and 209-fold higher compared with the human primary hepatocytes, respectively. Chemical induced cytotoxicity of HepG2/CYPs5-13, HK-2 and Huvec cells on the 24 well IdMOC plate were much higher than those of HepG2/CYPs5-13, HK-2 and Huvec cells which were separately plated on 24 well plates. Cyclosporine and admiodarone induced cytotoxicities in HepG2/CYPs5-13 cells were similar compared with human primary hepatocytes and about 10 to 15% higher than those in HepG2/Mock cells. The IC50 values of amiodarone in the HepG2/Mock cells (21.6 μM, 15.5 μM and 14.0 μM exposed for 24h, 48h and 72h, respectively, in 96 well plate) were higher than those in HepG2/CYPs5-13 cells (6.3 μM, 5.7 μM and 4.8 μM exposed for 24h, 48h and 72h, respectively, in IdMOC system). These results suggested that this *in vitro* model system using IdMOC plate have a potential to determine the multiple organ chemical toxicity for human.

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ABSTRACT FINAL ID: 2471 Poster Board -103
TITLE: Evaluation of the Eye Irritation Potential and Dermal Compatibility of an Adhesive Containing a Natural Rubber Latex Derived from Guayle (*Parthenium argentatum*) Used to Apply False Eyelashes

AUTHORS (FIRST INITIAL, LAST NAME): R. Labib[1], K. Edgar[1], A. Harrington[1], D. Goassai[1], R. von Stein[1], E. A. Gilberti[1], S. D. Gettings[1]
INSTITUTIONS (ALL): 1. Product Safety and Integrity, Avon Products, Suffern, NY, United States.
KEYWORDS: Latex, Allergy, Alternatives

ABSTRACT BODY: "Latex allergy" is a significant public health concern. In some individuals, exposure to commonly used latex materials derived from *Hevea brasiliensis* may cause a Type 1 anaphylactic reaction which can be life-threatening. The prevalence of latex allergy in the general population has been reported to be <1 to 6%. *Parthenium argentatum* Extract ("Guayule latex") is a highly purified latex derived from the bark of the Guayule plant (*Parthenium argentatum*). One of its primary advantages is that it does not contain antigenic proteins which cross-react with proteins found in latex materials derived from *Hevea brasiliensis*. Individuals sensitized to *Hevea*-derived latex do not react to Guayule latex (GL) and it has been used successfully in a number of applications including medical devices where flexibility and significant skin contact is anticipated. We report the results of the evaluation of the eye irritation potential and dermal compatibility of an adhesive containing GL used to apply false eyelashes. 100% GL was non-irritating using EpiDerm™. A prototype cosmetic formulation containing 40% GL was non-irritating in a clinical primary irritation test (occlusive patch, n=18). The eye irritation potential of a similar formulation containing up to 43.75% GL was evaluated using both EpiOcular™ and CAMVA and in each case was either non-irritating or minimally irritating. There was no evidence of sensitization associated with a formulation containing 25% GL in an HRIPT (n=100). 100% GL was non-phototoxic in the 3T3 NRU phototoxicity test. The formulation containing 40% GL was not phototoxic and there was no evidence of photosensitization in human photopatch tests (n=10 and n=26, respectively). An ophthalmologist-supervised clinical-use study (n=61) was conducted with the formulation containing 25% GL used as the adhesive to apply false eyelashes (≥ 3 X week for 4 weeks). No adverse effects were observed in or around the eye.

ABSTRACT FINAL ID: 2472 Poster Board -104
TITLE: Investigation of Potential Drugs' Carcinogenity Using Genetic and Functional Markers in Human Lymphocyte Culture

AUTHORS (FIRST INITIAL, LAST NAME): I. S. Kolesnikova[1]
INSTITUTIONS (ALL): 1. FSU RIHOPHE FMBA RF, Saint-Petersburg, Russian Federation.
KEYWORDS: Carcinogene, AURKA, Peripheral Blood Lymphocytes

ABSTRACT BODY: An alternative model of carcinogenity evaluation using human lymphocyte culture is presented. The idea of the approach is to reveal possible cell malformation on slides using FISH with probe specific to AURKA gene, which overexpression as well as/or an increased copy number was observed in many types of malignant cells including blood cancer, as a genetic marker and replication synchronity of homologous loci (that is disturbed in cells with genetic imbalance including malignant cells) as a functional marker of possible malignization or predisposition to it. Human peripheral blood lymphocytes were cultured during 96 h. An intact lymphocyte culture was established as a control. For system testing known carcinogenes NiCl2 (final concentration 50 mcg/ml) and N-nitroso-N-methylurea (final concentrations 50 and 100 mcg/ml) were used. Amorphous SiO2 nanoparticles (final concentrations 100 and 200 mcg/ml) influence on peripheral blood lymphocytes was investigate using this system, because there is a lack of data about their carcinogenity. Using FISH with 20q13 (AURKA) probe following parameters were analyzed: AURKA copy number; replication synchronity of homologous loci (20q13). In in presence of known carcinogens as well as SiO2 nanoparticles increased amounts of cells with multiple AURKA copy number and desynchronization of 20q13 replication compared with intact culture were observed. These results are consistent with data about NiCl2 and N-nitroso-N-methylurea carcinogenity. Essential influence of SiO2 nanoparticles on lymphocytes was also visible, ant this influence was analogous to known carcinogenes action. SiO2 nanoparticles in high concentrations may have possible carcinogenic activity, although additional tests are to perform. The

results of experiments performed show that the chosen approach can be informative for drugs potential carcinogenity testing, although subsequent experiments are required.

ABSTRACT FINAL ID: 2473 Poster Board -105
TITLE: Evaluation of Mainstream Cigarette Smoke-Induced Lung Epithelial Cells Oxidative Stress by Whole Smoke Exposure

AUTHORS (FIRST INITIAL, LAST NAME): X. Li[1], S. Zhang[1], K. Liu[1], F. Xie[1], H. Liu[1], J. Xie[1]
INSTITUTIONS (ALL): 1. Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou, Henan, China.
KEYWORDS: Cigarette Smoke, Air-Liquid Interface, Oxidative Stress

ABSTRACT BODY: Previous research on the hazards of cigarette smoke mainly focused on biological effects induced by cigarette smoke condensates or extracts, which ignores the physiological processes of smoking and cigarette smoke aging. Therefore, we performed air-liquid interface exposure studies, which ensures exposure mimic to *in vivo* inhalation and allows for the use of native and unmodified smoke. Mainstream cigarette smoke was generated from 3R4F reference cigarettes using a VC10 smoking robot under the ISO regimen (35/60/2 without blocking of filter ventilation). Lung epithelial cells (A549 cells) were exposed to fresh whole smoke at the air-liquid interface in the VITROCELL® modules. The results indicated that whole cigarette smoke induced A549 cells damage in cytotoxicity assays. Likewise, oxidative stress marker levels changed after whole smoke exposure. The ratio of reduced and oxidized glutathione (GSH/GSSG) decreased, where asmalondialdehyde (MDA), 4-hydroxy-2-nonenal (HNE), extracellular superoxide dismutase (EC-SOD) and 8-hydroxyl deoxyguanosine (8-OHdG) levels increased. Therefore, we observed whole smoke-induced oxidative stress in A549 cells based on the air-liquid interface exposure conditions.

ABSTRACT FINAL ID: 2474 Poster Board -106
TITLE: Evaluation of TRPV1 Activity to Assess the Eye Stinging Potential of Cosmetic Formulations

AUTHORS (FIRST INITIAL, LAST NAME): A. Gill[1], W. Chen[1], K. Norman[2], L. Krawiec[2], A. H. Dang[1], C. Gomez[1]
INSTITUTIONS (ALL): 1. Product Safety, Mary Kay Inc., Dallas, TX, United States. 2. Institute for *In Vitro* Sciences, Inc., Gaithersburg, MD, United States.
KEYWORDS: Eye Stinging, Cosmetics, Receptor

ABSTRACT BODY: The Transient Receptor Potential Vanilloid type 1 (TRPV1) receptor is one of the most well characterized pain-inducing receptors and has been recently identified as a valuable tool to predict eye stinging potential of surfactant based formulations. In this study we sought to predict eye stinging of non-surfactant based cosmetic formulations by studying TRPV1 activity using the NociOcular assay. In the NociOcular assay, TRPV1 expressing neuroblastoma cells are exposed to test substance and TRPV1 activity is measured by acute increases in intracellular calcium. Three of the formulations induced stinging in the human test and were also positive in the NociOcular assay. The other four formulations evaluated were classified as stinging in the human test, but a conclusive determination could not be made in the NociOcular assay as the formulations were not fully soluble in assay buffers. The formulations were also evaluated in the EpiOcular assay, an established *in vitro* model for eye irritation utilized by cosmetic industry. The Epiocular assay results did not correlate with the human sting data. Our data support that the NociOcular assay may be a valuable additional *in vitro* tool to predict human eye stinging sensation for cosmetic formulations. This data and the assay seeks to expand the applicability of the assay to product types other than surfactant based formulations.

ABSTRACT FINAL ID: 2475 Poster Board -107
TITLE: Do Synthetic Pyrethroids Have Subcellular Effects Other Than Sodium Channel Blockade? A Mechanistic Study Using RTG Cells

AUTHORS (FIRST INITIAL, LAST NAME): B. Yurdakok Dikmen[1], D. Vejselova[2], H. Kutlu[2], A. Filazi[1], F. Erkoc[3]

INSTITUTIONS (ALL): 1. Department of Pharmacology and Toxicology, Ankara University Faculty of Veterinary Medicine, Ankara, Ankara, Turkey. 2. Department of Biology, Anadolu University, Eskisehir, Eskisehir, Turkey. 3. Biology Education, Gazi University, Ankara, Ankara, Turkey.
KEYWORDS: Pyrethroids, RTG2, Cellular Effects

ABSTRACT BODY: Short-term, chronic and biological activity tests for registration process depict pyrethroid toxicity mechanism as sodium channel blockade. Recently other mechanisms of action have been proposed but potential subcellular and EDC effects remain unclear. In this study, cellular and subcellular effects of four synthetic pyrethroids on RTG2 trout cell line is assessed. Mitochondrial function/cell viability and membrane integrity were measured by MTT and LDH release assays. Mitochondrial dysfunction followed by cell death and increased LDH activity was recorded. Cells were exposed for 24 h to IC50 concentrations (µg/L) of permethrin (105.3, Type I), cypermethrin (23.7, Type II), tetramethrin (66.8, Type I) and deltamethrin (38.2, Type II). Light (H&E) and confocal microscopy (acridine orange and phalloidin double staining) results as major morphological changes were: treated cells had fragmented morphology and condensed nuclei, fragmentations with hole formation in cytoskeleton, pyknotic nuclei and micronucleus formation, shrinkage of the cells and nuclei. Generation of cytoplasmic vacuoles, prominent induction of cellular pleomorphism and hydropic degeneration were recorded as compared to controls showing fusiform cells and central nucleus by H&E staining. Results clearly indicate these widely used pyrethroids to effectively alter cell morphology at 24 h IC50 exposure, independent of effect class. Using RTG2 cell line to reliably detect/monitor potential cellular effects of pyrethroids provided valuable insight into their mechanisms of toxic action.

ABSTRACT FINAL ID: 2476 Poster Board -108
TITLE: Correlation Analysis of Chemical Toxicity and Ecotoxicity for Alternative Testing

AUTHORS (FIRST INITIAL, LAST NAME): B. W. Xu[1], X. Gao[2]
INSTITUTIONS (ALL): 1. Bridgewater-Raritan High School, Bridgewater, NJ, United States. 2. Hunterdon Central Regional High School, Flemington, NJ, United States.
KEYWORDS: Ecotoxicity, Mammals, Acute Toxicity

ABSTRACT BODY: There have been increasing efforts to develop alternative methods to ensure the safety of chemicals for human use without testing on mammals for systemic toxicity. At present, there are no validated alternative methods able to completely replace the use of mammals in assessing acute toxicity. The acute toxicity data are used to classify and label chemicals for protection of public health, as required by government authorities around the world. In this study, a comprehensive database was compiled based on OECD's standardized measured acute toxicity data of structurally diverse chemicals, primarily using online databases to explore the acute toxicity in algae, *Daphnia*, and fish as an alternative to acute toxicity testing in rodents. Median lethal concentration (LC50) and dose (LD50) were molar- and log-transformed for correlation and regression analyses. Simple linear regressions revealed significant correlations (r = 0.7956) between fish and rats, expressed as 96-h LC50 and oral LD50, respectively. When octanol-water partition coefficient (log Kow), water solubility, and molecular weight were included with multiple regressions to consider the effect of uptake on toxicity, correlations improved. A toxicity correlation model on rat toxicity in relation to freshwater ecotoxicity was developed to predict acute rodent toxicity. The results provide good evidence of the applicability of using fish tests as a method to reduce the use of mammals in toxicity testing and predict acute rodent toxicity using freshwater ecotoxicity, which can be consequently used for classification and labeling of chemical substances.

ABSTRACT FINAL ID: 2477 Poster Board -109
TITLE: Comparative *In Vitro* Dermal Absorption Study with Benzoic Acid, Testosterone, and Caffeine Using Human and Rat Split-Thickness Skin in a Flow-Through Diffusion System

AUTHORS (FIRST INITIAL, LAST NAME): R. M. Nagane[1], N. N. Patel[1], K. E. Tendulkar[1], R. S.[1], M. V. Patel[1], A. D. Deshpande[1], M. Aggarwal[2], R. Billington[2]

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INSTITUTIONS (ALL): 1. Toxicology, Jai Research Foundation, Vapi, Gujarat, India. 2. Human Health Assessment, Dow AgroSciences Ltd, Abingdon, Oxfordshire, United Kingdom.
KEYWORDS: Dermal Absorption, Human Skin, *In Vitro*

ABSTRACT BODY: *In vitro* dermal absorption studies offer a valid alternative for *in vivo* studies and are conducted with skin from different species such as human, rat and pig. As these studies are performed on very small pieces of skin in isolation in a sophisticated instrument, mainly flow-through diffusion cells, they require a specific set of technical skills. The authors have optimized the experimental conditions for such studies and the present study was conducted to evaluate and validate comparative *in vitro* dermal absorption of 14-C labelled benzoic acid, testosterone and caffeine through human and rat skin. These reference compounds cover different physico-chemical properties of Log PoW and molecular weight that can influence absorption. Each test group included eight replicates from four donors (i.e., 2 replicates/donor). Split-thickness skin membranes (300-400 µm) were placed in flow-through diffusion cells with 0.64 cm2 exposure areas. After checking skin integrity, membranes were exposed to reference compounds (4 mg/mL) in independent experiments. The exposure time was 8h with post-exposure sampling for 16h and total study duration of 24h. Mass balance analysis was conducted from samples of receptor fluid, donor and receptor chamber washes, the residues remaining in/on the skin and in the stratum corneum by measuring radioactivity using liquid scintillation counting. Residue in different layers of stratum corneum was also determined by performing tape stripping. The mean total recovery of benzoic acid was about 93 and 102% in human and rat skin, respectively. The mean total recovery of testosterone was 95-97% in human and rat skin. The mean total recovery of caffeine was 97.7% and 98% in human and rat skin, respectively. The results of this study indicate that the reference compounds showed different absorption profiles through human and rat skin.

ABSTRACT FINAL ID: 2478 Poster Board -110
TITLE: Functional Characterization of Compounds by Their Ability to Modulate Farnesoid X Receptor Interaction with a Coregulator Motif Peptide Array Reveals Multiple Modes of Action

AUTHORS (FIRST INITIAL, LAST NAME): R. Houtman[1], C. Hsu[2], M. Xia[2], R. van Beuningen[1], J. P. Groten[1]
INSTITUTIONS (ALL): 1. PamGene International BV, Den Bosch, Netherlands. 2. NCATS, NIH, Bethesda, MD, United States.
KEYWORDS: Nuclear Receptors, Coregulators, Functional Proteomics

ABSTRACT BODY: The nuclear receptor (NR) Farnesoid X receptor (FXR) regulates the homeostasis of bile acids, lipids, and glucose. Because endogenous chemicals bind and activate FXR, it is important to examine which xenobiotic compounds would disrupt normal receptor function and cause potential toxicity effects including cholestasis and cancer. Upon compound binding, NR-dependent gene expression is dictated by the nature of, a multitude of, recruited coregulators that regulate the chromatin accessibility at the target gene locus. Therefore compounds that induce similar FXR-coregulator interaction profiles are expected to display similar pharmacology, while differential interactions are indicative for a different mode of action. In the current study, a set of 12 FXR antagonists (i.e. (Z)-guggulsterone, ivermectin, chlorophacinone and their analogs) identified previously from the Tox21 10K library screening using a cell-based human FXR β-lactamase (Bla) reporter gene assay were characterized for their ability to modulate the coregulator interaction of CDCA-activated FXR using a peptide micro array with 154 coregulator-derived motifs. We found that chlorophacinone and ivermectin (100 and 90% efficacy resp.) are the most efficacious inhibitors of FXR-SRC2-2 interactions, consistent with the results measured from an orthogonal coactivator assay. All of the identified FXR antagonists showed significant modulation of all CDCA-induced FXR-coregulator interactions, i.e. displacement of coactivators (gene transcription enhancers), and profiles allowed us to sub-classify the chemically related compounds. Interestingly, the ivermectin analogs distinctively displayed enhancement of corepressor interaction, which may explain their superior antagonism over the other compounds. These results suggest that FXR-coregulator interaction profiling provides a novel method to classify the mechanism of action for the structure related compounds.

2015 Society of Toxicology Annual Meeting
Late-Breaking Abstracts

ABSTRACT FINAL ID: 2479 Poster Board -111
TITLE: Combination of Cheminformatics and *In Vitro* Assays to Predict Skin Sensitization Potential and Potency

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KEYWORDS: Skin Senstization

ABSTRACT BODY: Skin sensitization is a complex biological process of interactions of several cell types, proteins and molecular events e.g. formation of protein conjugates. *In vitro* prediction of skin sensitizing potential and chemical potency became critical issues for the cosmetics industry due to increasing interest from the public to replace animal testing and the 7th amendment of European cosmetic directive introduced in 2013. Current *in vitro* assays to predict skin sensitization potential were evaluated in combination with pattern recognition modeling techniques and cheminformatics. Several available skin sensitization assays were used to determine if chemical molecular descriptors available using RDKit would further improve with machine based learning the predicitivty of these approaches. The Direct Peptide Reactivity Assay (DPRA), KeratinoSens and SenCeeTox have been shown to predict skin sensitizing potential of sensitizing chemicals with accuracies of 80%, 77% and 87%, respectively. Neither DPRA nor KeratinoSens was improved using molecular descriptors, however, structural based analysis improved the prediction of the skin sensitizing potential of the SenCeeTox to 90%. The accuracy of skin sensitizing potential was 78%. Use of molecular descriptors allowed modeling approaches to exclude known false positive compounds e.g. sodium dodecyl sulphate (SDS), which was previously classified as a false positive compound in the DPRA and SenCeeTox assays due to its strong ability to oxidize cysteine residues. In addition, ECVAM and OECD recommend the potential to combine testing strategies to have an integrated approach to cover more than one pathway in the skin sensitization process. Using pattern recognition techniques a model was developed to combine DPRA, SenCeeTox and KeratinoSens with and without the molecular descriptors this combination gave an accuracy of 77% without and 81% with the molecular descriptors.

ABSTRACT FINAL ID: 2480 Poster Board -112
TITLE: Progress on the Society of Toxicology-Colgate Palmolive 2014 Grant for Alternative Research: *In Vitro* Assay for Identification of Dermal Sensitizers

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KEYWORDS: Dermal Sensitization, IL-18, CD86

ABSTRACT BODY: To better predict dermal sensitization by immunotoxicants, here we report results on a dual cell culture model consisting of a 3D Reconstituted Human Epidermis skin model (RHE) cultured above a media suspension of Langerhans Cell-like differentiated CD34+ progenitor cells (pDCs). Test chemicals were topically dosed directly onto the RHE stratum corneum, mimicking the dermal route of exposure. We hypothesized that this dual model of human skin-like tissues and langerhans-like cells could allow for a more mechanistic and predictive *in vitro* sensitization assay. We measured two markers of sensitization response: IL-18 secretion into the culture media and CD86 expression on pDCs. Initially, we exposed RHE tissues to chemicals overnight and then transferred the conditioned media to pDCs cultured for an additional overnight period. This sequential treatment was compared to a co-culture method, where RHEs were cultured above pDCs and exposed to test chemicals by topical application to the RHE. Using the sequential treatment method, the strong sensitizer 4-Nitrobutyl bromide (NBB), induced a small 10% increase in CD86. In contrast, NBB induced a 240% increase in CD86+ pDCs using the co-culture method. We next tested three sensitizing chemicals: Isoeugenol, Hexylcinnamaldehyde, and Dinitrochlorobenzene, and two non-sensitizers: Lactic Acid and Phenol, in the co-culture model. Topical treatment induced dose-dependent increases of IL-18 secretion by all three sensitizers, but not by non-sensitizers. The three sensitizers, and the non-sensitizer Phenol also induced significant increases in CD86 expression on pDCs, but Lactic Acid did not. These studies demonstrate that RHEs cultured with pDCs show promise as a dual *in vitro* sensitization assay, allowing cell-cell communication between two skin-resident cell types, critical for dermal sensitization.

2015 Society of Toxicology Annual Meeting
Late-Breaking Abstracts

ABSTRACT FINAL ID: 2481 Poster Board -113
TITLE: A Transcriptomic Analysis of Obesogens in Zebrafish

AUTHORS (FIRST INITIAL, LAST NAME): S. Kalasekar[1], C. W. McCollum[1], P. Jonsson[1], J. Gustafsson[1], M. Bondesson[1]
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KEYWORDS: Obesity, Gene Regulation, Zebrafish

ABSTRACT BODY: As of 2010, in the USA alone, more than one third of adults and more than 17% of children and adolescents were obese, and the numbers of overweight or obese individuals continues to rise. Apart from genetic and lifestyle factors, such as excessive intake of dietary fats and lack of exercise, environmental chemicals are now known to contribute to obesity. These chemicals, called obesogens, have been known to alter cellular and organismal metabolism and act through various mechanisms to alter adipose tissue function. The biocide tributyltin (TBT), and flame retardants tetrabromobisphenol A (TBBPA) and tetrachlorobisphenol A (TCBPA), are known to act as obesogens by interfering with nuclear receptor signaling (Retinoid X Receptor (RXR) and Peroxisome Proliferator-activated Receptor gamma (PPARg)), in mammals and in zebrafish, respectively. In order to further understand the molecular mechanisms behind these obesogenic chemicals, we exposed 2 month-old zebrafish to TBT, TBBPA or TCBPA for 48 hours. Post treatment, the animals were sacrificed, and their RNA was processed for transcriptomic analysis on Agilent 44K zebrafish microarray chips. The genes with changed expression were annotated to their human homologues and used for bioinformatics analysis using Pathway Studio. The analysis showed that the expression of 789 genes was regulated by TBBPA, 584 genes by TCBPA and 1435 genes by TBT exposure. In common, the three obesogens regulated the expression of 200 genes. Gene ontology annotation indicated that these genes cluster in several different biological pathways, including those related to lipid metabolism. Moreover, expression of genes related to fat cell differentiation was significantly affected by the chemicals. In addition to indicating the obesogenic action of TBT, TBBPA and TCBPA in zebrafish, our results also suggest that the zebrafish model is suitable for investigating the mechanism of action of obesogens.

ABSTRACT FINAL ID: 2482 Poster Board -114
TITLE: Development of an *In Vitro* DNA Synthesis HCS Assay Using HepatoPac® Micropatterned Cocultures

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KEYWORDS: *In Vitro* Methods, *In Vitro* Alternative Models, Constitutive Active/Androstane Receptor

ABSTRACT BODY: Recently, it has been shown that primary hepatocytes organized in micropatterned co-cultures (HepatoPac®) maintain high levels of liver functions for prolonged culture periods. Rat and human HepatoPac® co-cultures exhibit better biological fidelity for a number of chemical-induced toxicity pathways and are highly amenable to high-content screening (HCS) approaches. As such, this novel model system represents an exciting new approach for assessing the long-term effects of compounds for their metabolic and non-genotoxic effects across species. The overall goal of this project is to establish HCS parameters necessary to assess the effect of non-genotoxic tumor promoters on induction of DNA synthesis and proliferation in rat HepatoPac® co-culture plates. We determined optimized HCS parameters for cell culture, EdU labeling index, cytotoxicity, and CYP2B induction. Concentration range-finding and exposure optimization experiments for four prototype CAR agonists (phenobarbital, CITCO, TCPOBOP, oxazepam) were also performed over short- (48 hour) and long-term (144 hour) cultivation. Optimal concentrations of PB and oxazepam for eliciting CAR activation (CYP2B induction) in rat HepatoPac® were identified as >130 and >50 µM, respectively. Compound dependent increases in EdU labeling or cell proliferation were not observed, including with EGF, with the exception of the highest concentrations of oxazepam (>1 mM), which also caused significant hepatocellular injury. By contrast, there was a ~2-fold increase in the total percent responders in hepatocytes at 144 hours compared to those at 48 hours with no increase in total cell counts, suggesting an enhanced basal rate of S-phase progression in long-term culture. Overall, this work demonstrates the

potential for the HepatoPac® culture model to be utilized for long-term effects of nuclear receptor agonists using HCS strategies.

ABSTRACT FINAL ID: 2483 Poster Board -115
TITLE: Human Respiratory Platform for *In Vitro* Drug Development

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KEYWORDS: Organ-on-a-Chip, Pulmonary System, Rapid Fabrication

ABSTRACT BODY: Recapitulating the complex cellular and functional environment of a lung into an *in vitro* platform would provide critical advantages over conventional pulmonary toxicity models. However, the human lung is a complex organ system that contains numerous cells types and performs multiple mechanical/physiological functions. To capture multiple critical features of the human lung system *in vitro*, we have undertaken a stepwise approach to engineer a complex microfluidic platform. Our current focus was to develop a platform that facilitates the growth of at least three different types of primary cell lines including bronchiolar, alveolar, and microvascular cells. We also focus on integrating functional aspects such as bifurcation in the bronchiolar air ways, mechanical deformation in the alveolar sacs and air liquid interfaces to mimic the human respiratory system. To reconstitute the complex lung physiology and the microenvironment that maintains cell differentiation, we fabricated our human lung organ platform by integrating both bronchiolar and alveolar lung compartments. This lung platform contains porous polyester membrane (to mimic the bronchioles) and inflatable polydimethylsiloxane (PDMS) membranes (to mimic the alveoli). The platform was fabricated by stacking laser patterned modules with silicone based adhesive transfer tapes. Human lung tissue was reconstructed by culturing human bronchial epithelial cells (BEAS-2B) in the lumen of the hollow fibers, while human alveolar epithelial cells (AT1) and human lung microvascular endothelial (HLMVE) cells were cultured on both sides of the PDMS membranes after 5 days of cell culture. The toxicity model was established by introducing camptothecin, a pro-apoptotic anti-cancer drug into the lumen of hollow fiber covered with bronchiolar cells for 2 days. The cytotoxicity was evaluated from 50 % to 85 % when the concentration of camptothecin increased from 0.1 µM to 10 µM. The lactate dehydrogenase (LDH) result concludes that the cell apoptosis was observed after exposure to camptothecin.

ABSTRACT FINAL ID: 2484 Poster Board -116
TITLE: DNA Damage Analysis in *C. elegans*

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KEYWORDS: DNA Damage, *C. elegans*

ABSTRACT BODY: *Caenorhabditis elegans* (*C. elegans*) is a soil nematode commonly used as a model for both biomedical and toxicological research. *C. elegans* are often used for genetic studies, but background endogenous DNA damage levels are currently not known. *C. elegans* DNA extraction methods often require phenol, which induces DNA damage, are not standardized, or result in low DNA yields. To address these issues, we systematically investigated methods for *C. elegans* disintegration and DNA extraction. To extract DNA from *C. elegans*, the tough skin must be broken apart mechanically, chemically or enzymatically. Non-caustic methods to disintegrate the worm were identified in literature; seven were tested. Disintegration efficacy was measured via crude DNA measurement and microscopic imaging and/or flow imaging. Freezing and thawing in liquid nitrogen, homogenization with a tissue grinder, and freeze/thaw plus homogenization had little change from control; worms were largely intact and crude DNA measurements were 32.4 (N=4), 3.5 (N=12) and 1.2 (N=2) times control. Grinding in N2 resulted in the most worm fragments and DNA (334 x control, N=7). Three DNA extraction methods were tested: A *C. elegans* method with high-salt protein precipitation instead of phenol separation, a high-salt mammalian cell protocol, and DNeasy kit by Qiagen. The modified *C. elegans* protocol resulted in bad separation of DNA from proteins (82% of the DNA in protein pellet, 18% in aqueous phase). The high-salt cell protocol did not effectively

separate DNA from worm lysate. The Qiagen protocol was effective at isolating DNA, especially when used with worms ground in N2 and enzymatically digested. We are currently optimizing the protocol further and will next detect DNA lesions with isotope-dilution on both gas chromatography-tandem mass spectrometry (GC-MS/MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS).

ABSTRACT FINAL ID: 2485 Poster Board -117
TITLE: Transport of a Mn/Zn Ethylene-bis-Dithiocarbamate Fungicide Occurs via SMF-3, but Not Neurotransmitter Transporters, in *C. elegans*

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KEYWORDS: *C. elegans*, Mancozeb, Parkinson's Disease

ABSTRACT BODY: Data suggest that exposure to Mancozeb (MZ), a Mn/Zn ethylene-*bis*-dithiocarbamate-containing fungicide, leads to neurodegeneration in *Caenorhabditis elegans* (*C. elegans*). Thus, we wanted to ascertain if MZ enters neurons via pre-synaptic neurotransmitter transporters and/or a *C. elegans* divalent metal transporter homologue, smf-3. Worm strains with various green fluorescent protein (GFP)-labeled neurons were pre-treated with antagonists for dopamine (DAT), serotonin (SERT), or GABA (GAT) transporters to determine if this would ameliorate neurodegeneration. Worms were then exposed to various MZ concentrations for 30 min (acute) or 24 h (chronic). Fluorescence was measured 24 h post MZ exposure. When worms with GFP-labeled DAergic neurons were pre-treated with a DAT antagonist in the chronic paradigm, no statistically significant difference in pre-treated worms compared to controls was observed. When pan-neuronal::GFP worms were pre-treated with a SERT antagonist in the chronic MZ paradigm, a statistically significant decrease ($*p < 0.05$) in fluorescence was observed compared to non-pre-treated worms. When GABAergic neuron::GFP worms were pre-treated with a GAT antagonist followed by acute MZ exposure, statistically significant increases in fluorescence ($*p < 0.05$) were measured for mid-range MZ concentrations, but a statistically significant decrease ($*p < 0.05$) was found at the highest MZ concentration. When these studies were repeated in a chronic paradigm, there was no statistically significant differences in fluorescence compared to controls. Since studies suggest reduced Mn toxicity in *smf-3* knock-out (KO) worms, we also treated them with varying MZ concentrations, followed by an ATP assay. Interestingly, smf-3 KO worms in both the acute and chronic MZ treatment paradigms showed complete rescue of ATP levels compared to those measured in MZ-treated wild-type worms. Taken together, these data indicate that DAT, SERT, and GAT do not play a significant role in MZ neurodegeneration, while smf-3 apparently regulates MZ transport.

ABSTRACT FINAL ID: 2486 Poster Board -118
TITLE: Universal Media Development for Human Organ Constructs

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KEYWORDS: Organ Culture, Media Refinement, Universal Media

ABSTRACT BODY: *In vitro* human organ constructs (HOCs) have the potential to revolutionize toxicology because the function of multiple tissue types in concert could be utilized to gain a more accurate picture of pharmacokinetics compared to two-dimensional cell culture. However, a major limitation to HOCs is that there exists no common media that can be used to culture multiple organ models or tissue types at the same time. For example, there are no reports in the literature of interconnected well-differentiated HOCs due to the differences in media requirements between different organs. Our goal is to design a universal serum-free media that can be utilized for multiple organ types at once. Achieving our goal will allow us to connect four organ HOCs: heart, lung, liver, and kidney using a common media. We analyzed each of the media formulations currently in use for culturing these four HOCs. Of the 106 media components that go into the four organs, only

38 components are unique to only one of the four organs. From this observation, we developed several formulations for testing and optimization. Assays of primary cells, stem cells, and tissues indicate that several tissue types are easily adaptable, and can maintain organ-specific functionality for up to two weeks in media that contains nutrients and growth factors for multiple organs. Other tissue types are less adaptable and will require semi-factorial optimization of the media formula. These studies show that a universal media for HOCs may be within reach. A successful universal media formulation will have a major impact on *in vitro* human organ models and the potential that these models represent for improving toxicology research.

ABSTRACT FINAL ID: 2487 Poster Board -119
TITLE: Simultaneous PK/PD by Automated Blood Sampling and Radiotelemetered Cardiovascular Measurements in Macaques

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KEYWORDS: PK/PD, Radiotelemetry, Cardiovascular

ABSTRACT BODY: Cardiovascular safety studies commonly use radiotelemetry in animals such as cynomolgus macaques (cynos) but require toxicokinetic sampling in separate animals to avoid disruptions in physiologic measurements. Challenges with use of cynos include signal interference during procedures, and increases in stress markers (e.g., cortisol, heartrate) due to handling. A proof of concept study was conducted whereby automated blood sampling (Culex-L®) was coupled with radiotelemetry (DSI) to compare automated versus manual blood sampling during physiological measurements. Four cynos implanted with telemetry devices and intra-carotid catheters were dosed twice in a crossover design with 175 mg/kg of moxifloxacin (Moxi), a drug with QT prolongation liability. Samples for measurement of Moxi and cortisol were collected either manually or via the Culex-L® on different days, while cardiovascular measurements were recorded. Moxi concentrations were similar for both sampling techniques. Cortisol concentrations and heartrate were markedly elevated during manual collection. Furthermore, Moxi-related QT prolongation was obscured during manual collection but detectable during automated collection. Automated collection was associated with fewer room disturbances. Challenges encountered with Culex-L® and radiotelemetry included missed plasma samples, resolved by revising component connections, and telemetry data drop-out due to loss of digital signal to receivers, addressed by adjusting receiver placement. This study established the feasibility of collecting simultaneous blood samples by the Culex-L® while recording physiological measurements by radiotelemetry in caged, tethered cynos. Less animal stress was incurred by automated sampling as demonstrated by lower cortisol concentrations, lower heartrate, and fewer physiological data dropouts. Using combined methods, animal use numbers can be reduced by half, and refinement of experimental procedures can result in less animal stress and more robust data.

ABSTRACT FINAL ID: 2488 Poster Board -120
TITLE: Social Housing of Rats in Long-Term Studies: Effects on Food Consumption, Body Weight, and Survival

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KEYWORDS: Chronic Study, Group Housing, Rodent Model

ABSTRACT BODY: Social housing of social species, including rodents, is the expected housing of laboratory animals. While this has been the standard in Europe for decades, this expectation has only recently begun in the United States. The Guide for the Care and Use of Laboratory Animals (2011, 8th Edition) states that the housing environment needs to meet the animals' needs in order to prevent developmental and behavioral disorders and maintain animal wellbeing and scientific validity. The creation of this type of environment includes: social interactions with members of the same species, safety,

appropriate flooring (solid for rodents), bedding or structures for sleep, and appropriate size. Two years ago, our lab transitioned from single-housing in wire-bottom cages to social housing in solid-bottom cages. However, analysis of the health benefits of this housing method has not been done for long-term rodent studies for our lab. The purpose of this analysis was to compare food consumption, body weight, and survival of social- or single-housed Sprague Dawley rats. We assessed vehicle control groups of two-year oral gavage studies since 2008. In this time, changes to rat housing include: social housing, type of caging (wire-bottom or polycarbonate solid bottom), and feed (14 or 16% protein). Mean food consumption decreased in male and female rats housed in solid bottom cages, but was not affected by diet or social housing. There was a trend towards decreased mean body weight in male and female rats given a 14% protein diet compared to 16% protein diet. Type of diet and social housing did not affect mean body weight. Mean percent survival did not change as a result of social-housing, diet, or caging. While we did not find an increase in survival with social/solid-bottom housing, we do not have the data to assess the effect of social housing on mental health. In conclusion, social-housing did not result in overt health benefits over single-housed animals. Food consumption decreased in rats housed in solid bottom cages, and body weight decreased in animals fed a 14% diet.

ABSTRACT FINAL ID: 2489 Poster Board -121
TITLE: Two-Pore Potassium Channel Expression Analysis in Human, Rabbit, Canine, and Cynomolgus Heart Tissue Used to Guide Selection of Cardiovascular Preclinical Test Species

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KEYWORDS: Animal Model, Potassium Channel, Cardiovascular

ABSTRACT BODY: Task-1 (KCNK3), Task-3 (KCNK9) and Trek-1 (KCNK2) are three of the 15 member two-pore potassium channel family of proteins. These proteins are potential drug targets for several conditions including depression (Task-1, Task-3), atrial fibrillation (Task-1, Trek-1) and pulmonary hypertension (Task-1). Task-1, Task-3 and Trek-1 are notably expressed in several tissues, including the adrenal and pineal glands, the cerebellum and the heart atrium and ventricle. Because these channels have known important effects in cardiac tissue, determining the animal models used in early stage cardiovascular testing is an important consideration for pre-clinical safety assessment. RNA expression in several species was therefore used as a basis to select the model with an expression pattern nearest to that of the human as a basis for early stage cardiovascular safety testing. Real time PCR with TaqMan chemistry was performed and the gene expression of Task-1, Task-3 and Trek-1 evaluated in RNA isolated from human, rabbit, canine and Cynomolgus monkey adrenal cortex, cerebellum, cardiac atrium and cardiac ventricular tissues. Of the three species tested we determined that the targets in rabbit heart were most closely correlated to the human expression pattern. Comparitively, Task-1 was expressed at 2 fold higher levels in the atrium than the ventricle in both species while Task-3 was expressed at nearly equivalent levels and Trek-1 was expressed in slightly higher levels in the atrium than the ventricle. The overall levels of expression of the three genes were also closest between the rabbit and human. The canine expression pattern was similar to the human but overall expression levels of all three genes were much greater. The monkey expression pattern and levels for all three genes were not similar to human. Based on these results we concluded that the rabbit was the most appropriate animal model for our early stage cardiac testing.

ABSTRACT FINAL ID: 2490 Poster Board -122
TITLE: Investigating the Autosomal and an X-Linked Gene Mutation in Mice: A Pilot Study

AUTHORS (FIRST INITIAL, LAST NAME): H. Lin[1], M. G. Pearce[1], J. A. Bhalli[1], M. Pacheco-Martinez[1], V. N. Dobrovolsky[1]
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KEYWORDS: Gene Mutation, CD24-Deficient RBCs, Chromosomal Damage

ABSTRACT BODY: The Pig-a assay is a novel approach for measuring the induction of mutations at a specific gene location, the X-linked Pig-a gene, caused by genotoxic effects of treatment with drugs or other chemicals. However, the Pig-a gene mutation assay is relatively insensitive to detection of some specific mutagens, such as azidothymidine (AZT), and even radiation. CD24 is a GPI-anchored glycoprotein expressed on the surface of many types of cells, including murine erythrocytes and human B cells. The CD24 protein is also used as a marker for measuring Pig-a gene mutations of mice. In the current study, we developed an *in vivo* mutation model for the detection of mutation in the autosomal CD24 and the X-linked Pig-a genes of CD24+/- mice using flow cytometry. Wild-type (WT) and CD24+/- heterozygous (HZ) C57BL/6 mice were treated once with 0, 10, 40 or 160 mg/kg of N-ethyl-N-nitrosourea (ENU) dissolved in the vehicle, phosphate buffered saline. We found that ENU caused a similar dose-dependent increase in the frequency of micronucleated reticulocytes in both WT and HZ animals, indicating that ENU is equally potent in inducing chromosomal damage in mice of both genotypes. The increased frequency of CD24-deficient RBCs was overtly observed in a dose-dependent manner in both WT and HZ animals from 2 weeks to 8 weeks after the treatment, but it was consistently higher in HZ mice than in WT mice. The different patterns of anti-CD24 and FLAER RBC labeling in animals of different genotypes in blood samples and the frequencies of CD24-deficient RBCs imply that in WT mice all CD24-deficient cells are expected to be Pig-a mutants, while in HZ animals, some CD24-deficient cells are Pig-a mutants and others are CD24 mutants. These results suggest that CD24+/- mice are capable of detecting hematopoietic cell mutation in both an autosomal gene (i.e., CD24) and X-linked genes. The mutations detected in the assay potentially, may be caused by gene mutation, large deletion and loss of heterozygosity.

ABSTRACT FINAL ID: 2491 Poster Board -123
TITLE: Steroidogenic Effects of Sertraline on Juvenile Fathead Minnow (*Pimephales promelas*)

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KEYWORDS: Sertraline, Fathead Minnow, Steroidogenesis

ABSTRACT BODY: Sertraline was the third most prescribed drug in 2011 with over 37 million prescriptions and generally enters the environment through post-consumer use. Due to the abundance of sertraline in waste water effluents and surface waters, it is imperative to determine the chronic effects of low-level exposure of sertraline. Given that serotonin transporters (SERT) in teleost fish, compared to humans, have nearly 75% sequence similarity, a teleost model to study sertraline toxicity is appropriate. Fathead minnows and other teleost fish are widely used as model organisms for various toxicological/pathophysiological conditions, including endocrine disruption. In this study, early-life stage steroidogenic effects of sertraline on juvenile fathead minnows (FHM) were analyzed via RT-qPCR. Larval FHM were exposed to 0.1, 1, and 10 µg/L sertraline for 28 days and screened for differential expression of 11β-Hydroxysteroid dehydrogenase (11β-HSD), 20β-Hydroxysteroid dehydrogenase (20β-HSD), aromatase (CYP19), and nuclear thyroid receptor alpha (TRα) mRNA. Larval FHM exposed to 0.1 µg/L sertraline had a significant upregulation of 20β-HSD (log(2) = 0.84) and TRα (log(2) = 0.41). In addition to mRNA transcript analysis, FHM survival and weight were also taken into consideration. Survival rates of exposed fish were >80% in all exposure groups and while a positive correlation between weight and sertraline dose was observed, no statistical significance was found. This data presents additional insight into the negative ramifications SSRIs pose on aquatic life and offers caution to possible downstream effects SSRIs may have on human health.

ABSTRACT FINAL ID: 2492 Poster Board -124
TITLE: Surgical Animal Models in Support of 3Rs

AUTHORS (FIRST INITIAL, LAST NAME): M. Taschwer[1], J. Nelson[1], R. Haas[1], V. Dinkel[1], D. L. McKenzie[1]
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KEYWORDS: Animal Model, CSF, Pharmacokinetics

ABSTRACT BODY: The 3Rs (replacement, reduction, refinement) are the foundation of animal welfare in research. Animal models are critical for studying xenobiotic metabolism, biomarkers, and pharmacological/toxicological effects on the body.

Alternatives for the replacement of animals in research are invaluable; however, there is still a need to use live animal models and refinement of methods which reduce the number of animals used are of critical importance. Acute surgical models are available which can be used for sampling of various matrices non-terminally, but these are limited in number of samples and robustness/duration of the model. We have developed surgical techniques for portal vein- and recirculating bile duct-cannulation in dogs and lumbar laminectomy in nonhuman primates (NHP) resulting in maintenance of animals in a colony setting for re-use without compromising animal welfare. We have combined multiple models (portal vein-cannulation and laparoscopic liver biopsies) to further decrease animal use while maintaining the highest quality standard of animal care. Portal vein-cannulated dogs have maintained bidirectional patency for >1 year; recirculating bile duct-cannulated dogs have maintained patency for 6+ months. Lumbar laminectomy NHPs have maintained bidirectional patency for 4+ months. In all cases, animals recovered completely following surgery, are BAR (bright, alert, responsive), eating and mobile on the same day of surgery and display no signs of pain or distress, indicating exceptional pain management. Animals are commingled in accordance with SOPs and there have been no significant changes in body weight or clinical pathology and no surgical complications. Study designs allow for serial samples of each matrix, as applicable, resulting in complete data sets for individual animals and overall animal use has been decreased substantially. Ultimately, these models provide refined surgical methods to reduce overall animal use without compromising welfare providing a significant advantage over current techniques and supporting the 3R philosophy.

ABSTRACT FINAL ID: 2493 Poster Board -125
TITLE: Wireless Implantable Micropump for Automated Infusion in Vivarium Cage System

AUTHORS (FIRST INITIAL, LAST NAME): T. Q. Hoang[1, 3], C. A. Gutierrez[1], C. Jones[1], S. Stronks[1], L. Rodriguez[1], G. Shackleford[2], E. F. Meng[1, 3]
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KEYWORDS: Micropumps, Infusion Pump, Dosing

ABSTRACT BODY: Tether-free automated drug administration has dramatic implications to toxicology research by minimizing animal handling, stress-inducing events, potential dosing errors, and manual recording errors. We present a battery-less dosing system comprising an implantable infusion micropump, an external controller for wireless power and programmability. This system is the first capable of both wireless-operation and on-demand changes to the dosing regimen in animals as small as a mouse. Software allowed automated pump operation and data logging. We previously demonstrated micropumps with repeatable delivery of specific doses or flow rates (nL-µL/min). This implantable platform allowed delivery of compounds in unaltered format; it consisted of a refillable fluid reservoir, flow regulation valve, and cannula. Micropumps were refilled percutaneously with a small gauge non-coring needle. An external controller in a base station form factor was placed underneath a standard rodent cage to control wirelessly using a single frequency. We present a novel, scalable telemetry system that is fully compatible with high-density vivarium caging systems and automates chronic dosing regimen. Experiments were performed in collaboration with Childrens Hospital Los Angeles to demonstrate wirelessly controlled dosing of luciferin in freely moving mice trasngenically-expressing luciferase. We present 1) the improved pump system architecture with integrated multi-frequency external controller in a cage-wrap form factor, 2) pump and controller performance including on-demand flow rate performance and wireless power characterization, and 3) bioluminescence imaging verifying *in vivo* dose time course. On-demand dosing capability was preserved and interference minimized through implementation of the multi-frequency controller topology. This enabled control of multiple cages while maintaining access to important environmental control parameters including water, air, and temperature in standard high-density caging systems.

ABSTRACT FINAL ID: 2494 Poster Board -126
TITLE: Do Rats Develop Taste Distortions When Subjected to a Targeted Therapeutic with Clinical Effects on Taste?

AUTHORS (FIRST INITIAL, LAST NAME): J. Glendinning[1], J. Hill[1], C. DeMaio[2], T. Wang[2]

INSTITUTIONS (ALL): 1. Barnard College , New York, NY, United States. 2. Novartis Pharmaceuticals Corporation, East Hanover, NJ, United States.
KEYWORDS: Taste, Rat

ABSTRACT BODY: Taste and smell distortions are potential side effects of treatment with certain targeted therapeutics rendering foods and beverages unpalatable, and thus reducing quality of life for patients undergoing treatment. A new compound that is in development at Novartis Pharmaceuticals Corporation has produced chemosensory alterations in patients. This study asked whether taste distortions would be evident in a rodent model: the Wistar Hannover rat. Two measures of chemosensory responsiveness were used: brief-access lick tests (to assess stimulus palatability) and taste nerve recordings (to assess peripheral taste responsiveness). The chemical stimuli activated the taste (sucrose, monosodium glutamate+inosine monophosphate, citric acid, quinine and NaCl), trigeminal (capsaicin and mustard oil), trigeminal/olfaction (ethanol), and olfactory (grape and cherry) systems. All rats exhibited robust licking responses to the chemical stimuli, but there were no significant differences between rats subjected to the control or treatment. The only effect of the treatment occurred when when multiple aversive taste stimuli were tested during the same test session. In this case, the highest NaCl concentration was less aversive, while the highest quinine concentration was more aversive. This latter observation results point to a contextually dependent effect of the compound, in which taste distortions occur only when rats are presented simultaneously with a complex array of aversive taste stimuli. To determine whether these contextually dependent effects were mediated in the peripheral taste system, recordings were obtained from the chorda tympani (CT) nerve during lingual stimulation with a battery of taste stimuli; the CT nerve innervates the fungiform taste buds in the cranial tongue. However, there were no significant differences between rats subjected to the control or test solution. Taken together, our study indicates that the targeted therapeutic treatment produced relatively subtle, context-dependent changes in the taste system of the rat.

ABSTRACT FINAL ID: 2495 Poster Board -127
TITLE: Respiratory Measurements in Rats As Indicator of Adverse Respiratory Reactions to Inhaled Drugs

AUTHORS (FIRST INITIAL, LAST NAME): M. H. Larsson[1], M. Gustavsson[1], C. Kärrman-Mårdh[1], S. Kirk[1], S. Öhlin[1], A. Österlund[1], P. Åberg[1]
INSTITUTIONS (ALL): 1. AstraZeneca R&D, Molndal, Sweden.
KEYWORDS: Head Out Plethysmography, Dry Powder Inhalation, LAMA

ABSTRACT BODY: At AstraZeneca (AZ) we have experienced two cases where inhalation of long acting anti-muscarinic (LAMA) drugs, AZD9164 and AZD8683 (derived from collaboration with Pulmagen) were associated with signs of bronchoconstriction in subjects with chronic obstructive pulmonary disease. The effects on FEV1 (15-45% reductions from pre-treatment baseline at 15-30 min) were transient and returned to baseline after 2-4Hrs, but met the pre-defined stopping criteria. In animal toxicological studies there were sparse reports of respiratory clinical signs, but no pathological changes in the airways. In the current study we explored the potential of these compounds to cause acute sensory irritation of the respiratory tract using a respiratory model in rat. Based on the principles of Alarie et al (*Crit Rev Toxicol* 1973), Head out plethysmography (HOP) in rats were used to measure breath rate (BR) and tidal volume (TV) before (20 min), during (10 min) and after (20 min) inhalation of selected compounds. Groups of rats (n=7/group) were exposed to dry powder aerosols by nose-only exposure of either lactose (placebo), Tiotropium, AZD9164, AZD8683 or AZD5802 (target lung dose of 200 µg/kg). No effects on either TV or BR were seen with Tiotropium. However, all three AZ compounds instantly at start of dosing and throughout the dosing period reduced the tidal volume by 31, 45 and 47% respectively. After dose the TV slowly returned towards baseline. While sensory irritants are typically expected to affect BR during exposure, we only observed post-dose changes, with increases of BR (10-25%) compared to vehicle. Since the AZ compounds, as opposed to Tiotropium, showed effects on respiratory parameters in both the clinical setting (FEV) and the rat (TV), we propose that recording of the TV in the rat HOP model may act as a surrogate marker for respiratory adverse events, such as bronchoconstriction. Sensory respiratory reflex reactions as well as regulation of airway tone is multifactorial, hence further studies are warranted to explore the association of these different responses.

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ABSTRACT FINAL ID: 2496 Poster Board -128
TITLE: Histopathological Effects of Enrofloxacin in the Chicken Gastrocnemius Tendon

AUTHORS (FIRST INITIAL, LAST NAME): U. Blas-Machado[1], W. Yu[1], J. Zhang[1], J. Halper[1]
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KEYWORDS: Chicken, Tendon, Decorin

ABSTRACT BODY: Introduction: The association between administration of fluoroquinolones and degenerative tendon disease was recognized during the early 1980's. The main objective of the current study was to confirm the results of our previous *in vitro* study, which used avian embryonic tenocytes, in an *in vivo* model for degenerative tendon disease by a fluoroquinolone antibiotic. Experimental Design: From a total of 60, 1 day-old, male Avian reovirus-free White Leghorns chickens were used as follows, 48 chickens were exposed to enrofloxacin either in drinking water or by injection for 7 consecutive days (experimental days 0 - 7), and 12 chickens were exposed to enrofloxacin-free drinking water and/or injected with physiological saline solution to serve as controls. Methods: Chickens were necropsied at experimental day 0, 14, and 42. The response of the gastrocnemius tendon (GT) to enrofloxacin was followed by clinical observation, necropsy, histopathology, and immunohistochemistry for decorin and collagen I. In a follow-up experiment, gastrocnemius tendon fibers from control and treated birds were examined ultrastructurally and their fiber diameter measured and compared. Results: Gross lesions were absent in all birds. The GT enthesis of enrofloxacin-treated chickens developed significant degenerative changes by day 14 regardless of the day sampled, dose, or treatment route. Immunohistochemical detection of decorin and collagen I was diminished in injured areas. Ultrastructurally, the mean fiber diameter between control and treated groups was statistically significant ($p < 0.0001$); the treated tendon fibers were in average 35.88 nm thicker than fibers in the untreated control group. Findings were consistent with our previous *in vitro* findings. Impact Statement: Our observations indicate that the chicken serves as an acceptable *in vivo* chemical model for the study of fluoroquinolone GT degeneration.

ABSTRACT FINAL ID: 2497 Poster Board -129
TITLE: Hepatic Metabolite Profiling of Atlantic Killifish (*Fundulus heteroclitus*) from PCB-Resistant and Sensitive Populations

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KEYWORDS: Metabolomics, AhR, Killifish

ABSTRACT BODY: Atlantic killifish inhabiting polluted sites along the east coast of the U.S. have evolved resistance to toxic effects of contaminants. One highly contaminated site is the Acushnet River estuary, near New Bedford Harbor (NBH), Massachusetts, which is characterized by very high PCB concentrations in the sediments and in the tissues of resident killifish. However, the mechanisms underlying this evolved resistance are poorly understood. In this study we compared the hepatic metabolite profiles between resistant (NBH) and sensitive populations (Scorton Creek (SC), Sandwich, MA) using targeted and untargeted metabolomics. Polar metabolites were extracted from adult fish livers and metabolites were quantified. Targeted metabolomics was done using high-performance liquid chromatography (LC) coupled to a triple stage quadrupole mass spectrometer and untargeted metabolite profiling was done using LC coupled to a Fourier transform ion cyclotron resonance mass spectrometer. Data was analyzed using the Xcalibur Quan Browser software and XCMS Online, respectively. Preliminary analysis revealed differences in the levels of several metabolites between fish from the two sites, including higher levels of several amino acids as well as molecules involved in oxidative stress response in NBH samples compared to SC fish. We also detected higher amounts of adenosine and sodium taurocholate in SC compared to NBH fish livers. We believe that some of these differences in the metabolite profiles might provide some clues in understanding the evolved resistance observed in NBH fish. Funding support for this work was provided by The Joint Initiative Awards Fund from the Andrew W. Mellon Foundation (WHOI Interdisciplinary Award).

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ABSTRACT FINAL ID: 2498 Poster Board -130
TITLE: Comparison of Histopathology Findings in Macaque (Rhesus vs Cynomolgus) Tissues following a Single Dose of Irradiation

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KEYWORDS: Hematopoietic Acute Radiation Syndrome, Cynomolgus vs Rhesus Macaque, Histopathology Background Data

ABSTRACT BODY: In continuing efforts to develop and characterize well defined animal models for the acute radiation syndrome under the FDA Animal Rule (21CR314, subpart 1), total body irradiation was applied to 6 cynomolgus macaques at a total dose of 7.55 Gray (Gy) and 8 rhesus macaques each at a total dose of 7.25 or 7.75 Gy (levels evaluated for proximity to the Rhesus LD50/60 of 7.43 Gy), using a bilateral 6 MV linear accelerator photon radiation source at 0.80 Gy min-1. Animals receiving full supportive care were evaluated for approximately 60 days for hematology, body weight, core temperature, food consumption, and clinical observations. At necropsy gross observations were recorded, organ weights were collected and specific tissues were collected and stained with hematoxylin and eosin (H&E) for histopathology analysis. Results: 3/8 Rhesus monkeys (38%) exposed to 7.25 Gy of irradiation and 6/8 Rhesus monkeys (75%) exposed at 7.75 Gy were euthanized on an unscheduled basis by Day 19. 3/6 Cynomolgus monkeys (50%) given 7.55 Gy of irradiation were euthanized on an unscheduled basis by Day 37. The remaining animals at these dose levels survived to scheduled necropsy (Day 59 or 60). Primary histopathology findings in both Rhesus and Cynomolgus monkeys were lymphoid organ and bone marrow depletion with resulting secondary multi organ hemorrhage and opportunistic infections. Ulceration and necrosis of the large intestinal mucosa and skin were also primary findings, but were observed more sporadically. There were no notable differences between the two species. Overall, responses of the Cynomolgus and Rhesus macaques to a single application of irradiation were similar, suggesting the cynomolgus macaques could be a viable species utilization in acute radiation syndrome studies.

ABSTRACT FINAL ID: 2499 Poster Board -131
TITLE: Activation of EGFR by Nitrogen Mustard Exposure and Therapies to Attenuate Injury

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KEYWORDS: Vesicant Injury, Corneal Wound Healing, *In Vitro* Cornea Model

ABSTRACT BODY: To identify therapies for corneal healing after mustard injuries, the cell biology ongoing in the injured tissue must be understood. ADAM17 is phosphorylated (i.e., activated) in corneal organ cultures exposed to nitrogen mustard (NM) and helps separate the epithelium from the stroma. ADAM17 also is a sheddase that releases surface-bound ligands of EGFR. We examined the activation of EGFR in corneal organ cultures wounded by NM. Organ cultures were exposed to NM for various times up to 2 hrs, then continued in culture, receiving either doxycycline, tobradex, the cosmeceutical AminoPlex, or a retro olvanil hydroxamate (NDH 4417) 4 times over the course of the subsequent 22 hr. Control corneas received medium as therapy. Sets of corneas were embedded in OCT, for immunofluorescence (IF) analysis of pERK, EGF, TGFα, and pEGFR. Others sets were analyzed by Western analyses or ELISAs. ADAM17 activation correlated with IF detection of EGF and TGFα after injury. Phosphorylated EGFR was intensely immunodetected in NM-exposed corneas, while detected only at low levels in unexposed corneas. Doxycycline, tobradex, the cosmeceutical AminoPlex, and a retro olvanil hydroxamate (NDH4417) greatly reduced the EGFR IF signal, dropping it to the level of unexposed controls.

When ADAM17 was activated, the "sheddase" released EGFR ligands EGF and TGFα from the cell surface. A phosphEGFR (pEGFR, i.e., activated EGFR) antibody showed pEGFR was expressed in cultures after NM exposure, but not in control cultures. Therapies such as doxycycline, an MMP inhibitor; AminoPlex, a nucleotide-containing cosmeceutical; and a retro olvanil 8 hydroxamate deriviative that sequesters Zn from ADAMs and MMPs, were all shown to reduce the level of pEGFR and improve the appearance of NM-injured corneas 24 hr after exposure, compared to NM-exposed corneas receiving only medium as therapy.

ABSTRACT FINAL ID: 2500 Poster Board -132
TITLE: A Novel Cyanide Antidote's pH Dependence and Its Potential Use in Oral Formulations

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KEYWORDS: Cyanide Antidote, Sulfur Donor

ABSTRACT BODY: Exposure to cyanide (CN) causes toxicity by inhibiting the cell's oxygen utilization. The endogenous sulfurtransferase enzyme, rhodanese, converts CN to the less toxic thiocyanate (SCN) but the rate of detoxification is slow. There are clinically available medications against CN but each has limitations. For this purpose there is a need for finding novel antidotes with better antidotal profiles. Previous studies demonstrated the effectiveness of a novel sulfur donor (SD), that is a naturally occurring garlic component, against CN intoxication. CN can enter the GI tract by ingesting certain plants or contaminated food, therefore it is important to develop orally administered antidotes to scavenge CN. Our aim was to determine the pH dependence of the reaction between SD and CN with and without rhodanese at different concentrations and to assess its applicability for oral administration. Phosphate buffer with various pH values (2.4, 3.4, 4.4, 5.4, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4) and rhodanese activities (0.1, 0.05, 0.025, 0.0125, 0.0042, and 0.0021 units/ml) were applied. The formation of SCN was followed spectrophotometrically after forming the colorful Fe(SCN)2+ by adding Fe(NO3)3 to the reaction mixture. The SD reaction with CN was pH dependent. The rate of SCN formation increased with increased pH between pH 6 and 8, but no SCN formation was detected below pH 6. Addition of rhodanese enzyme to the reaction mixture enhanced the CN conversion. The amount of SCN formed was rhodanese activity dependent. Rhodanese enzyme activity was highest at pH 7.5. Based on our results with SD, it does not look suitable for scavenging CN in the stomach following oral administration, unless the pH is increased (e.g. by NaHCO3) before SD is administered in an appropriate formulation.

ABSTRACT FINAL ID: 2501 Poster Board -133
TITLE: Reactivation of Sarin-Inhibited Acetylcholinesterase by MINA and 2-PAM in Zebrafish

AUTHORS (FIRST INITIAL, LAST NAME): T. Dao[1], J. Koenig[1], R. K. Kim[1], L. R. Kapus[1], J. L. Leuschner[1], R. K. Kan[1]
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KEYWORDS: Zebrafish, Sarin, Reactivators

ABSTRACT BODY: Zebrafish (*Danio rerio*) are used in toxicity studies to predict human risk and facilitate drug discovery. As an animal model, zebrafish are ideal to study the toxic effects of organophosphorus nerve agent (NA) and the reactivation of NA-inhibited acetylcholinesterase (AChE) because zebrafish only express AChE. In the present study, cardiotoxicity was assessed in conjunction with reactivation efficacy of monoisonitrosoacetone (MINA) and pralidoxime (2-PAM) following exposure to sarin. To study cardiac toxicity induced by sarin, larval zebrafish at 6 days post-fertilization (dpf) were exposed to 50 µM sarin for 1 hour. Changes in heart rate as a measure of cardiac dysfunction were quantified at 1 hr after exposure. Larvae exposed to sarin showed a significant decrease in heart rate from 148 beats per minute (BPM) to 125 BPM. To evaluate the effectiveness of MINA or 2-PAM to reactivate sarin-inhibited AChE, 6 dpf larvae were exposed to 50 µM of sarin and then treated with various concentrations of MINA or 2-PAM when a lack of locomotion was observed. This typically occurred one min post-exposure. Following 20 mins of treatment, larvae were collected and their AChE activity

was measured by the Ellman assay. The percentage of AChE inhibition without MINA or 2-PAM treatment was 98.9%. MINA or 2-PAM given at 0 to 25, 50, 100, 200 or 400 µM increased AChE activity: 1.1% to 1.7% vs 4.5%, 2.4% vs 5.0%, 4% vs 9.3%, 8% vs 15%, or 17% vs 23%, respectively. At equimolar concentrations, 2-PAM is a better reactivator than MINA because of its efficiency in reactivating sarin-inhibited AChE. These observations provide strong evidence that the zebrafish is a suitable animal model system for *in vivo* evaluation of NA-induced cardiac toxicity and reactivity of novel reactivators to identify reactivators with more reactivation potency than the currently used AChE reactivators.

ABSTRACT FINAL ID: 2502 Poster Board -134
TITLE: Zebrafish Larvae As a Novel Animal Model System for Exposure to the Chemical Nerve Agent Soman

AUTHORS (FIRST INITIAL, LAST NAME): J. Koenig[1], T. Dao[1], R. K. Kim[1], T. A. Shih[1], R. K. Kan[1]
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KEYWORDS: Zebrafish, Soman, 2-PAM

ABSTRACT BODY: The current *in vivo* rodent models for studying medical countermeasures against acetylcholinesterase (AChE) inhibiting organophosphorus nerve agent (NA) exposure lack high throughput capabilities. Zebrafish are an already well-established animal model system for biomedical research, are genetically similar to humans, develop quickly as easily observable embryos, and exclusively express AChE. These characteristics, in addition to their rapid reproduction and relative inexpensiveness, make them an ideal model for the high throughput study of NA exposure and evaluation of possible therapeutics. The present study aimed to identify the LC50 of soman (GD) for various exposure times, measure the time-course of AChE inhibition, and evaluate the reactivation efficacy of the oxime 2-PAM in six days post-fertilization (6 dpf) larval zebrafish. The Ellman assay was utilized to determine AChE activity. Zebrafish were exposed to various concentrations of GD for 15, 30, 60, 90, or 120 min and screened at 24 hrs for mortality as determined by cessation of heartbeat. Both time- and concentration-dependent mortality was observed, and the LC50 for 15, 30, 60, 90, and 120 min was calculated as 78.2, 21.8, 4.0, 2.3, and 1.3 µM, respectively. Greater than 97% AChE inhibition was observed within one min of exposure to the 60 min LC50 of GD, with maximal inhibition (>99%) achieved within two min. To evaluate 2-PAM efficacy, zebrafish were exposed for two min to the 60 min LC50 of GD, rinsed two times in fresh zebrafish medium, and then placed in various concentrations of 2-PAM, ranging from 25 µM to 400 µM. 2-PAM proved ineffective at reactivating GD-inhibited AChE. These results show that larval zebrafish react to GD exposure in a time- and concentration-dependent manner and demonstrate 2-PAM efficacy similar to the established rodent model, lending support for the use of this model animal as a high throughput *in vivo* system for evaluating NA toxicity and the efficacy of potential therapeutics.

ABSTRACT FINAL ID: 2503 Poster Board -135
TITLE: Acute Adenosine A1 Agonist N6-Cyclopentoadenosine (CPA) Treatment Protects Acetylcholinesterase after Exposure to Soman Nerve Agent in a Rat Model

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KEYWORDS: Nerve Agent, Adenosine , Acetylcholinesterase

ABSTRACT BODY: Nerve agents (e.g., soman) pose a significant threat to military and civilian personnel. New therapeutics are needed to more effectively treat the seizure-inducing effects of central acetylcholinesterase (AChE) inhibition. Stimulation of central adenosine receptors via CPA may be an effective therapeutic mechanism. While CPA is believed to inhibit hyper-active neurons via pre- and post-synaptic effects, the exact protective mechanism has yet to be determined. In this study, we investigate the effects of CPA treatment on AChE activity after exposure to soman in a rat model. AChE activity was measured in rats exposed to saline or soman (1.0 x LD50, SC) and treated immediately with saline or CPA (60 mg/kg, IP). One day prior to exposure, baseline blood samples were taken. Group 1 served as the control and was exposed to saline and received saline treatment. Group 2 was exposed to saline and received CPA. Group 3 was exposed to GD and

received saline treatment. Group 4 was exposed to soman and treated with CPA. N=8 for each group. All rats received atropine methylnitrate (2 mg/kg, IM) as pretreatment for promoting survival. Rats were euthanized 45 min later; brain (cortex, striatum, hippocampus, midbrain, cerebellum, brain stem, spinal cord) and peripheral tissues (red blood cell, heart, diaphragm, skeletal muscle) were harvested, processed and assayed for AChE activity. The results indicate that acute CPA treatment protects both peripheral and central AChE from soman-induced inhibition. Whereas 85-98% of central AChE activity was inhibited by soman in rats receiving saline treatment, only 11-26% was inhibited with CPA treatment. CPA by itself did not significantly decrease AChE. These data suggest that CPA could also be a reversible AChE inhibitor. CPA may temporarily dock to AChE and thus prevent soman from binding. Further investigation is needed, but these data suggest that CPA has great therapeutic potential with multiple neuroprotective mechanisms.

ABSTRACT FINAL ID: 2504 Poster Board -136
TITLE: Efficacy of the Antihistamine Cyproheptadine in Rats Exposed to the Chemical Nerve Agent Soman

AUTHORS (FIRST INITIAL, LAST NAME): J. L. Winkler[1], J. A. Leuschner[1], T. Dao[1], S. W. Kaski[1], L. J. Shumway[1], R. K. Kan[1]
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KEYWORDS: Soman

ABSTRACT BODY: The present study was designed to evaluate the effectiveness of the antihistamine cyproheptadine against the toxic effects of the chemical nerve agent soman. Rats were pretreated with the oxime reactivator HI-6 (125 mg/kg, ip) 30 min prior to soman intoxication (2.0 LD50 or 225 ug/kg, sc) and were then treated one minute after soman challenge with atropine methylnitrate (AMN, 2.0 mg/kg, im). Cyproheptadine (10, 13, 16 or 20 mg/kg, ip) was given 1 min after soman intoxication or at the onset of soman-induced seizures. Controls received an equivalent volume of sterile water instead of cyproheptadine. All rats (100%) without cyproheptadine treatment developed seizures and died within 24 hr after exposure. Cyproheptadine at a dose of 10, 13, 16 or 20 mg/kg given 1 min after soman exposure had lower incidences of seizures 60%, 0%, 67% and 60%, respectively. In addition, seizures in cyproheptadine-treated animals were spontaneously terminated, and no deaths were observed within the 24 hr after exposure. When cyproheptadine at a dose of 10, 13, 16 or 20 mg/kg was administered at the onset of seizure, seizures were terminated in 80%, 89%, 100% and 100% of the animals, respectively. The mortality rate with treatment at the onset of seizure was 0%, 22%, 0% and 0%, respectively. These observations indicate that cyproheptadine treatment can effectively control seizures and improve survival following exposure to 2.0 LD50 of soman. Disclaimer:The views expressed in this abstract are those of the author(s) and do not reflect the official policy of the department of Army, Department of Defense, or the U.S. government. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. This research was supported by the Defense Thread Reduction Agency-Joint Science and Technology Office, Medical S&T.

ABSTRACT FINAL ID: 2505 Poster Board -137
TITLE: Development of a 96-Well Method for the Detection of Monoisonitroacetone (MINA) in Monkey Plasma by Solid Phase Extraction and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

AUTHORS (FIRST INITIAL, LAST NAME): R. Pennington[1], T. M. Myers[1], B. R. Capacio[1]
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KEYWORDS: Chemical Weapons, Countermeasures, LC-MS/MS

ABSTRACT BODY: Tertiary oximes, i.e., MINA, readily enter the brain and have been shown to be effective in reducing lethality due to organophosphorus (OP) nerve agent exposure in guinea pigs and rats by reactivating OP-inhibited cholinesterase in the brain. The blood brain barrier (BBB) penetrability of MINA is in contrast to quaternary oximes, which do not reactivate central cholinesterase. This work focuses on the development of a method to determine the

concentration of MINA in plasma via LC-MS/MS utilizing solid phase extraction (SPE) cartridges and its direct comparison to a more efficient method using a 96-well plate SPE setup and the same solid phase. This method was also used for analysis of plasma drawn from cynomolgus monkeys exposed to MINA and compared to pharmacokinetic (PK) data generated with ultraviolet-visible assay (UV-Vis). A method was successfully developed for the detection of MINA in non-human primate plasma for PK determination. This method utilizes C18 SPE cartridges and has been fully validated per FDA guidance. Calibration curves (15 to 4000ng/ml) were generated in plasma and run via the cartridge and 96-well plate methods. The calculated precision (%CV) for all concentrations ranged from 12.7 to 4.23 for the cartridges and from 16.88 to 4.33 for the plate method; the lowest precision (16.88%) corresponded to the lowest concentration calibrator. Interday precisions ranged from 7.74 to 11.4 for the cartridge SPE method and from 5.7 to 10.52 for the 96-well plate method. Intraday precisions ranged from 1.66 to 3.43 for the cartridge SPE method and from 3.58 to 9.87 for the 96-well plate method. This method was applied to determining MINA PK in cynomolgus monkey plasma following intramuscular dosing. These results match those determined by UV-Vis. With its high throughput and slightly better precision, the 96-well plate method is more beneficial than the cartridge method for analysis of future samples.

ABSTRACT FINAL ID: 2506 Poster Board -138
TITLE: Bronchiolitis Obliterans and Interstitial Fibrosis in Rats after Sulfur Mustard Inhalation

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KEYWORDS: Bronchiolitis Obliterans, Sulfur Mustard, Lung Fibrosis

ABSTRACT BODY: RATIONALE: Sulfur mustard (SM) inhalation causes pulmonary fibrosis and airway fibrosis >6 months after exposure in human casualties. The most prominent fibrosis after SM exposure is bronchiolitis obliterans (BO) and pulmonary interstitial fibrosis. Morbidity and mortality due to progressive dyspnea and hypoxemia are common symptoms of these chronic pulmonary sequellae. To date, there is no known therapy to prevent development of pulmonary fibrosis in survivors. Here, we developed a rat model of SM inhalation injury that mimics human disease after SM exposure, causing histologic evidence of both interstitial fibrosis and bronchiolitis obliterans, as well as increased mortality and morbidity due lung fibrosis. METHODS: Sprague-Dawley rats (250 g), intubated, under anesthesia, inhaled SM ethanolic vapor (0.5-3.8mg/kg). Pulse oximetry (pOx) measurements were obtained once daily for 28 days, and animals were euthanized if they met IACUC-directed euthanasia criteria. Lung were then removed, fixed, microdissected, and processed for histology. Trichrome stain was used to identify collagen deposition, and help guide in Ashcroft scoring for pulmonary fibrosis, and scoring of BO lesions. RESULTS: After 14 days of exposure, rats had progressive hypoxemia and death after SM exposure (1.0-1.4 mg/kg) due to lung interstitial fibrosis and airway obstruction from constrictive bronchiolitis obliterans. Both lesions stain position for collagen deposition. Fibrosis was worse with higher SM dosing (1.4 mg/kg), and it developed earlier after exposure. CONCLUSIONS: SM dose of 1.4 mg/kg gives reliable, severe, high grade, interstitial fibrosis, as well as very significant constrictive bronchiolitis. This model can be used to further study the mechanism of lung fibrosis development after SM exposure, and to help develop new therapies that can prevent progression to pulmonary fibrosis complication after SM inhalation exposure. Moreover, it is a reliable model to help test novel therapies to prevent and/or treat bronchiolitis obliterans in an animal model.

ABSTRACT FINAL ID: 2507 Poster Board -139
TITLE: Sensing of New Potential Cyanide Countermeasures by Surface-Enhanced Raman Spectroscopy

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KEYWORDS: Cyanide, Sensing, SERS

ABSTRACT BODY: In the present Cyanide (CN) therapies of NithiodoteTM [sodium thiosulfate (TS) and sodium nitrite (SN)]; TS facilitates the conversion of CN to the less toxic thiocyanate by acting as a sulfur donor. However, TS has a strong dependence on the localized enzyme rhodanese, and its efficacy is limited by challenges in reaching the enzyme. Novel reactive SDs, (SDX), are being explored as candidates that have the potential to overcome the limitations of TS. SD are commonly analyzed by HPLC and GC/MS. In the context of cyanide antidotal research it would advantageous to have a more rapid and portable method of analysis that has the ability to examine the contents of bottles nonperturbatively, and to rapidly assay the SD content of blood or tissue samples. We are working to develop a method for detecting SD's by headspace sampled surface enhanced Raman spectroscopy (SERS) with the aim of detecting SDx in the biologically relevant micromolar concentration range. We report proof of principle experiments that demonstrate rapid detection (<3min post exposure) of SD sampled from the air above a 5mM SDx solution using a gold Silmeco SERS sensor. A 785 nm excitation laser, operated below 10mW with a 120 micron diameter spot was used as the excitation source in all measurements. Based on the signal to noise ratio of the experiment, an upper bound for the SD limit of detection of 125 micromolar was established. This is sufficiently sensitive to be useful in detecting SD's in pharmacokinetic studies, and it is likely that this limit of detection will be pushed lower with modifications to future experiments.

ABSTRACT FINAL ID: 2508 Poster Board -140
TITLE: The Role of Cytochrome P450 Catalyzed Steroid Biotransformation in Diet of Coral-Consuming Fish in Hawaii versus Australia

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KEYWORDS: Cytochrome P450, Butterflyfish, Allelochemical

ABSTRACT BODY: Cytochrome P450 monooxygenase (CYP) is the primary enzyme system responsible for detoxification of xenobiotics including dietary chemicals and pollutants. CYP was found in higher concentration in butterflyfish that preferentially feed on allelochemically rich corals (specifically CYP3 and CYP2). Little is known about the biotransformation and detoxification of allelochemicals derived from dietary products in marine organisms. Certain species of butterflyfish of the genus *Chaetodon* have been shown to feed on several species of chemically-defended corals. Regulation of CYP may be affected by various factors including age, sex, reproductive status, diet, species, and environmental conditions. This study examines the effects of diet on the regulation of CYP in butterflyfish in Hawaii versus Australia. The butterflyfish investigated in this research included *Chaetodon kleinii*, which consumes chemically defended soft coral in Australia but facultatively feeds on plankton and hard corals in Hawaii. *C. lunulatus* consumes hard coral *M. captata* in Australia and hard coral *P. lobata* in Hawaii. *Chaetodon auriga* is a generalist in Hawaii and *C. auriga* is also a generalist in Australia. The CYP3A and CYP2 expression profiles and catalytic activities were compared between locations to assess induction in butterflyfish with different feeding strategies. Testosterone hydroxylase (TOH) at the 16 β, 16 α, 6 β positions was higher in *C. kleinii* and *C. auriga* from Australia versus Hawaii. These results may indicate that species consuming soft coral in their diet have significantly more CYP3 and CYP2 catalytic activity. In addition, for hard coral feeding *C. lunulatus*, the higher TOH activities in Hawaii suggest difference in allelochemical content and potency within the dietary items.

ABSTRACT FINAL ID: 2509 Poster Board -141
TITLE: Aquatic Environmental Impact Evaluation of Raw Materials Used in Cosmetics Products

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KEYWORDS: Ecotoxicity, Cosmetic Ingredients, Aquatic Toxicity

ABSTRACT BODY: The concern of cosmetic industries about supply chain, ingredient's sustainability and biodiversity, increases the need to ensure the lowest environmental impact of finished goods. Whereas few studies are published on ecotoxicity of cosmetics' raw materials, the aim of this work was to propose relevant internationally recommended parameters by analyzing database and following the principle of the three R's. This study considered 50 common raw materials used in rinse-off products, due to their greater environmental exposure and gray water concern by defining parameters for risk assessment and prediction of the effects on aquatic organisms. After database access and review of international legislations, it was considered the guidelines established by the international agencies. To analyze the aquatic environmental impact of ingredients four parameters were evaluated: Bioaccumulation; Biodegradation, Toxicity and PEC/PNEC, which is the risk quotient that evaluates environmental exposure and aquatic toxicity. Aiming to quantify the environmental risk, four different classes of raw materials were analyzed. The results showed that among the emollients, the mineral oil and petrolatum had higher environmental risk than glycerin and propylene glycol; among the surfactants despite the few data available, decyl glucoside seems to have the lowest risk; among the sunscreens studied most of them have a high bioaccumulation and biodegradability and toxicity should be taking into account to make a comparison and among the preservatives triclosan has much higher environmental impact than phenoxyethanol and benzyl alcohol. This study concluded that it is possible to obtain ecotoxicity information about raw materials through databases, thus predicting the aquatic toxicity to replace ingredients with higher environmental risks, following the main international guidelines and making ecofriendly cosmetic products.

ABSTRACT FINAL ID: 2510 Poster Board -142
TITLE: Developmental Abnormalities and Differential Expression of Genes Induced in Oil and Dispersant-Exposed Menidia beryllina Embryos

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KEYWORDS: Crude Oil, Embryotoxicity, *Menidia beryllina*

ABSTRACT BODY: Exposure of fish embryos to relatively low concentrations of oil has been implicated in sub-lethal toxicity. However, the effect of oil and commonly used dispersants should be more thoroughly evaluated to better understand and anticipate ecological impacts. Oil (1ppm) and dispersants (0.1ppm, Corexit 9500 or 9527) were weathered singly and in combination in 25ppt saltwater for 7 days. The water accommodated fraction (WAF) post-weathering was diluted at 1:5 (200 ml WAF: 800 ml 25ppt saltwater). Thereafter, 35-40 *Menidia beryllina* embryos at 30-48hrs post-fertilization were exposed to diluted WAF in quadruplicate for 72 hours as follows: Control (1 liter 25 ppt Saltwater), WAFs, weathered Oil (200ul/L), C9500 (20ul/L), C9527 (20ul/L), Oil/9500 (200/20ul/L) and Oil/9527 (200/20ul/L). Mortality, heartbeat, embryo normalcy score and abnormality types were recorded. The QPCR assay was used to quantify abundances of transcripts of target genes: Vitellogenin, CYP1A, HSP90, StAR, GhR, CYP19b, IGF-2, AMH, DMRT1 and Choriogenin L; GAPDH served as the housekeeping gene. Mortality was not significantly different (p=0.68). Heartbeat and normalcy scores were significantly different (p=0.002 and 0.007, respectively). The lowest heartbeats and normalcy scores respectively were recorded in Corexit 9500 (67.5beats/min and 60%) and 9527 (67.1beats/min and 44%) exposed embryos compared with control (82.7beats/min and 100%). Significantly more embryos were in a state of deterioration, with arrested tissue differentiation compared with control (p=0.008). Dispersants and the dispersant-oil mixtures induced aberrant expressions of all the genes, with StAR being significantly down-regulated and CYP1A up-regulated in all exposures except for embryos exposed to 9500 alone where CYP1A was significantly down-regulated. Molecular endpoint could therefore be an early indicator of the long term effects of oil spill and dispersants usage.

ABSTRACT FINAL ID: 2511 Poster Board -143
TITLE: Perfluorooctanoic Acid Exposure for 28 Days Affects Glucose Homeostasis in Mice

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KEYWORDS: Perfluorononanoic Acid, Glucose Homeostasis, Insulin Resistance

ABSTRACT BODY: Perfluoroalkyl acids (PFAAs) are widely used in a number of applications due to their unique physical and chemical characteristics. Due to the increasing prevalence of metabolic syndromes such as obesity, dyslipidemia, and insulin resistance, concern has risen about the roles environmental pollutants may play in this trend. Earlier epidemiologic studies have shown a potential association between perfluorooctanoic acid (PFOA) and glucose metabolism, but how PFOA influences glucose homeostasis still unknown. In this study, male mice were exposed to 0, 0.08, 0.31, 1.25, 5, and 20 mg/kg/day of PFOA for 28 days. We analyzed the modulation of PI3K-AKT pathway in livers of mice after exposure, and compared with normal mice, PFOA exposure induced AKT activation along with decreased expression of the PTEN protein. Tolerance tests implied PFOA exposure increase insulin sensitivity in mice and higher levels of phosphorylated AKT in livers and muscles from PFOA exposed mice after insulin injection further supported this hypothesis. Biochemical analysis revealed PFOA exposure reduced hepatic glycogen synthesis and this may be caused by gluconeogenesis impairment. After serum proteomic analysis using iTRAQ labeling combined with two-dimensional liquid chromatography and tandem mass spectrometry (2DLC–MS/MS), several circulating proteins levels were found to be altered after PFOA exposure, including proteins that have been reported to be potentially related to diabetes or liver diseases. Our results suggest that PFOA affects glucose metabolism and induces insulin hypersensitivity in mice.

ABSTRACT FINAL ID: 2512 Poster Board -144
TITLE: The Interaction of Perfluorononanoic Acid with Human and Rat Peroxisome Proliferator-Activated Receptor Alpha

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KEYWORDS: Perfluoroalkyl Acids, PPARα Ligand Binding Domain, Interaction

ABSTRACT BODY: Perfluoroalkyl acids (PFAAs) are highly persistent and bioaccumulative, resulting in their broad distribution in the human body and the environment. PFAAs can activate Peroxisome proliferator activated receptor alpha (PPARα), which plays a key role in lipid metabolism, thereby inducing hepatic toxicity. However, the activation process of PPARα in human and rodents have differences in quality and quantity. In this study, we characterized and compared the binding of PFAAs to the human PPARα ligand binding domain (hPPARα LBD) and rat PPARα ligand binding domain (rPPARα LBD), and identified critical structural features in their interaction. The binding interactions of PFAAs with hPPARα LBD/rPPARα LBD and four hPPARα LBD variants (Phe273, Cys276, Tyr314 and His440) were determined by Circular dichroism spectroscopy (CD), fluorescence displacement and isothermal titration calorimetry (ITC) assay and molecular docking methods. The results did not show significant differences between the interactions of PFAAs binding with hPPARα LBD and rPPARα LBD. CD results showed an obvious change of secondary structure caused by PFNA/PFDA. Fluorescence displacement and ITC measurement revealed that PFDA displayed a moderate affinity for hPPARα LBD/rPPARα LBD at a 1:1 molar ratio, a weak affinity for PFNA and PFOA, and the interactions was mainly mediated by electrostatic attraction and hydrogen bonding. Our findings suggest that the binding of PFAAs to hPPARα LBD/rPPARα LBD do not show significant differences and that Phe273, Cys276, Tyr314, and His440 play a pivotal role in these interactions.

ABSTRACT FINAL ID: 2513 Poster Board -145
TITLE: Plasma Cholinesterase Activity As a Biomarker for Quantifying Exposure of Green Sturgeon (Acipenser medirostris) to Carbaryl following Applications to Control Burrowing Shrimp in Washington State

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KEYWORDS: Cholinesterase, Sturgeon, Carbaryl

ABSTRACT BODY: Willapa Bay (Washington State, USA) is a rare intertidal location where large-scale pesticide applications occur. Since 1963, carbaryl, a cholinesterase inhibitor, has been applied to control burrowing shrimp that decrease commercial oyster productivity. The carbaryl application poses an unknown hazard to the ESA-listed green sturgeon (*Acipenser medirostris*), for which the Bay is critical habitat. To assess the unknown hazard, the congeneric white sturgeon (*Acipenser transmontanus*, seawater adapted), which shares similar life histories and habitat requirements, was exposed to 0, 30, 100, 300, 1,000 and 3,000 µg L^{-1} carbaryl for 6 h. Post-exposure brain acetylcholinesterase (AChE) and plasma butyrylcholinesterase (BChE) activities were measured to assess the impact of carbaryl. Enzyme recovery was measured in an additional cohort exposed to 1,000 µg L^{-1} for 6 h. AChE activity was reduced ($p \leq 0.05$) at concentrations at or above 100 µg L^{-1} with recovery in the 1,000 µg L^{-1} cohort by 72 h. Surprisingly, BChE activity was greater than controls at concentrations at or above 100 µg L^{-1} ($p > 0.05$); a finding confirmed with an additional cohort exposed to 3,000 µg L^{-1} for 6 h ($p \leq 0.05$). Plasma samples were collected non-lethally from free-living green sturgeon before and 4-5 d after application of carbaryl in Willapa Bay. BChE activity post application was reduced 37% indicating exposure to the pesticide. However, the relationship between BChE inhibition and AChE activity is unclear in this species. Additional studies would be required to better understand the magnitude and effects of carbaryl exposure in green sturgeon.

ABSTRACT FINAL ID: 2514 Poster Board -146
TITLE: Analysis of Pb and Cu in Macrocystis pyrifera in the Coastal Area of Playas de Tijuana

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KEYWORDS: Lead, Cooper, Macroalgae

ABSTRACT BODY: In recent years the concentration of heavy metals in the coastal areas of Baja California has been increasing due to anthropogenic activities that discharge to sea coasts, causing impairment in human health and aquatic environments. Heavy metals in ionic form are distributed and landed in the hydrosphere. Possible toxicity of metals may occur under concentrations ranging from 0.10 to 10 mg / mL. Some metals are elements essential for life, but some others do not, such as Hg and Cd, where concentrations ranging from .001 to .10 mg / ml to show toxic effects. In this paper is analyzed bioaccumulation of two metals: Pb and Cu in the macroalgae in greater proportion in the coastal area of Tijuana, BC, *Macrocystis pyrifera*. To count as an indicator of the quality conditions of seawater in the area mentioned. Fresh *Macrocystis pyrifera* samples randomly, followed by freeze-drying techniques in the fresh sample temperature controlled oven (Thermoline) acid reaction was conducted subsequently for digestion were collected. Samples were analyzed on atomic absorption equipment with specific lamp for lead and copper. The analysis of the samples, throw the presence of metals in concentrations such that, according to studies reported, allows us to establish that the conditions of this coastal area has a high concentration of Pb and Cu metals. The proportions of metals obtained from Cu and Pb (574 ppb and 825.26 ppb) It is noteworthy that this study will form the basis for further studies that allow us to have a larger number of indicators of bioaccumulation, toxicity and possible biomagnification of metals in coastal waters which are used for recreation. As a secondary theme, highlights the need to assess the driving systems of wastewater treatment and discharge into water bodies and the degree of compliance of these with the applicable regulations.

ABSTRACT FINAL ID: 2515 Poster Board -147
TITLE: Investigation on Systemic Effects of Korean Traditional Fermented Soybean Products Intake on Overall Immunity of Mice

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KEYWORDS: Fermented Soybean, Cell-Mediated Immunity, NK Cell Function

ABSTRACT BODY: Two Korean traditional fermented soybean food ingredients, Doenjang (DJ) and Cheonggukjang (CGJ), have been reported to be beneficial for prevention of various metabolic diseases. Immuno-stimulatory effects of the ingredients were further investigated through feeding mouse chow mixed with 5% DJ or CGJ for four weeks to four week old male or female BALB/c mice. Using K562 human leukemia cell line as a target, splenic natural killer (NK) cell activity was evaluated, which resulted in significant enhancement of NK cell function in the mice fed with DJ or CGJ compared with the control mice fed with a normal mouse chow. No significant difference was found in serum IgA and IgE levels between the mice fed with the experimental diet and the control mice. Furthermore, intake of the two ingredients did not enhance the serum level of IgG isotype (G1, G2a, G2b, and G3) compared to the control mice. These results may indicate no potential upregulation of soybean peptide-mediated aberrant antibody production. Splenic T cells were activated with immobilized anti-CD3 mAb for 48 hours, and levels of interferon-gamma (IFNγ) and interleukin-4 (IL-4) in the culture supernatants were quantitated. Significantly higher production of IFNγ was obtained from the experimental diet-fed mice than the control mice. In addition, IFNγ versus IL-4 ratio, an indicator for relative enhancement of type-1 helper T cell activity compared with type-2 helper T cell activity, was significantly higher in the experimental diet-fed mice than the control mice. Proportion of peripheral neutrophil was enhanced, but that of basophil was lowered in the experimental diet-fed mice than the control mice. Overall, this study suggests that intake of the two Korean traditional fermented soybean food ingredients could improve cell-mediated immunity. [supported by CJ Food R&D Center 0F13-JF11-RD and BK21plus project]

ABSTRACT FINAL ID: 2516 Poster Board -148
TITLE: Comparing the Anorectic Potential of Simple Trichothecenes Using Benchmark Dose Methodology

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KEYWORDS: Trichothecenes, Mycotoxin, Benchmark Dose

ABSTRACT BODY: Cereal products are often simultaneously contaminated by multiple *Fusarium* spp. fungi which subsequently produce a cocktail of trichothecenes in food. These mycotoxins jointly contribute to the observed adverse effects such as anorexia, emesis and immunosuppression but to varying extents. The relative potencies depend on their chemical structure and functional groups which either increase or decrease the toxicity of the molecule. Therefore, a clear understanding of the relative potency is important for risk assessment following exposure to a mixture of such mycotoxins. This study investigated the potential of Deoxynivalenol (DON), 3-acetyl Deoxynivalenol (3-ADON), 15-acetyl Deoxynivalenol (15-ADON), Nivalenol, Fusarenon-X, T-2 and HT-2 mycotoxins to cause feed refusal in a mouse model. The US-EPA benchmark dose (BMD) method for continuous data was used to calculate BMD and potency of each trichothecene relative to DON. The time course effects of each toxin were studied by calculating the incremental area under the curve and percent decrease in feed intake as compared to controls over time. The potency of the trichothecenes following oral exposure was: T-2 > Fusarenon-X > HT-2 > Nivalenol > 15-ADON ≈ 3-ADON ≈ DON. For intraperitoneal exposure, the order of potency was: T-2 > HT-2 > Nivalenol > 3-ADON > DON > Fusarenon-X > 15-ADON. DON caused significant feed refusal within 30 minutes of exposure and lasted 3 hours while 3-ADON and 15-ADON, congeners of DON, induced feed refusal within 30 minutes of exposure that lasted 6 hours. Oral exposure to Nivalenol caused about 30 minutes delay in response, but overall, Nivalenol had a higher potency than DON. The anorectic effects of Nivalenol, Fusarenon-X, T-2 and HT-2 toxins lasted up to 16 hours. This is the first study to employ the benchmark dose method to calculate relative potency of trichothecenes. Our findings will contribute towards improved assessment of risk due to simultaneous exposure to mycotoxins in food.

ABSTRACT FINAL ID: 2517 Poster Board -149
TITLE: Expanded Evaluation of Carcinogenic Polycyclic Aromatic Hydrocarbons (PAHs) in Gulf Seafood

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KEYWORDS: Polycyclic Aromatic Hydrocarbons , Gulf Seafood, Cancer Risk

ABSTRACT BODY: Current methods to assess cancer risks from exposure to PAHs are based upon quantifying seven carcinogenic PAHs of varying potency. Recently, the U.S. EPA has identified an additional 16 carcinogenic PAHs and provided relative cancer potency estimates for each. The importance of consideration of risks from these newly designated carcinogenic PAHs is unclear as there is almost no information in the literature on the extent to which they are found in PAH-contaminated environmental media. Analytical standards were obtained for 12 of these additional carcinogenic PAHs, and methods for detecting and quantifying them in the low ppb range in biological samples were developed using gas chromatography-mass spectrometry. As part of an ongoing project examining the presence of PAHs in Gulf fin- and shellfish, 32 fish tissue samples with one or more detected "conventional" carcinogenic PAHs were analyzed for the expanded suite of PAHs. Although some of the newly designated PAHs were detected in the fish samples, only benzo(b)/(j)fluoranthene and benz(e)/(j)aceanthrylene were present above their practical laboratory quantitation limit in a single sample (7 and 6 ppb, respectively). None of the fish samples obtained to date for this NIEHS-funded study, including fish samples taken shortly after the Deepwater Horizon oil spill, have shown substantial PAH contamination based upon the standard suite of chemicals used for regulatory risk assessment. This preliminary assessment suggests that this conclusion holds even when considering the full range of PAH compounds for which cancer risks can be estimated. Supported in part by a grant from the National Institute for Environmental Health Science (U19 ES020683).

ABSTRACT FINAL ID: 2518 Poster Board -150
TITLE: AFB1 Decontamination Potential and Safety of Neutral Electrolyzed Oxidizing Water

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KEYWORDS: NEW, AFB1, Maize

ABSTRACT BODY: Aflatoxin B1 (AFB1) is one of the most potent carcinogens present in staple foods such as corn. Neutral electrolyzed oxidizing water (NEW) has the potential for degradation of this compound and seems to be a safe and economical alternative to other chemicals used for these purposes. In this study, we evaluated the effects of NEW (Esteripharma, SA de CV) on aflatoxin degradation and the safety of 3 NEW solutions (10, 40 and 60 ppm of free chlorine) on maize seeds using germination analyses and root growth measurements. For AFB1 experiments, 20 and 100 ppm AFB1 solutions were prepared and incubated in a 1:1 proportion with SES 60 for 20 minutes. After that, samples and controls were run with UPLC (Waters). Mobile phase consisted in water:acetonitrile:methanol in a 64:18:18 proportion. For the germination and root length analysis, maize seeds (AS-900, ASPROS) were used. Seeds were washed with 10% bleach and rinsed with sterile distilled water before experiments. Groups of 25 seeds with 4 replicates were used for each treatment that consisted on: controls [distilled water; 0.4 polyethylene glycol (PEG); 0.2 PEG; bleach], NEW 10, NEW 40 and NEW 60. All solutions were passed through 0.22 µm filters. Seeds were incubated in Petri dishes and germination was evaluated at 48, 72 and 96 hours. Root length measurement was done at 96 hours. Toxin degradation studies at 20 and 100 ppm showed 100% transformation of AFB1 to a new compound compatible with previously reported data by Xiong et al., 2012, while germination studies and root length measurements showed no significant differences ($p \leq 0.05$) among treatments and controls using non-parametric analysis. These results suggest that NEW has potential to reduce AFB1 contamination and does not affect seed germination parameters in maize. Funding: CONACYT-PROINNOVA 212523.

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Late-Breaking Abstracts

ABSTRACT FINAL ID: 2519 Poster Board -151
TITLE: Analytical Toxicology of PAHs from Inshore-Harvested Seafood from the Gulf of Mexico: Studies to Support Seafood Safety Post-Deepwater Horizon Oil Spill

AUTHORS (FIRST INITIAL, LAST NAME): A. S. Kane[2, 1], J. W. Munson[1], M. O. James[3, 1], E. B. Overton[4], M. Kozuch[1], R. Brooks[2], N. Odezulu[1], S. M. Roberts[1]
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KEYWORDS: Polycyclic Aromatic Hydrocarbons , Seafood Safety, Deepwater Horizon

ABSTRACT BODY: In response to community concerns regarding seafood safety after the DWH oil spill, we filled critical gaps left by NOAA and US FDA by measuring locally-caught and consumed seafood types, and seafood from locations not surveyed by state or Federal agencies. Over 1,000 finfish, shrimp, blue crab and oyster samples from coastal waters of Florida, Alabama and Louisiana, between November 2010 and February 2013, and were field-processed, homogenized and prepared using dispersive solid phase extraction, and analyzed using GC/MS-SIM. Analysis of the sum of parent polycyclic aromatic hydrocarbons (PAHs), and associated C1-3 alkyl homologs, revealed that 74% of samples were below detection limits; 23% were between 0.1-0.9 ng/g; and 3% were between 1.0 and 48.0 ng/g wet weight. Further, the ratio of parent PAHs to alkyl homologs, when present, were typically >1.0 indicating that these low-level analytes were not petrogenic, i.e., not associated with the DWH oil spill. Based on PAHs measured in Gulf seafood thus far, contaminant levels are remarkably low and indicate that edible portions of inshore-sampled seafood species do not have elevated contaminant body burdens. These data, combined with consumption patterns of coastal high-end consumers of Gulf seafood, are being used to refine outreach and resiliency programs, and develop probabilistic, community-based risk assessments.

ABSTRACT FINAL ID: 2520 Poster Board -152
TITLE: Gastrointestinal Protective Efficacy of Kolaviron (a Bi-Flavonoid from Garcinia kola) following Single Administration of Sodium Arsenite in Rats: Biochemical and Histopathological Studies

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KEYWORDS: Sodium Arsenite, Kolaviron, Chemoprevention

ABSTRACT BODY: Arsenic intoxication is known to produce symptoms including diarrhea and vomiting, which are indications of gastrointestinal dysfunction. We investigated whether Kolaviron (KV) administration protects against Sodium arsenite (NaAsO2)-induced damage to gastric and intestinal epithelium in rats. Control rats (Group I) were given a daily oral dose of corn oil. Rats in other groups were given a single dose of NaAsO2 (100mg/kg; intraperitoneal) alone (group II) or after pre-treatment for 7 days with KV at 100mg/kg (group III) and 200mg/kg (group IV). Rats were sacrificed afterwards and portions of the stomach, small intestine and colon were processed for histopathological examination. Hydrogen peroxide (H2O2), Reduced Glutathione (GSH), Malondialdehyde (MDA) concentrations as well as activities of Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPX), Glutathione S-transferase (GST) and Myeloperoxidase (MPO) were measured in the remaining portions of the different GIT segments. NaAsO2 caused significant increases ($p<0.05$) in MDA levels and MPO activity, with significant reductions ($p<0.05$) in GST, GPX, CAT and SOD activities in the stomach and intestines. KV significantly reversed the changes ($p<0.05$) in a largely dose-dependent manner. The different segments had marked inflammatory cellular infiltration, with hyperplasia of the crypts, which occurred to much lesser degrees with KV administration. The present findings showed that KV might be a strong potent product for mitigating sodium arsenite toxicity in the gastrointestinal tract.

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ABSTRACT FINAL ID: 2521 Poster Board -153
TITLE: Lead (Pb), Iron (Fe), Zinc (Zn), Copper (Cu), and Iodine (I) Levels in a Popular Herbal Preparation in Nigeria: A Pilot Study

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KEYWORDS: Yoyo Bitters, Pharmacotherapeutic, Iron

ABSTRACT BODY: "Yoyo Bitters" (YYB) is one of the popular herbal drugs in Nigeria produced conventionally like orthodox drugs. Aside from the fact that the drug's claim of multi-pharmacotherapy is equivocal, there are no pharmacokinetics or toxicokinetics data to support the claims. This work set out to examine the level of toxic trace metal [lead (Pb)] and micronutrient constituents [Iron (Fe), Copper (Cu), Zinc (Zn) and Iodine (I)] in this popular herbal preparation with a view to determining the scientific basis for its pharmacotherapeutic claims. This popularly consumed herbal product were purchased from one of the pharmaceutical markets within the metropolis after ascertaining and authenticating the drugs through its approved NAFDAC number, manufacturer's batch and serial numbers, date of manufacture and expiration. Twelve bottles of YYB was purchased and levels of Iron, Copper, Zinc, Lead and Iodine were determined using standard methods. Analysis of the product following standard laboratory practice showed that the herbal product contained mean levels of: Lead (0.04±0.01mg/dl); Iron (1.26±0.35mg/dl); Copper (0.02±0.00mg/dl); Zinc (0.03±0.00mg/dl); and Iodine (14.55±3.26mg/dl) respectively. Although permissible levels of copper and lead were observed in the preparation, possible iodine and iron toxicities observed in the preparation could be associated with heavy use of the product. The need to exercise caution to avoid possible Iodine and Iron overload due to excessive consumption of this herbal product was highlighted in this work.

ABSTRACT FINAL ID: 2522 Poster Board -154
TITLE: Effects of Enantia chlorantha Extract on the Development of the Cardiovascular System in Zebrafish

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KEYWORDS: Medicinal Plants, Teratogenicity, Zebrafish

ABSTRACT BODY: Studies have shown that numerous medicinal plant extracts exhibit medicinal properties against diverse ailments. One such medicinal plant is *Enantia chlorantha*, commonly used by pregnant women in Lagos, Nigeria, as an anti-malarial agent—despite the fact that its teratogenic potential is not well characterized. The aim of the current study was to determine the effects of aqueous *E. chlorantha* extract on the development of the cardiovascular system in zebrafish. Transgenic zebrafish embryos were exposed to extracts (0.08, 0.16 and 0.32mg/ml) between 4-144 hours post-fertilization and assessed for morphological and cardiovascular developmental abnormalities. qPCR was used to assess the relative expression of angiogenesis (flt4 and tal1) and erythropoiesis (haeb1)-related genes as a factor of treatment. Results showed a significant ($p<0.01$) decrease in the number of circulating erythrocytes and their relative velocity in the vessels of treated Tg(gata1:dsRed)sd2/+(AB) eleutheroembryos compared to controls. However, there was no significant ($p>0.05$) change in the total number of blood cells in the whole eleutheroembryo. In addition, the use of Tg(Fli-1a:nEGFP)y7/+(AB) zebrafish revealed a significant disruption in the morphology of the intersegmental blood vessels. qPCR results showed a statistically significant 3-fold increase ($p<0.01$) in the relative expression of flt4 in eleutheroembryos exposed to 0.32 mg/ml extract compared to all other experimental groups. These preliminary results suggest that *E. chlorantha* extract may disrupt cardiovascular development in zebrafish via a mechanism that involves the upregulation of flt4.

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ABSTRACT FINAL ID: 2523 Poster Board -155
TITLE: Hepatoprotective Activity of Dietes bicolor Leaf Extract: Role of Vitexin

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KEYWORDS: *Dietes bicolor*, Vitexin, Hepatoprotection

ABSTRACT BODY: *Dietes bicolor* (DB) is an evergreen perennial belonging to family Iridaceae. This family has been known as a rich source of flavonoids. The phytochemical and pharmacological properties of DB are not known yet. This study aimed to investigate the potential hepatoprotective activity of DB leaf extract and fractions, in addition to the isolation and structural elucidation of the major compound of the biologically active fraction using UV, ESI-MS, 1H and 13C NMR. Total leaf extract and its fractions (n-hexane, dichloromethane and n-butanol) were tested for hepatoprotective activity at 3 different concentrations (50, 100, 200 µg/mL). This was achieved *in vitro* using HepG2 cells challenged with carbon tetrachloride (CCl4) as a model. Pretreatment with DB leaf extract and fractions exhibited a promising hepatoprotective activity against CCl4-induced damage. Butanol fraction (Fr-C) (100 µg/mL) showed the maximum protection as evidenced by a reduction in the leakage of alanine transaminase (ALT) and aspartate transaminase (AST) in the culture medium by 72% and 64% ($P<0.001$) compared to CCl4. The extract and fractions showed potent antioxidant effect confirmed by an increase in reduced glutathione (GSH) levels as well as superoxide dismutase (SOD) activity. Fr-C exhibited the most potent antioxidant effect by boosting GSH and SOD by about 2 folds ($P<0.01$) and 2.4 folds ($P<0.001$) versus CCl4. Fr-C protected against CCl4-induced apoptosis, as indicated by reduced Bax/Bcl-2 ratio and caspase-3 activity by 19% ($P<0.001$) and 52% ($P<0.01$), compared to CCl4. The same fraction also reduced CCl4-induced increase in PGE2 levels by 52% ($P<0.001$). Phytochemical analysis revealed that the main constituent of Fr-C is vitexin. This flavone C-glycoside was shown to contribute to the observed hepatoprotective and anti-apoptotic activity.

ABSTRACT FINAL ID: 2524 Poster Board -156
TITLE: Modulation of Estrogen Depurinating DNA Adduct by Sulforaphane in ACI Rats: Implication for Breast Cancer Prevention

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KEYWORDS: Estrogen Depurinating DNA Adduct, Sulforaphane, Breast Cancer Prevention

ABSTRACT BODY: The goal of this study is to test the hypothesis that sulforaphane (SFN), a isothiocyanate found in broccoli, may alter estrogen metabolism to protect against estrogen-mediated carcinogenesis in ACI rats. SFN is a potent inducer of detoxification enzymes such as NAD(P)H:quinone oxidoreductase (NQO1) and glutathione-S-transferases (GST). NQO1 reduces the carcinogenic estrogen metabolite, catechol estrogen-3,4-quinone, while GSTs can detoxify it through nucleophilic addition. Both pathways may lead to reduced levels of estrogen-DNA adducts. Female ACI rats were treated with either SFN or vehicle three times a week for 7 weeks. One week later rats were implanted with estradiol (E2) pellets. The doses of SFN and E2 were 150 µmole/kg and 3mg, respectively. The urinary levels of key estrogen metabolites and depurinating DNA adducts collected from ACI rats were measured by using ultra-performance liquid chromatography coupled with tandem mass spectrometer. The protein/activities of key estrogen metabolism enzymes in hepatic tissue were assayed by using western blot and activity assays. There were significantly higher levels of hepatic NQO1 and GST activities after the 7 weeks of treatment with SFN; NQO1 expression levels were also significantly higher compared with vehicle treatment ($p<0.05$). The levels of depurinating estrogen-DNA adducts excreted in urine 6 weeks after E2 implantation, expressed as the sum of, 4-OHE1/2-1-N3Adenine and 4-OHE1/2-1-N7Guanine, was significantly lower (~75%) in the animals treated with SFN than that of the vehicle. We conclude that SFN might be an effective chemoprevention agent against

breast cancer through its actions on estrogen metabolism. Supported by DoD BCRP Postdoctoral Fellowship103928 and the Breast Cancer Research Foundation.

ABSTRACT FINAL ID: 2525 Poster Board -157
TITLE: Antineoplastic Potential of Bioactive Fractions of Rhus trilobata on Colon Cancer Cells CaCo-2

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KEYWORDS: *Rhus trilobata*, Cancer, Antineoplastic

ABSTRACT BODY: Colorectal cancer (CaCo) is the most common malignancy of the gastrointestinal tract. In 2008, it was reported that Chihuahua State occupies the second place in mortality by CaCo, with a rate of 3.1 per 100,000 habitants. Recently, studies done by our research group demonstrated that aqueous and methanol crude extracts of *Rhus trilobata* (Rt) decrease the proliferation of both CaCo-2 cells and SKOV-3 ovarian cancer cells by an apoptotic-dependent pathway. The objective of this work was to demonstrate the antineoplastic activity (ANAc) of fractions from aqueous (AE) and methanol (ME) extracts of Rt on CaCo-2 cells. In an attempt to isolate the main compound, both Rt crude extracts were fractionated by solid phase extraction (SPE) on a C-18 column, fractions were tested for ANAc using the MTT assay for IC50 calculation. Bioactive fractions were subsequently fractioned by HPLC and tested for viability, cytotoxicity and apoptosis using the kit ApoTox-Glo®. Results demonstrated that fractions 02 and 03 from both extracts contained and important ANAc with IC50 values < 10 µg/mL, HPLC analysis showed that fractions 02 and 03 contained the highest concentration of compounds resulting in inhibition of CaCo-2 cells mitosis by 45.5% at 2.5 µg/mL. Apoptosis analysis showed that ANAc is more related with induction of apoptosis, than with cytotoxicity mediated by membrane cell rupture. These results support the use of *Rhus trilobata* extracts as alternative treatment against cancer, due to its proapototic ability. Additional studies are being carried out to purify the main compound presents in fraction 02 and 03. Supported by FOMIX CHIH-2010-C01-147532.

ABSTRACT FINAL ID: 2526 Poster Board -158
TITLE: Potential Role of Metallothionein in Manganese-Induced Cellular Aggresomes

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KEYWORDS: Manganese, Metallothionein, a-Synuclein

ABSTRACT BODY: Manganese (Mn) exposure induces α-synuclein (Scna) aggregation in the brain and such synucleinopathies are a feature of several neurodegenerative disorders. Mn induces metallothionein (MT), a metal binding protein. In prior work we found Scna and MT were important components of lead-induced aggresome-like inclusion bodies. Thus, we used MT-I/II double knockout (MT-null) and parental wild-type (WT) cell lines to see if Mn-induced Scna aggregations involved MT. Mn exposure (MnCl2; 30 µM) for 48 hrs produced more visible aggresomes in WT cells (9.0 ± 3.7/100 cells) than MT-null cells (2.0 ± 0.4 /100 cells). Considering Mn-induced aggresomes may be like disease-related synucleinopathies, Scna transcript was also assessed. Mn treatment increased Scna over time in WT cells with maximal increases at 48 hrs, when aggresomes were clearly formed. In contrast, Mn treatment increased Scna transcript at later time points and to a much lesser extent in MT-null cells. Transfection of MT-I into MT-null cells restored some expression of Scna transcript (2-fold). Mn increased MT transcript in WT cells with maximal transcript increases at 16 hrs which then fell back to control levels by 48 hrs. However, MT protein levels decreased after Mn exposure in WT cells, indicating free MT protein was going into forming aggresomes. In MT-null cells, MT transcript and protein were undetectable. Mn was less cytolethal in WT cells (LC50 = 71 ± 6 µM) than in MT-null cells (LC50 = 43 ± 3 µM). MT-null cells accumulated more Mn than WT cells after 48 hrs of Mn treatment. Transfection of MT-I into MT-null cells partially reduced Mn accumulation towards

WT levels. Mn efflux did not show a difference among WT, MT-null and MT-I transfected MT-null cells. Mn increased expression of oxidant stress defense genes GCL and Nrf2 to much higher levels in WT cells than MT-null cells. Thus, Scna is likely a component of Mn-induced aggresomes and MT may play a role in the process of their formation.

ABSTRACT FINAL ID: 2527 Poster Board -159
TITLE: Bioaccumulation and Biochemical Responses of African Catfish (*Clarias gariepinus*) to Sublethal Concentrations of Lead and Zinc

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KEYWORDS: Metal Toxicity, Bioaccumulation, Biomarkers

ABSTRACT BODY: There is great need for routine monitoring of aquatic ecosystems to assess risks of metal accumulations in indicator species. This study investigated the bioaccumulation and biochemical responses of *Clarias gariepinus* exposed to sublethal concentrations of Pb(NO3)2 and ZnCl2 using laboratory bioassays. The 96hr-LC50 values of Pb and Zn against *C. gariepinus* were found to be 55.12 mg/l and 32.15 mg/l respectively. Post juveniles of C. gariepinus were exposed to 1% 96hr-LC50 values of Pb and Zn in a single metal bioassay, for 30 days. *C. gariepinus* accumulated (Pb and Zn) in their liver, muscle and gill with the highest concentration of the heavy metals occurring in muscle tissue. Biochemical analyses showed that the level of reduced glutathione (GSH), and the activities of superoxide dismutase (SOD), glutathione-s-transferase (GST), and catalase (CAT) in the liver of the *C. gariepinus* were reduced significantly ($p < 0.05$) when exposed sublethal concentrations of Pb and Zn. However, increase in the level of malondialdehyde (MDA) in liver of the *C. gariepinus*, after the 30 days exposure was not significant ($p > 0.05$) compared to controls. This study showed that SOD, GSH, GST and CAT are useful molecular responses that can be used as biomarkers in the assessment of metal accumulation and toxicity.

ABSTRACT FINAL ID: 2528 Poster Board -160
TITLE: The Tibetan Medicine Zuotai and Cinnabar Are Much Less Nephrotoxic Than Mercury Chloride and Methylmercury in Mice

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KEYWORDS: Metal, Kidney, Mercury

ABSTRACT BODY: Zuotai is mainly composed of metacinnabar (beta-HgS) and is included in many popular Tibetan medicines; cinnabar (alpha-HgS) is also included in 30 patent Chinese medicines. Both Zuotai and cinnabar are mercury compounds, and mercury is well known for many toxic effects, especially nephrotoxicity, and their safety is of concern. The aim of this study was to compare the nephrotoxic potential of Zuotai and cinnabar (HgS) with environmental mercury compounds mercury chloride (HgCl2) and methylmercury (MeHg). Mice were orally administrated with Zuotai (30 mg/kg, 5-fold of clinical dose), cinnabar (30 mg/kg), HgCl2 (33.6 mg/kg, equivalent Hg content as Zuotai) and BrMeHg (4.6 mg/kg, 1/10 Hg of Zuotai) for 7 days, and nephrotoxicity was examined. Animal body weights were decreased by HgCl2 and to a less extent by MeHg, while kidney weights were increased 58% and 30% by HgCl2 and MeHg, respectively. HgCl2 and MeHg produced widespread renal tubular vacuolation, degeneration, renal interstitial inflammation and cell death, while such pathological lesions are absent or mild in Zuotai and HgS-treated mice. Renal Hg contents reached 250-300 ng/mg kidney in HgCl2 and MeHg groups; while Hg contents were about 2 ng/mg in Zuotai and HgS groups. The expression of renal injury biomarkers, kidney injury molecule-1 (Kim-1) and neutrophil gelatinase-associated lipocalin (Ngal), were increased after HgCl2 and MeHg, but unchanged after Zuotai and HgS. The expression of renal influx transporters such as Oatp4c1 was decreased, while the expression of renal efflux transporter such as Mrp2 was increased following HgCl2 and MeHg, but

were basically unaltered by Zuotai and cinnabar. In conclusion, the Tibetan medicine Zuotai and cinnabar have less nephrotoxic potentials than mercury chloride and methylmercury, indicating that chemical forms of mercury are a major determinant of their disposition and toxicity in traditional medicines.

ABSTRACT FINAL ID: 2529 Poster Board -161
TITLE: Linkage Analysis of Urine Arsenic Species Patterns among Persons without Diabetes in the Strong Heart Family Study

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KEYWORDS: Toxicogenetics, Arsenic, Arsenic Metabolism

ABSTRACT BODY: This linkage study explored possible genetic determinants of arsenic kinetics in the Strong Heart Family Study. We included participants with urine arsenic species above detection limit (0.1 µg/L) measured by high-performance liquid chromatography inductively-coupled plasma mass spectrometry (HPLC-ICPMS), who were not taking diabetes medications, had fasting glucose < 126 mg/dL, and who had data on ~400 genome-wide microsatellite markers spaced ~10 centimorgans apart. The sample size was n=2,189: 683 from Arizona, 684 from Oklahoma, and 822 from North or South Dakota. We logit-transformed % arsenic species (proportion of inorganic, monomethylarsonate [MMA] or dimethylarsinate [DMA] contributing to their sum), and used the inverse-normalized residuals from polygenic heritability analysis, controlling for age, sex, geography, body mass index, education, smoking, drinking, and arsenic exposure level, for a multipoint variance components linkage analysis. The strongest evidence for linkage in this study was seen on chromosome 10 (LOD 4.12 for %MMA, 4.65 for %DMA). A conditional linkage analysis among participants with genotyped arsenic III methyltransferase (AS3MT) polymorphisms rs17878846 and rs10509760 (n=2,165) suggested that this peak may not be fully explained by tagged variation in AS3MT. Some loci were suggestive or significant for one study center, but not across all centers, indicating possible locus heterogeneity by center. Additional fine-mapping may help identify variants relevant for arsenic kinetics in human populations.

ABSTRACT FINAL ID: 2530 Poster Board -162
TITLE: Ameliorative Effect of Ethanolic Extract of *Annona muricata* (Sour sop) against Sodium Arsenite-Induced Hepatotoxicity in Wistar Rats

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KEYWORDS: Arsenic, Antioxidant, Hepatoxicity

ABSTRACT BODY: Ingestion of arsenic in drinking water causes cancer at multiple tissues and there is no cure. Researches are therefore directed at chemoprevention using medicinal herbs for the management of arsenicosis. In this study, we evaluated the *in vitro* antioxidant and protection offered by *Annona muricata* L. (AM) against sodium arsenite induced hepatotoxicity in rats. Anti-oxidant and radical scavenging activities of AM were compared to vitamin C and butylated hydroxytoluene (BHT). Proximate and phytochemical analyses were also carried out. Hepatoprotective study was investigated with six groups of rats that received water (Control), 5.0 mg/kg bwt NaAsO2, 250 mg/kg bwt AM, 500 mg/kg bwt AM, NaAsO2 plus 250 mg/kg AM, NaAsO2 and 500 mg/kg AM. The NaAsO2 was given once on days 7, 14 and 21, while AM was administered orally daily for 21 days. Serum transaminases and alkaline phosphatase activities were determined

and liver histopathology carried out. AM contained 2.00% ash, 1.94% crude fat, 25.65% crude fibre, 2.88% protein and 58.62% carbohydrate. Photochemical analysis indicated the presence of alkaloids, flavonoids and cardiac glycosides. The reducing power and metal chelating ability was in the order Vit C > BHT > AM, while for DPPH scavenging ability AM > Vit C > BHT. The NaAsO2 significantly ($p < 0.05$) increased the liver function enzymes relative to control. However, AM treatment markedly reduced the marker enzymes and restores the severe vaculation of hepatocytes in the NaAsO2 group to near normal. Our findings suggest that AM may constitute a remedy against arsenic induced hepatic injuries.

ABSTRACT FINAL ID: 2531 Poster Board -163
TITLE: Reversal Effect of Vitamin E against Arsenic-Induced Oxidative Stress

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KEYWORDS: Arsenic Toxicity, Vitamin E, Oxidative Stress

ABSTRACT BODY: The present study aimed at the effect of arsenic (As) on oxidative enzymes and gene expression in rat liver, kidney and heart. The young albino rats (3 months) were exposed to As (2.5 and 5.0 mg/kg body weight) through intraperitoneal injection daily for 3 weeks. After the period of dosage, the As exposed animals were divided into two groups of which one group of both the doses were given vitamin E at a dose of 20 mg/kg body weight for a period of one week. In this study, we have examined the activities of Mn-Super oxide dismutase (Mn-SOD), Cu/Zn Super oxide dismutase (Cu/Zn-SOD), catalase, m-RNA expression of Mn-SOD and levels of lipid peroxidation and protein content. The results showed a significant decrease in Mn-SOD activity, Cu/Zn-SOD activity, catalase activity, Mn-SOD m-RNA expression and protein content while lipid peroxidation increased in low and high As exposed rats. The exposure to vitamin-E, however, showed recovery and increase in the Mn-SOD activity, Cu/Zn - SOD activity, catalase activity, Mn-SOD m-RNA expression and protein content. As treated rats showed significant increase in the lipid peroxidation over control rats. The exposure to vitamin-E, As showed recovery and decrease in the lipid peroxidation levels was observed. The effect was more pronounced in high dose As exposed animals. The result may be attributed to the reason that the As exposure disrupts the functioning of liver, kidney and heart and this could be due to oxidative deficits. Vitamin-E is an antioxidant an effective chelating agent reduces the As burden in the tissues like liver, kidney and heart. Hence there was decreased oxidative damage in the As exposed rats treated with vitamin E. Key words: Arsenic, Vitamin-E, Oxidative stress, m-RNA Expression.

ABSTRACT FINAL ID: 2532 Poster Board -164
TITLE: Protective Role of Vitamin E in Cadmium-Induced Oxidative Stress in Rat Brain

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KEYWORDS: Cadmium Toxicity, Oxidative Stress, Vitamin E

ABSTRACT BODY: Acute exposure to moderately high concentrations of freshly generated cadmium oxide fumes (200-500 µg cadmium/m3) may cause symptoms similar to the metal fume fever. The present study addressed the effect of cadmium (Cd) on oxidative system in rat brain. Three months old rats were exposed to Cd intraperitoneally at a concentrations of low dose (1.5 mg / kg body weight) and high dose (3 mg / kg body weight) for a period of three weeks. A separate batch of low dose and high dose of Cd exposed rats received Vitamin-E 20mg/kg body weight intraperitoneally. In this study, we assessed the biochemical end points indicative of oxidative stress in mitochondrial fraction of three brain regions: cerebral cortex, cerebellum, and hippocampus. We measured the activities of glutathione peroxidise (GPX), glutathione reductase (GRD), glutathione –S- transferase (GST), xanthine oxidase(XO), and levels of protein content showed a significant decrease, whereas the lipid peroxidation showed significant increase over control rats in a dose-dependent manner. The exposure to vitamin-E, however, to low and high dose of Cd showed recovery which was observed in the increased activities

of GPX, GRD, GST, XO and increased levels of protein content. Similarly decreased levels of lipid peroxidation was observed. The results may suggest that Cd-induced functional deficitsin the brain mayinvolve oxidative stress. As an antioxidant, Vitamin-E acts as a peroxyl radical scavenger, preventing the propagation of free radicals in tissues, by reacting with them from a tocopheryl radical, which will then be reduced by a hydrogen donor and thus return to its reduced state. As it is incorporated into cell membranes, which protects them from oxidative damage. Thus Vitamin-E lessened the Cd burden in the brain as effective chelating agent decreasing the oxidative stress.

ABSTRACT FINAL ID: 2533 Poster Board -165
TITLE: Activation of Line1 Retrotransposon by Arsenic: A Novel Mechanism of Genomic Damage and Instability

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KEYWORDS: Arsenic, Line1 Retrotransposition, Nrf2

ABSTRACT BODY: Exposure to environmental arsenic is associated with increased risk of cancer, cardiovascular disease and neurological diseases. Arsenic toxicity is mainly mediated by strong genotoxicity causing chromosomal aberrations and mutations. Line1 retrotransposon is a group of mobile DNA elements that comprise 17% of the human genome. Activation of Line1 contributes to increased genetic instability by insertional mutagenesis and extensive chromatin remodeling. Whether arsenic genotoxicity is partially mediated by Line1 activation remains unknown. Here we present data showing that arsenic induces the expression of both Line1 ORF-1 and ORF-2 mRNAs, starting 2hr after exposure and peaking at 8hr, in cardiac progenitor cells. This induction is associated with increased expression of Hmox1 mRNA indicating that Line1 activation may associate with arsenic-mediated ROS production. Dual luciferase assays show that the transcriptional activity of Line1 promoter is induced by arsenic in a time- and dose-dependent manner. Nrf2 may play an important role in arsenic-mediated Line1 activation as a Line1 element carrying a mutated ARE site exhibits an overall decrease in transcriptional activity compared to the wild type element. Overexpression of Nrf2 shows synergistic L1 induction effects with arsenic, and this response is significantly blocked by Nrf2 siRNA. In addition, increased nuclear translocation of Line1-ORF2 is detected following arsenic exposure in a dose-dependent manner. Nuclear accumulation of truncated Line1-ORF2 is detected after 30 min of arsenic exposure, suggesting potential genomic insertion events. In summary, arsenic exposure leads to Nrf2 dependent induction in Line1 transcription, increased Line1 gene expression and Line1 ORF-2 protein nuclear accumulation. These observations demonstrate a novel mechanism of arsenic associated genomic damage and instability involving activation of Line1 retrotransposon.

ABSTRACT FINAL ID: 2534 Poster Board -166
TITLE: Interplay of Arsenic Exposure and Circadian Clock in Energy Metabolism

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KEYWORDS: Arsenic, Diabetes, Circadian

ABSTRACT BODY: Inorganic arsenic present in the earth's crust is one the most ubiquitous toxic metalloid in the environment. Most previous toxicological studies on arsenic have been focused on its role in carcinogenesis. Recently, several epidemiological studies showed correlation between arsenic exposures and development of type 2 diabetes (T2D), a disease and its associated metabolic disorders including obesity have reached pandemic levels with over 35% adult population affected. Transcription of many metabolic genes is regulated by the internal circadian clock and display robust

oscillation in many tissues including liver. Misalignment of the internal clock and feeding behavior underlies the pathogenesis of T2D. Here we show that arsenic exposures disrupt the hepatic circadian clock and exacerbate insulin resistance in a mouse diabetes model with a reverse-phase feeding regimen. These observations provide novel insights into how the environmental toxin arsenic interferes with the internal circadian clock in the regulation of metabolic homeostasis.

ABSTRACT FINAL ID: 2535 Poster Board -167
TITLE: Proteomic Analysis in the Lung and Brain of Rats following Silver Nanoparticle Inhalation

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KEYWORDS: Nanotechnology, Neurotoxicity, Mass Spectrometry Imaging

ABSTRACT BODY: Silver nanoparticles (AgNP) are widely used in industrial, household and diagnostic products, as well as in antimicrobial applications. Due to its high production volume there is potential for occupational and environmental exposure. Here, we investigated the potential toxic effects associated with AgNP both in the lung and brain. Male Sprague-Dawley rats were exposed by inhalation to 1 mg/m3 AgNPs (20 nm diameter, 0.3% PVP coated), or filtered air (control), for 4 h/d for 14 work days. At 1 and 28 d post-exposure, rats were humanely sacrificed, perfused with saline, and lungs and brains were harvested and sectioned for histology-guided mass spectrometry to determine localization of elemental silver in tissues and associated alterations in protein profiles. Digital microscopy images of the stained sections were annotated. After merging annotated and serial unstained section images, protein and metal ion mass spectra were collected from the annotated areas. Data were analyzed for differences between AgNP treated animals and controls. Focal areas in airways and parenchyma were analyzed from the lung. Several proteins were found to be differentially expressed at 1 and 28 d post-exposure. A peak consistent with thymosin β4 was decreased and calcyclin increased in lungs of AgNP-exposed animals. Minimal changes were observed in brain cortex; however, significant differences in protein expression were observed in the striatum and hippocampus at 28 d after AgNP exposure. Specifically, in the hippocampus, myelin basic protein (14.1 kDa) was greatly elevated. Using matrix free laser desorption ionization, a signal consistent with elemental silver was detected in the lung but not the brain at 1 and 28 d post-exposure. Taken together, our findings suggest persistent alterations in both the lung and brain proteome following AgNP exposure, warranting further investigation of AgNP-related toxicity to avert or reduce potential human health risks.

ABSTRACT FINAL ID: 2536 Poster Board -168
TITLE: Diagnostic Utility of Liver and Kidney Specimens for the Determination of Lead and Mercury Exposure and Toxicity in Bald Eagles

AUTHORS (FIRST INITIAL, LAST NAME): K. E. Huff[2], W. K. Rumbeiha[3], K. Stuart[2], T. Cooley[4], J. G. Sikarskie[5], A. Lehner[2], J. P. Buchweitz[1, 2]
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KEYWORDS: Lead, Mercury, Eagles

ABSTRACT BODY: An animal's liver has generally been the preferred diagnostic specimen type for post-mortem determination of ante-mortem nutrient mineral status and/or heavy metal exposure. In the absence of sufficient liver sample as a result of scavenging, decomposition, other diagnostic testing, etc. other tissues (e.g. kidney) may be submitted in lieu of liver. The purpose of this study was to evaluate the diagnostic utility of kidney vis-à-vis liver in *Haliaeetus leucocephalus* (bald eagle) for assessing lead (Pb) and mercury (Hg) toxicosis. Between 2001 and 2009, a total of 202 birds

were collected for analysis from around the state of Michigan, primarily the Upper Peninsula. Eagles submitted for necropsy and testing represented both sexes and all age groups and body conditions. Pb residues ranged from below detection to 65.5 ppm in liver and to 39.791 ppm in kidney. Pb residues indicative of exposure or toxicosis in liver did not correlate well with kidney concentrations in the same animal suggesting temporal differences in distribution and detection. In an opposite manner, Hg residues ranged from below detection to 71.173 ppm and 352.933 ppm in liver and kidney specimens, respectively. The molar ratio of mercury to selenium was 0.603 ± 0.341 (linear correlation 0.5377) in liver and 0.743 ± 0.233 (linear correlation 0.8456) in kidney. Utilization of the Hg:Se ratio from kidney provided a more concise range for diagnosing mercury toxicity. Results indicate that kidney was less effective than liver when diagnosing lead exposure/toxicity but is a superior choice for diagnosing mercury exposure/toxicity.

ABSTRACT FINAL ID: 2537 Poster Board -169
TITLE: Determination of As, Cu, Pb, and Hg in Muscle and Liver of Fish by ICP-MS

AUTHORS (FIRST INITIAL, LAST NAME): L. V. Saldívar[1], C. M. Meza[1], M. G. Gutierrez[1], C. E. Marquez[1], M. G. Espejel[1]
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KEYWORDS: Trace Elements, Fish, ICP-MS

ABSTRACT BODY: Fish is a major source of animal protein and it also contains vitamins [1]. Fish is a good indicator of trace metal pollution in aquatic ecosystems within this group of elements are chromium and selenium, these are present in hormones, vitamins, enzymes and other proteins with specific biological functions. The objective of this research was to quantify arsenic, copper, lead and mercury. For this purpose the following performance parameters will be evaluated: accuracy and precision, limit of detection, limit of quantification, linear range and range of work. In general, values of relative error and RSD [Relative Standard Deviation] less than 20% in the two certified reference materials (DORM-4 [Fish protein] and DOLT-4 [Dogfish liver]) were achieved. Therefore, the method proved to be accurate and precise in the determination of four analytes. The concentrations of arsenic, copper, lead and mercury were determined in thirty muscle samples and liver of *Oreochromis niloticus* from Presa Requena of Hidalgo, Mexico. Metal concentrations were determined by ICP-MS [inductively coupled plasma mass spectrometry] after microwave-assisted acid mineralization using 2 mL of HNO3 (concentrated) and 1 mL of H2O2 (30% V/V) [2]. The average content of metals in fish muscle samples was [0.014-0.035], [0.120-0.5], [0.023-0.084] and [0.080-0.248] mg/kg for arsenic, copper, lead and mercury, respectively. In the other hand, the average content of metals in liver samples was [0.051-0.204], [36.92-300.2], [0.116-0.675] and [0.085-0.325] mg/kg for arsenic, copper, lead and mercury, respectively. Acknowledgements.This research was partially supported by PAL 3400-02. References.[1] Nuray, E; Özkan, O. (2006). Proximate composition and mineral contents in aqua cultured sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*) analyzed by ICP-MS, *Food Chemistry*, 102, 721-725. [2] Shailini Ashoka, et al.(2011). Distribution of trace metals in a ling (*Genypterus blacodes*) fish fillet, *Food Chemistry*, 125, 402-409.

ABSTRACT FINAL ID: 2538 Poster Board -170
TITLE: Possibility to Reduce Human PCBs Exposure by Intake of Fruits and Vegetables

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KEYWORDS: PCBs, Exposure, Human

ABSTRACT BODY: Health effects by fetal exposures to PCBs or dioxins have been concerned. The major source of exposure to PCBs/dioxins has been reported as food such as fish in Japan. Our previous studies also revealed that colestimide (fiber; a medication prescribed for hyperlipidemia) was effective to reduce dioxins/PCBs levels in the human body. It is important to know how PCB contamination level is affected by eating habit. In this presentation, the correlation between the results

from Food Frequency Questionnaire (FFQ) and blood PCB contamination level of the participants in Japan Environment and Children's Study (JECS) will be reported. JECS is a long term cohort study of children with participation of their parents. Blood PCB levels in 197 fathers who were recruited in Chiba Regional Center, one of the 15 Regional Centers of JECS were analyzed and calculated by Packed Column Gas Chromatography Electron Capture Detector (GC/ECD). The answers of FFQ were analyzed and the relationship between food items, frequencies and blood PCB levels was discussed with Partial Least Squares (PLS) analysis and Bayesian ridge regression. The PCB levels expected from the answers in FFQ and the actual blood PCB level correlated by PLS analysis and Bayesian ridge regression (r2 = 0.50 and predictive concordance = 0.953, respectively). It was found that it would be possible to estimate blood PCB level from FFQ and age data. Moreover, consumption of fruits and vegetables showed significantly negative correlation with PCB concentration. These results indicate that intake of fruits and vegetables which contain fibers might reduce PCBs in human. The current FFQ data contain tentative data and final data will reveal even more detailed and important information. (This study was conducted as an adjunct study of JECS. The findings and conclusions are solely the responsibility of the authors).

ABSTRACT FINAL ID: 2539 Poster Board -201
TITLE: Evaluation of "M Series" PhysioTel Digital Telemetry for Cardiovascular Data Acquisition in Group-Housed Dogs: Comparison to Jacketed External Telemetry

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KEYWORDS: Safety Pharmacology, Telemetry, Cardiovascular Safety

ABSTRACT BODY: Introduction. Collection of high fidelity cardiovascular data during repeat dose toxicology studies is routinely achieved through the use of minimally invasive indwelling telemetry blood pressure devices in conjunction with jacketed external telemetry (JET) for BP and ECG collection. However, the jacketing procedure is time consuming and involves extensive animal handling. Newly developed M series implantable telemetry devices now enable both ECG and BP data collection without the use of a jacket. This study evaluated the performance of the M series compared to standard telemetry and JET combination for the collection of ECG and BP data in socially housed dogs following moxifloxacin and L-NAME administration. Methods. Female dogs (n=6) were pair-housed and dosed orally in an ascending design. Cardiovascular data were recorded continuously from 1h pre-dose to 20h post dose. Evaluation of gross pathology and histopathologic effects was performed following 9 weeks of M series implantation. Results. M series effectively detected the expected dose-dependent increases in rate-corrected QT with moxifloxacin (90 mg/kg: 23.7±7.6 ms) and BP with L-NAME (10 mg/kg: 25.2±6.3 mmHg). Drug-effects were quantitatively comparable between the M series and JET data sets. Following 9 weeks of implantation, the histopathological assessment did not detect any related findings in the major target organs and tissues. Conclusions. M series telemetry devices provided high-fidelity cardiovascular data and effectively detected expected effects of reference drugs. The subcutaneously implanted devices employing femoral catheterization were well tolerated, which enabled the unambiguous interpretation of histopathologic endpoints. The current study demonstrates that M series implants provide for a suitable alternative to external methods, facilitating robust toxicological assessments while removing the need to jacket the animals.

ABSTRACT FINAL ID: 2540 Poster Board -202
TITLE: Urban Fine-Particulate Matter and Ozone Induce Differential Effects on HDL Antioxidant Function

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KEYWORDS: HDL Cholesterol, Fine-Particulate Matter, Ozone

ABSTRACT BODY: Short-term exposures to ambient particulate matter (PM) are associated with increased morbidity and mortality. PM2.5 (< 2.5 µm) and ozone exposures have been shown to associate with carotid intima media thickness in humans. Animal studies support a causal relationship with atherosclerosis and identified adverse effects on HDL functionality. We aimed to determine whether brief exposures to PM2.5 and/or ozone could induce effects on HDL anti-oxidant and anti-inflammatory capacity in humans. Subjects were exposed to concentrated ambient fine particles (PM2.5 ~ 150 µg/m3), ozone (120 parts per billion), fine particles plus ozone, and filtered air for 2 hours on 4 different occasions, at least two weeks apart, in a double-blinded randomized, crossover design. Blood was obtained before, after and 24 hours after exposure. Plasma HDL anti-oxidant/anti-inflammatory capacity and paraoxonase activity were determined. HDL anti-oxidant/anti-inflammatory capacity was expressed as HDL oxidant index (HOI) units. We observed that PM2.5 and ozone exerted differential effects on the HOI that were significantly different after exposure ($p<0.05$) but not 24 hours after ($p= 0.19$). PM2.5 induced an increase in HOI compared to filtered air ($p< 0.05$), indicating decreased HDL anti-oxidant/anti-inflammatory capacity, when baseline HOI was lower (i.e., < 1.5 or 2.0). The change in HOI showed a trend to associate with particle mass concentration and significantly associated with the slope of systolic blood pressure during exposures. Brief exposures to concentrated PM2.5 elicited immediate effects on HDL function, which could indicate a potential mechanism for how particulate air pollution induces harmful cardiovascular effects.

ABSTRACT FINAL ID: 2541 Poster Board -203
TITLE: Evaluation of Blood Pressure, Heart Rate, Temperature, and Activity Using New Digital Rodent Telemetry Technology

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KEYWORDS: Cardiovascular, Blood Pressure, Telemetry Implant

ABSTRACT BODY: Radio-telemetry is an invaluable technology for obtaining high quality *in vivo* cardiovascular data in experimental research. The purpose of this study was to evaluate the performance of a new digital HD-S10 rat telemetry implant from Data Sciences International (DSI) for acquisition of blood pressure, heart rate, activity and temperature data. Data obtained with the HD-S10 units were compared to data obtained with C50-PXT telemetry transmitters using a compound known to alter blood pressure, heart rate and body temperature. Male Wistar Han rats were implanted with HD-S10 rodent telemeters and the pressure catheter was placed in the abdominal aorta. PF-X (500 mg/kg) or vehicle (0.5% methylcellulose) was orally administered to conscious, unrestrained rats, and cardiovascular telemetry parameters (mean, systolic and diastolic pressures, heart rate, activity and body temperature) were obtained continuously for 24 hours post-dose. A single-dose crossover design was employed using 8 animals. Oral administration of PF-X produced test article-mediated increases in blood pressure and heart rate in the study using the HD-S10 transmitter that were similar to that of a previous study using the C50-PXT transmitter (10 vs. 18 mmHg; 20 vs. 38 bpm, respectively). Likewise, decreases in body temperature observed using the HD-S10 transmitters were similar to data obtained with the C50-PXT technology (-1.7 vs. -1.9°C, respectively). In summary, the changes in heart rate, pressures and temperature obtained using the 2 different telemeters were similar in magnitude and duration, indicating that the new digital HD-S10 rat telemetry implant produced comparable results to that of the C50-PXT implant.

ABSTRACT FINAL ID: 2542 Poster Board -204
TITLE: DOXORUBICIN-INDUCED CARDIOTOXICITY DETECTION AND MONITORING IN A MOUSE MODEL BY FUNCTIONAL AND MOLECULAR POSITRON EMISSION TOMOGRAPHY IMAGING

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KEYWORDS: Cardiotoxicity, Cardiac Imaging, PET

ABSTRACT BODY: Doxorubicin, a cytotoxic chemotherapy drug used to treat many cancers, can induce a number of serious secondary effects, including progressive congestive heart failure. Hence, it is of paramount importance to detect signs of cardiotoxicity before irreversible damages occur. In this work, a mouse model of doxorubicin-induced cardiotoxicity was used to identify early biomarkers of heart failure using positron emission tomography (PET) imaging for the noninvasive assessment of the heart function and metabolism. Methods: Cardiotoxicity was induced in Balb/c mice (n=9) by administration of 40 mg/kg cumulative dose of doxorubicin over 7 weeks. Control (n=9) and treated mice were followed by PET during 17 weeks to detect and monitor any change in the left ventricular function or myocardial perfusion and metabolism. ^{18}F-FDG was used to measure the myocardial uptake rate of glucose (K_i) and left ventricular ejection fraction (LVEF), while ^{11}C-acetoacetate, a ketone body tracer, was used to determine indexes of myocardial perfusion and oxygen consumption. Results: Normal values of perfusion and oxygen rates were 3.4±0.4 min^{-1} and 1.3±0.2 min^{-1}, respectively, decreasing significantly in treated animals after 9 weeks to reach 2.3±0.3 min^{-1} and 0.8±0.1 min^{-1}. These changes were shortly followed by a gradual drop of LVEF from a normal average of 79.4±4.9% before week 10 to 49.3±2.5% at the end of the follow-up period, reflecting a significant increase of ventricular volumes. This was paralleled by a steep K_i increase becoming significant relative to normals around the 11th week. These findings were confirmed by histopathology at the end of follow-up. Conclusion: PET imaging provides a potentially useful tool to noninvasively detect early signs of cardiotoxicity by monitoring several cardiac parameters in subjects undergoing doxorubicin chemotherapy.

ABSTRACT FINAL ID: 2543 Poster Board -205
TITLE: A Comparison of Baseline Heart Rates, Left Ventricular, and Systolic Pressure in Group-Housed versus Single-Housed Beagle Dogs and the Effects of Housing on Sensitivity to Detect Changes in Contractility following Atenolol Administration

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KEYWORDS: Heart Rate, Contractility, Blood Pressure

ABSTRACT BODY: The ICH S7A and S7B guidelines require that effects of test substances on the cardiovascular system be assessed with respect to blood pressure, heart rate and electrocardiogram intervals and that effect on contractility only be assessed as supplementary study when potential adverse effects are identified. This is despite data to support that the incidence of contractility modification noted in phase 1 trials is greater than QT prolongation. Historically assessments of contractility have been limited to snap-shot echocardiography or single housed telemetry assessments of dp/dt max. However with advances in cardiovascular technology it is feasible to conduct cardiovascular assessments under group housing conditions to improve animal welfare. The purpose of this study was to evaluate baseline cardiovascular parameters, within a group housed environment compared to historical baseline data from a single housed environment and to demonstrate that the model retained sensitivity of the traditional single housed implantable telemetry. In this study four animals were instrumented with DSI HD-L21 implants for continuous 24 hour assessment of systemic pressures, left ventricular pressures, heart rate and electrocardiograph intervals. The results showed that group housing in European caging had no influence on baseline cardiovascular assessments, compared to historical data from single housed animals and that treatment with Atenolol induced decreased dp/dt max in-line with historical data. In conclusion, the collection of cardiovascular data in male dogs co-housed in European caging showed stable cardiovascular baseline, was sensitive to detect the expected changes in Atenolol and therefore it was considered that this approach allows for assessments in line with ICH S7A and S7B guidance, provides secondary information on potential contractility effects whilst providing improved animal welfare through the introduction of social housing.

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Late-Breaking Abstracts

ABSTRACT FINAL ID: 2544 Poster Board -206
TITLE: Multiple Ion Channel Effects (MICE) Explain Vanoxerine's Efficacy As an Antiarrhythmic Drug

AUTHORS (FIRST INITIAL, LAST NAME): C. Obejero-Paz[1], A. Bruening-Wright[1], M. Tatalovic[1], H. Dittrich[2], A. M. Brown[1, 2]
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KEYWORDS: Cardiotoxicity, Cardiac Safety, Arrhythmias

ABSTRACT BODY: Vanoxerine is a potent dopamine transporter inhibitor that has been in clinical trials for Parkinsonism, depression and cocaine addiction. The drug produced no serious adverse events (SAEs) but lacked efficacy. Subsequently it has been repositioned for treatment of atrial fibrillation and flutter (AF/AFL), serious arrhythmias for which there is no satisfactory medical treatment. Despite being a potent blocker of hERG we showed that the drug terminated AF/AFL in an animal model (Matsumoto et al, 2010) and subsequently a clinical trial (Dittrich et al, 2014) without associated SAEs. Vanoxerine exemplifies the value of the new regulatory approach to assess proarrhythmic liability of drugs through the Comprehensive InVitro Proarrhythmia Assay (CIPA). Here we describe how the drug's MICE account for its safety. We used: 1) manual patch clamp and step-ramp voltage protocols at 1 Hz to measure block of hERG, hCav1.2,and peak and late hNav1.5 currents expressed heterologously in cell lines; 2) the O'Hara-Rudy model of the human ventricular action potential to simulate block at relevant clinical exposures (~10 nM) and, 3) experiments on human iPSC-derived cardiomyocyte action potentials for comparison. Vanoxerine blocked hERG (IC50: 10.3 ± 1.3 nM (95% CI: 7.8-13.4)) and hCav1.2 (12.2 ± 2.0 nM (95%CI: 7.5-17.1)) currents with comparable potencies. Peak and late hNav1.5 currents were blocked at 28.9 ± 2.9 nM (95%CI: 22.5-37.1) and 92.7 ± 9.4 nM (95%CI: 72.5-117.2) respectively. Both vanoxerine and dofetilide a selective hERG blocker, prolonged the ventricular action potential. Unlike dofetilide vanoxerine did not produce arrhythmias experimentally or in simulations at concentrations up to 100 nM. Our results provide a quantitative description of how vanoxerine's MICE properties overcome its hERG liability and validate the regulatory CIPA initiative.

ABSTRACT FINAL ID: 2545 Poster Board -207
TITLE: Influence of Simvastatin Nanocarriers on Hypercholesterolemia-Induced Eryptosis

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KEYWORDS: Hypercholesterolemia, Eryptosis, Simvastatin-Nanocarriers

ABSTRACT BODY: PURPOSE: In hypercholesterolemia erythrocytes become cholesterol enriched, and oxidatively modified, this accelerate eryptosis, and foam cells formation. Simvastatin was decreased oxidative stress and apoptosis. Nanocarriers may increase therapeutic effect of simvastatin. The objective of this study was to determine whether simvastatin or simvastatin nanoparticles can modulate simvastatin erythrocytes level and eryptosis. Methods: Simvastatin nanoparticles were prepared. Erythrocytes were isolated and divided into 4 groups as follows: Group1: erythrocytes served as control, they were suspended in plasma. Group 2: hypercholesterolemia, in which erythrocytes were incubated with cholesterol enriched plasma. Group 3: hypercholesterolemia treat with simvastatin in which erythrocytes were incubated in cholesterol enriched plasma plus simvastatic acid. Group 4: hypercholesterolemia treat with nanoparticles in which erythrocytes were incubated in cholesterol enriched plasma plus simvastatin solid lipid nanoparticles. Afterward, samples were subjected to the following analysis. A- Simvastatin uptake was assessed by HPLC methods. B- Cholesterol inclusion was measured by cholesterol oxidase method. C-Eryptosis was assessed using flow cytometry analysis. Results: 1-Incubation of erythrocytes with cholesterol enriched plasma resulted in a significant increase of cholesterol inclusion, hemolysis and eryptosis compared to control. 2- Incubation of erythrocytes with cholesterol rich plasma in the presence of simvastatin solid lipid nanoparticles or simvastatin decrease cholesterol inclusion, hemolysis and eryptosis. 3- Effect of simvastatin nanoparticles on the measured parameters was more pronounced than simvastatin. Conclusions: Simvastatin nanoparticles increase simvastatin accumulation into erythrocytes and decreased cholesterol inclusion compared to simvastatin solution. Therefore , they decreased eryptosis and foam cells and atherogenic risk Oxi-RBCs. Acknowledgment: The authors acknowledge NSTIP No-(12-MED2563-02) in the KSA.

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ABSTRACT FINAL ID: 2546 Poster Board -208
TITLE: Work-Loop Assay: A Novel Cardiac Contractility Assay Predicts the Adverse Cardiac Effects of Checkpoint Kinase Inhibitors

AUTHORS (FIRST INITIAL, LAST NAME): M. Gharanei[2, 1], R. Wallis[1], M. A. Babba[1], A. Varcianna[2], H. L. Maddock[2, 1]
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KEYWORDS: Cardiotoxicity, Cardiac Contractility, Safety Pharmacology

ABSTRACT BODY: Adverse effect of drugs on the cardiovascular system (CVS) is a major cause for compounds failing in both non-clinical and clinical studies. Although adverse drug effects on the CVS may be due to many effects, one area of great concern is the effect of drugs on the force of contraction of the heart. This is of particular importance in oncology in which a number of effective therapeutics have adverse effects on cardiac function in man. One class of compounds aimed at enhancing the efficacy of both conventional chemotherapy and radiotherapy in the treatment of cancer include checkpoint kinase inhibitors (Chk). Recently AZD7762 and SCH90076 failed in clinical trials due to adverse CVS related effects. Thus, there is a need for new non-clinical assays to better characterise cardiac effects of oncology products prior to clinical trials. We tested a range of Chk inhibitors to investigate whether a new assay - the rat myocardial work-loop assay had the potential to provide a more predictive model of heart muscle dynamics. Methods: Rat papillary muscles were isolated and superfused with modified cardiac ringer in an organ bath and gassed. Muscle length and stimulation amplitude was optimised to develop maximum isometric developed force. Active work-loops were undertaken and measured in which net power outputs were calculated. Muscles were allowed to stabilise and perfused in the absence or presence of Chk inhibitors, AZD7762 (1µM), SCH90076 (5µM), Chir-124 (1.5µM) and PF477736 (10µM) or vehicle controls (n=3-4). Results: The work-loop assay predicted the relative negative inotropic effects and also the rank order of potency for the Chk inhibitors. Conclusion: This is the first study to demonstrate the utility of work-loop assay to predict cardiotoxicity hazard identification for Chk inhibitors and provide insight into the intricate mechanisms implicated in contractility related cardiotoxicity.

ABSTRACT FINAL ID: 2547 Poster Board -209
TITLE: Evaluation of a Digital Implantable Telemetry in Multiple Social Housing Paradigms for Cynomolgus Monkey

AUTHORS (FIRST INITIAL, LAST NAME): S. D. Tichenor[1], D. Regalia[1], H. Holzgrefe[1], B. Lilly[1], A. Wilcox[2], R. A. Kaiser[1]
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KEYWORDS: Telemetry, Safety Pharmacology, Social Housing

ABSTRACT BODY: Proactive efforts to socialize laboratory animals are a contemporary initiative for enhancing animal welfare. Telemetry-implanted cynomolgus monkeys have been traditionally considered exclusionary criteria for socialization during repeat-dose or standalone safety pharmacology toxicology studies with safety pharmacology endpoints. Our objective was to evaluate the effect of implementing cynomolgus monkeys implanted with a digital L-11 PhysioTel device (Data Sciences International) in different housing paradigms. Six animals were randomized and rotated (control for time bias) into three different housing conditions (standard individual [SI], quad-paired caging [QP], and European-compliant paired caged [EU]) and dosed on consecutive days with sterile water and subsequently with 100 mg/kg moxifloxacin. Cardiovascular parameters were recorded for 24 hours on each occasion using PhysioTel-Digital and hematology parameters were measured. All animals demonstrated diurnal rhythms in heart rate (HR) and blood pressure (BP) parameters, indicating similar acclimation in each type of caging. HR indicated variation that was dependent on housing type (resting HR for SI =97-106 BPM; QP=117-121 BPM; EU=125-132 BPM) with a similar trend in mean BP (MAP, SI= ~70 mmHg; QP and EU=~78 mmHg). Leukocyte profiles did not indicate differences in stress responses between housing conditions (total white cell counts were within 10% regardless of housing condition). Moxifloxacin administration resulted in an anticipated 20 ms prolongation in individually rate corrected QT (QTca) with no notable differences between

housing conditions. These data support the sensitivity of PhysioTel digital to detect measurable pharmacological and/or physiological changes in the ECG and hemodynamic parameters. Interestingly, SI was associated with slightly lower HR and MAP compared to social housing but no notable hematological changes and no difference in the sensitivity to detect hemodynamic or ECG interval changes (namely QTca).

ABSTRACT FINAL ID: 2548 Poster Board -210
TITLE: Characterization of the Sensitivity and Distribution of Potential Rate-Dependent T-Wave Detection Bias in Telemetered Dogs

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KEYWORDS: ECG Analysis, ECG Telemetry, ECG Intervals

ABSTRACT BODY: Integration of telemetered electrocardiographic assessments in toxicology studies is an emerging trend which can offer detailed information on potential cardiovascular safety issues that are only identified with repeat dosing. Ponemah automated ECG acquisition and analysis (Data Sciences International, St. Paul, Minn.) is an enabling technology for which intrinsic heart rate-dependent T-wave detection sensitivity has not been previously evaluated in the dog, a species with pronounced sinus arrhythmia and beat-to-beat heart rate variability. Accordingly, beagle dogs (n=8, 10±1.2 kg) were instrumented with jacketed external telemetry (JET) devices. Lead II ECGs (24 h) were continuously digitized (500 Hz) in a quiet radio frequency environment and retrospectively analyzed in beat-to-beat increments (Ponemah software, ver. 5.2, DSI). For the current cohort, 729,947 detected beats were analyzed (mean RR 620 ms, mean beat-to-beat variability 358 ms). All RR intervals were grouped in 10 ms bins and T-wave detection efficiency characterized as the T-wave % (T%) expressed as the proportion of total waves (TW; detected R waves) for all bins with ≥ 200 beats. The associated RR range and distribution (210-1640 ms) reflected the typical sinus arrhythmia in the dog. Data are presented as mean±std. Over the stated RR range, T-wave detection efficiency remained extremely uniform (93.8±1.8%) where the T%-TW linear regression yielded a slope of 0.0007 and associated r2 of 0.022 confirming the lack of any rate-dependent T-wave detection bias over the physiologically normal range of heart rates in this species. These data confirm the absence of any rate-dependent T-wave detection bias with the Ponemah ECG algorithm and confirm its suitability for use in the presence of pronounced sinus arrhythmia and associated QT hysteresis.

ABSTRACT FINAL ID: 2549 Poster Board -211
TITLE: Low-Dose Intravenous Opioid Toxicity in Gottingen Minipigs

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KEYWORDS: Opioids, Minipig, Fentanyl

ABSTRACT BODY: Fentanyl is utilized as an adjuvant in surgeries at a recommended dose of 50µg/kg intravenously (IV). The current study was undertaken to determine if fentanyl given at this therapeutic dose can result in an increase in cardiac troponin I (CTnI). Duel-lumen cannulas were surgically implanted into the external jugular vein of young adult male Gottingen minipigs. After a 1 week recovery baseline (BL) whole blood samples were collected through one lumen of the jugular cannula. CTnI was quantified using CTnI diagnostic cartridges and an iStat analyzer. iStat CTnI cartridges are often fielded in emergency rooms to assist in the diagnosis of cardiac injury. Because no BL CTnI values are available for patients, the 99th percentile value (0.08) is used as an indicator of cardiac injury. In our study, the mean BL CTnI value (n=4) was 0.0125 ng/dL ±0.0025 SEM. Fentanyl citrate was purchased from a commercially available source and solubilized in saline. Unanaesthetized animals were dosed IV with 50 µg/kg given as a single bolus into the 2nd lumen of the cannula. Serial blood samples were collected at various time points following dosing. All animals dosed (n=4) had peak CTnI values above the 99th percentile. Mean peak CTnI was 13.865ng/dL ±10.06 SEM (range 2.09 - 43.87 ng/dL). In order to determine if the

increase in cardiac troponin was due to the direct injection of the fentanyl into the superior vena cava, via the external jugular cannula, a subset of animals were injected with the same dose via the marginal ear vein. While the mean peak CTnI values (2.882 ±1.190 ng/dL) in these animals was not as elevated as that seen when the dose was given via the jugular cannula, all of these pigs had CTnI values above their own BL values. Three out of 4 pigs had values above the 99th percentile. Finally, in order to determine if the elevation in CTnI was the result of receptor mediated binding, a subset of minipigs were pretreated with the opioid receptor antagonist naloxone (0.5mg/kg IV) prior to fentanyl dosing. None of these animals showed an increase in CTnI above the 99th percentile.

ABSTRACT FINAL ID: 2550 Poster Board -212
TITLE: Cigarette Smoke Inhibits Nitric Oxide-Dependent Dilation in Mouse Mesenteric Arterioles

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KEYWORDS: Cigarette Smoke, Vasodilation, Nitric Oxide

ABSTRACT BODY: Cigarette smoking is a major independent risk factor for cardiovascular disease. Endothelial dysfunction, as measured by brachial flow mediated dilation (FMD), precedes the development of many cardiovascular diseases (CVD) in smokers. FMD is impaired not only in smokers with CVD, but also in young, healthy smokers. The goal of this project was to elucidate the mechanism by which cigarette smoke (CS) impairs FMD. We hypothesized that there would be a loss of nitric oxide (NO)-dependent vasodilation when stimulated by either acetylcholine (ACh) or increases in flow in CS-exposed mice compared to filtered air-exposed mice. Methods. Four month old C57BL/6 mice were exposed to mainstream CS (~300 TPM/m3) or filtered air for 5 days (n=7/group). First order mesenteric vessels were isolated and cleaned of connective tissue and fat. All vessels were cannulated and pressurized to 60mmHg (ACh) or 75mmHg (FMD). Vessels were pre-constricted to 55% of basal diameter with U46619 (a thromboxane analog) and then dilated with ACh (10-9 – 10-4M) ± LNNA (NO synthase inhibitor) or stepwise increases in pressure (0-40mmHg) resulting in increases in flow ± LNNA. Results. CS-exposure resulted in impaired ACh-mediated dilation (area under the curve (AUC): air: 256±24; CS: 184±16, $p<0.05$), which was, in part, due to loss of NO (% NO-dependent dilation: air: 24±6%; CS: 8±3%, $p<0.05$) Similarly, FMD was also impaired in CS-exposed mice (AUC: air: 815±152; CS: 371±56, $p<0.05$) due to loss of NO (% NO-dependent dilation: air-exposed, 22±6%; CS-exposed, 6±2%, $p<0.05$). Conclusions. These data suggest that CS impairs ACh-dilation and FMD by decreasing NO-bioavailability in mice. This may represent a therapeutic target to prevent or delay onset of CVD in smokers.

ABSTRACT FINAL ID: 2551 Poster Board -213
TITLE: Repeat Inhalation Exposure to Traffic-Derived Particles Exacerbates Ischemic, Left Ventricular Hemodynamic, Sympathetic, and Arrhythmic Responses to Psychosocial Stress and Decreases Contractility Index in Conscious Rats

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KEYWORDS: Particulate Matter, Heart Failure, Cardiovascular

ABSTRACT BODY: Vehicular traffic is a major source of fine particulate matter ($PM_{2.5}$), a ubiquitous air pollutant associated with increased cardiovascular morbidity and mortality. Inhalation of traffic-derived $PM_{2.5}$ (TPM) might exert cardiotoxicity by increasing sympathetic neural influence over cardiovascular function, but this mechanism remains unconfirmed and the dose-effect relationship poorly understood. Psychological stress is a common trigger of adverse cardiac events that acutely increases sympathetic regulation and may compound or unmask the toxicity of TPM exposure. We chronically implanted rats with left ventricular (LV) pressure catheters to test whether a repeat 16-day exposure to TPM (250 $\mu g/m^3$, 5 h/day, 4 d/wk, 4 wk) would alter LV performance, heart rate (HR), heart rate variability (HRV), and electrocardiogram (ECG) during exposure and during two acute 20-min psychosocial stress relative to filtered air-exposed rats (n=4/group). During stress

after 4 days of exposure, TPM caused ST interval depression, possibly suggesting ischemia, and increased inotropic, lusitropic, hemodynamic, and HR responses, suggesting enhanced sympathetic regulation. Immediately before exposure on day 16, TPM-exposed rats had decreased contractility index relative to air-exposed rats. One day later, TPM caused ST depression and greater arrhythmias and declines in time-domain HRV parameters during social stress relative to air exposure. Our findings suggest repeat exposure to traffic-derived PM initially increases LV hemodynamic responses to psychosocial stress, and eventually decreases LV performance while exacerbating arrhythmic and sympathetic responses to stress. These findings will guide deeper mechanistic investigations into how repeat PM exposure may potentiate stress-induced arrhythmia and precipitate heart failure.

ABSTRACT FINAL ID: 2552 Poster Board -214
TITLE: Hypoxia-Induced Pulmonary Arterial Hypertension Augments Ozone Lung Injury and Airway Reactivity

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KEYWORDS: Pulmonary Arterial Hypertension, Ozone, Hypoxia

ABSTRACT BODY: BACKGROUND: With the recent EPA proposal to implement stricter ozone (O3) standards, O3-related health effects are a growing public concern. Pre-existing respiratory diseases are likely to be exacerbated by inhaled O3. In patients with chronic obstructive pulmonary disease (COPD), hypoxia-associated pulmonary hypertension (HPH) is a frequent comorbidity that is difficult to treat clinically yet associated with increased mortality and frequency of exacerbations. In this study, we hypothesized that established HPH would confer vulnerability to acute O3 pulmonary toxicity. METHODS: O3 effects were assessed after HPH was established. C57BL/6 mice were randomly assigned to 4 different treatment groups: normoxia and filtered air (Nx,FA), hypoxia and filtered air (Hx,FA), normoxia and O3 (Nx, O3), and hypoxia and O3 (Hx,O3). Mice were exposed to 1 ppm O3 or FA for 4 h immediately following 3 wks of continuous hypoxia (10% O2) or normoxia. Outcomes including lung weight/body weight (LW/BW), right ventricle/left ventricle-septum (RV/LVS), lung resistance and compliance during methacholine challenge, lung water and dry lung weights, bronchoalveolar lavage fluid (BALF), BALF total protein, and lung water/dry lung ratio were evaluated. All statistics were computed using a 2-way ANOVA and statistical significance was determined at $P \leq 0.05$. RESULTS: Hypoxia alone significantly increased RV/LVS, LW/BW, and lung water and dry lung weight. O3 exposure alone significantly increased LW/BW, lung water and dry lung weights, lung water/dry lung ratio, lung resistance, BALF cell counts, and total BALF protein. However, combined O3 and Hx resulted in the greatest response and significant interactions in terms of LW/BW, lung resistance, lung water and dry lung weights and BALF cell counts. CONCLUSIONS: These results suggest that exposure to O3 in an HPH model induces an exacerbated pulmonary toxic response compared to hypoxia or O3 exposure, alone. Further research is needed to decipher the mechanism behind this interaction.

ABSTRACT FINAL ID: 2553 Poster Board -215
TITLE: Activation of Neuronal Irritant Receptors and Respiratory Irritation Responses by Electronic Cigarette Vapor and Flavor Constituents

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KEYWORDS: Electronic Cigarettes, Flavoring Agents, Irritation

ABSTRACT BODY: The physiological effects and potential toxicities of flavorants in traditional tobacco products and electronic cigarettes remain poorly understood. Among electronic cigarette users minty (menthol, carvone), fruity (linalool) and cinnamon (cinnamaldehyde) flavors are highly popular. Users of cinnamon flavored electronic cigarette fluids reported adverse respiratory effects and burning sensations, raising concern about irritant effects and toxicity of some flavorants

when inhaled. The effects of dilutions of flavored electronic cigarette fluids on the cloned human sensory irritant receptors, TRPV1 and TRPA1, were examined by fluorescent calcium imaging in HEK293 cells. Cinnamon flavored solutions strongly activated TRPA1, even at high dilution factors. Carvone- and methyl salicylate-containing solutions, activated both TRPV1 and TRPA1 receptors. These effects occurred also with nicotine-free flavored solutions. Minty solutions also activated the cold/menthol receptor, TRPM8, known to suppress respiratory irritation. In mice, plethysmography experiments showed that electronic cigarette vapors activate respiratory irritation responses, characterized by increases in the time of breaking and lowered respiratory rates. Vaporized polyethylene glycol, one of the major solvent constituents of electronic cigarette liquid, also produced respiratory irritation in mice. Taken together, these data show that flavorants in electronic cigarette fluids activate sensory irritant receptors *in vitro* and respiratory irritation *in vivo*. Smokers switching to electronic cigarettes likely prefer high concentrations of flavorants since their effects mimic the sensory impact of irritants in tobacco smoke. However, while flavorants are generally recognized as safe for oral consumption, inhalation of flavorants may promote chronic irritation, inflammation and exert toxic effects.

ABSTRACT FINAL ID: 2554 Poster Board -216
TITLE: Subchronic Inhalation Toxicity Study of Polyhexamethyleneguanidine Phosphate in Sprague-Dawley Rats

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KEYWORDS: Biocide, Inhalation, Lung

ABSTRACT BODY: Potential possibility exposed to polyhexamethyleneguanidine phosphate (PHMG-P) via inhalation was increased because of its wide application into various household chemical products even including the humidifier disinfectant in Korea. In recent, it was reported adverse health effects like fatal pulmonary disease. In this study, 90-days repeated inhalation toxicological effect was evaluated in compliance with GLP guideline. Generated PHMG-P particles were exposed to rats using whole-body inhalation chamber. Eight-week-old Sprague-Dalwey rats (control; fresh-air, low-dose; 30 μg/m3, middle-dose; 90 μg/m3 and high-dose; 300 μg/m3) were exposed to PHMG-P particles for 6 hr/day, 5 day/week, and for 13 weeks. Observation on mortality, clinical signs, body weight and food consumption was performed during exposure periods. Clinical pathology analysis, organ weight measurement and macro and microscopic examination were also conducted. Biochemical analyses in BAL fluid were further conducted. Found dead animals of high-dose group were directly affected by repeated inhalation exposure of PHMG-P particles. Both sexes of high-dose group showed significant decrease in body weight and food consumption. Toxicological effects like pulmonary fibrosis, alveolar emphysema, exudate and etc. were observed in lungs of middle and high dose group, and these changes were related with the increase of lung weight and ballooning or focus/foci observed from macroscopic examination. PHMG-P related changes were also observed in nasal cavity, larynx and trachea. Pulmonary inflammation or injury biomarker levels in BAL fluid were significantly changed in high-dose group. It was concluded that respiratory tract was adversely affected via inhalation exposure of PHMG-P, and NOAEL (no observed adverse effect level) is considered to be 30 μg/m3 at both sexes of rats under this study condition.

ABSTRACT FINAL ID: 2555 Poster Board -217
TITLE: Investigation of Acute Pulmonary Deficits Associated with Biomass Fuel Cookstove Emissions in Rural Bangladesh

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KEYWORDS: Biomass, Particulate Air Pollution, Pulmonary Function

ABSTRACT BODY: The use of solid biomass fuels in cookstoves has been associated with long term chronic health impacts that disproportionately affect women worldwide. The immediate effects on acute pulmonary function, however, have not

been investigated. This study measured the respiratory impact of exposure to biomass fuel emissions for 12 females within the age range 18-65 in rural Bangladesh. Solid fuel stoves that use wood, litterfall, and cow dung are common in rural Bangladesh and cooking is done in a separate room from the main house. Pulmonary function was measured with spirometry before and during cooking to assess changes in respiratory function associated with exposure to cookstove emissions. Specifically, the forced expiratory volume in one second (FEV1), forced vital capacity (FVC), FEV1 over FVC ratio, and peak expiratory flow (PEF) were compared. Cookstove emissions were characterized using continuous measurements of particulate matter (PM) concentrations at a 1 second time resolution. Exposure metrics were calculated from the measured PM data to estimate the acute exposure to cookstove emissions for each study participant. The exposure estimates include the 3-minute maximum, 30-minute average, and the time weighted average PM concentration for the cooking time period. Litterfall (i.e., leaves and twigs) and household material waste (i.e., paper and plastic wrappers) emissions have the highest 3-minute maximum PM concentration. Larger or open cooking areas have lower PM concentrations due to increased ventilation. Participants that have been preparing meals for more than 20 years in enclosed cooking areas with elevated PM concentrations displayed lower FEV1/FVC ratios, consistent with an enhanced risk of chronic obstructive pulmonary disease (COPD). Preliminary results also indicate that PEF decreased during cooking for 58% of the participants, and FEV1/FVC decreased during cooking for 67% of respondents.

ABSTRACT FINAL ID: 2556 Poster Board -218
TITLE: Subchronic Exposure to PM2.5 Induces Aldosterone System Response and Metabolic Cardiac Fetal Gene Reprogramming

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KEYWORDS: Particulate Matter, Cardiopulmonary Damage, Aldosterone

ABSTRACT BODY: Exposure to particulate matter with an aerodynamic diameter ≤ 2.5μm (PM2.5) has been associated with morbi-mortality of cardiopulmonary diseases. During the pathological state of cardiopulmonary diseases a localized over-activation of the renin-angiotensin-aldosterone system (RAAS) and a "maladaptative" heart response has also been reported. We tested the hypothesis that subchronic PM2.5 exposure induces an aldosterone response from the RAAS as well as a consequent metabolic cardiac fetal gene reprogramming; since previously we reported the induction of angiotensin receptor type-1 from PM2.5 exposure. Male Sprague-Dawley rats were exposed to PM2.5 and filtered air (FA), for 5 h/d, 4 d/wk for 8 wk. We evaluated: arterial blood pressure, aldosterone synthase (CYP11B2) and subcellular distribution of the mineralocorticoid receptor (MR) protein levels in lung and heart and serum aldosterone at the end of the exposure. mRNA and protein levels of GLUT-4 and PPAR-α as markers of metabolic cardiac fetal reprogramming were also evaluated. We observed that PM2.5 exposure increased diastolic and mean blood pressure. Also, the exposure induced an increment in CYP11B2 levels in the lung but not in heart, which probably contributed to the observed increase in serum aldosterone. The MR subcellular distribution indicated an increment in the nuclear fraction in the lung and heart, with respect to the cytoplasmic fraction. An increment in metabolic cardiac fetal reprogramming GLUT-4 and PPAR-α mRNA were observed, although protein levels were lower compared to FA. Our results indicate that subchronic PM2.5 exposure induced an increase in arterial blood pressure, the induction of aldosterone response mediated by CYP11B2 and MR, which could consequently contributed to the metabolic cardiac fetal gene reprogramming. Our results support that the exposure to PM2.5 promotes aldosterone response involving RAAS, a mechanism that may be related to cardiovascular damage.
(Funding: Conacyt 167778).

ABSTRACT FINAL ID: 2557 Poster Board -219
TITLE: A Comparison of Respiratory Minute Volume of Nonhuman Primates When Using Different Restraint Systems

AUTHORS (FIRST INITIAL, LAST NAME): A. Viau[1], M. Freke[1], C. Copeman[1]
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KEYWORDS: Respiratory Minute Volume, Restraint System, NHP

ABSTRACT BODY: Respiratory minute volume of Non human Primates can be measured in conscious animals and is used to calculate the achieved dose on inhalation studies. Non human primates need to be restrained during dose administration by the inhalation route in order to allow the delivery system to remain well positioned on the animal to inhale the aerosol at a determined concentration for the given period of time. This poster will describe the different restraint systems used along with a comparative of the respiratory minute volume obtained under these different restraint procedures. Respiratory minute volume ranged from 1.20 to 2.86 L/min in Non human primates when restrained on a sling, and ranged from 1.41 to 4.86 L/min when restrained on a chair. Both restraint systems have been routinely used on inhalation studies and results demonstrate slightly higher respiratory minute volumes for animals restrained in a chair compared to those on sling. The difference in the position of the animal and the extent of chest compression experienced for each of the restraint devices may contribute to these differences. This emphasizes the importance of maintaining a consistent restraining approach when assessing respiratory minute volumes to calculate achieved doses.

ABSTRACT FINAL ID: 2558 Poster Board -220
TITLE: Cisplatin-Induced Acute Kidney Injury Potentiates in Sprague-Dawley Rats by Pretreatment with Thioacetamide

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KEYWORDS: Cisplatin, Acute Kidney Injury, Hepatotoxicant

ABSTRACT BODY: Cisplatin (CDDP)-mediated acute kidney injury (AKI) was compared in rats after treatment with or without thioacetamide. Tioacetamide (150 mg/kg) was administered orally to Sprague-Dawley male rats for 3 days prior to cisplatin (5 mg/kg, i.p.) treatment. All animals were sacrificed 3 days after the last treatment and various urine or blood biomarkers were measured. No significant changes in serum and urinary biomarkers were observed in cisplatin-treated group. However, significant increases in serum ALT and AST levels as well as BUN and creatinine levels were observed in thioacetamide-treated groups. In addition, urinary levels of Kim-1, NAGL, HMGB1, and Netrin-1 proteins were markedly increased in the cisplatin-treated group. These adverse effects were potentiated in cisplatin-treated group by pretreatment with thioacetamide. In addition, thioacetamide pretreatment led to a significant increase in the urinary levels of 3-indoxyl sulfate (3-IS), early biomarker for renal toxicity. Furthermore, histopathological examinations indicated that pretreatment of thioacetamide had a severe renal toxicity against cisplatin-induced proximal tubular damage. From these results, cisplatin-induced nephrotoxicity was slight, while its nephrotoxicity was more potentiated in hepatic dysfunction.

ABSTRACT FINAL ID: 2559 Poster Board -221
TITLE: Nonalcoholic Fatty Liver Disease Potentiates Disinfection Byproduct-Induced Proximal Tubular Immunotoxicity by Mesangial Cell-Mediated Suppression of NKT Cells

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KEYWORDS: NAFLD, Mesangial Cells, NKT

ABSTRACT BODY: Obesity and nonalcoholic fatty liver disease (NAFLD) are associated with the development and progression of chronic kidney disease (CKD). We recently showed that obesity and NAFLD induce CYP2E1 and potentiate bromodichloromethane (BDCM), a disinfection byproduct of drinking water, induced hepatotoxicity. The present study examined the effects of BDCM hepatotoxicity in the presence of NAFLD on CYP2E1 mediated oxidative stress and renal mesangial cell activation. Mice were fed a high fat diet for 12 weeks to induce obesity with NAFLD. Then mice were exposed to BDCM by intraperitoneal injection for 4 weeks. NAFLD+BDCM increased CYP2E1-mediated lipid peroxidation in proximal

tubular cells compared to mice with NAFLD alone or BDCM treatment alone. The increased lipid peroxidation caused mesangial cell activation as evidenced by increased a-SMA immunoreactivity, which was blocked by the CYP2E1 inhibitor diallyl sulphide (DAS). In addition, CYP2E1-induced mesangial cell activation increased release of pro-inflammatory mediators IL-1b, IFN-g, TNF-a, osteopontin, PDGF-2 and CTLA4 at the mRNA and protein levels, but decreased NK1.1 mRNA levels in the kidney. Mice lacking NK cells but not NKT cells revealed decreased production of pro-inflammatory mediators while mice lacking NKT cells (CD1d KO) showed elevated (>10-fold) mesangial cell activation and pro-inflammatory mediator release in the kidney. Finally, CD1D KO mice treated with NAFLD+BDCM exhibited increased HMGB1 and FasL levels, and TUNEL positive nuclei indicating higher cell death. In summary, mesangial cell-NKT cell crosstalk plays a major role in renal inflammation induced by BDCM in the presence of NAFLD. NIH R00ES019875 to S.C.

ABSTRACT FINAL ID: 2560 Poster Board -222
TITLE: Erythrophagocytosis of Lead-Exposed Erythrocytes by Renal Tubular Cells May Contribute to Lead-Induced Nephrotoxicity

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KEYWORDS: Lead, Nephrotoxicity

ABSTRACT BODY: Nephrotoxicity associated with lead poisoning has been frequently reported in epidemiological studies; but the underlying mechanisms have not been fully elucidated. We examined the role of erythrocytes, one of the major bodily lead reservoirs, in lead-associated nephrotoxicity. Co-incubation of lead-exposed erythrocytes with renal proximal tubular cells (HK-2) significantly potentiated renal tubular cytotoxicity, reflecting a role of erythrocytes in lead-induced nephrotoxicity. Morphological and flow cytometric analysis revealed that HK-2 actively phagocytized lead-exposed erythrocytes, mediated by phosphatidylserine (PS) externalization on erythrocyte membrane and generation of PS-bearing microvesicles. Increased oxidative stress and expression of nephrotoxic biomarkers such as NGAL or KIM-1 were observed in HK-2 cells undergoing erythrophagocytosis. Moreover, TGF-β, a marker of fibrosis, was significantly up-regulated in HK-2 cells by erythrophagocytosis. The significance of erythrophagocytosis in lead-induced nephrotoxicity was examined *in vivo* in rats exposed to lead via drinking water for 12 weeks. Increased iron deposition and generation of oxidative stress in renal tissues were found in lead-exposed rats, which matched well the histo-pathological alteration, fibrosis and expression of KIM-1, NGAL and TGF- β. Our data strongly suggest that erythrophagocytosis and iron deposition could significantly enhance nephrotoxicity following lead exposure, shedding a new light on the understanding of lead-associated kidney damage.

ABSTRACT FINAL ID: 2561 Poster Board -223
TITLE: Symmetric Dimethylarginine (SDMA) Increases Earlier Than Serum Creatinine in Dogs and Cats with Chronic Kidney Disease (CKD)

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KEYWORDS: SDMA, LCMS, Kidney

ABSTRACT BODY: SDMA is a dimethylated derivative of arginine resulting from posttranslational methylation and subsequent metabolism of proteins. It is eliminated primarily by renal clearance; therefore, concentrations of SDMA are correlated to glomerular filtration rate (GFR). In humans, SDMA has been shown to be a useful biomarker for diagnosing CKD with better sensitivity and specificity compared with serum creatinine. The aim of the current study was to develop an LC/MS assay to assess the utility of SDMA as a biomarker for CKD in dogs and cats. A highly sensitive and specific LC/MS method was developed and validated per FDA and CLIA guidelines. Validation included establishing sensitivity, interferences, recovery, linearity, accuracy and precision. All metrics were within established guidelines. A nine point

standard curve demonstrated linearity with R2>0.99; intra and inter day CV were found to be <3%. Lower limit of quantitation (LLOQ) was found to be 1.56 µg/dL. No significant interference from hemolysis, lipemia or icterus was observed and related compounds such as arginine, MMA and ADMA had no significant impact on performance. In a retrospective study, banked serum samples from 10 dogs and 21 cats with CKD were analyzed for SDMA and creatinine concentrations. In all dogs, SDMA increased above the reference limit of 14 µg/dL before creatinine increased above its reference limit of 1.6 mg/dL. In dogs, this occurred at mean of 17 months (range 11 to 26 months) before creatinine increased. Cats were found to have the same upper SDMA reference limit as dogs and they also showed an earlier increase in SDMA (mean, 14.6 months; range, 40 days to 4 years). In both species the upper reference limit for SDMA represents an approximate 25 to 40% reduction in GFR. Our results suggest that serum SDMA is indeed an earlier biomarker than serum creatinine for diagnosing and monitoring CKD in dogs and cats.

ABSTRACT FINAL ID: 2562 Poster Board -224
TITLE: A Fully Automated Platform for the Efficient and Accurate Prediction of Nephrotoxicity

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KEYWORDS: Drug-Induced Nephrotoxicity, Predictive *In Vitro* Model, High Throughput

ABSTRACT BODY: The kidney is a major target for drug-induced toxicity. Due to the limited predictability of current pre-clinical models nephrotoxicity is typically detected only late during drug development. Recently, we have developed the first *in vitro* model that predicts nephrotoxicity in humans with high accuracy (Li et al., *Toxicol. Res.*, 2013; Li et al., *Mol. Pharm.*, 2014). This model employed human primary renal proximal tubular cells (HPTC) or stem cell-derived HPTC-like cells and endpoint was increased expression of interleukin (IL)6 and/or IL8 detected by qPCR. However, the throughput of this model was low and it was not suitable for the screening of large compound libraries. In order to solve this problem we developed a fully automated screening platform. By using high content screening and HPTC we screened a library of 43 compounds with well-characterized effects on human kidneys. Subsequent automated data analysis was performed with the cellXpress platform (Laksameethanasan et al., *BMC Bioinformatics*, 2013) and 44 cellular features were assessed. The values obtained with respect to sensitivity, specificity and balanced accuracy were 90%, 87% and 88%, respectively. After determining the predictive performance in a retrospective study with well-characterized compounds we screened a library that consisted of a novel class of antimicrobial compounds. Based on the result we would predict that this novel class of antimicrobials is nephrotoxic in humans. All of the individual compounds exhibited toxic effects on HPTC, although to variable extents. Therefore, it would be recommendable to develop applications with these antimicrobials that would not involve systemic exposure. Together, our results show that our fully automated HCS platform allows the accurate prediction of nephrotoxicity in humans in a highly efficient manner at an early stage of compound development.

ABSTRACT FINAL ID: 2563 Poster Board -225
TITLE: Unraveling the Mechanism of Propiverine-Induced DAAO Accumulation in Rat Kidney: Manipulation of DAAO Trafficking *In Vitro*

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KEYWORDS: Protein Trafficking, DAAO, Site-Directed Mutagenesis

ABSTRACT BODY: Abnormal protein aggregation and aberrant protein localization can change cell physiology and contribute to the pathogenesis of diverse forms of cancer and human disorders. Chemical-induced mislocalization and accumulation of a non-disease-specific protein in rat was reported for the peroxisomal enzyme D amino acid oxidase (DAAO) following treatment with various pharmaceuticals e.g. Propiverine, a common medication for urinary incontinence. Propiverine leads to cytosolic and nuclear aggregation of DAAO in rat kidney by a so far unknown mechanism. Since we

observed a loss of peroxisomal localization of rat DAAO (rDAAO) after Propiverine administration *in vivo*, we hypothesized that the drug may interfere with the cellular protein trafficking machinery involved in DAAO transport and subcellular targeting. Thus, we manipulated DAAO trafficking in HEK293 cells by mutating specific signal sequences of EYFP-rDAAO via site-directed mutagenesis. We observed that deletion of the peroxisomal targeting signal 1 (PTS1) completely prevents peroxisomal transport leading to a diffuse cytosolic distribution while concomitantly resulting in an extensive nuclear mislocalization of rDAAO. A similar effect was observed after insertion of a prototypical nuclear localization signal (NLS) into rDAAO. These findings highly suggest the presence of a currently unknown active nuclear import mechanism specific for rDAAO despite the inherent absence of a classic NLS. Indeed, we identified a potential nuclear translocation signal (NTS) variant in rDAAO, which was previously shown to be involved in stimulus-dependent nuclear protein import in CHO, COS7 and HeLa cells. Consequently, the nucleo-cytoplasmic trafficking of rDAAO was evaluated by mutating this NTS sequence. In conclusion, the present data suggest that Propiverine may intervene with the processes underlying normal peroxisomal targeting and/or nuclear import of rDAAO.

ABSTRACT FINAL ID: 2564 Poster Board -226
TITLE: Cytoplasmic pH As an Early Screen for Renal Cytotoxicity

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KEYWORDS: Kidney, Cytotoxicity, Intracellular pH

ABSTRACT BODY: Alpha-7 agonists are attractive targets for the management of schizophrenia but one compound (BMS-863736) showed renal proximal tubular degeneration and necrosis along with acute drug-induced renal failure (DIRF) after a single dose in rats and dogs. *In vitro*, several molecules also showed hepatic cytotoxicity paralleling renal toxicity *in vivo*. Two compounds: BMS-863736 (Pka1 = 3.60, pKa2 = 8.10) and BMS-870838 (pKa1 = 5.80, pKa2 = 8.50) showed CC50 values of 30 µM and ≥ 200 µM respectively. The mechanistic basis of the cytotoxicity was hypothesized as secondary to subcellular organelle disruption due to amphipathicity in this chemotype. Because subcellular organelle toxicity often involves pH gradients within the cell, we postulated that pH homeostasis was disrupted. If true, observing pH changes may allow screening to identify problematic molecules. Cytosolic pH was evaluated using the human renal proximal tubule cell line HK-2 and canine primary proximal tubule cells. BMS-863736 (10 µM) caused immediate cytosolic pH decreases (maximal change at 50 µM) sustained for least 20 minutes in both cell types as the concentration approached cytotoxic concentrations. BMS-870838 did not cause noticeable changes in the cytosolic pH at physiologically relevant concentrations in either cell type. Eight commercially available standards with known renal effects along with BMS compounds BMS-863736, BMS-824430 and BMS-902483 were evaluated to test the power of the pH analysis to correlate pH with subcellular organelle toxicity in both HK-2 cells and canine primary proximal tubule cells. We found that by using BCECF-AM and a fluorescent plate reader we were able to quickly measure changes in cellular pH when either human HK-2 cells or primary canine proximal tubular cells were exposed to known renal toxicants and BMS compounds.

ABSTRACT FINAL ID: 2565 Poster Board -227
TITLE: High-Throughput Microfluidic Platform for Culture of 3D Kidney Tissue Models

AUTHORS (FIRST INITIAL, LAST NAME): M. Vormann[1], H. Lanz[1], K. Wilschut[1], R. van Vught[1], B. Trietsch[1], J. Joore[1], P. Vulto[1]
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KEYWORDS: 3D Kidney Tissue Model, High-Throughput Microfluidic Platform, *In Vitro* Model

ABSTRACT BODY: Drug toxicity remains a major issue in drug discovery and stresses the need for better predictive models. Here, we describe the development a perfused renal proximal tubule cell (RPTC) model in the Mimetas' OrganoPlate™ (1) to predict kidney toxicity. OrganoPlates™ are microfluidics-based culture plates consisting of 96 microchambers chips, enabling high-throughput culturing and screening of predictive organ and tissue models. In OrganoPlates™, extracellular

matrix gels can be freely patterned in chips through the use of PhaseGuide™ technology. PhaseGuides™ (capillary pressure barriers) define channels within chips that can be used for seeding cells in or against extracellular matrix, or for medium perfusion. The goal of this study is to reconstruct viable and leak tight boundaries from RPTCs to perform cytotoxicity and metabolism assays, as well as, transport and efficacy studies. Towards this end, RPTCs were seeded in the OrganoPlate™ against collagen I and were analyzed by phase contrast imaging. The RPTCs cells formed a highly viable boundary in more than 80% of the chips. Confocal imaging furthermore revealed that all RPTCs grew a mono-layer against collagen I and the wall of the chips, showing a tubule like structure. RPTCs stained positive for ZO-1 (tight junctions) and acetylated tubulin (a polarization marker). Interestingly, cilia pointed in the direction of the lumen of the tubules. The tightness of RPTC boundaries were assessed in leakage experiments using a FITC-dextran dye. Boundaries of RPTCs showed leak tight barriers and were maintained for several days. Therefore, leakage assays in the RPTC model in OrganoPlates™ enable the direct screening of compound induced toxicity, which can be performed in high-throughput and monitored over time. In addition, the RPTC model is highly suitable to study transport and efficacy of (potential) therapeutic compounds. Ref.(1) S.J.Trietsch et al., *Lab on a chip*, 2013, 8, 3548-3554.

ABSTRACT FINAL ID: 2566 Poster Board -228
TITLE: Modeling Nephrotoxicity Using a Novel Human Kidney Microphysiological System

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KEYWORDS: Human Kidney Proximal Tubule Cells, Animal Alternative Models, Microphysiological Systems

ABSTRACT BODY: Background: There is a critical need to accurately model the human kidney in order to improve our understanding of drug and xenobiotic toxicity. Proximal tubule epithelial cells (PTECs) play a vital role in the renal excretion of endogenous toxins and xenobiotics and are a primary target of toxicant-induced injury. However, conventional *in vitro* cultures of human PTECs fail to fully recapitulate physiological function and response *in vivo*. Methods: We have developed and assessed the functionality of a 3D culture model of primary human PTECs in a biocompatible microfluidic device (microphysiological system or MPS). In addition to biochemical and metabolic characterization, response was assessed using a panel of drugs/xenobiotics with known nephrotoxic properties. Results: Human PTECs in the MPS self-assemble and exhibit intrinsic properties of the proximal tubule, including cell polarity, synthetic function and secretory activities. In the MPS, PTECs expressed sodium-glucose transporter 2 (SGLT2) and lacked expression of the validated injury biomarker, kidney injury molecule 1 (KIM-1). However, variable levels of expression were observed in 2D culture, which confirms that in the MPS, PTECs are able to maintain their normal *in vivo* phenotype. In response to cisplatin and gentamicin exposure, human PTECs in 3D culture displayed altered morphology after timed exposure to these prototypical nephrotoxic drugs. Exposure to the heavy metal cadmium chloride (25 µM for 48 hr) also induced nephrotoxicity as observed by immunofluorescent staining for cell-associated heme-oxygenase (HO-1) and KIM-1, with HO-1 displaying a higher level of induction than KIM-1. Conclusions: We have recapitulated kidney proximal tubular epithelium structure *ex vivo* in an MPS and characterized it with regard to metabolic functions and response to prototypical nephrotoxins.

ABSTRACT FINAL ID: 2567 Poster Board -229
TITLE: Purinergic Receptor X7 Modulates Glut-4 Induction and Stellate Cell Activation following Disinfection Byproduct-Mediated Potentiation of NAFLD

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KEYWORDS: Purinergic Receptor, Glut4, NAFLD

ABSTRACT BODY: Epidemiological studies report that environmental toxins including disinfection byproducts of drinking water exacerbate obesity and metabolic syndrome. We have shown that exposure of bromodichloromethane (BDCM), a disinfection byproduct of drinking water exacerbates liver injury in an underlying condition of NAFLD. In the present study we explore the role of P2X7r in activating natural killer T cells which in turn induce Glut 4 protein to cause stellate cell proliferation *in vivo* following BDCM exposure. Using mice models of NAFLD, where high fat diet (60%kcal) induces steatosis at 16 weeks, we show that BDCM exposure over 4 weeks in mice that are steatotic cause lipid peroxidation and hepatocyte necrosis. Mice that were co-exposed to BDCM and high fat diet (NAFLD+BDCM) had significantly increased expression of P2X7r in the liver that was CYP2E1 dependent. Increased P2X7r expression correlated well with increased Glut4 mRNA and protein in the livers since P2X7r KO mice had significantly decreased Glut4 levels. Mechanistically, P2X7r activated NKT cell-mediated IFNg release and Glut4 induction since both P2X7r KO and CD1d KO mice that have depleted NKT cell population in the liver had significantly decreased Glut4 levels. Finally, CD1D KO and P2X7r KO mice had significantly decreased stellate cell activation as shown by a SMA levels. In conclusion, we show that environmental toxin BDCM modulates innate immune mediators P2X7r and NKT cells to cause higher Glut 4 transporter protein levels in hepatic stellate cells to sustain higher glucose transport into the cells needed for its activation and potentiation of hepatocellular injury. The higher Glut 4 expression might be due to the paracrine effects of P2X7r-triggered release of IFN-g from activated NKT cells. NIH R00ES19875 to S.C

ABSTRACT FINAL ID: 2568 Poster Board -230
TITLE: Effect of Benzo[a]pyrene on Sirt1 Expression in HepG2 Cells

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KEYWORDS: Benzo[a]pyrene

ABSTRACT BODY: Benzo[a]pyrene (B[a]P) is an environmental contaminant mainly studied for its toxic/carcinogenic effects. B[a]P is the most studied dangerous polycyclic aromatic hydrocarbon for its hepatotoxic, carcinogenic, mutagenic, teratogenic, and immunosuppressant effects, which can affect both wild and farmed marine fish through the trophic chain. Sirtuin 1 (Sirt1) is a nicotinamide adenine dinucleotide-dependent class III histone deacetylase. It reportedly can repress cellular apoptosis and senescence to affect DNA repair, stress response and aging. Notably, previous data have indicated that Sirt1 is both a tumor promoter and a tumor suppressor in tumorigenesis. However, Sirt1 expression by B[a]P in human hepatocytes HepG2 cells remains unknown. In the present study, we examined the effect of B[a]P on SIRT1 gene expression and analyzed the molecular mechanism of its activity in HepG2 cells. B[a]P dose-dependently increased SIRT1 protein and mRNA levels in HepG2 cells. Additionally, B[a]P activated the transcription factors nuclear factor-κB (NF-κB), AP-1, and mitogen-activated protein (MAP) kinases. These results demonstrated that B[a]P induced SIRT1 expression via NF-κB activation through MAP kinase pathways.

ABSTRACT FINAL ID: 2569 Poster Board -231
TITLE: Evaluation of the 3D InSightTM Human Liver Microtissues for the Detection of Compounds That Cause Drug-Induced Liver Injury in Humans

AUTHORS (FIRST INITIAL, LAST NAME): A. Foster[1], C. Summers[1], K. Oldman[1], P. Hockings[1], S. Messner[2], J. Sidaway[1], P. Morgan[1], J. Haugstetter[2], T. Rutt[2], D. Williams[1]
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KEYWORDS: Hepatotoxicity, Spheroid, Microtissue

ABSTRACT BODY: Drug-induced liver injury (DILI) is a major cause of attrition during drug development and drug withdrawal. Hence the need for predictive toxicology screening assays which enable early identification and deselection of compounds that have the potential to cause DILI. A novel and promising *in vitro* model is the 3D InSightTM primary human

hepatocyte and non-parenchymal co-culture model (hLiMT)[1]. In this study we assessed ATP depletion as an indicator of cytotoxicity towards the hLiMTs for 53 marketed drugs reported to cause DILI, liver enzyme elevations or no reports of DILI in man. Test compounds were administered to the hLiMT on days 0, 5 and 9 and cytotoxicity was determined on days 5 and/or 14 using the ATP end-point assay. The top concentration tested was ≥ 100 fold plasma Cmax or the limit of solubility. Biomarker release was assessed (alpha-Glutathione S Transferase, HMGB1 & miR122) in selected incubations. Dose and time dependent cell toxicity was observed with 4 out of 6 compounds for which cell viability was determined at day 5 and day 14. When the day 14 ATP IC50 values were expressed relative to the human plasma Cmax concentrations, for those compounds assigned DILI severity category 1, 2 or 3 (1. withdrawn from clinical use due to DILI or had been given DILI Black Box Warnings, 2. associated with rare cases of liver failure or 3. cases of liver injury) 12 out of 15 had IC50 values less than a threshold of 100 fold human plasma Cmax concentration. Only 5 of 20 compounds with no reports of DILI in man, and 4 of 13 compounds associated with liver enzyme elevations, had IC50 values below this threshold. We conclude that the hLiMT represent a relevant model to perform chronic *in vitro* hepatotoxicity investigations. Reference: 1. Messner, S., Agarkova, I., Moritz, W., Kelm J.M. (2013) Multi-cell type human liver microtissues for hepatotoxicity testing. *Archives in Toxicology* 87, 209–213.

ABSTRACT FINAL ID: 2570 Poster Board -232
TITLE: The Elucidation of Poly(ADP-Ribose) Polymerase in Drug-Induced Toxicity

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KEYWORDS: Drugs, Cocaine, Acetaminophen

ABSTRACT BODY: An overdose of a drug may produce a number of adverse health effects, including liver injury. The induction of these events may lead to DNA damage and the induction of the repair protein, poly (ADP-ribose) polymerase (PARP), in the liver. A study of this protein mechanism in the liver was undertaken using known hepatotoxicants: cocaine and acetaminophen (APAP). While the mechanism for such hepatotoxicity is not fully understood, both are known to activate PARP in liver tissues undergoing DNA fragmentation. A dosing analysis for cocaine concluded that a dose as low as 20 mg/kg resulted in elevated ALT and AST levels. A much higher dose of 60 mg/kg was tested for analyses but resulted in severe hemorrhaging. The dosing analyses for APAP resulted in no elevated liver enzyme levels for a 75 mg/kg and 150 mg/kg dose. A 200 mg/kg dose resulted in a 10 fold increase in ALT levels. A dose of 50 mg/kg for cocaine, and a dose of 300 mg/kg for APAP were used to analyze temporal trends for both toxicants. Both cocaine and APAP produced incremental increases in liver enzymes at 2hr, 6hr, 12hr, 18hr, and 24hr time points, respectively. An activation of PARP was analyzed for a 50 mg/kg dose of cocaine at each time point with the highest levels observed at the 2hr and 6hr time points. Decreases in PARP activity levels were observed beyond the 6 hr time point. PARP activity levels for a 300 mg/kg dose of acetaminophen were observed at each time point with the highest levels observed at the 18 hr time point. A 50 mg/kg dose of cocaine and a 300 mg/kg dose of acetaminophen resulted in hepatotoxic events that lead to apparent liver damage to tissues. The activation of PARP levels indicate damage to DNA that has occurred in hepatocytes. This widespread damage to DNA may further result in cell death and further damage to liver tissue.

ABSTRACT FINAL ID: 2571 Poster Board -233
TITLE: A Quantitative Measure of Hepatic Stellate Cell Activation Using Impedance-Based Technology

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KEYWORDS: Hepatic Stellate Cells and Fibrosis, *In Vitro* Assays, Cell Impedance

ABSTRACT BODY: Hepatic stellate cells (HSCs) are the primary effector of fibrosis making them a target for anti-fibrotic therapy and an entity by which to screen potential fibrogenic agents. HSCs exhibit a dynamic phenotype in response to injury; their transition from a quiescent to activated phenotype is critical to fibrogenesis. This response is characterized by a host of changes, including cell spreading, pro-fibrotic/inflammatory mediator production and the synthesis/deposition of extracellular matrix. Standard assays and imaging over short periods of time only provide brief snapshots and limited insight into the activation process making it difficult to clarify the full range of temporal changes that occur. Elucidating the morphological and biochemical changes during this period will help define key events that drive HSC activation and provide a means to understand how chemicals alleviate or exacerbate this process. We have established a label free, non-invasive, and real-time measure of primary rodent and human HSC activation using the ACEA Biosciences iCELLigence platform. Utilizing this impedance-based approach to inform cell status over time in culture, we hypothesized that the extent of cell spreading corresponds to the degree of HSC activation. Cell spreading, consistent with a time-dependent increase in electrical impedance, corresponded with increases in activation markers (α-smooth muscle actin, collagen 1a1), collagen content, and an approximate 50-fold shift in the ratio of pro- and anti-inflammatory cytokines towards a pro-inflammatory state. In the presence of a potent fibrogenic cytokine (10 ng/mL TGF-β1), the ≥2-fold increase in impedance recording 24 hours post-treatment demonstrates the responsiveness of the culture and sensitivity of this approach to discern differences in treatment groups at early time points. The iCELLigence platform represents a novel approach to monitor HSC activation over time and a promising means by which the short-term and long-term effects of therapeutic agents and potential fibrogenic compounds can be studied.

ABSTRACT FINAL ID: 2572 Poster Board -234
TITLE: Hyperthermia Exacerbates MDPV-Induced Hepatic Oxidative Stress

AUTHORS (FIRST INITIAL, LAST NAME): M. D. Bastos[1], M. Valente[1], A. Araújo[1], F. Carvalho[1], P. Guedes de Pinho[1], M. Carvalho[1, 2]
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KEYWORDS: MDPV, Hepatotoxicity, Hyperthermia

ABSTRACT BODY: Synthetic cathinones have emerged in recreational drug markets as legal alternatives for classical amphetamines. Though currently banned in several countries, 3,4-methylenedioxypyrovalerone (MDPV) is one of the most commonly abused derivative [1;2]. A prominent symptom of acute intoxication by amphetamines is hyperthermia, which has also been observed after MDPV-induced intoxications [3;4]. The aim of this study was to evaluate the mechanisms underlying the hepatotoxicity of MDPV, both at normo- and hyperthermic conditions. Cultured rat hepatocytes were exposed to 0.2–1.6 mM MDPV for 24 and 48h, at 37oC or 40.5oC, simulating the rise in body temperature that follows MDPV intake. Cell viability was measured through the MTT reduction and LDH leakage assays. The oxidative potential of MDPV was evaluated via the production of reactive oxygen and nitrogen species (ROS and RNS), and measurement of intracellular levels of ATP and reduced glutathione (GSH). Data on MTT reduction showed that MDPV induces a concentration-dependent cell death, more pronounced at 40.5oC, which was corroborated by the LDH leakage assay. MDPV induced a time- and concentration-dependent decrease in GSH levels, aggravated under hyperthermic condition. Similarly, a decline in ATP levels was observed at both time points, more noticeable at 40.5oC. A concentration-dependent increase of ROS and RNS production was also observed at both temperatures, being more significant at 40.5oC. These data show that oxidative stress plays a key role in MDPV-induced hepatic injury, and that the rise in body temperature after MDPV consumption contributes to exacerbated end-organ toxic effects. [1] A.M. Araujo, M.J. Valente, M. Carvalho, D.D. da Silva, H. Gaspar, F. Carvalho, M.L. Bastos, P.G. de Pinho, *Arch Toxicol* (2014). [2] M. Coppola, R. Mondola, *Toxicol Lett* 208 (2012) 12-5. [3] H.A. Borek, C.P. Holstege, Ann *Emerg Med* 60 (2012) 103-5. [4] B.L. Murray, C.M. Murphy, M.C. Beuhler, *J Med Toxicol* 8 (2012) 69-75.

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ABSTRACT FINAL ID: 2573 Poster Board -235
TITLE: Leptin-Mediated Hepatic Steatosis and Inflammation in Binge Alcohol-Exposed HIV Transgenic Rats

AUTHORS (FIRST INITIAL, LAST NAME): A. Banerjee[1], M. A. Abdelmegeed[1], S. Jang[1], B. Song[1]
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KEYWORDS: Leptin, Binge Alcohol, Alcoholic Liver Disease

ABSTRACT BODY: Globally, people infected with HIV have increased to pandemic proportions. Currently, about 1.2 million people in the United States are living with HIV infection. A vast majority of these HIV positive people consume alcohol. However, the effect of alcohol on the progression of liver damage in HIV-infected individuals is not known. This study was aimed to investigate the effect of binge alcohol on the progression of liver disease in HIV transgenic rats. Female wild-type (WT) and HIV transgenic rats were treated with three doses of binge ethanol (EtOH) (3.5 gm/kg body weight oral gavage at 12-h intervals) or dextrose (Control). Compared to the WT-EtOH treated group, the HIV-EtOH treated rat had significantly higher hepatic steatosis and inflammation, as evident by histology, F4/80 staining and hepatic triglyceride levels. These changes were accompanied by increase in serum endotoxin level. Real-time PCR analysis revealed that hepatic levels of toll like receptor 4 (TRL4) and leptin were significantly up-regulated in the HIV-EtOH treated rats, as compared to the other groups. In addition, a significant increase in monocyte chemoattractant protein 1 (MCP-1) was observed in the HIV-EtOH treated group. Kupffer cells isolated from HIV rats treated with EtOH, showed increased MCP-1 expression upon stimulation with leptin. Hepatocyte apoptosis was also significantly higher in the HIV-EtOH treated group. In conclusion, these data collectively suggest that endotoxin and leptin mediated upregulation of MCP-1 in HIV-EtOH treated rats make them more susceptible to binge alcohol-mediated liver damage.

ABSTRACT FINAL ID: 2574 Poster Board -236
TITLE: Transcriptional Profiling Suggests That Nevirapine Mediates Effects on Primary Human Hepatocytes Necessary for Immune-Mediated Toxicity

AUTHORS (FIRST INITIAL, LAST NAME): Y. Terelius[2], B. Blackman[1], R. Figler[1], S. Marukian[1], S. Collado[1], M. Lawson[1], D. Manka[1], B. R. Wamhoff[1], A. Dash[1]
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KEYWORDS: Nevirapine, Liver, Toxicity

ABSTRACT BODY: Nevirapine is the most common antiretroviral agent that causes serious, clinical liver injury. The mechanisms of clinical hepatotoxicity are poorly understood and suspected to involve immune-mediated responses as well as direct toxic effects on hepatocytes. *In vitro* hepatocyte cultures require drug exposures at significantly higher concentrations than clinical plasma Cmax levels to elicit induction and toxicity responses, making mechanistic pathway changes difficult to interpret. We previously described a system that uses liver-derived hemodynamic blood flow and transport parameters to restore primary human hepatocyte morphology, biology, and efficacy or toxicity responses to drugs at concentrations relevant to clinical/*in vivo* exposure levels. Using this system, hepatocytes were treated with the antiviral drugs Nevirapine (non-nucleoside reverse trancriptase inhibitor) and Ritonavir (protease inhibitor) at clinically relevant exposures for 48 hours. Whole genome transcriptomics was performed by RNAseq. Nevirapine induced the genes CYP2B6, CYP3A4 and UGT1A1, reflective of CAR activation. TGF-β signaling and extracellular matrix-related genes were suppressed, suggestive of reduced fibrosis. In contrast to Ritonavir, Nevirapine treatment resulted in upregulation of 1) MHC Class I (cytotoxic T cell activator), 2) gluconeogenesis pathway and Kreb's cycle, 3) bile acid synthesis without a change in bile acid transporters and 4) Glucose-6-phosphate dehydrogenase, the rate limiting enzyme of the pentose phosphate pathway. Unlike Nevirapine, Ritonavir upregulated 1) fatty acid synthesis, 2) fatty acid oxidation with mitochondrial uncoupling and 3) the unfolded protein response. These findings collectively support clinical reports that Nevirapine therapy may have a lower propensity of steatosis and progression of fibrosis, and suggest hepatocellular toxicity mechanisms that involve immune cell-mediated toxicity in addition to direct cholestatic effects.

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ABSTRACT FINAL ID: 2575 Poster Board -237
TITLE: Comparison of Regular and Cryopreserved HepaRG Cells for Studies of Acetaminophen Toxicity

AUTHORS (FIRST INITIAL, LAST NAME): J. L. Weemhoff[1], M. R. McGill[1], H. Jaeschke[1]
INSTITUTIONS (ALL): 1. University of Kansas Medical Center, Kansas City, KS, United States.
KEYWORDS: Acetaminophen, Hepatocytes, *In Vitro* Models

ABSTRACT BODY: Acetaminophen (APAP) overdose is the most common cause of drug-induced liver injury in the US. The mechanism involves reactive metabolite formation, protein binding and mitochondrial damage. Primary human hepatocytes (PHH) are the gold standard for *in vitro* drug hepatotoxicity studies. However, limited availability and loss of viability in cryopreservation hinder their use. While the human liver cell line HepaRG has been shown to be a suitable alternative, the lengthy growth and differentiation process limits their usefulness for some applications. Pre-differentiated cryopreserved HepaRG (cHepaRG) cells have been developed; however, no comparison between HepaRG and cHepaRG cells has been done. Thus, we sought to determine the suitability of cHepaRG cells for APAP toxicity studies. HepaRG cells were grown and differentiated in our laboratory according to the manufacturer's instructions. cHepaRG cells were thawed and immediately plated for experiments. Cells were washed and treated with 20mM APAP in Williams' E Media without DMSO for 0, 3, 6, 18, 24, or 48h then were harvested and subjected to biochemical assays. Cell death (% LDH release) increased over time in both HepaRG and cHepaRG cells (61.5±4.2% and 73.5±0.5% at 48h for HepaRG and cHepaRG, respectively), though the increase began earlier in cHepaRG cells. GSH depletion, an indicator of CYP450-mediated reactive metabolite formation, showed similar changes (32% and 46% depletion over 24h in HepaRG and cHepaRG, respectively). Similarly, APAP-protein adduct formation was comparable between the two, with a rapid initial increase between 3 and 6h. Finally, the JC1 assay was performed to determine loss of mitochondrial membrane potential. Both HepaRG and cHepaRG cells had a rapid decrease in potential between 0 and 3h (32.1% and 32.3%, respectively), which persisted until later time points. These results demonstrate similar mechanisms of APAP toxicity between the two cell lines. Conclusion: cHepaRG cells are a convenient alternative to PHH and HepaRG cells for studies of drug toxicity.

ABSTRACT FINAL ID: 2576 Poster Board -238
TITLE: Effect of Decreased Expression of CYP2E in Constitutive Androstane Receptor (CAR)-Knockout Mice on Hepatocarcinogenesis of Diethylnitrosamine

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KEYWORDS: Constitutive Androstane Receptor (CAR), Hepatocarcinogenesis, CYP2E1

ABSTRACT BODY: In our previous studies, a decrease of diethylnitrosamine (DEN)-induced hepatocellular adenomas and foci of altered hepatocytes were repeatably observed in CAR-knockout mice, compared with wild-type mice (Sakamoto et al., 2013 and unpublished data). To clarify CAR involvement in the hepatocarcinogenesis of DEN, 6-week old male CARKO and C3H (wild-type) mice were intraperitoneally injected with 90 mg/kg body weight (bw) of DEN and histopathology. Thereafter, CYP2E1 expression and activity in the liver were determined sequentially. On Day 1, significant changes such as the paling of cytoplasm of the hepatocytes in the centrilobular area and a decrease of Cyp2e1 expression and CYP2E1 activity were observed and these changes were more pronounced in CARKO mice than in wild-type mice. To confirm the increased susceptibility of CYP2E1 reduction by DEN in CARKO mice, male CARKO and wild-type mice were injected with DEN on Day 0 and then treated with 300 mg/kg bw acetaminophen (APAP) intraperioneally on Day 1 (DEN+APAP group). Mice in both genotypes were treated with saline on Days 0 and1 (untreated group), DEN only on Day 0 (DEN group) or APAP only on Day 1 (APAP group), and mice in all groups were euthanized on Day 2. In the APAP group, hepatocellular necrosis and CYP2E expression in wild-type mice were more evident compared with those in CARKO mice. These results in the APAP group indicate that CYP2E, a metabolic enzyme that converts APAP to its toxic form, might be diminished in CARKO mice. Interestingly, in the DEN+APAP group, single cell necrosis of the hepatocytes and CYP2E expression in the centrilobular area were comparable between wild-type and CARKO mice and these changes were similar to the DEN group. These results were

different from our speculation that the effect of APAP after DEN injection might become milder in CARKO mice than in wild-type mice. Taken together, the diminishment of tumor induction by DEN might be caused by less expression of CYP2E in CARKO mice.

ABSTRACT FINAL ID: 2577 Poster Board -239
TITLE: Modificatons Required for ATP and Caspase Detection Assays Applied to 3D Cell Spheroids

AUTHORS (FIRST INITIAL, LAST NAME): T. L. Riss[1], M. P. Valley[1], K. R. Kupcho[1], C. A. Zimprich[1], D. Leippe[1], A. L. Niles[1], J. Vidugiriene[1], B. Hook[1], J. J. Cali[1], D. F. Lazar[1]
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KEYWORDS: Cell Viability, Apoptosis, 3D Cell Culture

ABSTRACT BODY: The design and validation of *in vitro* assay methods to interrogate cytotoxicity markers from large aggregates of cells has lagged behind the development of systems to culture cells in physiologically relevant three dimensional (3D) structures. The rationale for this study was to investigate whether cell viability and apoptosis assays designed for measuring parameters from 2D monolayers of cells could be applied to 3D spheroid models. The experimental procedures included a comparison of acid extraction and detergent extraction of ATP as a marker of viable cells in 3D microtissues. Because acid extraction or high detergent concentration is not compatible with caspase-3 activity, we compared physical disruption procedures using an orbital shaker to extract caspase-3 from 3D microtissues. The data from 6 different cell lines cultured with Matrigel to form 3D structures indicated we extracted an average of 29% of the ATP using an unmodified commercially available reagent whereas we extracted an average of 100% of the ATP (compared to acid extraction control) using a reagent formulation containing an increased and optimized amount of detergent. The results comparing procedures to extract caspase-3 activity from 350µm spheroids of HCT116 cells treated with 10µM panobinostat for 24 hours demonstrated shaking plates for 5 min (instead of the 30 seconds recommended for 2D cultures) resulted in a 64% increase in recovery of caspase-3 activity. We found that published protocols developed for *in vitro* assays for cell viability and apoptosis were not adequate for extraction of the desired markers from cells grown in 3D structures. Modification of protocols to improve the lytic capacity of reagents by increasing detergent concentration or applying more rigorous physical disruption is recommended as a first approach for verifying performance of *in vitro* assays on 3D microtissues.

ABSTRACT FINAL ID: 2578 Poster Board -240
TITLE: Study on the Regulation of the Mycotoxin-Induced Toxicity Response Process in *A. thaliana*

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KEYWORDS: Mycotoxin, Toxicity, *A. thaliana*

ABSTRACT BODY: Mycotoxin, as a secondary metabolite produced by fungi, is nephrotoxic, hepatotoxic and genetoxic to animals and human. The toxicity induced by mycotoxin to plant such as *Arabidopsis thaliana* and how this happen is not so clear. A comparative analysis of the phytotoxic action of representative mycotoxin, the growth and morphology of *A. thaliana* growing on media containing these compounds was investigated. The inhibition of germination of *A. thaliana* seeds decreased in the following order: T-2 toxin>AFB1>OTA>DON>ZEN. Ochratoxin A (OTA) exposure inhibited plant growth obviously, especially the roots and leaves, DON preferentially inhibited root elongation, and T-2 toxin caused dwarfism. Trypan blue staining for measuring of cell death, 3,3'-diaminobenzidine (DAB) staining for reactive oxygen species (ROS), method of Nakano for measuring of SOD,CAT and POD activities were used. It showed that OTA or DON exposure could cause necrotic lesions in detached leaves and ROS production was stimulated, suggesting mycotoxin is toxic to *A. thaliana* and ROS pathways involved in the mycotoxin-induced toxicity. Ultrastructural examination of *A. thaliana* leaves exposed to OTA showed that the separation of the plasma membrane from the cell wall, fold formation, chromatin

condensation, destruction in the structures of the mitochondria and chloroplasts, uneven thickness of the membrane, and distorted nuclear membrane and nuclei. When *A. thaliana* seedlings exposed to DON, the number of starch granules and peroxisome increased. qRT-PCR was used for the relative expression level of the salicylic acid-inducible marker gene PR1, aminocyclopropane carboxylate synthase ACS6, respiratory burst oxidase homologue AtrbohC and AtrbohD, and ascorbate peroxidase APX. A continuous increase in the ROS content with the increase of toxin concentration was observed. The application of toxin to excised *A. thaliana* leaves significantly accelerated the increase in MDA.

ABSTRACT FINAL ID: 2579 Poster Board -241
TITLE: Loss of α(E)-Catenin-Fscn2 Signaling Increases Cisplatin-Induced Apoptosis in Aged Kidney

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KEYWORDS: Alpha-catenin, Nephrotoxicity, Cisplatin

ABSTRACT BODY: Aging patients are highly susceptible to acute kidney injury. Previous studies in our laboratory demonstrated a dramatic decrease of α(E)-catenin expression in proximal tubular epithelium in the aged kidney. We created stable α(E)-catenin knock-down NRK-52E (C2) cells (NT3 is the non-targeted control) and observed a significant loss of viability in C2 cells as compared with NT3 cells after cisplatin challenge. In this study, we aimed to delineate the pathway by which loss of α(E)-catenin increases cisplatin injury. Increased caspase-8 and -9 activation, BID cleavage and cytochrome C release were observed in C2 cells after cisplatin treatment. Blocking apoptosis, using caspase-8 or -9 inhibitors, completely abolishes the increased susceptibility of C2 cells. Interestingly, the expression of fascin actin bundling protein 2 (Fscn2) is decreased in α(E)-catenin knock-down cells. Re-expression of Fscn2 in C2 cells attenuates the increased apoptosis following cisplatin challenge. Furthermore, our *in vivo* study showed a significant increase in serum creatinine, KIM-1 and *in situ* apoptosis levels at 72 hr after a single dose of cisplatin in 24-month-old rats, but not in 4-month-old rats. The expression of Fscn2 was also decreased in aged kidney. Taken together, these results suggest that loss of α(E)-catenin-Fscn2 signaling increases cisplatin-induced apoptosis in aged kidney.

ABSTRACT FINAL ID: 2580 Poster Board -242
TITLE: Perfluorooctanoic Acid Impairs Autophagy at the Late Stage in the Liver

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KEYWORDS: Toxic Liver Injury, Perfluorooctanoic Acid (PFOA), Autophagy

ABSTRACT BODY: Perfluorooctanoic acid (PFOA) has been shown to cause hepatotoxicity and other toxicological effects, but the mechanism of PFOA induced hepatotoxicity still remains unclear. *In vivo* studies were performed using mice exposed to PFOA for 28 days at doses of 0, 0.08, 0.31, 1.25, 5, and 20 mg/kg/d, and *in vitro* studies were also performed using HepG2 cells exposed to PFOA for 24, 48, or 72 hours at doses of 0, 50, 100, and 200 μM. We observed accumulation of autophagosomes using western blotting in both mice livers and HepG2 cells after PFOA exposure and immunofluorescence analysis in HepG2 cells further confirmed the results. However, results from histopathological analysis and apoptosis related factors expression demonstrated that no significant apoptosis occurred in mouse livers after PFOA exposure. Further study *in vitro* revealed that the accumulation of autophagosomes was not caused by autophagic flux stimulation or lysosome dysfunction. Proteomic analysis of the crude lysosomal fractions (CLFs) from HepG2 cells exposed to 200 μM PFOA for 72 hours using isobaric tags for the relative and absolute quantification (iTRAQ) method revealed that 63 differentially expressed proteins are related to autophagy or vesicular trafficking and fusion. Among these proteins, the expression levels of N-ethylmaleimide-sensitive factor attachment protein α (NAPA) and V-ATPase V0 a3 (TCIRG1), both suggested by earlier reports to play critical roles in autophagy and vesicle traffic, were reduced. This was further validated in CLFs of both cells *in vitro* and livers *in vivo* after PFOA exposure, which revealed an impairment at the late stage of

autophagy after PFOA exposure. In conclusion, these findings demonstrate that autophagy impairment rather than apoptosis plays an important role in PFOA induced hepatotoxic effects.

ABSTRACT FINAL ID: 2581 Poster Board -243
TITLE: Evaluation of the Potential Cytotoxic Effects of Vanadyl Sulphate on Human Breast Cancer Cells

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KEYWORDS: Vanadyl Sulphate, Breast Cancer, Cytotoxicity

ABSTRACT BODY: The anticarcinogenic and cytotoxic effects as well as other biological activities of different metal drugs were investigated in a number of *in vivo* and *in vitro* studies. One of the well investigated metal agents is vanadium as well its different compounds. The compounds of vanadium showed to have chemopreventive and antitumor effects on a number of cancer cell types. The effects of vanadium compounds on human breast cancer cells (MCF7) morphology remain unclear. Herein, the potential cytotoxic and apoptotic effects of vanadyl sulphate on MCF7 cells were investigated via confocal microscopy. For this manner MCF7 cells were incubated with IC50 value of vanadyl sulphate for 24 hours previously detected to be 85 µM. Treated cells were stained with Alexa fluor-488 Phalloidine and acridine orange and observed and photographed under confocal microscope. Morphological alterations detected on our confocal micrographs are damaged cytoskeleton as hole formation, shrinked cells and fragmented and condensed nuclei as apoptotic sparks. According to our results, vanadyl sulphate caused important changes in MCF7 cells morphology as well as cytotoxicity in low concentration and this may serve as an important finding for further investigation of vanadyl sulphate for its effects on different cancer cell lines and its usage as anticancerogenic agent.

ABSTRACT FINAL ID: 2582 Poster Board -244
TITLE: Testing a Novel Real-Time Cell Viability Assay: Comparison to ATP Assay and Compatibility for Multiplexing

AUTHORS (FIRST INITIAL, LAST NAME): A. Landreman[1], S. Duellman[1], B. Hook[1], W. Zhou[1], J. Vidugiriene[1]
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KEYWORDS: Cell Viability, Real-Time Cellular Assay, Pathway Analysis

ABSTRACT BODY: Recently developed novel assay technology makes it possible to use multi-well plate readers to measure the number of live cells in culture in real time over a period of days. Live cells can be measured by adding a reagent containing a shrimp-derived luciferase and a pro-substrate directly to the culture medium. Only viable cells can convert the pro-substrate into a luciferase substrate and generate light. Cells remain viable following recording of data which enables many options for multiplexing additional assays. The rationale of this study was to test whether the novel real time assay technology correlated with the gold standard ATP assay for measuring potency of a toxin. We also tested whether the presence of the pro-substrate and shrimp luciferase in culture medium affected the results from firefly luciferase (Fluc) reporter assays and RNA extraction procedures. Parallel samples of cells were cultured with or without the presence of the pro-substrate and luciferase, then tested using: 1) an orthogonal cell viability assay measuring ATP, 2) an Fluc reporter assay, and 3) extraction of RNA using both manual and automated methods. Comparison of the IC50 values for measuring the potency of thapsgargin-treated A549 cells using the real time or ATP assays were 10.0nM and 7.6nM, respectively, and Fluc reporter gene activity showed similar results in the presence of the shrimp-derived luciferase and a pro-substrate (1.61 +/- 0.15 x 106 RLU) vs vehicle control (1.64 +/- 0.05 x 106 RLU). Extraction of RNA from multicellular spheroids of HEK-293 cells showed similar yields in the presence of the shrimp-derived luciferase and a pro-substrate (45 ng) vs vehicle control (38 ng). The new assay technology to measure viable cells in real time in cell culture correlated well with measuring ATP as a viability marker. The novel real time assay reagents do not interfere with the other assay chemistries tested and enable subsequent multiplexing with a broad range of downstream applications, such as genetic signaling pathway analysis, from the same sample.

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Late-Breaking Abstracts

ABSTRACT FINAL ID: 2583 Poster Board -245
TITLE: Mechanisms Underlying Acrolein-Mediated Inhibition of Chromatin Assembly

AUTHORS (FIRST INITIAL, LAST NAME): C. Jin[1], L. Fang[1], D. Chen[1], C. Yu[2], H. Li[1], L. Huang[2]
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KEYWORDS: Epigenetics, Chromatin, Acrolein

ABSTRACT BODY: Acrolein (Acr) is a major component of cigarette smoke. It has been implicated in cancer and neurodegenerative diseases. Previously we reported that Acr compromises chromatin assembly, however, underlying mechanisms have not been defined. Post-translational modifications and histone chaperones are two major regulators of chromatin assembly. In the present study, we found using mass spectrometry that Acr reacts with histone lysine residues including 5 and 12 on H4, which are important for chromatin assembly. HAT (histone acetyltransferase) assays demonstrated that Acr-modified histones are resistant to acetylation, suggesting that reduced H4K5,12 acetylations that we detected previously in the cells exposed to Acr are likely due to the formation of Acr-histone lysine adducts. This was further supported by the observation that Acr treatment does not change HAT activities. H4K5,12 acetylations are required for the interaction between histone and histone chaperone ASF1B. Accordingly, co-immunoprecipitation assays demonstrated that the association of H3/H4 with ASF1B was disrupted in cells following Acr exposure. Interestingly, plasmid supercoiling assays, the method frequently used to test *in vitro* nucleosome assembly activity, revealed that treatment either of histones or ASF1B with Acr, had no effect on formation of plasmid supercoiling, indicating that Acr-protein adduct formation itself does not directly interfere with nucleosome assembly. To get mechanistic insight, we further utilized a unique RSF (Remodeling Space Factor) chromatin assembly assays. This assay requires histones are acetylated for efficient assembly. Notably, exposure of histones to Acr prevented histones from being acetylated by HAT, leading to the inhibition of RSF-mediated chromatin assembly. These results suggest that Acr compromises chromatin assembly via reacting with histone lysine residues at the sites critical for the chromatin assembly and preventing these sites from being physiologically modified.

ABSTRACT FINAL ID: 2584 Poster Board -246
TITLE: Epigenetic Changes in Population Exposed to Polycyclic Aromatic Hydrocarbons (PAHs)

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KEYWORDS: Epigenetics, PAHs

ABSTRACT BODY: Indoor exposure to air pollutants causes significant damage to health world wide especially in developing countries. Around 3 billion people cook and heat their homes using biomass fuel (i.e. wood, charcoal, coal, dung, crop wastes) on open fires or traditional indoor stoves. Such inefficient cooking and heating practices produce high levels of household air pollution which includes a wide range of health damaging pollutants such as fine particles, carbon monoxide and PAHs The exposure to different PAHs had been associated to the development of neoplastic processes, asthma, genotoxicity, altered neurodevelopment and interestingly highlight the proinflammatory effects. Recently, it has been reported the increased expression of TNF-α, IL-1β and IL-8 in mononuclear cells in culture exposed to BaP. So far, the effects on the induction of proinflammatory cytokines are attributed to the activation of the aryl hydrocarbon receptor (AhR). However, the molecular mechanisms which the PAHs produce proinflammatory effects does not known with certainty. Interestingly, the main epigenetic mechanisms such as DNA methylation, histone modification and microRNAs change in response to environmental contaminants exposure such as PAHs that may modify epigenetic regulatory mecanism and it could be associated with the risk of disease development. We performed an study in two human Mexican population exposed to PAHs where 1-hydroxy-pyrene (1-OHP) was determined on urine as a biomarker of exposure. Furthermore, the relative expression of TNF-α, IL1- β, IL-8, DNMT1, AhR, CYP1B1, miR-125b, and 155 were quantified by real-time PCR in peripheral blood mononuclear cells (PBMCs). TNF-α and CYP1B1 shown augmented expression in high

exposed population with respect to the population with lower levels of PAHs, and positive correlation between the expression of TNF-α and activation of AHR were obtained. In addition, methylation of the TNF-α promoter and the expression of miR-125b and 155 were evaluated in study population.

ABSTRACT FINAL ID: 2585 Poster Board -247
TITLE: Investigation of Bilirubin's Potential Protective Effects on Myocardial Injuries Induced by Isoproterenol in Rats

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KEYWORDS: Bilirubin, Myocardial Injury, Rat

ABSTRACT BODY: The cytoprotective effects of bilirubin have been demonstrated in many *in vitro* studies, but limitedly studied *in vivo*. The purpose of this study was to assess the *in vivo* effects of bilirubin on the myocardial injuries induced by isoproterenol (ISO) in rats. Unconjugated bilirubin (BIL) was administrated once daily by intravenous (IV) or intraperitoneal (IP) injection to male rats for five days at doses ≤70 mg/kg. ISO (0.1 mg/kg) was administrated by a single subcutaneous (SC) injection to these rats at 0.5 hour after the first BIL injection to induce cardiotoxicity. The results showed that, in the ISO control group, there were mild myocardial injuries (multifocal myocardial degeneration and mononuclear cell infiltrations, etc.), and mild increases in serum lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) at 2 hours after the ISO injection. In the BIL treated groups, there were dose related increases in serum total bilirubin, indirect bilirubin, and bilirubin/albumin (bil/alb) molar ratios. However, similar levels of the heart 4-hydroxynonenal (4-HNE) protein adduct and similar myocardial injuries were noted for the BIL treated groups in comparison with the ISO control group. Potential reasons for the inconsistent results of bilirubin's *in vivo* cytoprotective effects compared with *in vitro* could be related to differences in exposure levels of free bilirubin in cell culture versus in the rat hearts. In conclusion, the study results didn't demonstrate the *in vivo* protective effects of bilirubin on the ISO induced myocardial injuries in this study.

ABSTRACT FINAL ID: 2586 Poster Board -248
TITLE: Lutein Attenuates STZ-Induced Testicular Damage and Oxidative Stress in Diabetic Rats

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KEYWORDS: Lutein, Diabetes, Testis

ABSTRACT BODY: Background and Aims: Diabetes mellitus with the successive generation of reactive oxygen species signifies a major risk factor for testicular dysfunction. Antioxidants supplements are one of the best options to prevent such disorder. In present study, lutein as dietary supplement has used to explore the potential protective effects against diabetic-induced oxidative stress in testicular cells. Methods: Diabetes was induced using a single IP injection of streptozotocin (STZ). Lutein content diets (40, 80 and 160 mg/kg diet) was prepared in pellet form and supplemented to diabetic rats for 5 weeks. Serum testosterone levels were estimated. In testicular cells, thiobarbituric acid reactive substances (TBARS), total sulfhydryl groups (T-GSH), non-protein sulfhydryl groups (NP-SH), superoxide dismutase (SOD) and catalase (CAT) activities were measured. Pro-inflammatory mediators like tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) were also estimated in testicular cells. Cytotoxicity of testicular cells was measured by estimating nucleic acids and total protein (TP) levels. Histopathological changes were evaluated in testis. Results: Lutein significantly inhibited the diabetic induced decrease in serum levels of testosterone in dose dependent manner. The levels of TNF-α, IL-1β and TBARS were significantly increased, while T-GSH, NP-SH, DNA, RNA and TP levels decreased in diabetic rats. Lutein supplementations markedly attenuation the elevated levels of TNF-α, IL-1β and TBARS and decreased T-GSH and NP-SH levels in dose dependent manner. The inhibited testicular enzymatic activities and nucleic acid levels of diabetic rats were increased with lutein supplementation. Histopathological evaluation revealed protection the damage in testicular cells of

diabetic rats by lutein supplementation. Conclusion: These findings showed that lutein has potentially beneficial effects on diabetic-induced testicular damage, probably through its antioxidant and anti-inflammatory properties.

ABSTRACT FINAL ID: 2587 Poster Board -249
TITLE: Genotoxic and Oxidative Stress Effects of 2-Amino-9H-pyrido [2, 3-b] indole in Human Hepatoma G2 (HepG2) and Human Lung Alveolar Epithelial (A549) Cells

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INSTITUTIONS (ALL): 1. Zhengzhou Tobacco Research Institute, Zhengzhou, China.
KEYWORDS: 2-Amino-9H-pyrido [2,3-b] indole (AαC), Genotoxic Effects, Oxidative Stress

ABSTRACT BODY: 2-Amino-9H-pyrido[2,3-b]indole (AαC) has been reported as a probable human carcinogen that is present in high quantities in cigarette smoke. However, rare studies were reported on the genotoxicity and oxidative stress AαC-induced. In this study, the genotoxic effects of AαC in human hepatoma G2 (HepG2) and human lung alveolar epithelial (A549) cells were investigated using comet assay. Significant increases of DNA fragment migration indicated that AαC cause serious DNA damage in HepG2 and A549 cells. In order to clarify the role of oxidative stress in the mechanism of AαC-induced genotoxicity, the level of the intracellular reactive oxygen species (ROS), GSH/GSSG ratio and the formation of 8-hydroxydeoxyguanosine (8-OHdG), a marker for oxidative DNA damage were measured. The results showed that levels of ROS, 8-OHdG increased and GSH/GSSG ratio decreased. And 8-OHdG was positively related to DNA damage. Considering all the results, it is inferred that AαC could induce genotoxicity and oxidative stress, and AαC probably exerts genotoxicity in HepG2 and A549 cells through the oxidative DNA damage ROS-induced. This is the first report of AαC-induced genotoxic and oxidative stress effects in HepG2 and A549 cells.

ABSTRACT FINAL ID: 2588 Poster Board -250
TITLE: Dinitrotoluene-Induced Oxidative Stress, Genotoxicity, and Apoptosis in Human Liver Carcinoma (HepG2) Cells

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KEYWORDS: Dinitrotoluenes, Genotoxic Stress, Apoptosis

ABSTRACT BODY: Dinitrotoluenes (DNTs) [CH3C6H3(NO2)2] are nitroaromatic compounds that are produced industrially and released into the environment as a result of human activities. DNTs appear as pale yellow crystalline solids and exist as a mixture of 2 to 6 isomers, among which 2,4-DNT and 2,6-DNT are the most significant. Previous research from our laboratory demonstrated that DNTs are cytotoxic, and able to induce stress-related genes/proteins in human liver carcinoma (HepG2) cells. To further elucidate their molecular mechanisms of toxicity, we have in the present study investigated their potential to induce oxidative stress (OS) as well as their genotoxic and apoptotic effects. OS, genotoxicity, and apoptosis were assessed respectively by lipid peroxidation assay, single cell gel electrophoresis (Comet) assay, and flow cytometry assay of annexin V and caspase 3 activities in HepG2 cells exposed to 2,4- and 2,6-DNT. Data obtained from the lipid peroxidation assay indicated that there is a strong dose-response relationship between malondialdehyde production and chemical exposure; indicating that DNTs are able to cause OS in HepG2 cells. The results of Comet assay indicated a gradual increase in the comet tail length and percentage of DNA damage, with increasing doses of 2,4- and 2,6-DNT. The mean percentages of DNA damage were 2.92 ± 0.45%, 7.04 ± 2.27%, 19.79 ± 5.36%, and 28.18 ± 4.12% in 0, 100, 200, and 300µg/mL of 2,4-DNT, and 6.09 ± 7.43%, 9.07 ± 16.53%, 18.75 ± 17.47%, and 36.25 ± 3.83% in 0, 100, 200, and 300µg/mL of 2,6-DNT respectively. Similar trends were observed with the mean values tail length in DNT-treated cells compared to control cells. Flow cytometry results from annexin V and caspase 3 experiments indicated that the levels of both early and late apoptosis in HepG2 cells increased in a dose-dependent manner within the range of 0-300 µg/mL but only reached a maximum of 3%; indicating minimal apoptotic damage compared to necrotic cell death.

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ABSTRACT FINAL ID: 2589 Poster Board -251
TITLE: Global Proteomic Profiling of Acetaminophen-Exposed 3D Human Liver Microtissues Identifies Novel NAPQI Adduct Sites

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KEYWORDS: Acetaminophen, NAPQI, Proteomics

ABSTRACT BODY: During the last decades it was shown that Acetaminophen (APAP) induced hepatotoxicity requires metabolic activation of APAP to N-acetyl-p-benzoquinone imine (NAPQI), which is mediated by Cytochromes P450 2E1 (CYP2E1) and partly 1A2 (CYP1A2). NAPQI subsequently binds to amino acid residues of proteins, which is thought to mediate APAP-induced hepatotoxicity. However, despite intensive research, no exact NAPQI adduct binding sites on human liver proteins were so far mapped. Here, we report the use of Hyper Reaction Monitoring (HRM), a novel mass spectrometric approach for reproducible and accurate proteomic profiling of 3D Human Liver Microtissues exposed to various Acetaminophen concentrations. With a starting material of only 12,000 cells per sample, the abundance of 2,830 proteins was quantified over an APAP concentration range after 72 hours exposure. The results revealed significant changes of protein abundance at low, physiologically relevant (4.6 µM) drug concentrations. APAP-induced differential protein expression was observed for several phase I (CYP2E1, CYP1A2, CYP3A4) and phase II enzymes (UGT1-1, UGT1-7, UGT1-7) in a dose dependent manner. In addition, we identified six novel NAPQI-cysteine protein adducts on mitochondrion oxidative stress related proteins (GATM, PARK7, PRDX6 and VDAC2) and two other proteins (ANXA2 and FTCD). These results identify for the first time the exact adduct sites of NAPQI on human liver proteins, which might play a key role in APAP-mediated hepatotoxicity.

ABSTRACT FINAL ID: 2590 Poster Board -252
TITLE: Hepatic Oxidative Stress in Pregnant and Nursing Female Mice Exposed to Bisphenol A and High-Fat Diets

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KEYWORDS: BPA, Oxidative Stress, Nonalcoholic Fatty Liver Disease (NAFLD)

ABSTRACT BODY: Hepatic oxidative stress is associated with chronic liver disease, including non-alcoholic fatty liver disease (NAFLD). Bisphenol A (BPA) exposure and high fat diet (HFD) have been linked to NAFLD, but it is unknown whether different types of HFDs in combination with chemical exposures, modify oxidative stress. Investigation of the hypothesis that BPA and HFD, particularly Western HFD, increase hepatic oxidative stress was carried out via an *in vivo* mouse model. Isogenic female mice (93% C57BL/6; n=31) were exposed to 1 of 6 diets for eight weeks spanning preconception, gestation, and lactation: Control (Con), Mediterranean HFD (Med), Western HFD (West), Control + 50ug BPA/kg chow (CBPA), Med + 50ug BPA/kg chow (MBPA), and West + 50ug BPA/kg chow (WBPA). Hepatic redox potentials were determined via measurement of oxidized and reduced thiols using high performance liquid chromatography (HPLC). One-way analysis of variance (ANOVA) indicated that mean hepatic redox potentials (GSH:GSSG) significantly differed across the 6 exposure groups (p=0.029). Redox potentials were more oxidative in WBPA (-206.01 mV) compared to Con (-218.09 mV, p=0.047) and CBPA (-219.30 mV, p=0.016) dams. While BPA did not significantly alter redox potentials in dams consuming a Con diet or a Med HFD, the addition of BPA to a Western HFD (-206.01 mV) resulted in a more oxidizing redox potential in dams compared to West (-216.42 mV). Protein S-glutathionylation levels were unexpectedly lower in WBPA dams relative to Con, which is indicative of adaptation of the dams' hepatic cells to a more oxidized state. Consistent with redox potentials, body weight gain from time of exposure to mating was significantly higher in WBPA than in Con (p=0.008). Taken together, these results suggest that a Western HFD may induce hepatic oxidation, and that BPA may potentiate this effect in pregnant

female mice. Funding for this study includes NIH: P01 ES02284401, P30 ES017885, T32 ES007062, T32 HD079342, and EPA: RD83543601.

ABSTRACT FINAL ID: 2591 Poster Board -253
TITLE: RNA-Seq and Bioinformatics Reveals That TCDD-Regulated Genes Are Highly Associated with Glucocorticoid and Progesterone Signaling Pathways in Human MCF7 Breast Cancer Cells

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KEYWORDS: AhR, TCDD, RNA-Seq

ABSTRACT BODY: 2,3,7,8, tetrachlorodibenzo-p-dioxin (TCDD) is a ubiquitous environmental toxicant that is formed during industrial processes. The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that mediates the toxic effects of TCDD. Many different studies have shown that TCDD disrupts estrogen and androgen signaling, however less attention has been directed at the potential effects of TCDD on glucocorticoid or progesterone signaling. The objective of this report was to investigate whether TCDD interacts with glucocorticoid or progesterone signaling pathways. RNA-Seq expression profiling on human MCF7 breast cancer cells (BCCs) was performed to identify TCDD-regulated genes (TRGs). The results revealed that 10 nM TCDD (6 hr) regulated the expression of approximately 100 genes, and that nearly 600 genes were regulated by 100 nM TCDD (6 hr). In order to determine functions and pathways regulated by TRGs, we analyzed the TRGs set using the Ingenuity Pathway Analysis (IPA). These TRGs were significantly correlated with cancer-related pathways including: cancer, cell morphology, cellular growth and proliferation, cell cycle and tumor morphology. The IPA Upstream Regulator Analysis tool was used to determine if the TRGs were connected through a common upstream regulator. This analysis revealed that TRGs were enriched in beta-estradiol, NOTCH1, TGFB1 progesterone, progesterone receptor, WNT3A and glucocorticoids. The principle conclusions of this study are that TCDD affects cancer by regulating the expression of specific cohorts of genes that are linked to discrete cancer pathways. Discovering that TRGs are highly associated with progesterone and glucocorticoid signaling opens up a new line of study that will investigate how TCDD interacts with these steroid hormones.

ABSTRACT FINAL ID: 2592 Poster Board -254
TITLE: Prenatal Exposure of Mice to the Human Liver Carcinogen Aflatoxin B1 Reveals a Critical Window of Susceptibility to Genetic Change

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KEYWORDS: Aflatoxin B1, Prenatal Exposure, DNA Adducts

ABSTRACT BODY: It has become axiomatic that critical windows of susceptibility to genotoxins exist and that genetic damage *in utero* may be a trigger for later life cancers. Data supporting this critical window hypothesis are remarkably few. This study provides a quantitative bridge between DNA damage by the liver carcinogen aflatoxin B_1 (AFB_1) during prenatal development and the risk of later life genetic disease. AFB_1 was given to pregnant C57BL/6J mice, carrying F1 gestation day 14 (GD14) embryos of the B6C3F1 genotype. Ultra-high performance liquid chromatography and mass spectrometry (UPLC-MS) using aflatoxin-$^{15}N^5$-guanine adduct standards afforded measurement of the AFB_1-N7-Gua and AFB_1-FAPY adducts six hours post dosing in liver DNA of mothers and embryos. A parallel cohort gave birth and the livers of the F1 were analyzed for mutations in the gpt gene at three and ten weeks of age. The data revealed mutational spectra dominated by G:C to T:A

mutations in both the mother and offspring that are characteristic of AFB_1 and distinct from background. It was shown that adducts in GD14 embryos were 20-fold more potent inducers of mutagenesis than adducts in parallel-dosed adults. This sensitivity enhancement correlated with Ki67 staining of the liver, reflecting the proliferative potential of the tissue. Taken together, these data provide insight into the relative genetic risks of prenatal and adult exposures to AFB_1. Early life exposure, especially during the embryonic period, is strikingly more mutagenic than treatment later in life. Moreover the data provide a baseline against which risk prevention strategies can be evaluated.

ABSTRACT FINAL ID: 2593 Poster Board -255
TITLE: A Transmission Electron Microscopic Analysis of the Effects of Vanadyl Sulphate on the Ultrastructure of MCF7 Cells

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KEYWORDS: Cancer Therapy, Apoptosis, TEM

ABSTRACT BODY: Vanadium compounds as well as other metal based agents have been used in treatment of different diseases including cancer disease. The different effects of vanadium compounds on a variety of cell lines were reported of which anti-tumorigenic activity is more investigated. The potential cytotoxic and apoptotic effects of these compounds on breast cancer cells (MCF7) ultrastructure is not investigated yet. In this study we investigated the potential cytotoxic effects of vanadyl sulphate (a vanadium salt) on MCF7 cells as well as its effects on this cell ultrastructure. For detecting the ultrastructural alterations caused by the 50% inhibition concentration (IC50) of vanadyl sulphate, MCF7 cells were incubated in flasks for 24 hours then treated with IC50 concentration for 24 hours. After the incubation, treated and untreated MCF7 cells were fixed in glutaraldehyde overnight at +4 °C. Following fixation, post fixation was realized in osmium tetroxide and the samples were dehydrated in ethanol then embedded in EPON 812 epoxy. Sectioned samples were stained in uranyl acetate and lead citrate then observed under a transmission electron microscope (TEM) and photographed. Our results demonstrated that vanadyl sulphate is highly cytotoxic in low dosages in MCF7 cells and caused important alterations and damages in the cells ultrastructure like ondulations and blebbings on the cell membrane, condensation and fragmentations of the nuclei. These alterations showed that vanadyl sulphate is effective in causing apoptotic cell death of MCF7 cells in low concentration and this may exert big importance for drug designing for cancer treatment but further investigations are required.

ABSTRACT FINAL ID: 2594 Poster Board -256
TITLE: Aminoflavone-Loaded Unimolecular Micelles Demonstrate Antitumor Activity in Triple Negative Breast Cancer Models

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KEYWORDS: Nanoparticle, Aminoflavone, EGFR

ABSTRACT BODY: Triple negative breast cancers (TNBCs) lack expression of common therapeutic targets, and they are associated with a poor prognosis. Therefore, development of targeted drug delivery systems to improve treatment outcome is necessary. Our lab has found that aminoflavone (AF, NSC 686288) is growth inhibitory in two TNBC cell lines. Despite *in vitro* efficacy, pulmonary toxicity may be associated with AF treatment *in vivo*. To address this, we have designed a nanoparticle (NP)-based drug delivery system specifically targeting TNBC to circumvent AF-associated toxicity. Because overexpression of epidermal growth factor receptor (EGFR) is common in TNBC, an EGFR-binding peptide (GE11) was used for selective targeting. Spherical morphology and uniform size distribution of the NPs was confirmed using transmission electron microscopy. Using flow cytometry and confocal microscopy, we demonstrated that GE11-conjugated NPs exhibited

enhanced cellular uptake in the EGFR-amplified TNBC cell line MDA-MB-468. Further, GE11-conjugated AF-loaded NPs displayed significantly greater cytotoxicity in this model compared to NPs lacking GE11. This data suggests that GE11-conjugated unimolecular micelle NPs efficiently target EGFR and deliver AF in an *in vitro* model of TNBC. Further, mice bearing MDA-MB-468 xenografts were subjected to treatment with control, free AF, or AF-loaded NPs either containing or lacking GE1. Tumor volumes decreased most significantly in mice treated with AF-loaded GE11-conjugated NPs. This data suggests that GE11-conjugated NPs loaded with AF exhibit anti-tumor activity in an *in vivo* model of TNBC. Overall, our work suggests that unimolecular micelles are effective tools for specific delivery of anti-cancer drugs, such as AF, to TNBC tumors.

ABSTRACT FINAL ID: 2595 Poster Board -257
TITLE: Characterization of a SF129 "Humanized" Mouse Transgenic for Human Cytochrome p450 1B1

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KEYWORDS: CYP1B1, Transgenic Animal Model, PAH Metabolism

ABSTRACT BODY: Cytochrome p450 1B1 (Cyp1B1) are xenobiotic metabolizing enzymes with endogenous roles in retinal development. Targeted substrates include exceptionally hydrophobic compounds, such as polycyclic aromatic hydrocarbons (PAH). Cyp1B1 metabolically activates several species of environmentally relevant procarcinogenic PAHs to reactive carcinogens. PAHs are AhR agonists, leading to Cyp1B1 upregulation. Though Cyp1B1 is highly conserved across species, wild type mouse cyp1b1 differs from human Cyp1B1 in several ways resulting in only 5 of the 11 xenobiotic responsive elements found in mouse being conserved in humans. We hypothesize that a transgenic "Humanized" Cyp1B1 mouse model can be generated and validated for tissue specific expression of Cyp1B1. This model would be used to ethically study human-like metabolism of carcinogenic, or suspected carcinogenic compounds, such as PAHs, in exposure related disease. A wild type control mouse (B6 129 sF1 female x 129s male) was used as a positive control for native mouse expression. Mixed litters containing experimental hemizyogous Cyp1B1 and negavite control cyp1b1/Cyp1B1 null mice were generated by crossing a hemizygous Cyp1B1 female (null for mouse cyp1b1) and a null Cyp1B1 and cyp1b1 male. Inclusion in study group was determined experimentally via PCR genotype. mRNA expression was determined by tissue type (gonad, thymus, lung, Liver) via qRT-PCR. Current data indicates that mRNA expression in humanized mice may be lesser than that of wild type mice. Potential applications of model include translational model of mouse to human risk from environmental exogenous lipophilic contaminant exposure.

ABSTRACT FINAL ID: 2596 Poster Board -301
TITLE: Use of Spirometry for Medical Clearance and Surveillance in Occupations Requiring Respirator Use

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KEYWORDS: Occupational Health, Respiratory Protection, Workplace Safety

ABSTRACT BODY: Medical certification of workers for respirator use is an important activity of occupational medicine health professionals. Spirometry is a diagnostic tool to evaluate respiratory distress/insufficiency that may affect respirator use. In this study, we analyzed the pulmonary function data of 337 workers from different occupations who required medical evaluation to wear a respirator. The American Thoracic Society (ATS) and National Fire Protection Association criteria were used to evaluate workers. Of 337 workers who were cleared for respiratory use on the basis of medical questionnaires for respirator compliance, 14 (4.15%) failed to pass respirator compliance on the basis of NFPA criteria and 5 (1.48%) failed to pass respirator compliance criteria on the basis of ATS criteria. We compared the use of different Spirometric equations to evaluate these criteria and we found the Crapo equation cleared more workers for respirator use when compared to the Knudson and NHANES III equations. We also measured repeated Forced Expiratory Volume in 1st

Second (FEV1) and Forced Vital Capacity (FVC), and compared the results longitudinally over time. Age was the only significant factor affecting the reduction in the lung function in longitudinal analyses. Longitudinal spirometry results suggested that workers were protected while using a respirator in the workplace, but age is a significant factor in reducing their lung function. Since, some workers were able to qualify for respirator use based on a questionnaire alone, but failed respirator clearance subsequent to pulmonary function testing, it is recommended that spirometry be used to evaluate clearance for all workers who will use a respirator in the workplace. As well, using different spirometric equations can affect the outcome on passing or failing clearance for respirator use and this should be considered in a respiratory medical certification program.

ABSTRACT FINAL ID: 2597 Poster Board -302
TITLE: Exposure Doses Rates to Indoor Ultrafine Particles in Primary Schools Environments

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KEYWORDS: Indoor Air Quality, Ultrafine Particles, Primary Schools

ABSTRACT BODY: Children attending primary schools may be largely exposed to the ultrafine particles (UFPs) present in the classroom's indoor air, which may lead to severe health consequences resultant from their increased susceptibility. The current study aimed to estimate the UFP exposure dose rates in Portuguese children attending public primary schools. Ultrafine particles were sampled in 10 primary schools located in Porto, Portugal. Exposure dose rates were estimated for 488 children aged 8 to 10 years. The estimated mean of exposure dose rates in children were 4.06 x 108 ± 0.11 x 108 part/kg.day. Specific indoor activities as well as outdoor environment conditions appeared to be associated with increased indoor UFP number concentrations and, consequently, with higher exposure doses in children attending those schools. Overall, children showed at least two times higher UFP exposure dose rates when compared to occupationally exposed adults (i.e. teachers and school staff). This work was supported by Fundação para Ciência e Tecnologia through ARIA project (PTDC/DTP-SAP/1522/2012).

ABSTRACT FINAL ID: 2598 Poster Board -303
TITLE: Oxidative Stress and Lung Histopathology following Subacute Exposure to Natural Dust from the Nellis Dunes Recreation Area

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KEYWORDS: Arsenic, Oxidative Stress, Risk Assessment

ABSTRACT BODY: Exposure to particulate matter containing heavy metals can induce adverse health effects through industrial exposures; however, little data exist on effects associated with natural exposures. We evaluated markers of oxidative stress and lung histopathology following sub-acute exposure to metals-containing dust collected from a natural setting used heavily for ORV recreation. Adult female B6C3F1 mice were exposed to dust collected from seven surface types at the Nellis Dunes Recreation Area. Dust representative of each surface type was prepared with a median diameter of ≤4.5µm, suspended in PBS, and given by to oropharyngeal aspiration at 0.01 - 100 mg of dust/kg body weight. Four exposures a week apart over 28-days mimicked a month of weekend exposures. Lungs for histopathology and blood for evaluation of oxidative stress markers were collected 24 hours after the final exposure. These data were compared to similar measures collected from mice exposed to titanium dioxide (TiO2), a particle devoid of a complex metal mixture. No

single surface type consistently induced markers of oxidative stress. Two highest concentrations of dust from one surface type increased two markers of oxidative stress; results of other surface types were inconsistent. These observations were relatively consistent with TiO2; a significant change at the highest exposure was observed in one measure of oxidative stress. All surface types induced some level of lung inflammation, typically at highest doses. These results indicate that exposure to these natural, mineral dusts, concomitant with our exposure scenario, while are unlikely to considerably increase the risk of oxidative damage systemically, may induce local effects of lung inflammation in exposed individuals.

ABSTRACT FINAL ID: 2599 Poster Board -304
TITLE: Wearable Technology Products and Allergic Contact Dermatitis: A New Risk Assessment Challenge

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KEYWORDS: Risk Assessment, Allergic Contact Dermatitis, Wearable Technology Products

ABSTRACT BODY: Wearable products with electronic components are being introduced to consumers without formal biocompatibility testing or health risk assessment. Some products involve prolonged skin contact with plastic and metal components under occluded conditions. Recent media reports describe occurrences of wearable technology products causing skin reactions, due to allergic contact dermatitis (ACD). There is no standardized methodology for measuring chemical leaching or assessing ACD elicitation risk from these types of wearable products. To fill this gap, a quantitative risk assessment model was developed that incorporates both estimates of dermal exposure and ACD elicitation risk. Product testing involves using an artificial sweat solution, for varying time periods, to reflect product-specific exposure-use scenarios and analyzing leachate for sensitizing chemicals. A potential applied dermal dose or load is assessed as µg/cm2/unit time. Initially, an ACD elicitation risk model was developed based upon nickel elicitation data as reported in published human patch test studies (as % of sensitized user population expected to have ACD reactions at specified dermal loads). Distribution of population sensitivity to nickel was applied to the patch test dose-response data for other sensitizing chemicals with limited patch test data, to characterize sensitized population response. Results indicate that the sensitizing metals, nickel, chromium and cobalt, and sensitizing organics, primarily acrylate and epoxy compounds, are leached from a variety of tested wearable product prototypes. Dermal loads were estimated to range from <1 to >50 µg/cm2/week with chemical loads potentially posing a wide range of risks of ACD reaction in sensitized users, with <0.01% to >10% of the sensitized users expected to react. This methodology can help manufacturers to identify wearable technology products at high risk of leaching and causing ACD reactions, prior to introduction into consumer markets.

ABSTRACT FINAL ID: 2600 Poster Board -305
TITLE: Proposed Inhalation Reference Exposure Levels for Toluene Diisocyanate and Methylene Diphenyl Diisocyanate

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KEYWORDS: Noncancer Risk Assessment, Inhalation Risk Assessment, Human Health Risk Assessment

ABSTRACT BODY: Toluene diisocyanate (TDI) and methylene diphenyl diisocyanate (MDI) are known pulmonary irritants and sensitizers used in the manufacture of polyurethane. Acute human exposure to 10 ppb TDI resulted in an asthmatic reaction in non-sensitized asthmatics. Long-term occupational exposure to TDI resulted in accelerated pulmonary function loss at 1.9 ppb, and a NOAEL of 0.9 ppb. For acute 1-hr TDI Reference Exposure Level (REL) derivation, a total uncertainty factor (UF) of 30 was applied to the LOAEL of 10 ppb, resulting in a proposed acute REL of 0.3 ppb. Time–adjustment for an 8-hr REL included 7 d per week exposure, and continuous exposure for the chronic REL. For both RELs a subchronic UF of v10 and an intraspecies UF of 100 (for toxicogenomic variability) was applied, resulting in RELs of 0.002 (8-hr) and 0.001

ppb (chronic). MDI RELs were based on animal studies. Rats exposed to 0.7 mg/m3 MDI for 6 hrs resulted in transient increased protein in lavage fluid. Time adjustment to a 1-hr exposure based on c x t data, and a human equivalency concentration (HEC) adjustment from rodent to human exposure generated an adjusted LOAEL of 7.2 mg/m3. UFs of √10 for LOAEL-to-NOAEL, 6 for interspecies and 30 for intraspecies resulted in a proposed acute REL of 12 µg/m3 (1.2 ppb). The 8-hr REL is based on a 2-yr study in rats exposed 6 hrs/d resulting in bronchiolo-alveolar hyperplasia, and the chronic REL is based on a 2-yr study in rats exposed 18 hrs/d resulting in pulmonary fibrosis. Benchmark dose modeling produced points of departure (POD) of 0.12 (8-hr) and 0.026 (chronic) mg/m3. Following HEC and time-adjustment, the adjusted PODs were 0.095 (8-hr) and 0.046 (chronic) mg/m3. Cumulative UF of 600 (6 for intraspecies; 100 for interspecies) was applied to both resulting in proposed 8-hr and chronic RELs of 0.16 and 0.08 µg/m3 (0.015 and 0.008 ppb), respectively.

ABSTRACT FINAL ID: 2601 Poster Board -306
TITLE: Implementation of Toxicokinetics in toxicity Studies—Example of 4-Methylanisole

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KEYWORDS: Risk Assessment, Alternatives to Animal Testing, Toxicokinetics

ABSTRACT BODY: The current risk assessment of compounds is generally based on external exposure and effect relationships. External doses are often not representative for internal exposure concentrations. The aim of this study was to show how the implemention of toxicokinetics in a scheduled toxicity study contributes to improved data interpretation without additional use of animals and to the three goals of the 3R principles for animal testing. Toxicokinetic analyses were implemented in a rat developmental immunotoxicity study with 4-methylanisole without interfering with the outcome of the study and without the use of additional animals. 4-Methylanisole and its metabolites were analysed in plasma of adult rats and in pups at postnatal day 10. 4-Methylanisole has a short half-life in adult animals and the plasma concentrations increased more than proportional with increasing dose. The metabolic profile appeared to be different at low dose as compared to high dose. This information on the dose proportionality of the internal exposure is crucial for the interpretation of the toxicity data and helps to identify the toxic agent and the appropriate dose metric. The metabolism was comparable in adult and juvenile animals. Large inter-individual variability in adult animals, as observed for 4-methylanisole, may hamper dose-response analyses of the results. In addition, 4-metylanisole was excreted via milk, but concentrations in the juvenile animals appeared to be 20-100-fold lower than via direct gavage exposure. The toxicokinetic parameters support the data interpretation, among others by providing better insight into internal exposures. Subsequently, it will help to prevent testing of irrelevant exposure scenarios and exposure concentrations. Overall, implementation of kinetics with limited effort provides useful information to support the interpretation of toxicological data and can contribute to reduction and refinement of animal testing.

ABSTRACT FINAL ID: 2602 Poster Board -307
TITLE: Empirical Approach to Mathematical Modeling of Carcinogenic Potency for Various Mineral Types of Asbestos Fibers

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KEYWORDS: Asbestos, Risk Assessment, Mathematical Modeling

ABSTRACT BODY: Objective: Epidemiological studies of mesothelioma mortality in asbestos exposed cohorts and associated meta-analyses have demonstrated different potency factors. The hypothesis that asbestos mineral type significantly affects mesothelioma risk has been proposed by many scientists. The objective of this paper is to demonstrate correlation of mesothelioma potency factors with measurable characteristics of various mineral types of asbestos. Methods: Mesothelioma potency factors associated with Quebec chrysotile, African amosite, Libby amphibole, as well as Australian and South African crocidolite, as determined by Hodgson and Darnton (2000, 2010), were modeled as a function of asbestos fiber type chemical composition and observed half-life of the fibers in human lungs. Results : It was demonstrated

that mesothelioma potency can be adequately quantified as a function of pulmonary biopersistence as well as both average Fe3+ and Mg2+ content of each fiber type, with significant correlation between observed and modeled potency values (R=0.9993, P=0.002). The model also yields plausible estimations of the potency for tremolite, actinolite, erionite, balangeroite, anthophyllite, Bolivian crocidolite, as well as chrysotile from Russian and Zimbabwe mines. The model allows to conditionally consider asbestos fiber toxicity as a combination of two main (and potentially interrelated by themselves) factors: 1. Biopersistence of the asbestos fibers, and 2. Their ability to produce chemical destabilization of the cell environment. High correlation of the mesothelioma potency with Fe3+ content in the fibers and negative correlation with Mg2+ content can be suggestive of specific toxicological modes of action. Conclusions: Mathematical modeling can be instrumental in assessment of hidden modes of actions, requiring additional studies and systematic validation. The proposed modeling approaches, in spite of their explicit empirical character, may constitute a basis for additional targeted studies that can serve as a basis for further discussion of asbestos-related cancer mechanisms.

ABSTRACT FINAL ID: 2603 Poster Board -308
TITLE: P-Chloronitrobenzene-Induced Methemoglobinemia: A Potential Biomarker

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KEYWORDS: Risk Assessment, Biomarker, p-Chloronitrobenzene

ABSTRACT BODY: p-Chloronitrobenzene (PCNB) (CASRN 100 00 5) is used in the synthesis of industrial chemicals, drugs, and pesticides. Exposure may occur in occupational settings, and, in the general public through contamination of environmental media. PCNB can enter the body through oral, inhalation, and dermal routes, and is then bio transformed in the liver to p chloroaniline and its N hydroxy metabolite. PCNB exposure has been associated with effects on hematopoietic system (methemoglobinemia, anemia, and unspecified vascular tumors), spleen and liver (hematopoiesis, hemosiderosis, and hemangiosarcomas), kidney (hemosiderosis), and bone marrow (hyperplasia). Some evidence for reproductive (oligospermia, and reduced fertility), and developmental toxicities (decreased fetal weight, and increased skeletal anomalies, and resorptions) also exists. Of all the toxicological effects associated with PCNB exposure, the critical effect is methemoglobinemia with LOAELs for oral subchronic and chronic exposure of 3.0 and 0.7 mg/kg-day, respectively, in both sexes of rats, and an inhalation subchronic LOAEL of 1.7 mg/m3 in both sexes of rats. The selection of methemoglobinemia in both sexes of rats as the critical effect is supported by dose-dependent increases in subsequent toxic effects on other organs (e.g., spleen, liver, kidney, and bone marrow), including paleness of extremities and cyanosis. The proposed toxic mode of action involves the transport of the N hydroxy metabolite from the liver to erythrocytes where the N hydroxy metabolite is oxidized and covalently binds to protein sulfhydryl groups leading to oxidative stress, erythrocyte cytotoxicity, and concomitant formation of methemoglobin. Nonneoplastic and neoplastic lesions in other organs are considered to be a progression associated with erythrocyte destruction and methemoglobinemia. These data support the hypothesis that methemoglobinemia is an early biomarker of PCNB exposure [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]

ABSTRACT FINAL ID: 2604 Poster Board -309
TITLE: Risk Assessments at Closed Small Arms Shooting Ranges Need to Consider Lead in Soil and in Lead Bullet/Shot Sources

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KEYWORDS: Risk Assessment, Lead, Munitions

ABSTRACT BODY: Lead bullets/fragments/shot (i.e., lead munitions debris (MD)) account for most of the lead remaining at shooting ranges which continue to weather to more soluble lead compounds in a site-specific manner (Rooney and McLaren 2000; Rooney et al., 2007). Each .30 caliber (Cal) bullet initially contains ~8000 mg lead. The complete release and mixing of lead from one .30 Cal bullet would bring 100 kg of soil to the 80 mg/kg lead screening level for residential use in California. However, there is only about a 1 in 100,000 chance the bullet would be analyzed using grab sampling because EPA Method 3050B/6010B only digests 1 gram of soil. This is not sufficient to assess risk of future use, since over 99.9% of the samples would miss the bullet and underestimate total lead. We developed a screening-level survey approach that allows a metal detector to rapidly screen the top 6 inches of soil for density of lead MD that would exceed 80 mg/kg total lead after complete MD dissolution. Lead bullets were not visible at several shooting ranges that had been closed for 40 to 70 years, but were easily detected in the top 6 inches of soil with a Whites TDI SL metal detector. Screening rifle and pistol ranges with a TDI SL detector easily identified many locations with a high density of lead bullets and over 10,000 mg/kg total lead that had been missed by gridded sampling for lead using X-ray fluorescence (XRF). Therefore, shooting ranges should also be screened for density of lead MD with a suitable metal detector. For detecting No. 9 lead shot, a Whites GMT detector was suitable at up to 2 inches and could also aide in screening for potential bird ingestion exposure. To account for total lead, we recommend collecting large samples where spent ammunition is most concentrated, sieving the entire sample to recover and quantitate lead gravimetrically. Lead should also be measured in fine sieved soil by XRF or EPA Method 3050B/6010B. Cleanup and risk management decisions regarding future use should be made on total lead in combined lead MD and fine soil.

ABSTRACT FINAL ID: 2605 Poster Board -310
TITLE: Does Agglomeration State Influence Titanium Dioxide Nanoparticle Kinetics after Intravenous Injection in Rats?

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KEYWORDS: Titanium Dioxide, Nanoparticles, Agglomeration State

ABSTRACT BODY: Nanoparticles (NPs) tendency to agglomerate prompts investigations of the impact of agglomeration state on kinetic time courses. This has yet to be evaluated for titanium dioxide (TiO2) NPs. The aim of this study was to determine the effects of two different agglomeration states of intravenously administered TiO2 NPs on the kinetics in blood, tissues and excreta. Adult male Sprague-Dawley rats were injected with 1 mg/kg bw of commercial anatase TiO2 NPs (~20 nm core size), suspended in physiological saline (high agglomeration state) or Emulphor 1% solution (lower agglomeration state). Blood, urine and feces were collected at 0, 2, 4, 8, 12, 24 and 72 h post-dosing (n = 5 rats per exposure condition) and tissues (lung, liver, kidney, spleen) were excised in groups of rats sacrificed after 1, 3, 7 and 14 days (n = 5 per group). TiO2 hydrodynamic diameters (dH) in suspended solutions were determined by dynamic light scattering (DLS). Biological Ti levels were quantified by ICP-MS. DLS characterization confirmed a high agglomeration of TiO2 in saline solution with dH> 2.5 µm, while it is lower than 1 µm in Emulphor solution. There were no clear differences in time courses of TiO2 in blood between the high and low agglomeration state (on average 0.012-0.072 and 0.018-0.048 µg/ml, respectively), except for slightly higher concentrations at 72 h post-dosing for the high agglomeration state. Lung and kidney profiles were similar following both exposure conditions while spleen and liver time courses showed that peak values were reached more rapidly with the high agglomeration state (at 72 h compared to 14 days). There were also apparent differences in excretion profiles of TiO2 in urine, with mean total excretion (± SD) over 72 h post-dosing reaching 3.7±0.9 and 1.2±0.2% for the high and low agglomeration state, respectively. It is thus necessary to consider agglomeration state of TiO2 NPs for a proper interpretation of its kinetics.

ABSTRACT FINAL ID: 2606 Poster Board -311
TITLE: Application of a Tiered Surrogate Approach to Identify Toxicity Surrogates for Human Health Risk Assessment

AUTHORS (FIRST INITIAL, LAST NAME): P. R. Dodmane[1], L. E. Lizarraga[1], J. Kaiser[2], S. C. Wesselkamper[2], J. Zhao[2]

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KEYWORDS: Surrogate, Human Health Risk Assessment, Structure Activity Relationship

ABSTRACT BODY: Chemicals with insufficient toxicity data pose a challenge for human health risk assessment. In such cases computational toxicological methods can be used to inform derivation of reference values for these chemicals based on toxicological information for their corresponding surrogates. In this study, a tiered surrogate approach (Wang et al., 2012) was applied to identify potential surrogates for cis- and trans-nonachlor, oxychlordane, dichlorodiphenyldichloroethane (4,4-DDD) and dichlorodiphenyldichloroethylene (4,4-DDE). Available toxicity literature was compiled by searching PubMed, DSSTox, and ChemIDplus. Applying the tiered surrogate approach, structure relationship evaluation via DSSTox and ChemIDplus identified structural analogs. Only structural analogs with chronic reference values from IRIS and/or PPRTV assessments were evaluated further. The metabolic information and toxicity profile for the structural analogs were then compiled and analyzed. Ultimately, based on weight-of-evidence, the final surrogates were identified. Oxychlordane is the major toxic metabolite of nonachlor and chlordane. 4,4-DDD and 4,4-DDE are major metabolites of dichlorodiphenyltrichloroethane (4,4-DDT). The metabolites have similar organ toxicity profiles as that of parent compounds. Our analysis suggests that chlordane is an appropriate surrogate for both cis- and trans-nonachlor and oxychlordane. Additionally, 4,4-DDT is a viable surrogate for both 4,4-DDD and 4,4-DDE. This study exemplifies how the tiered surrogate approach combines structural, metabolic, and toxicity information to identify potential surrogates, thus informing potential toxicity and human health risk for data-poor chemicals. The views expressed in this abstract are those of the author and do not necessarily reflect the views and policies of the U.S. EPA.

ABSTRACT FINAL ID: 2607 Poster Board -312
TITLE: Toxicological Review of Biocides Used in Hydraulic Fracturing—2015 Update

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KEYWORDS: Fracking, Unconventional Oil and Gas Exploration, Antimicrobial

ABSTRACT BODY: The use of hydraulic fracturing (i.e., fracking) to extract gas and oil form shale formations has maintained its exponential growth in US and worldwide. Hydraulic fracturing consists of injecting water, friction reducers, proppants, disinfectants, surfactants, thickeners, scale inhibitors, corrosion inhibitors, and acids to promote flow of hydrocarbons otherwise bound in impermeable matrices. The rapid explosion of volume and extent of fracking exploration has led to community, regulator, and health practitioner concerns over the potential health effects associated with exposure to fracking fluid constituents. Many of the disclosed ingredients are cited as harmless. However, there are certain chemical groups, such as antimicrobials, that have specific (by design) adverse biological activity. This presentation focuses on the identity and toxicity of fracking fluid constituents listed as having a disinfectant function. The methodology consisted of querying publicly-available databases used by the oil and gas industry to disclose chemical information. Data fields associated with chemical name, CAS number, additive concentration, and fracking fluid concentration were extracted for each disclosed biocide. Next, retrieved records were matched with toxicity data from open literature. The information on the stock as well as the formulated fluid concentrations, in concert with toxicity thresholds, was used to calculate Potential for Exposure Quotients (PEQs) under an accidental release scenario. Results for 2013 show that nearly 100 hydraulic fluid additives are categorized as a biocide and/or an antimicrobial agent. Some of the more commonly listed disinfectants are glutaraldehyde (CAS 111-30-8), naphthalene (CAS 91-20-3), ethoxylated nonylphenol (CAS 9016-45-9), and tetrakis hydroxymethyl-phosphonium sulfate (CAS 55566-30-8). Aldehydes were found to be the most common (N=1,678) toxic (lowest RfC of 8.0E-05 mg/m3) fluid ingredients with the highest PEQs (average fracking fluid concentration of 2.5%). For the purpose of this presentation, all findings are presented as summary tables for ease of reference and sharing of information.

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Late-Breaking Abstracts

ABSTRACT FINAL ID: 2608 Poster Board -313
TITLE: Communication of Uncertainty in Human Health Risk Assessments

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KEYWORDS: Risk Assessment, Communication, Uncertainty

ABSTRACT BODY: A systematic risk assessment (RA) is a time consuming and very detailed process that can often contain hundreds of pages and in depth discussion of scientific details. However, the culmination of such a human health RA is often a single point of departure (PoD) based on the most sensitive endpoint. In the best case, multiple PoDs are developed and presented for all relevant endpoints. What are often brought forward from these assessments for decision making/communication are the single point estimates, while the assumptions and uncertainties of the assessment are lost, which may therefore provide a false sense of precision. Many factors can influence the final PoD including experimental variability, quality of data, and underlying regulatory tenets (e.g. precautionary principle). All of these influencing factors and many others encompass the underlying uncertainty of the RA, which is not reflected in the PoD. Though this uncertainty is often articulated within a RA, and the importance is clearly recognized, the approach to account for uncertainty and communicate the level of uncertainty that exists in a given assessment is something with which the RA community continually grapples. The Center for Advancing Risk Assessment Science and Policy (ARASP) held a workshop in Nov 2013 “Workshop Informing Risk Assessment: Understanding and Communicating Uncertainty in Hazard Assessment” that evaluated different methods for communicating uncertainty in a regulatory assessment of chemicals using IRIS assessments for Acrylamide and Carbon Tetrachloride. The results of that workshop are being presented at a Tues. AM SoT Workshop Session entitled “Understanding and Communicating Uncertainty in Hazard Assessment and Dose Response”. In this presentation, the methods developed at the ARASP workshop are expanded to current RAs from other regulatory frameworks, along with a discussion of their utility, clarity, and suggestions for further refinement. These approaches offer promise as an improved means of communicating and conveying uncertainty to regulatory decision makers, risk managers, and the public.

ABSTRACT FINAL ID: 2609 Poster Board -314
TITLE: Derivation of a Human Equivalent Concentration for Chronic Inflammation in the Bronchial and Bronchiolar Epithelium of the Lung following Inhalation Exposure to Diacetyl

AUTHORS (FIRST INITIAL, LAST NAME): M. E. Glynn[1], R. Adams[1], E. M. Beckett[1], J. S. Pierce[1], P. K. Scott[1], B. L. Finley[1]
INSTITUTIONS (ALL): 1. Cardno ChemRisk, Chicago, IL, United States.
KEYWORDS: Diacetyl, Benchmark Dose, Human Equivalent Concentration

ABSTRACT BODY: Severe respiratory disorders such as bronchiolitis obliterans (BO), a condition that results from inflammation of the deep lung, have been alleged in some food and flavorings workers following exposure to diacetyl and other flavoring compounds. Some investigators have proposed ‘exposure thresholds’ for diacetyl that represent 8-hr exposures, below which there is little to no risk of respiratory effects. The purpose of this analysis was to derive human equivalent concentrations (HECs) for selected endpoints using data from the Morgan et al. (2008) subchronic mouse study, and to build upon previous threshold analyses using refined parameters and methods. Two endpoints were selected, as they were the effects that occurred in the deepest portion of the lung in the study animals: 1) peribronchial lymphocytic inflammation (PLI), and 2) peribronchiolar lymphocytic inflammation (PRLI). The diacetyl concentrations associated with a 10% excess risk (BMC_{10}) and its 95% lower confidence bound ($BMCL_{10}$) were estimated for both endpoints using EPA guidelines, BMDS software, and the available dose-response models for a dichotomous outcome. The estimated $BMCL_{10}$ values were adjusted to HECs in the following manner: 1) adjustment of the $BMCL_{10}$ estimates to rat-equivalent estimates using a regional gas dose ratio, and 2) application of a region-specific time-weighted human:rat ratio based on the Gloede et al. (2011) CFD-PBPK model. The selected models yielded BMC_{10} ($BMCL_{10}$) values that ranged from 7.30 (1.98) ppm to 13.5 (6.36) ppm for PLI and 70.2 (35.1) ppm to 86.0 (35.5) ppm for PRLI. The associated HECs were estimated to range from

0.582 ppm to 1.87 ppm and 6.47 ppm to 6.54 ppm for PLI and PRLI, respectively. Our results were consistent with those reported by Maier et al. (2010), who calculated an HEC of 1.8 ppm for minimal to mild PLI. These HECs should be considered when evaluating the risk of developing BO and other serious respiratory diseases following diacetyl exposure.

ABSTRACT FINAL ID: 2610 Poster Board -315
TITLE: Refined Derivation of a Human Equivalent Concentration for Hyperplasia of Bronchiolar Epithelium following Airborne Diacetyl Exposure

AUTHORS (FIRST INITIAL, LAST NAME): E. M. Beckett[1], M. E. Glynn[1], R. Adams[1], J. S. Pierce[1], P. K. Scott[2], B. L. Finley[3]
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KEYWORDS: Diacetyl, Benchmark Dose, Human Equivalent Concentration

ABSTRACT BODY: Bronchiolitis obliterans (BO) and other severe respiratory diseases have been alleged to occur among food and flavorings workers as a result of exposure to diacetyl and other flavoring compounds. Some investigators have proposed 'exposure thresholds' for diacetyl that represented 8-hr cumulative exposures, below which there was little risk of deleterious respiratory effects. Unpublished results from a 2008 National Toxicology Program (NTP) study, in which rodents were exposed to concentrations of airborne diacetyl for 6 hours per day, 5 days per week, and for approximately 90 days have recently become available; this data has subsequently been analyzed by NIOSH in a recent BMC analysis. The aim of our study was to perform an updated analysis using these data and refined model input parameters to derive human equivalent concentrations (HECs) for hyperplasia in the bronchiolar epithelium (HBE), the effect that occurred in the deepest portion of the lung in the study animals. The diacetyl concentrations associated with a 10% excess risk (BMC_{10}) and its 95% lower confidence bound ($BMCL_{10}$) were estimated for HBE according to EPA guidelines and using the BMDS software; all available dose-response models for a dichotomous outcome were considered. The estimated $BMCL_{10}$ values from selected models were adjusted to HECs by the application of a time-weighted human:rat ratio, which incorporated: 1) study-specific rodent body weights, 2) minute volumes, and 3) measured tissue diacetyl concentrations in bronchiolar epithelium that were obtained from the Gloede et al. (2011) CFD-PBPK model. The analysis yielded BMC_{10} ($BMCL_{10}$) values that ranged from 66.9 (53.6) ppm to 92.9 (60.4) ppm for HBE, and the corresponding HEC was estimated to range from 1.35 to 1.53 ppm. Given that HBE is thought to occur in the early stages of BO, these HECs should be considered in evaluating the risk of BO and other serious respiratory diseases following diacetyl exposure.

ABSTRACT FINAL ID: 2611 Poster Board -316
TITLE: Dose Response from High-Throughput Gene Expression Studies and the Influence of Time and Cell Line on Inferred Mode of Action by Ontologic Enrichment

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KEYWORDS: High Throughput, Dose Response, Ontologic

ABSTRACT BODY: Gene expression with ontologic enrichment and connectivity mapping tools is widely used to infer modes of action (MOA) for therapeutic drugs. Despite progress in high-throughput (HT) genomic systems, strategies suitable to identify industrial chemical MOA are needed. The L1000 is a HT genomics platform that measures 1000 landmark genes to then computationally predict expression across a whole human genome equivalent array. We used the L1000 system with visualization tools to assess gene expression changes and ontologic enrichment for 9 agrochemicals at 9 concentrations across 5 cell lines at 6 and 24 hr after treatment. The cell lines were the metabolically competent HepaRG line and 4 cancer cell lines: A549 (lung), A673 (bone), HT29 (colorectal) and MCF7 (breast). Genes significant for a monotonic dose response (log likelihood ratio test with permutations) were used for analyses. Differential gene expression levels varied significantly

across cell lines and times, with no one cell line consistently responsive for any chemical at both times. A broad assessment of the 9 chemicals showed that A549 and HT29 cells (at 6 hr) and HepaRG and A673 cells (at 24hr) were typically the most responsive. At 6hr, A549 and HT29 cells tended to show significant gene enrichment of cell cycle mitotic processes. At 24hr, A673 cells tended to show gene enrichment of immune response, disease and cellular metabolic pathways. Comparison of HepaRG and A673 cell responses (at 24hr) for fenbuconazole, associated with liver effects, showed enrichment of common parent pathways (DNA repair, cell signaling) in both, but several child categories of these were identified only in HepaRGs. These findings suggest selection of appropriate time points and the use of multiple cell models should be considered in HT genomics strategies designed to inform chemical MOA. This abstract does not necessarily reflect U.S. EPA policy.

ABSTRACT FINAL ID: 2612 Poster Board -317
TITLE: The Impact of Rodent Reflex Bradypnea on Human Health Assessments of Inhaled Irritants

AUTHORS (FIRST INITIAL, LAST NAME): J. E. Whalan[1], J. Pauluhn[2], A. D. Kraft[1], S. L. Makris[1]
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KEYWORDS: Formaldehyde, Inhaled Irritants, Isocyanates

ABSTRACT BODY: Reflex bradypnea (RB) is a protective sensory reflex that allows rodents—but not humans—to markedly reduce their exposure to inhaled upper respiratory tract (URT) irritants such as aldehydes, isocyanates, and pyrethroids. When an irritant stimulates trigeminal nerves in the URT, rodents experience a rapid and sustained decrease in ventilation (as much as 90%) so they inhale a much lower chemical dose than if they were breathing normally. This bradypnea is accompanied by decreases in body temperature (as much as 14°C), metabolic rate, heart rate, and activity, and altered acid-base status. These protective physiological effects may be misconstrued as adverse "systemic" outcomes. This poster will demonstrate that a health or risk assessment that fails to account for a reduced inhaled rodent dose may be biased to understate the true human risk. It will also demonstrate that behavioral and developmental effects in rodents experiencing or recovering from RB-induced hypothermia may not be relevant to humans. For example, RB-induced hypothermia can impede learning and motor function tests (swim maze, rotarod, etc.). RB in pregnant dams can result in fetal hypothermia, altered placental transfer of O2 (hypoxia) and CO2 (hypercapnia), developmental disturbances, and other effects that may be erroneously attributed to a test article. The impact RB can have on health and risk assessments has not received the attention it deserves from toxicologists and risk assessors, largely because current testing guidelines do not require examination of RB-related endpoints. This analysis shows the major impact RB can have on the interpretation of findings, and it demonstrates why it may be necessary to adjust points-of-departure in risk assessments of inhaled irritants (e.g., formaldehyde) to make them health protective for humans. The views expressed are those of the authors, and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency or Bayer Pharma AG.

ABSTRACT FINAL ID: 2613 Poster Board -318
TITLE: Diisocyanate Exposures from the Application of Spray Polyurethane Foam (SPF) Insulation: A Risk Assessment

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KEYWORDS: Isocyanates, MDI, Spray Polyurethane Foam

ABSTRACT BODY: Methylene diphenyl diisocynate (MDI) is a known respiratory irritant and can induce occupational asthma. Exposure to diisocyanates in spray polyurethane foam (SPF) insulation has been reported to cause adverse health effects including respiratory irritation, burning eyes, nausea, dizziness, and asthma. In March 2014, the Cal EPA DTSC listed SPF as one of their first Priority Products over concerns of the health effects of uncured diisocyanates. Given the increasing use of SPF, this analysis sought to evaluate the potential health risks bystanders exposed to MDI during application. A systematic review, following PRISMA guidelines, of literature identified from PubMed and TOXNET was conducted with "spray polyurethane foam" and the following search terms: "health," "exposure," "isocyanates" and "toxicity". Industry

white papers, MSDSs, and government reports were also considered. Specific eligibility criteria were applied to all relevant documents identified. Identified exposure concentrations were grouped by job type and distance from the application and compared to regulatory standards including the RfC, ACGIH 8hr TWA, OSHA ceiling limit and NIOSH 8hr REL and ceiling limit. In total, 45 documents matched our search terms; 7 papers assessed the exposure, toxicity, or health risk of SPF isocyanates, and 3 papers with adequate data were included in this analysis. Our results suggest that bystanders 6 to 30 meters from the application were exposed to average MDI concentrations of 0.037 mg/m3. This concentration is 62 times the RfC, but is 70% of ACGIH 8hr TWA, 18% of the OSHA and NIOSH ceiling limits, and 75% of the NIOSH 8hr REL. Our findings can be used to assess potential health risks of bystanders occupationally exposed to SPF application. It should be noted that the RfC for MDI is based on minimizing the risk of olfactory epithelium hyperplasia. The ACGIH TLV, however, is set to minimize the likelihood of pulmonary decrements and respiratory tract sensitization, which is a more pertinent endpoint.

ABSTRACT FINAL ID: 2614 Poster Board -319
TITLE: Using Google Trends As a Surveillance Tool for Health Concerns Related to Unconventional Resource Development

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KEYWORDS: Shale, Google Trends, Risk Communication

ABSTRACT BODY: The rapid increase in unconventional resource development has generated scrutiny by policymakers, researchers and the general public. One of the most commonly cited concerns is the potential for adverse health effects on the community where development is occurring. Google Trends (GT) have the potential to offer insights that could precede conventional triggers to human health risk assessments and provide information to the risk communication community. Monitoring the public's information searching behavior has been shown to detect changing population concerns, and public health events. For example, GT have been used to monitor influenza outbreaks. The premise is that there is a relationship between people searching for information on flu symptoms and those experiencing symptoms. In this case, GT were used to determine if public information searches can be used to reveal changes in community health due to unconventional resource development. Three longitudinal data-sets from January 2011 to December 2013 were used: LexisNexis (media coverage); Web of Science (Peer-reviewed studies); and Google Trends (Public information needs). Database search terms were selected based on exploring Google trends. Pearson's correlation coefficients was calculated between data-sets. Public queries about unconventional resource development and health showed positive correlations with media coverage (Pearson's r ranges of 0.3 to 0.7). No correlation was found between health related queries and peer-reviewed journal articles (lag-times not considered). An analysis of query terms revealed that the public searched for information on effects or consequences but not on potential symptoms. By monitoring GT it is possible to determine the public's information needs and also the public perception of unconventional resource development. However, it was not possible to determine if community health changes were occurring. That information requires a high incidence of health symptom searches to trigger a GT analysis. Currently, the public is not searching for symptom specific information.

ABSTRACT FINAL ID: 2615 Poster Board -320
TITLE: A Meta-Analysis of Paraoccupational Asbestos Exposures from Occupational Simulation Studies of Asbestos-Containing Encapsulated Products

AUTHORS (FIRST INITIAL, LAST NAME): A. Madl[1], K. Devlin[2], C. Poteete[1], D. M. Cowan[1]
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KEYWORDS: Asbetos, Exposure, Paraoccupational

ABSTRACT BODY: Paraoccupational exposures and asbestos-related lung disease among family members often involve laundering clothing worn by a working family member who experienced significant asbestos exposure themselves. In most household cases, workers who bring asbestos home were exposed to raw asbestos or friable asbestos-containing products

at relatively high concentrations in mining, manufacturing or shipyard workplace settings and most often involved amphiboles or amphibole-contaminated chrysotile. In contrast, handling chrysotile-containing encapsulated asbestos products (e.g., gaskets, packing, brakes, clutches) result in not only low worker exposures, but also low or negligible airborne asbestos concentrations when handling work clothes worn during these activities. Simulation studies have attempted to characterize potential paraoccupational exposures after working with a variety of asbestos-containing encapsulated products. A meta-analysis was conducted of 5 simulation studies to determine whether a common quantitative factor can be derived in determining how occupational work with encapsulated products translates to household exposures from laundering work clothes. Occupational and clothes handling exposure data were converted to 8 hr and 40 hr time-weighted averages; duration and number of clothing articles handled as well as method for assessing non-detectable measurements were also considered. Across 5 published simulation studies, exposures experienced while handling clothing worn during work with different encapsulated products were on average about 1 to 2% of the occupational exposures. The available data associated with handling and laundering clothing worn during work with encapsulated asbestos-containing products indicate that airborne asbestos concentrations are low and estimated to be in range with lifetime cumulative doses associated with ambient exposures.

ABSTRACT FINAL ID: 2616 Poster Board -321
TITLE: Assessment of 5-Aminotetrazole (5-AT) Toxicity in Acute and Subacute Oral Exposures to Male and Female Rats

AUTHORS (FIRST INITIAL, LAST NAME): V. H. Adams[1], M. R. Way[1], M. A. Bazar[1], W. S. Eck[1]
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KEYWORDS: Safety, Tetrazole, Perchlorate

ABSTRACT BODY: Perchlorate is an important oxidizer used by the US Army in explosives and propellants. The Army Environmental Quality Technology (EQT) Ordnance Environmental Program (OEP) is dedicated to finding replacements for perchlorate that have a lower probability of causing adverse environmental impacts and will reduce or eliminate the potential health risks from exposure. Perchlorate is very water soluble and known to interfere with iodide uptake in the thyroid gland. Due to environmental contamination, state regulators and the USEPA are revising and adopting new perchlorate drinking water standards and other regulations. Thus, there is a need for identifying a suitable replacement for perchlorate that will not degrade mission readiness and increase training costs due to site contamination. 5-aminotetrazole (5-AT) is being considered as a perchlorate replacement and is currently used in propellant formulations for commercial airbags. The purpose of this study was to determine the oral acute and subacute toxicity in the rat and compare the toxicity of 5-AT to other compounds being considered as replacements. In the oral LD50 test, 5-AT was not toxic and no mortalities were observed at the limit dose of 2000 mg/kg. In the 14-day study, there were no clinical signs of toxicity nor morbidity up to 623 mg/kg-day; the highest dose tested. No differences were observed in hematology, clinical chemistry, organ weight, body weight, food consumption, or DNA damage (peripheral blood micronucleus assay). Histopathological findings were negative and no dose or group differences were noted. Due to the lack of toxicity and observed effects, derivation of an LD50, determination of the Lowest Observed Adverse Effect Level, and derivation of the Benchmark Dose were not possible. The resulting No Observed Adverse Effect Level (NOAEL) was 623 mg/kg-day, the highest dose used in the subacute exposure test.

ABSTRACT FINAL ID: 2617 Poster Board -322
TITLE: Acute and Subacute Oral Toxicity Assessment of the Explosive Methylnitroguanidine in Rats (Rattus norvegicus)

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KEYWORDS: Methylnitroguanidine, Rat, Toxicity

ABSTRACT BODY: The U.S. Army is currently evaluating the use of alternative explosive compounds to replace 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), both of which can contribute to significant

environmental contamination at manufacturing and training sites. TNT and RDX are also toxic following both acute and chronic exposures. Methylnitroguanidine (MeNQ) is a munition currently undergoing evaluation as a component of DEMN, a new explosive formulation anticipated to have significantly reduced environmental impact as well as fewer health hazards to munitions workers and Soldiers. The acute and sub-acute oral toxicity of MeNQ was evaluated in Sprague-Dawley rats (*Rattus norvegicus*). For the acute test, male and female rats were exposed to a single dose of MeNQ with an upper limit of 2,000 mg/kg and observed for a total of 14 days. Based on these results, male and female rats were then exposed to MeNQ for 14 days at 100, 210, 415, 830 and 1250 mg/kg-day. Blood was taken at the end of the study for hematology, clinical chemistry and the micronucleus assay (males only). No clinical signs of toxicity throughout the studies and at necropsy were observed. The micronucleus assay was negative for DNA damage in males. In females, thymus weights were significantly decreased in the 415 mg/kg-day dose group, while the percent neutrophils significantly decreased (1250 mg/kg-day) and percent lymphocytes significantly increased across the three highest dose groups. Given the overall results, it is likely that these changes are a stress response to the physical nature of the compound and the dosing suspension, as opposed to a chemical effect of MeNQ on the immune system.

ABSTRACT FINAL ID: 2618 Poster Board -323
TITLE: Pilot Study on the Utility of Generational DART Toxicity Studies in Safety Assessments for Infant Food Packaging

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KEYWORDS: Infant, Safety, Food Packaging

ABSTRACT BODY: Infants (0-6 months of age) represent an age group of potentially increased vulnerability to the effects of food packaging migrants due to differences in their physiology and chemical exposure relative to adults (Neal-Kluever et al., 2014). The Neal-Kluever et al. review also indicated that the generational (extended 1 generation or 2 generation) developmental and reproductive toxicity (DART) assays may capture the greatest number of endpoints relevant to infant product safety assessments. To evaluate the utility of these studies in the context of infant safety, we conducted a systematic review of single and multi-generation DART studies within the Office of Food Additive Safety (OFAS) archives. We limited our analysis to FDA-reviewed studies where the test article was administered through oral gavage, diet, or drinking water. We assessed chemical sensitivities between offspring and parental generations using both qualitative and quantitative means by comparing the lowest observed effect levels (LOELs) of each generation per sex. We harvested data from 31 studies (22 multi-generational) covering 29 chemicals. Overall, the chemicals presented a low toxicity profile, with roughly 20% of the multi-generational studies containing chemicals eliciting no adverse effects on either generation. Of the studies with chemicals that elicited adverse effects, the majority of these had similar effects on both generations. In a few cases, only the parental or offspring generation was sensitive to the test article, likely indicative of differences in toxicological susceptibility between the two groups. Overall, the DART studies provide information that indicates whether there may be a toxicological concern in the offspring. Further work such as comparing these DART studies with other juvenile toxicity study paradigms may assist the optimization of protocols to be used in the context of infant product safety assessment.

ABSTRACT FINAL ID: 2619 Poster Board -324
TITLE: Risk Evaluation of Printed Electronics Using Nanosilver Ink

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KEYWORDS: Silver Nanoparticles, Printed Electronics, Risk Evaluation

ABSTRACT BODY: Nanotechnology is increasing and the society is surrounded by the risks it may have. Many people are exposed to these manufactured nanomaterials in occupational environments. Currently, there are many products in the market that contain silver nanoparticles (AgNPs). These products include various household products such as yoga mats, cutting boards, running shirts, and socks. Studies are continuous in finding levels of in other products and in workplaces. As a result, the focus of this experiment will be the release of AgNPs in printed electronics. Using an exposure simulation chamber as a setting for the experiment, various instruments such as a nanoparticle collector, SMPS (scanning mobility particle sizer), CPC (condensation particle counter), and OPC (optical particle counter) and MCE (mix cellulose esters) filters are connected to the chamber to measure the exposure levels of AgNPs when utilizing printed electronics. Very small amount of AgNPs were released during printed electronics operations and the number of particles during printed electronics operation in the simulation chamber was less than outside of chamber. When potential risks to consumer and workers were evaluated by margin of exposure (MOE) approach with target MOE 1000, the printed electronics operation exceed far over the target MOE in both simulation study and actual workplace personal exposure study. Taken together, printed electronics operation using nanosilver ink has less concern of risk.

ABSTRACT FINAL ID: 2620 Poster Board -325
TITLE: A 14-Day Toxicity Repeat-Dose Study of PSOA by Oral Gavage in Rats

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KEYWORDS: PSOA, Repeat-Dose Study

ABSTRACT BODY: This study is a follow-up to prior work assessing the repeat-dose toxicity of peroxy sulfonated oleic acid (PSOA). PSOA was dosed daily via gavage to Sprague-Dawley rats (10/group, 5 males and 5 females) for 14 days at 0, 100, 300 and 1000 mg/kg/day. The following study endpoints were evaluated: clinical signs, body weight, food and water consumption, clinical pathology, organ weight, gross pathology and histopathology. Mortality occurred in the mid (10%) and high (30%) doses with body weight loss observed for all unscheduled deaths. Clinical signs were noted at all PSOA doses, with a dose-response of incidence and severity. Rats surviving to study termination in the control, low and mid-dose groups gained body weight throughout the study. Surviving animals at the high-dose all gained weight from Day 0, but weight loss was observed periodically. Decreased food and water consumption occurred in the mid (one rat) and high doses (all rats). Clinical pathology endpoints were largely unaffected aside from select parameters at the mid and high doses, which had questionable biological significance or appeared as adaptive responses. Liver weight effects for high-dose animals appeared to be the result of nutritional status. Other organ weight differences from the control group were confined to the high-dose and attributable to decreased body weights. Gross pathology was unremarkable, with all non-gastric findings considered unrelated to PSOA. Histopathological lesions were confined to the stomach and were observed in all high-dose rats and three of four surviving mid-dose females. No stomach lesions were observed in the mid-dose males or in rats at the low-dose or control. Overall, effects were observed at all PSOA doses. However, given that the only effects observed at the low-dose were clinical signs of minimal severity in a minority of rats, a study NOAEL of 100 mg/kg/day was identified. Based on the study findings, the effects observed for PSOA are related to local gastric irritation and not the result of systemic toxicity.

ABSTRACT FINAL ID: 2621 Poster Board -326
TITLE: The Use of Structural Alerts in Alternatives Assessment: An Organophosphate Case Study

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KEYWORDS: Alternative Assessment, Flame Retardants, Hazard Assessment

ABSTRACT BODY: The EPA's DfE Alternatives Assessments (AA) program provides information to decision makers to understand and compare hazard concerns associated with potential alternatives to a chemical under evaluation. The AA

assigns hazard designations for persistence, bioaccumulation, aquatic toxicity, and human health endpoints. Populating the hazard designation summary table requires a comprehensive review of the chemical and toxicological data available for each alternative and subsequent comparison to the DfE AA criteria. Where data gaps exist, DfE alternative assessment methodology permits the use of structural alerts, EPA-approved QSARs, or read-across analogs. Methodology for designating the hazard concern from a structural alert associated with chemical classes, functional groups or substructures that are linked to a particular endpoint was applied, focusing on the mechanism of the initiating event. Of the 6 flame retardant AAs completed or on-going to date, half contain members with an organophosphate or organophosphorus structural alert. There are 4 chlorinated alkyl phosphates, 2 branched alkyl, 4 aromatic phosphates, 4 polymers, brominated alkyl phosphate and 8 phosphonates. DfE evaluated organophosphates with and without experimental data. For those that did not have experimental data, the starting point for the hazard designation was the EPA's Sustainable Futures "Noncancerous Screening Protocol" neurotoxicity structural alert for organophosphorus and organophosphates. Strict adherence to the structural alert would result in an estimated Moderate designation, although the AA process undertook a more rigorous mechanistic-based evaluation. Several organophosphates and organophosphorus compounds induce neurotoxicity through the well-known inhibition of acetyl cholinesterase (AChE) by the parent molecule or a metabolite. The degree of AChE inhibition was important in determining the neurological and developmental toxicity designations. In addition, this work also considered the potential adverse effect of the resulting phosphate ester metabolites.

ABSTRACT FINAL ID: 2622 Poster Board -327
TITLE: Preclinical Safety Assessment of CMX16669, an Orally Available Lipid-Antiviral Conjugate for Prevention of Serious Cytomegalovirus and BK Virus Infections

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KEYWORDS: Nucleoside Analog, Bone Marrow Transplantation, CMV Infections

ABSTRACT BODY: Cytomegalovirus (CMV) and BK virus infections are a source of significant morbidity and mortality in immunocompromised transplant patients. Antiviral drugs currently used to treat these infections, ganciclovir, cidofovir and foscarnet, are limited by a significant risk of serious, potentially life-threatening toxicities. Due to toxicity, none of these drugs are ideal for prevention of CMV infection in stem cell transplants; however prevention rather than treatment, would be expected to produce improved patient outcomes. CMX16669 is a novel, acyclic nucleoside analog with *in vitro* activity against CMV, BK virus and a potential for a much improved safety profile compared to existing drugs. After the activity and selectivity of CMX16669 was demonstrated *in vitro*, non-GLP dose escalating studies were conducted, followed by 7 day toxicology and toxicokinetic studies in rats and dogs. Oral doses of up to 300 mg/kg/day were well tolerated, exhibiting good systemic exposure in both species; without significant, dose-limiting clinical or anatomic pathology findings. Additionally, a Stemina DevTOXqp™ *in vitro* assay suggested a low teratogenicity risk of CMX16669, a selection criterion for the compound. Accordingly, CMX16669 was designated for development as an IND candidate. GLP-compliant 14-Day oral dose toxicology and toxicokinetic studies with 4 week post-treatment periods were performed in rats and dogs with doses ranging from 3 to 100 mg/kg/day. Standard IND-enabling genotoxicity and safety pharmacology studies were also conducted. Data reflected that doses of up to 100 mg/kg/day were well tolerated with findings of decreased (< 10%) body weight and food consumption in both species. Overall, the results of the IND-enabling toxicology studies are expected to support an acceptable safety factor for the initiation of Phase 1 clinical trials for the development of CMX16669 for the prevention of serious infections caused by CMV and BK virus.

ABSTRACT FINAL ID: 2623 Poster Board -328
TITLE: Assessment of Vehicle Effect(s) and Computing Methods while Compiling Clinical Pathology Historical Control Data

AUTHORS (FIRST INITIAL, LAST NAME): A. Vander Wal[1], K. Hill[1], E. Ziemer[1], J. Waldschmidt[1], T. Vidmar[2], L. L. Freshwater[2], D. Dandekar[1]

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KEYWORDS: Clinical Pathology, Historical Control Data, Statistical Analysis

ABSTRACT BODY: In toxicology studies, interpretation of clinical pathology (CP) data is a crucial aspect in determining compound-related findings. In addition to results from a concurrent control group, historical control data (HCD) are used to determine biological significance of compound. We assessed a) potential effect(s) of various dose formulation vehicles on HCD and b) three statistical approaches for compilation of CP HCD. We selected six serum chemistry parameters (gluc, trig, chol, AST, ALT, ALP) collected from male Sprague-Dawley rats (8-10 weeks of age) from 11 sub-chronic studies where 9 dose formulation vehicles were used via oral or IV route. HCD ranges were computed using three methods: M1) Grubb's outlier test followed by ±2SD; M2) exclude values beyond Mean ±3SD followed by ±2SD and M3) structured analysis using Shapiro-Wilks=>Kruskal-Wallis=>Dunnett's=> resulting outliers excluded=>Harrell-Davis procedure for 95% confidence level. The M1 and M2 represent most commonly used methods across labs and M3 is a structured statistical approach. For comparison purposes, data collected from the 0.5% Methylcellulose (MC) group was chosen as a reference control. The M3 approach indicated one or more parameters for 7 of 10 vehicles compared with MC were affected and the affected parameter for that particular vehicle was excluded from the HCD calculation This resulted in differences in HCD range when the data was compiled using the three methods listed above. Low- and high-HCD range for Glucose using M1 was 68.73 – 175.50; M2 was 69.90 – 173.09 and M3 was 86.27 – 131.65, indicating similar lower range across all methods and higher value for the upper range using M1 and M2. Similar impact was noted for Triglycerides. Based on the results of this study using 6 analytes, it is evident there is no single best method for compiling HCD and therefore, a cautious scientific approach is warranted before deriving meaningful scientific conclusions from studies when using HCD.

ABSTRACT FINAL ID: 2624 Poster Board -329
TITLE: Safety Assessment of Extractables/Leachables from a UV-Curing Ink Label for Large-Volume Parenteral Drug Product

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KEYWORDS: Safety Assessment-Pharmaceutical Drug Development, Extractables/Leachables, Drug Safety

ABSTRACT BODY: A systemic extractables/leachables study and safety evaluation for a UV-curing ink was conducted to demonstrate the safety of UV-curing ink for labeling the large volume parenteral (LVP) drug products packaged in the plastic containers. The UV-curing inks are composed of several chemical starting materials such as photo-initiators, modifiers, monomers for photo-polymerization through UV curing. During ink printing and curing process, some of these components will be consumed to form polymer layer and/or changed to their by-products after initiator polymerization reaction. The residual components and their potential byproducts from the ink curing process may migrate into the finished products and cause safety concerns for patients and/or compatibility, efficacy, and quality issues for drug product formulations. The safety evaluation studies for the UV-ink were performed by using a phase-appropriate, progressing from extractables testing for screening, selection and qualification of materials, leachables testing of final drug products through animal toxicity studies, and then risk-based analysis to meet regulators and product safety requirements for its intended use. The several extractables were detected from the UV-curing ink extractions by High Performance Liquid Chromatography with a Diode Array Detector and Mass Spectrometry Detector (HPLC/PDA/MS) and Gas Chromatography with flame ionization detector and Mass Spectrometry Detector (GC/FID/MS). The increased amount of leachables from the UV-ink were observed through product shelf life with highest amount up to 1.23µg/mL. The amounts of leachables were below their Maximum Tolerable Leachables Concentration, which were calculated following ISO 10993-17 and PQRI Guidance. The 28-day repeat dose animal toxicity study for total leachables following daily intravenous administration in mice showed no statistical difference ($P<0.05$) between the control group and test group. It is concluded that the leachables found from the UV ink have neither toxicological concerns on patients and nor quality impact on LVP drug products.

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ABSTRACT FINAL ID: 2625 Poster Board -330
TITLE: Transfer of Tetravalent Dengue Vaccine during Gestation and Lactation in Mice

AUTHORS (FIRST INITIAL, LAST NAME): R. Guillaume[1], A. Rogue[2], F. Spezia[2], R. Forster[2], N. Mantel[1], S. Gould[1]
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KEYWORDS: Vaccine Safety, Reproductive Toxicology, Biodistribution

ABSTRACT BODY: To support the licensure of a recombinant, live, attenuated tetravalent dengue vaccine, a developmental and reproductive toxicity (DART) program was initiated to investigate the potential risk for women of childbearing potential (WOCBP) and their offspring following vaccination. In designing a DART program for a live attenuated vaccine, the selection of the appropriate species is key. The species must be relevant and develop a detectable viremia and humoral response post-vaccination with transfer to the offspring. Based on available preliminary data with the dengue vaccine in non-pregnant animals, the mouse was selected as an appropriate species for the DART studies. A sensitive method was developed to investigate transfer of the RNA vaccine virus to embryos and fetuses through the placenta and to the offspring through the milk in the mouse. The Quantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-qPCR) techniques specific to the vaccine virus sequence were established in maternal serum, milk, embryos and pup serum matrices and fully validated according to current regulatory guidelines. *In vivo* studies were then conducted to investigate the exposure and transfer of the vaccine in mice. Mouse dams were given one dose of the dengue vaccine during either gestation or lactation. The dams were administered the test vaccine by the intravenous route to maximize the exposure to the virus. Maternal serum, whole embryos, lactating milk and serum of pups were sampled and RNA was extracted using customized extraction procedures. RNA copy numbers were calculated by interpolation from a calibration curve. RNA was detected by qRT-PCR at low level in serum of pregnant females and in embryo samples. Vaccine RNA was not detected in the milk of dams or the serum of pups. These results illustrate the feasibility of techniques for evaluation of placental and milk transfer of the dengue vaccine to offspring and were further extended to the rabbit for the purpose of the development of another live attenuated vaccine.

ABSTRACT FINAL ID: 2626 Poster Board -331
TITLE: *In Vitro* Assessment of Toxicity and Selectivity Index of Selected South African Medicinal Plants

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KEYWORDS: Phytomedicine, Cytotoxicity, Selectivity

ABSTRACT BODY: Background: A number of plants with effective biological properties have not been assayed or developed further due to their levels of toxicity. The plants evaluated have been used in South Africa to treat parasitic infections in livestock and humans. In this study, we determined whether in addition to the antiparasitic properties of these plants, there are other unexplored properties of the plants; specifically, the cytotoxic and antibacterial properties of their leaf acetone extracts. The selectivity indices (therapeutic indices) of these extracts in combating bacteria were also calculated. Materials and Methods: Cytotoxicity was determined by evaluating the viability of cells in the presence of the plant extracts using the tetrazolium-based colorimetric assay against Vero African Green monkey kidney cells, antibacterial activity was determined by using a serial micro dilution method against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis*. Results: All the acetone leaf extracts from the plants showed low toxicity. *Antizoma angustifolia* and *Cassia (Senna) italica* had good bacterial inhibition properties with *A. angustifolia* having MIC value of 0.02 mg/ml against *S. aureus* and *C. (S.) italica* having MIC values of 0.08 mg/ml against *S. aureus* and *E. coli*. The plant with the highest selectivity index was *Cleome gynandra* against all the bacterial pathogens followed by *Antizoma angustifolia* against *S. aureus*. Conclusions: Our work has indicated that all the nine South African medicinal plants used in this study showed low toxicity. In view of this, subsequent *in vivo* experiments may be carried as these plant extracts present little or no risk of toxicity. In addition, two of the plants have good antibacterial properties which are noteworthy. Further work will prove the

usefulness of these plants and their extracts, or whether pure compounds from these plants may be of pharmacological values.

ABSTRACT FINAL ID: 2627 Poster Board -332
TITLE: Comparison of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes from Two Major Suppliers

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KEYWORDS: iPSC-CMs, Cardiotoxicity, Proarrhythmia

ABSTRACT BODY: Drug-induced proarrhythmia is a major safety issue in drug development. Sensitive *in vitro* assays that can predict drug-induced cardiotoxicity have been the focus of toxicology research for several decades. Recently, human induced pluripotent stem cell derived-cardiomyocytes (iPSC-CMs) have become a popular model because they largely resemble the electrophysiological behaviors of human ventricular myocytes. However, human iPSC-CMs are derived from individuals with diverse genetic backgrounds, and different laboratories/suppliers may use different differentiation processes and various conditions to culture these cells. Therefore, the responses of different iPSC-CMs to cardiotoxic drugs may vary. In this study, we compared human iPSC-CMs from two major suppliers: Cellular Dynamics International (CDI) and Axiogenesis. We found that the two lines of cells had different sensitivities to the hERG channel blocker dofetilide: 3 nM of dofetilide was sufficient to induce irregular beats in iCells from CDI, but a dose of 30 nM was required to induce arrhythmia in Cor.4U cells manufactured by Axiogenesis. Moreover, the expression levels of cardiac ion channel genes were different between the two lines of cells: iCells had higher expression levels of SCN5A, CACNA1C, and KCNJ2, while Cor.4U cells have more transcripts of KCNE1 and KCNIP2 genes. The expression profiles of cardiac-specific genes and cardiac differentiation genes were also different. The difference in cardiac ion channel gene expression profiles and sensitivity to proarrhythmic drugs between iCells and Cor.4U cells can significantly affect the data interpretation for utilizing these cells for drug safety assessment; a comprehensive characterization of iPSC-CMs models is needed.

ABSTRACT FINAL ID: 2628 Poster Board -333
TITLE: Proof of Concept for a Biologics Potency Banding Scheme

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KEYWORDS: Biologics, Cross-Contamination, Potency

ABSTRACT BODY: With increasing development of various types of biologic therapies, the potential for manufacturing biologics in multi-purpose facilities raises concerns regarding cross-contamination, even with appropriate controls. The present work was performed to assist with deriving levels of concern for various types of biologics, to help determine need for a dedicated *vs.* a multi-purpose facility. Specifically, in order to affirm a recently proposed banding scheme theory (M. Carver, Fujifilm Diosynth Biotechnologies), research was conducted to identify publicly available data on representative biologics (noted in brackets) as follows (with potency/toxicity proposed to decrease with each band): Band A - lethal toxins (botulinum and diphtheria toxins); Band B - toxins and apoptosis signals (crotoxin, TNF-α); Band C - cytokines and growth factors (interferons, interleukins, growth factors); and Band D - antibodies, antibody fragments, scaffold molecules, and insulins (insulin, collagen, mAbs). Critical review and summary of the identified information confirmed the potency/toxicity of the representative substances as follows: Band A - low ng levels exert lethal effects; Band B - repeated administration of µg levels is tolerated in humans; Band C - endogenous substances and recombinant versions administered to patients in low (interferons), intermediate (growth factors), and high (interleukins) µg doses, often on a chronic basis; and Band D - endogenous substances present or produced in the body in mg quantities per day (insulin, collagen) or protein therapeutics administered in mg quantities per dose (mAbs). The major implication of this work is the confirmation that substances in

Bands A, B, C, and D are considered to represent very high, high, medium, and low concern with regard to risk of cross-contamination in manufacturing facilities. Overall, the results of this effort support the proposed banding scheme as a feasible approach to provide a basis for an initial practical evaluation of risks associated with unintended exposure to biologics.

ABSTRACT FINAL ID: 2629 Poster Board -334
TITLE: Effect of Animal Handling on Bodyweights in CD-1 Cruk Mice on Continuous Infusion Studies

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KEYWORDS: Continuous Infusion, Animal Welfare

ABSTRACT BODY: Intravenous (i.v) administration of test compound, in mice, can be given as a slow bolus injection by the tail vein or by a surgically implanted catheter in the femoral vein with tail cuff exteriorisation. Covance performs infusion studies using mice, implanted in-house, where daily repeat-dose administration of compounds at > 5 minutes per dosing occasion for > 7 consecutive days is required. Retrospective analysis of how the frequency of handling implanted animals impacted on their welfare, as determined by recovery in bodyweight, was performed where the mice on toxicology study A (28 day study) required frequent daily handling, post-surgery and on study, compared to study B (7 day study) where minimal handling was required. The average recovery given for A (192 mice), was 6.5 days, which resulted in an average bodyweight loss of -7.1% at day 1 of study, where 49 mice (25.5%) had a recovery bodyweight loss of > -10% of these 15 had > -15 % bodyweight loss (7.8%). Of the 15 mice, 6 didn't complete the study, 8 mice regained pre surgical bodyweight between 16-21 days post-surgery and the remaining 1 mouse showed a bodyweight loss of 4.9% at the end of study. For the 34 mice with a bodyweight loss >-10% but <-15% on study day 1, 7 mice didn't complete the study, 26 mice regained pre-surgical bodyweight approximately 21 days post-surgery; one mouse remained below original weight (-1%). The average recovery given for study B, (198 mice), was 7.5 days resulting in an average bodyweight loss of -2.1% at day 1 of study. 29 mice (14.6%) had a recovery bodyweight loss of >-10% only 1 mouse had >-15 % bodyweight loss (0.5%). Pre-surgery weights of the remainder were regained by day 1of study. At study Day 7 average bodyweights were +1.7% of pre-surgery weights. Conclusion: Additional handling for frequent examinations during the post-surgery recovery period, and on study, had a negative impact on the bodyweight recovery.Increased animal handling, for study procedures such as post dose observations, may result in further bodyweight loss on study, which may mask bodyweight loss related to toxic effects of the test compound.

ABSTRACT FINAL ID: 2630 Poster Board -335
TITLE: Evaluation of Humanized Chimeric Mouse Model As a Potential Screen for Human Hepatotoxicity

AUTHORS (FIRST INITIAL, LAST NAME): J. Dwyer[1], T. Ackerson [1], E. Poulton[1], J. Oliver[1], G. Lund[2], V. Olson[2], N. Kneteman[2, 3, 4], Z. Jayyosi[1]
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KEYWORDS: Humanized Mouse Model, Drug-Induced Liver Injury, Chimeric Mouse

ABSTRACT BODY: Drug induced liver injury (DILI) is a major cause of drug failure in preclinical and clinical drug development. Liver toxicity often goes undetected in preclinical species. Therefore, there is a need for better *in vivo* preclinical models for detection of DILI. One potential model is the chimeric mouse with a humanized liver where part of the mouse's native liver is replaced with functional human hepatocytes. A collaboration with KMT Hepatech was established to investigate the use of this model in safety assessment. SSR504734, a glycine transport I inhibitor, was selected as a specific human hepatotoxicant. No liver toxicity was observed with SSR504734 in preclinical species, however 2 out of 8 subjects showed liver enzyme elevations in phase I clinical trials. The objective was to determine whether the

hepatoxicity observed in humans could be reproduced in this model. A two week study with SSR504734 was performed in humanized mice. Liver and plasma samples were obtained for histopathology, clinical pathology and drug plasma exposure. Histological examination of mouse livers confirmed the presence of greater than 75% human hepatocytes. Transient increase in ALT and ALKP (2-fold) or total bilirubin (4-fold) was observed in mice treated with SSR504734 for 14 days at 2 hours following the final dose. However, 24 hours after the final drug treatment the group average values in the drug group had declined to near the control group average values. Microscopic observations revealed moderate vacuolation of human hepatocytes in control and SSR504734-treated mice. There was no difference in the human liver histopathology in SSR504734-treated mice as compared to vehicle control-treated mice. Further investigation is needed to confirm the potential of this model for the detection of human liver toxicity.

ABSTRACT FINAL ID: 2631 Poster Board -336
TITLE: Evaluation of Paracetamol-Induced Cytotoxicity and Metabolism in Primary Hepatocytes

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KEYWORDS: Hepatocyte, Clearance

ABSTRACT BODY: Current *in vitro* test systems comprise simple liver-derived cell-based models that are poorly predictive of toxicity of chemicals entering the systemic circulation. Cells cultured in bioreactors is an emerging *in vitro* system, enabling a more physiologically representative environment. Paracetamol (APAP) represents a well documented hepatotoxin, whereby toxicity is metabolism dependent. APAP has been utilised as a tool to investigate limitations of current hepatocyte *in vitro* systems with the aim of providing baseline parameters in current *in vitro* systems for comparison with a zonated hollow fibre bioreactor system. Hepatocytes were isolated from male Wistar rats (150-300g) by a two-step *in situ* collagenase perfusion method. Fresh hepatocyte suspensions were incubated for 6h with APAP (0-20mM), assessing cytotoxicity (trypan blue exclusion and ATP content). Hepatocytes were cultured for 72h, assessing APAP-induced cytotoxicity (0-20mM) through LDH leakage and ATP content. APAP-induced cytotoxicity was observed in rat hepatocyte suspensions at 6h (IC50 11.12mM). GSH depletion was observed at lower non-toxic concentrations of APAP (IC50 1.60mM), along with a decrease in ATP content. APAP-induced cytotoxicity in cultured rat hepatocytes indicated GSH depletion and ATP depletion at 24h, with LDH leakage observed at 48h and 72h. GSH conjugate formation was also evident. Determination of predicted *in vivo* clearance was determined through measurement of APAP (500µM) disappearance over time. Clearance for hepatocyte suspensions and hepatocytes in culture was found to be 2.96mg/ml and 1.37mg/ml respectively. To summarise, APAP-induced cytotoxicity was observed in both hepatocyte suspensions and culture. *In vitro* to *in vivo* extrapolated clearance, indicated an under prediction of over 50% when comparing freshly isolated rat hepatocytes to *in vivo*. This highlights metabolic limitations of current *in vitro* systems and will serve as baseline markers for the comparison with hepatocytes cultured in a zonated hollow fibre bioreactor system.

ABSTRACT FINAL ID: 2632 Poster Board -337
TITLE: Statistical Analysis of Relationship of Body Weight and Incidence of Testicular Leydig Cell Adenoma and Hyperplasia in the Rat

AUTHORS (FIRST INITIAL, LAST NAME): Q. Huang[1], J. Mikl[2], E. Gaillard[1], M. Bogdanffy[1]
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KEYWORDS: Testicular Leydig Cell Adenoma, Body Weight, Carcinogenicity Study

ABSTRACT BODY: Testicular Leydig cell adenomas are commonly observed in rat carcinogenicity studies. The incidence of Leydig cell (LC) adenomas differs markedly between strains. It has been reported that there is an inverse relationship between terminal body weight and the occurrence of LC adenomas in Wistar rats. To examine whether a similar

relationship exist in other strains of rat, the body weight data and the incidence of LC adenoma and hyperplasia were analyzed from two separate rat carcinogenicity studies with BI201335 (HCV protease inhibitor) and BI10773 (SGLT2 inhibitor) conducted in the Sprague Dawley and Wistar Han rats, respectively. The individual animal regression slope of body weight change over time was obtained through non-linear curve fitting. A logistic regression model was fitted to evaluate the association of individual body weight slope (BWS) and occurrences of LC adenoma or hyperplasia for each study. For BI201335 study there was an inverse relationship between BWS and the incidence of LC adenoma. With each increase of a single unit in BWS the estimated odds of tumor incidence decreased by a factor of 2.5. A similar inverse relationship existed for BWS and LC hyperplasia. For BI10773 study there was an inverse relation between BWS and the incidence of LC adenomas, with each increase of a single unit in BWS the estimated odds of tumor incidence decreased by a factor of 3.2. However, there was no statistically significant relationship between BWS and LC hyperplasia for BI10773. In summary, the statistical analysis of these two studies demonstrates a clear association between decreased body weight gain and increased likelihood of LC adenoma independent of the treatment group (treated or control). The conclusions and statistical models may be applicable to the data interpretation of other 2 year carcinogenicity rat studies.

ABSTRACT FINAL ID: 2633 Poster Board -338
TITLE: Targeting STAT3 DNA Binding in Glioblastoma with Small Molecule Therapeutics

AUTHORS (FIRST INITIAL, LAST NAME): S. L. Furtek[1], D. Backos[1], C. Matheson[1], P. Reigan[1]
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KEYWORDS: STAT3, Glioblastoma, Small Molecule

ABSTRACT BODY: Glioblastoma multiforme (GBM) is the most common and aggressive primary malignant adult human brain tumor with average survival rates in the range of 12–15 months. The constitutive activation of the signal transducers and activators of transcription 3 (STAT3) transcription factor has been detected in a number of human malignancies, including GBM, and leads to promotion of cell-cycle progression and cell survival, stimulation of angiogenesis, and reduction of immunological responses. A wide variety of STAT3 inhibitors exist, many of the inhibitors that directly target STAT3 bind to the Src Homology 2 (SH2) dimerization domain, which may not be effective as unphosphorylated dimeric STAT3 may be functional. Therefore, inhibiting the DNA-binding activity of STAT3 regardless of phosphorylation status may be a more effective approach. In order to identify compounds with the potential to bind the DNA-binding domain (DBD) of STAT3 a computational-based screen of the commercial SelleckChem compound libraries (approx. 4500 compounds) was performed using Maestro Glide docking (Schroedinger, Inc.) and 5 candidate inhibitors of STAT3 including niclosamide, one of the few compounds known to bind the DBD, were identified. GBM U87 cells were treated with niclosamide at 0, 1, 5, and 10μM concentrations for 4 hours and nuclear extracts were subjected to an electro-mobility shift assay. Consistent with reported findings in HeLa cells, niclosamide treatment showed a dose-dependent decrease in STAT3-DNA complexes. Treatment of IL-6 stimulated HeLa cells with 0, 6.25, 12, 25, 50, and 100μM raf265 derivative also demonstrated a dose-dependent decrease in STAT3-DNA complexes. These findings suggest the use of raf265 derivative as a novel STAT3 DBD inhibitor and as a lead compound for further inhibitor development to optimize interactions with the DBD and improve selectivity for STAT3.

ABSTRACT FINAL ID: 2634 Poster Board -339
TITLE: Predicting Compound Effects on Cardiac Repolarization and Detection of Proarrythmia Signals Using Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes

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KEYWORDS: Safety Assessment, Stem Cells, Cardiac

ABSTRACT BODY: After a series of drugs were withdrawn due to adverse cardiac effects, the FDA enacted guidelines for preclinical cardiac safety testing. This guidance document, known as S7B, specifically addressed the role of hERG blockade

in the development of compound induced arrhythmias and required specific preclinical hERG testing. However, it has been well established that hERG testing alone is not sufficient to predict the arrhythmogenic potential of a compound. Compounds that block hERG can, and do, block other channels simultaneously, which can counteract the effect of hERG blockade. Additionally, agonist/antagonist effects on ion channels other than hERG have the potential to induce arrhythmia. The FDA is reassessing S7B with the HESI/FDA Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative. As part of the CiPA initiative the use of human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM's) for cardiac safety testing is being assessed. hiPSC-CM's have emerged as a powerful model for predicting compound induced cardiac effects. Human iPSC-CM's express the full complement of ion channels, generate action potentials and calcium transients, and demonstrate contractile motion. These cells also possess most of the cardiac signaling pathways allowing for analysis of compound effects on mechanisms beyond ion channel block such as ion channel trafficking, calcium handling, and metabolism. Here we demonstrate how a high throughput cell-by-cell approach to calcium transient analysis can be utilized to make predictions concerning compound-induced QT interval changes and the generation of arrhythmia using hiPSC-CM's (Cor.4U, Axiogenesis AG). In this study we screened 40 compounds representing a wide array of potentially arrhythmogenic mechanisms. Consistent with the goals of the CiPA initiative we demonstrate that this approach accurately predicts compound effects on cardiac repolarization and detects proarrhythmia signals within clinically relevant concentration ranges.

ABSTRACT FINAL ID: 2635 Poster Board -340
TITLE: Quantification of Efflux Transporter Activity in 3D Human Liver Microtissues

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KEYWORDS: Efflux, BSEP, Cholestasis

ABSTRACT BODY: A functional impairment of hepatobiliary transporters such as bile salt export pump (BSEP) and multidrug resistance-associated protein 2 (MRP2) are strongly associated with an increased risk of liver injury. Currently mostly artificial models, such as BSEP expressing membrane vesicles, are used for studying efflux transporter function. However, they lack the integration of the functional complexity of the natural 3-dimensional (3D) liver environment. Here we describe the use of 3D human liver microtissues for assessing hepatobiliary transporter function. Confocal imaging was used to assess BSEP-mediated efflux of cholyl-lysyl-fluorescein (CLF) and MRP2-mediated efflux of 5-chloromethylfluorescein diacetate (CMFDA) into the bile canaliculi. Confocal imaging of 3D human liver microtissues revealed a fluorescent bile canaliculi network after CLF and CMFDA exposure. Fluorescent CLF area was decreased after adding BSEP inhibitor Sitaxentan, whereas fluorescent CMF network was unaffected by this compound. Cytochalasin, an actin inhibitor, induced a drop in fluorescent bile canaliculi area of both CLF and CMF. In support to the confocal microscopy, the fluorescence of 5(and 6)-carboxy-2', 7'-dichlorofluorescin diacetate (CDFDA) exposed human liver microtissues increased over time in the supernatant. The secretion of fluorescent CDF was inhibited by cyclosporine A and accompanied by an accumulation of intracellular fluorescent CDF. These results indicate that 3D human liver microtissues are a useful tool to study hepatobiliary transporter activity in a complex organotypic *in vitro* liver model system.

ABSTRACT FINAL ID: 2636 Poster Board -341
TITLE: Exposure Study to Examine Chemosensory Effects of Caprolactam in Healthy Male and Female Human Volunteers

AUTHORS (FIRST INITIAL, LAST NAME): G. Triebig[1], A. Dammert[1], I. Triebig[1], T. Bruckner[1]
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KEYWORDS: Caprolactam, Chemosensory Effects, NOAEC

ABSTRACT BODY: ε-Caprolactam (CL) is the chemical monomer used for polymerization to produce Nylon-6 fibers which are found in many products of daily life such as carpets, rugs, textiles. Exposure to high concentrations can cause upper

respiratory tract and eye irritation in animals and humans. The main objective of this study was to examine possible chemosensory effects of CL vapors in healthy male and female (non-pregnant)-nonsmoking volunteers with respect to smell as well as nasal and ocular symptoms and functions. The statistical analyses were completed recently and were not available before the official SOT Abstract Deadline. Methods: After written informed consent, 26 men and 26 women (20-50 years; non-pregnant) were exposed for 6 hours daily to CL vapor concentrations of 0.0, 0.05, 0.5 or 5.0 mg/m^3 in a repeated-measures crossover design. The study was approved by the Ethics committee of the University and performed according to the Declaration of Helsinki. The following parameters were measured before, during and after daily exposures: conjunctival redness, eye-blinking frequency, tear film break-up time, rhinomanometry, oflactory function, Protein and Interleukin 8 in nasal lavage fluid. The symptoms were recorded with a standardized questionnaire (SPES). Results: The symptom questionnaire indicates an unpleasant smell/perception of impure air at the end of daily chamber exposure in control and all CL concentration groups unrelated CL. A comparison of before and after exposure results showed few significant differences, without relation to CL exposures. Furthermore, statistical test results did not reveal any concentration-effect-relationships. Conclusion: CL vapors at concentrations of 0.05, 0.5 and 5.0 mg/m^3 did not cause any effects on parameters monitored during the study. The NOAEC for caprolactam vapor is 5 mg/m^3 for exposures up to 6 hours duration. Acknowledgement: The study was financially supported by European caprolactam and fiber producers and the U.S. Carpet and Rug Institute (CRI), USA.

ABSTRACT FINAL ID: 2637 Poster Board -342
TITLE: Ocular Coherence Tomography Supports an Investigational New Drug Application for a C3 Complement Inhibitor Targeting Macular Degeneration

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KEYWORDS: Optical Coherence Tomography, Age-Related Macular Degeneration, IND-Enabling Toxicology

ABSTRACT BODY: APL-2, a peptide conjugate that inhibits complement C3, was assessed for 9 months following monthly intravitreal (IVT) injection to cynomolgus monkeys at dose levels of 3.1, 12.4, and 24.8 mg/eye (50 or 100 µL). Vehicle (5% Dextrose, USP) and control article (unfunctionalized conjugate backbone) groups were included. The results presented cover an interim phase which included 3 doses. Clinical signs, body weights, ophthalmology, electroretinography (ERG), tonometry, spectral domain optical coherence tomography (SD-OCT), clinical pathology, and bioanalysis parameters (serum bioanalysis, anti-drug antibody, CH50) were evaluated. No interim necropsy occurred. Procedure-related ocular findings included clinical signs of eye opacity and pupil dilation, ophthalmic findings of aqueous flare and cells, and vitreous haze, and decreased intraocular pressure. All findings were in one eye of a single 24.8 mg/eye/dose animal, except for pupil dilation (also noted in one vehicle-treated animal). Cells were noted in the anterior vitreous of control article and APL-2 animals. No other parameters were affected. Increases in serum exposure for the 12.4 mg/eye group were slightly less than dose proportional relative to the 3.1 mg/eye group, but were generally dose proportional in the 24.8 mg/eye group were relative to the 12.4 mg/eye group. No sex differences or antigenicity were detected for APL-2 or the conjugate backbone. SD-OCT evaluation was used as the primary means of assessing the retina at the interim time point and demonstrated no test article-related retinal changes. The use of OCT was a pivotal component of an IND that was opened in November, 2014. On the basis of these findings, the Phase I clinical trials were initiated.

ABSTRACT FINAL ID: 2638 Poster Board -343
TITLE: The Use of Illicit Drugs in Nonfatal and Fatal Trauma in PR: A Snapshot with US and Europe

AUTHORS (FIRST INITIAL, LAST NAME): H. Jirau[1, 4], F. Colón[1, 4], M. Figueroa[3], G. Rodríguez[2], P. Rodríguez[2, 3], B. D. Jimenez-Velez[1, 4]

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KEYWORDS: Trauma, Drug, Alcohol

ABSTRACT BODY: Some types of illicit substances are involved in at least 45.9% of trauma cases in Puerto Rico, which also fall between ages of 20 to 40 years. We correlate the incidence in fatal and non-fatal trauma to the use of drugs and other substances and compare it to those occurring in the United States and Europe. Blood and urine toxicology assays were done in patients once they arrived at the PR trauma Center and the Forensic Institute. Data recollected from 2006 to 2013, was used to study the prevalence problem of drug of abuse in both fatal and non-fatal patients. Blood and urine toxicology indicate that the drugs of most common use in Puerto Rico are benzodiazepines (24.1% of drug related trauma) followed by cannabinoids (20.0%) and cocaine (18.6%). Studies in US show that the most prevalent illicit drugs related to trauma are cannabinoids (9.2%-34.2%) followed by cocaine (2.7%-18.7%). The prevalence of cocaine in US trauma patients is higher than in EU (2.7%-3.0%). Our data show that in fatal trauma approximately 55.6% of the subjects positive to drugs used cocaine, while non-fatal trauma patients only showed a 17.8% in comparison with those screened with at least one drug. It is interesting to notice that most trauma (fatal and non-fatal trauma) cases featuring drugs of abuse in the US are related to violent crimes and the use of cocaine while in PR they are related to self-intoxication. EU and the US share cannabis as the most used illicit drug, however they differ in the use of cocaine. We also found that alcohol is present in about 33.9% of all trauma patients and in 45.2% of traffic related trauma in PR. Alcohol combined with the high use of benzodiazepines in Puerto Rico is of great concern since this enhances impairment of the psychomotor performance in humans negatively influencing and exacerbating driving capabilities.

ABSTRACT FINAL ID: 2639 Poster Board -344
TITLE: Enhanced QSAR Models for Predicting Rodent Carcinogenicity

AUTHORS (FIRST INITIAL, LAST NAME): N. L. Kruhlak[1], D. Guo[1], L. Stavitskaya[1]
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KEYWORDS: QSAR, Carcinogenicity, *In Silico*

ABSTRACT BODY: Two-year rodent carcinogenicity studies are the most costly of the required toxicology studies for new drugs in both time and the number of animals used, and the outcome of these studies can significantly impact the marketability of a drug product. Through an FDA/PhRMA partnership, an initiative is currently underway to assess the ability of a battery of shorter duration tests to replace the two-year rodent bioassay. *In silico* screening of new pharmaceuticals for carcinogenic potential using structure-based methodologies may provide addition information to support this effort, potentially benefiting both industry and regulatory audiences. After developing a large, high-quality rodent carcinogenesis database (n=1682) covering a large number of structural alerts and characteristics of both genotoxic and non-genotoxic carcinogens, we constructed, optimized and validated a battery of quantitative structure-activity relationship (QSAR) models based on the non-proprietary portion (n=1518) to predict rodent carcinogenicity of untested chemicals. These models were constructed using a partial least-squares regression algorithm in conjunction with fragment-based descriptors derived from medicinal chemistry building blocks within a commercially available cheminformatics platform. Models were developed to predict four rodent carcinogenicity study groups: male rat, female rat, male mouse, and female mouse carcinogenicity. In external validation experiments using an additional set of 722 test compounds (49% positive) derived from the public sources, the models exhibited negative predictivity ranging from 82% to 83% and sensitivity from 70% to 82%, which are statistical parameters considered important for protecting patient safety. In addition to possessing higher underlying data quality, these models demonstrated good coverage, ranging from 85% to 88% for pharmaceutical and industrial chemical structures. These newer, higher-quality training datasets and models can provide a high-throughput assessment of carcinogenic potential for new drugs and components of drug products.

2015 Society of Toxicology Annual Meeting
Late-Breaking Abstracts

ABSTRACT FINAL ID: 2640 Poster Board -345
TITLE: Importance of Comprehensive Reporting of (Q)SAR Assessments under ICH M7

AUTHORS (FIRST INITIAL, LAST NAME): L. Stavitskaya[1], B. L. Minnier[1], M. W. Powley[1], N. R. Hartman[1], N. L. Kruhlak[1]
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KEYWORDS: QSAR, Mutagenicity, *In Silico*

ABSTRACT BODY: Pharmaceutical synthesis involves the use of reactive chemicals, such as reagents and intermediates, which often continue to be present at low levels in a finished drug substance and have the potential to present a mutagenic risk to patients. Regulatory agencies recommend that these impurities be controlled to an acceptable level (e.g., Threshold of Toxicological Concern) or shown to lack mutagenic potential. Mutagenicity assessment may be performed through an empirical Ames test or, under the newly finalized International Conference on Harmonisation (ICH) M7 guideline, through the use of two complementary (Q)SAR methodologies. Under ICH M7, pharmaceutical sponsors are submitting (Q)SAR model predictions for bacterial mutagenicity to regulators in order to qualify impurities as non-mutagenic. Historically, the content of these submissions has varied from a simple, single sentence conclusion to an in-depth discussion of the predictions with supporting raw model data. Although the content of a (Q)SAR report may differ depending on the complexity of the prediction, all reports should ideally include key information such as the version of software and models used, raw model predictions, and an explanation of any conclusions that are based on expert interpretation of the (Q)SAR data, particularly if those conclusions differ from the raw model output. Expert interpretation, or the application of expert knowledge, can enhance the overall accuracy of predictions. Specific examples of expert knowledge include verifying that the test chemical is within a model's applicability domain, confirming that there is an adequate number of relevant training set examples for a structural alert, and examining structurally analogous chemicals with known empirical data from external sources. This poster will describe the general processes, expert analysis steps, and reporting formats that could be utilized for regulatory (Q)SAR assessments under ICH M7 through the presentation of non-proprietary examples.

ABSTRACT FINAL ID: 2641 Poster Board -346
TITLE: Curation and Analysis of a Rodent Uterotrophic Database: Insights on Data Quality and Reproducibility

AUTHORS (FIRST INITIAL, LAST NAME): P. Ceger[1], N. Kleinstreuer[1], D. G. Allen[1], X. Chang[1], J. Strickland[1], Q. Zang[1], J. T. Hamm[1], E. Phillips[1], B. Jones[1], W. Casey[2]
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KEYWORDS: Endocrine Disruption, Literature Mining, Uterotrophic

ABSTRACT BODY: High-quality *in vivo* reference data are critical to understanding the biological relevance of Tox21 and ToxCast *in vitro* assay data. The rodent uterotrophic bioassay, validated by OECD as a short-term screening test for assessing the estrogenic potential of chemicals, is included in the EPA's Endocrine Disruptor Screening Program as an *in vivo* Tier 1 screen. We performed a comprehensive literature review for uterotrophic bioassays conducted on 1812 chemicals in the EPA ToxCast screening program. Over 700 articles were identified as potentially relevant. Protocols used in each article were evaluated by two independent reviewers for conformity to six predefined criteria based on EPA and OECD uterotrophic test guidelines, with overall compliance determined by consensus. Studies meeting all criteria were considered guideline-like (GL). Information on 442 GL bioassays extracted from 92 articles and containing data for 98 ToxCast chemicals was compiled into a database of uterotrophic outcomes. The database includes data on 42 descriptors, including species/strain, number of animals per group, route of administration, duration of dosing, number of doses, maximum dose tested, lowest effect level, and test outcome. The immature rat model was used for 80% of the reported studies, with 72% of these using injection as the route of administration. Active outcomes were more common in rat models (74% active) compared to mouse models (36% active). Of the 70 chemicals in the database with at least two reported GL uterotrophic bioassays, 18 (26%) had discordant outcomes, many of which may be attributable to differences in study design (e.g. injection vs. oral dosing). This database provides a valuable resource for evaluating the performance of *in vitro* assays that

measure key events in the estrogen receptor signaling pathway. This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract No.HHSN27320140003C.

ABSTRACT FINAL ID: 2642 Poster Board -347
TITLE: Development of Improved (Q)SAR Models for Predicting the Outcome of the *In Vivo* Micronucleus Genetic Toxicity Assay

AUTHORS (FIRST INITIAL, LAST NAME): J. Yoo[1], B. L. Minnier[1], N. L. Kruhlak[1], L. Stavitskaya[1]
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KEYWORDS: QSAR, *In Vivo* Micronucleus, Genetic Toxicity

ABSTRACT BODY: All drugs entering clinical trials are expected to undergo a series of *in vitro* and *in vivo* genotoxicity tests as part of their safety assessment. These tests, described in the International Conference on Harmonization (ICH) S2 Guideline, enable the identification of compounds that induce damage to DNA to assess their potential as a human carcinogen. Among the standard battery of genotoxicity tests used for pharmaceuticals, the *in vivo* micronucleus assay, which measures the frequency of micronucleated cells mostly from blood or bone marrow, is recommended for detecting clastogens and aneuploidy inducers. (Quantitative) structure-activity relationship [(Q)SAR] models may be used as early screening tools to assess genetic toxicity risk during drug development or to support regulatory safety decisions for drugs and drug products when experimental data are insufficient or absent. We used commercially available modeling software to construct (Q)SAR models for *in vivo* micronucleus induction from an in-house database of non-proprietary study findings in mice. The database was recently enhanced with new data harvested from publicly available FDA approval packages and the published literature. An earlier generation *in vivo* micronucleus (Q)SAR model built at CDER featured high specificity (90%) but low sensitivity (45%); however, the new models constructed here were tuned for higher sensitivity while maintaining a balance of other predictive characteristics, consistent with FDA's mandate to protect patient safety. Cross-validated performance statistics for the new models showed sensitivity of up to 75% and negative predictivity of up to 86% based on a training data set of 996 compounds. In addition, the models demonstrated cross-validated specificity of up to 74% and coverage of up to 94%. These new models will provide more reliable predictions and offer enhancement in the quality of drug safety assessment with regards to identifying potentially genotoxic compounds.

ABSTRACT FINAL ID: 2643 Poster Board -348
TITLE: Classification of Skin Permeability Potential following Dermal Exposure to Chemicals to Support Safety Assessment

AUTHORS (FIRST INITIAL, LAST NAME): A. S. Mostrag-Szlichtyng[1], C. Yang[1], J. Rathman[1, 2], B. Hobocienski[2], F. Steinmetz[3], J. Madden[3], M. T. Cronin[3]
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KEYWORDS: Skin Permeability, Classification Rules, Chemotypes

ABSTRACT BODY: Degree of dermal absorption/permeation of chemical has impact on its bioavailability and potential toxicity after topical exposure. We present a set of rules to categorize a query molecule based on skin permeability potential (low/med/high). Skin Permeability Database (developed in the EU COSMOS Project) contains >450 chemicals with data rigorously curated from existing databases and by harvesting literature/ regulatory sources. Systematic quality control was used to minimize concerns about data accuracy and reliability. For the rules formulation and validation we used 280 compounds (split into training/test sets) with data on 2 parameters key to understanding skin permeability: *in vitro* steady-state flux, J and permeability coefficient, Kp. Computational methods for classifying compounds as low/med/high with respect to J and Kp were developed; the descriptors used were structural fragments encoded with electronic properties (ToxPrint chemotypes) and selected physicochemical properties. Principle component (PC) analysis was used to identify differentiating descriptors and compensate for descriptors intercorrelations. The chemotype frequencies and mean values and ranges of properties were determined and used to develop profile for each category. For instance, chemotype-based

PC projection plots reveal the chemotypes useful for assigning the molecules to low J category (cyclic alkane/alkene ketones, cyclic alkanes, fused rings, alicyclic amines), while the physicochemical property-space plots indicated the usefulness of H-bond donors/acceptors number, polar surface area, McGowan volume, molecular weight, and logP for identification of high J category compounds. This research supports further modeling of dermal absorption/permeation and skin sensitization to assess safety of dermal exposure to chemicals.

ABSTRACT FINAL ID: 2644 Poster Board -349
TITLE: Methods for Determining Optimal Cell-Type Selections for Covering Biological Space in HTS Toxicity Testing

AUTHORS (FIRST INITIAL, LAST NAME): N. S. Sipes[1], D. L. Svoboda[2], D. Mav[2], R. Shah[2], R. S. Paules[1], R. Judson[3], S. S. Auerbach[1]
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KEYWORDS: Tox21

ABSTRACT BODY: The Tox21 high-throughput screening (HTS) collaboration is profiling the *in vitro* bioactivity of thousands of chemicals to identify target and pathway level activity associated with adverse health effects. Cell types utilized in the HTS assays need to reflect important aspects of human biological diversity. We hypothesize that a limited number of cell types can predict a majority of the relevant biology for toxicity testing. To test this hypothesis, we are building statistical associations based on gene expression (GE) and chemical cytotoxicity. The National Cancer Institute (NCI) CellMiner and the NextBio body atlas databases were mined for chemical induced GI50 (the concentration that inhibits 50% growth) and GE values, respectively for the NCI 60 cell line. Principle component analyses (PCA) for both the 597 chemical induced GI50 values and 22207 transcripts for GE profiles revealed that cell lines exhibiting tight clusters over 9 organ types, although the leukemia and melanoma cell lines were the most dissimilar, being spread farther from the cluster center than the other cell lines. Similarity matrices confirmed the PCA analyses with tight Pearson's correlation values (0.71-0.94 for GI50 and 0.84-0.97 for GE). In light of similar growth characteristics in response to chemicals and basal GE patterns for cell lines, we aimed to look at a broader coverage of biology. GE data were gathered for 1076 human biological samples (cell lines, tissues, primary and stem cells) from the NextBio database. PCA analyses revealed distinct clustering between the cell lines and tissues, with stem cells and primary cells filling the space in between. Greedy co-expression analysis of molecular phenotype revealed lymphocyte and melanoma cell lines, and primary lymphocyte, neural and stem cells as among the top biological samples that best represent the biological space. This approach has shown promise for cell identification toward chemical HTS to ensure adequate coverage of biological space. This abstract does not represent USEPA policy.

ABSTRACT FINAL ID: 2645 Poster Board -350
TITLE: Evaluating Expert Opinions for ICH M7 Compliant Regulatory Submissions Created Using an Automated Framework

AUTHORS (FIRST INITIAL, LAST NAME): K. P. Cross[1], G. J. Myatt[1], D. A. Bower[1]
INSTITUTIONS (ALL): 1. Leadscope, Inc., Columbus, OH, United States.
KEYWORDS: Computational Toxicology, International Conference on Harmonisation (ICH) M7, *In Silico* Prediction of Mutagenicity

ABSTRACT BODY: The International Committee on Harmonisation (ICH) has recently issued the M7 guideline to ensure that any drug impurities present pose a low-level of risk of causing cancer. The guideline focuses on identifying direct DNA reactive substances, usually detected using bacterial mutation assays. In the absence of laboratory carcinogenicity or bacterial mutagenesis testing data for impurities, a computational structure-activity analysis is permitted using two complementary *in silico* prediction methodologies: one expert rule-based and the other statistical-based. Any computational analysis should be accompanied by an expert human opinion, especially in cases where the prediction results are conflicting or are incomplete. We assessed the value of developing expert opinions by blindly evaluating 49 test compounds (30 Ames positive and 19 negative). We employed an automated framework that retrieves mutagenicity data,

in silico predictions, structural analogs, etc. and interactively guides the creation of an expert opinion (as part of a larger tool for semi-automatically generating submissions). Inclusion of an expert opinion reduced computationally-predicted false positives by 55% and false negatives by 22%. Overall accuracy improved by 13% and sensitivity, specificity, positive predictivity, and negative predictivity improved 7%, 21%, 12%, and 14% respectively. Several factors contributed to the improved accuracy of the expert opinion, particularly when reversing positive predictions. These included: 1) examination of close analog structures, 2) close scrutiny when conflicting alert/model predictions were made, 3) examination of non-alerting structural features with database examples, 4) examination of significant negative model features, and 5) test compound features similar to known alert mitigation features. We conclude that creation of an expert opinion is valuable in reducing false positives and that a standardized, semi-automated approach to evaluating the many variables results in submissions that are transparent, consistent and traceable.

ABSTRACT FINAL ID: 2646 Poster Board -351
TITLE: A Massively Orthogonal Search Engine for Mechanism of Action & Toxicity Studies

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KEYWORDS: Bioinformatics, Computational Toxicology, Secondary Pharmacology

ABSTRACT BODY: Better understanding of the mechanism of action & toxicity (MoA & MoT, respectively) of small molecules would lead to a more rational drug design and development process, and presumably also to safer and more efficacious drugs. Large amounts of data have been collected in service of this goal (biochemical activities, chemical proteomics, chemical genetics, toxicogenomics, etc.) however it remains difficult to assemble a "bottom line" conclusion about which mechanism(s) the data most strongly supports. To address this need, we designed a search engine called MoA Central. Based on an initial small molecule query, we identify structurally and phenotypically related compounds, and their putative targets. Compound-compound similarities and compound-target links can come from any number of appropriate data sources, analyses, and *in silico* approaches. The resulting network, which we call a "focal graph", can be analyzed by graph-theoretic techniques, including the PageRank algorithm made famous by Google™. Targets which are considered central to the graph represent putative mechanisms. 10s, 100s, or even orders of magnitude more data types could theoretically be used to build this graph, making it potentially a "massively orthogonal" approach. The results are transparent and scientifically interpretable: supporting evidence for particular hypotheses (the edges that connect the query compound to a given target) can be easily read from the graph. Targets and compounds with similar supporting evidence can be grouped together by community-finding algorithms, similar to those used by social networking sites. Targets and compounds, from the whole graph or from individual communities, can be analyzed by standard set enrichment methods to identify overlaps with genes/compounds linked to various levels of biology: from the biophysical (protein families, domains, complexes) to signaling pathways, toxicities, adverse events, and therapeutic uses.

ABSTRACT FINAL ID: 2647 Poster Board -352
TITLE: Gene Selection for Tox21 High-Throughput Transcriptomics

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KEYWORDS: High-Throughput Screening, Gene Expression, Transcriptomics

ABSTRACT BODY: To screen chemicals for potential adverse health concerns, the Tox21 consortium has used high-throughput (HT) *in vitro* screening in the first two phases, whereas the goal of the third phase is to improve biological diversity by incorporating high-content approaches and HT-Transcriptomics (HTT). The hypothesis is that HTT signatures can serve as surrogates to chemical-induced toxicity to prioritize chemicals for further testing and to shed more light on the mode of action of chemical-specific cellular responses. To generate HTT signatures on a large number of samples, HT Gene Expression (GEx) platforms capable of measuring activity of several thousand genes are being considered. To enable such HTT, one needs to identify the most relevant and biologically diverse set of 1500 genes that are (i) representative of highly diverse GEx changes reported to date, (ii) capable of predicting the GEx changes observed across rest of the transcriptome, and (iii) representative of all major biological pathways. To achieve this goal, we developed a bioinformatics method that uses publicly available human GEx data and computes a score for every gene where the score represents gene's importance in representing the transcriptional diversity, correlation of GEx with other genes, and known pathway annotation. We performed 20-fold cross validation and tested the performance using a variety of parameters including pearson correlation (GEx fold change values), concordance rate (pathway activity calls), and significance overlap (top differentially expressed genes), where the actual microarray data from the test set were compared to the extrapolated data generated by our method. Results indicate that our method can select 1500 genes with full pathway coverage and can predict the GEx of rest of the transcriptome with high accuracy (Avg Pearson correlation 0.79, concordance rate 0.84, significance overlap 0.5, all of which significantly exceeded the performance of randomly selected geneset of equal size).

ABSTRACT FINAL ID: 2648 Poster Board -353
TITLE: ToxCast Assay Network (TCAN) Viewer: A Visualization Tool for High-Throughput Assay Chemical Data

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KEYWORDS: ToxCast, Annotation Network, Spider Plot

ABSTRACT BODY: USEPA's ToxCast program has generated high-throughput bioactivity screening (HTS) data on thousands of chemicals. The ToxCast program has described and annotated the HTS assay battery with respect to assay design and target information (e.g., gene target). Recent stakeholder and use-feedback highlighted a need for improved and expanded assay information, as well as improved data visualization tools. We have added additional assay annotations (gene targets and assay vendors), associated quantitative parameters (e.g., activity concentration, efficacy). To foster easier data exploration and visualization we developed Shinyapps-based ToxCast Assay Network (TCAN) Viewer, an R software-based web application. TCAN is a visualization tool currently equipped with two primary views: animated spider plots and assay-gene network. These views enable the user to view data across assays grouped by one or more annotation features (e.g., gene target: PPARA) with respect to a quantitative parameter. The assay-gene network view displays connections across assays based on common gene targets and allows multilayered circles for each node to quickly indicate the response of a particular chemical across the tested concentration range. These animated spider plots display the modeled response (e.g., percent activity of positive control) across a set of assays with the variable of concentration being controlled by the user. The user can dynamically illustrate concentration responses for a set of related assays and chemicals demonstrating concordance across assays or a specific response profile as concentration changes. For example, a set of chemicals may similarly activate PPARA, but the animated spider plots will uniquely illustrate concentration differences between PPARA changes and other endpoints like cytotoxicity and oxidative stress. These new graphical enhancements improves access, analyses and visual display of the large and complex ToxCast data. This work does not necessarily reflect Agency policy.

ABSTRACT FINAL ID: 2649 Poster Board -354
TITLE: Late-Life Impairments in Brain Chemistry and Behavior in Rats following Developmental Exposure to Lead and/or Manganese

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KEYWORDS: Metal Mixture Toxicity, Brain Chemistry, Behavior

ABSTRACT BODY: Exposure to complex mixtures of metals can produce a broad range of health effects rather than individual compounds. The potential late life health effects associated with exposure to multiple metal hazards in general was ignored for long. We have examined the late life alterations in brain chemistry and behavior in rats following individual and combined exposure to lead (Pb) and manganese (Mn) in early life. Male rats were lactationally exposed to 0.2% Pb in drinking water of the mother from postnatal day 1 (PND 1) to PND 21. Mn was separately given to young rats (PND 28) at a low (2.5mg/kg) dose through intraperitoneal injection for a period of 4 weeks (3 alternative days/week). To study the combined effect of these metals, Pb exposed animals were exposed to Mn (2.5mg/kg body weight) from PND 28 for 4 weeks. The results showed synaptosomal acetylcholine and dopamine levels increased where as acetylcholinesterase and mitochondrial monoamine oxidase activities decreased in cortex, cerebellum and hippocampus following individual and combined exposures to Pb and Mn at PND 60, 12 and 18 months age group rats. Further, we found both Pb and Mn (individual and combined) altered the activities of mitochondrial superoxide dismutase, catalase and aconitase and increased the MDA levels in cortex, cerebellum and hippocampus in all age groups of rats. These alterations were greater in hippocampus following Pb exposure and in cortex of Mn and Pb+Mn exposed rats. The total locomotor activity, exploratory behavior and grip strength (forelimb and hind limb) performance were also decreased in age dependent manner. In conclusion, these data indicate that developmental exposure to Pb can continue to influence brain chemistry and behavior of rats whereas the late life health effects of Mn were very marginal suggesting co-exposure to these metals can significantly increase the late life impairments in brain chemistry as well as behavior compared with individual metal.

ABSTRACT FINAL ID: 2650 Poster Board -355
TITLE: Genotoxic Mixtures Give a Nonmonotonic DNA Damage Dose Response in Three-Dimensional Cell Culture

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KEYWORDS: Mixtures, 3D Cell Culture, DNA Damage

ABSTRACT BODY: Genotoxic carcinogens are present in the human diet, and two important examples are Benzo(a)pyrene (BaP) and 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). BaP is a polycyclic aromatic hydrocarbon generated by incomplete combustion of organic substances, thus contaminating numerous foodstuffs, and PhIP is a heterocyclic amine formed when meat is cooked. Genotoxicity testing of chemical carcinogens has focussed largely on individual chemicals, particularly in relation to diet, despite mixtures representing a more realistic exposure scenario. We have previously shown that exposure of MCL-5 cells to BaP-PhIP mixtures produces a TK mutation dose response that differs from the predicted additive response, using traditional regulatory-like two-dimensional (2D) cell culture. There is a large gap between 2D cell culture and the whole animal, and three-dimensional (3D) cell culture, shown to better represent *in vivo* tissue structure, may bridge the gap. The aim of the current study was to use 3D spheroids to characterise the DNA damage response following exposure to mixtures of the mammary carcinogens BaP and PhIP. Mammary MCF-7 cells were grown in 3D spheroids, exposed (24h) to BaP (0.01–10μM) or PhIP (0.1–100μM) (concentrations consistent with diet-borne human exposure) individually or in mixtures and DNA damage assessed by micronucleus (MN) formation. A dose-dependent increase in MN was observed for the individual chemicals in 3D cell culture. In line with our previous 2D TK mutation data, 3D mixture exposures gave a modified DNA damage profile from the individual chemicals, with a potent response at low dose combinations and a decrease in MN with higher concentrations of BaP in the mixture. Ethoxyresorufin-O-deethylase (CYP1A) activity increased with increasing concentration of BaP in the mixture, and for combinations with 10μM BaP, CYP1A1 mRNA induction was sustained up to 48h. These data suggest mixtures of genotoxic chemicals give DNA damage responses that differ considerably from those produced by the chemicals individually, and that 3D cell culture is an appropriate platform for DNA damage assays.

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ABSTRACT FINAL ID: 2651 Poster Board -356
TITLE: Exposure of Mammalian Cells to Air-Pollutant Mixtures at the Air-Liquid Interface

AUTHORS (FIRST INITIAL, LAST NAME): J. Zavala[1], R. Greenan[2], B. N. Chorley[1], N. M. Hanley[1], T. Krantz[1], C. King[1], L. Walsh[1], A. Ledbetter[1], D. Demarini[1], P. White[2], M. A. Higuchi[1], I. Gilmour[1]
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KEYWORDS: Air-Liquid Interface, Aerosols, Vitrocell

ABSTRACT BODY: It has been widely accepted that exposure of mammalian cells to air-pollutant mixtures at the air-liquid interface is a more realistic approach than exposing cell under submerged conditions. The VITROCELL® systems, are commercially available systems for air-liquid interface exposures. The VITROCELL® 6 CF system was used to expose BEAS-2B cells to diesel exhaust (DE), smog, and ozone (O3) for 1 hour. Markers of cytotoxicity, inflammation, and oxidative stress were assessed at 6 and 24 hours post-exposure. In order to use the system, extensive modifications to the aerosol delivery system were required to improve temperature and humidity control. DE particulate matter concentrations ranged from 0.5-2.0 mg/m3. No cytotoxicity or inflammation was observed, whereas levels of Hmox-1 were elevated at the highest concentrations for the 6 and 24 hours post-exposure. Cells were exposed to a simulated smog atmosphere (325µg/m3 SOA, 0.11ppm O3, and 0.17ppm NOX) generated using the EPA's Mobile Reaction Chamber. For this atmosphere, significant increases in IL-8 and TNF-α were observed at 6 hours post-exposure. Finally, cells were exposed to O3 concentrations of 0.5, 1, 2.5, and 5ppm. Significant cytotoxicity (measured by LDH release) and inflammation (measured by IL-6 and IL-8) was observed only at concentrations of 5 ppm. These results indicate that, with respect to the conditions examined, high concentrations of DE and O3 were needed to induce adverse effects, while lower concentrations of a reacted atmosphere were sufficient. A new in-house *in vitro* exposure system that accommodates 6-well and 24-well platforms is being developed to improve the delivery of both gas- and particle-phase pollutants to the cells. [Abstract does not necessarily reflect the views or policies of the U.S. EPA.]

ABSTRACT FINAL ID: 2652 Poster Board -357
TITLE: Evaluating the Impact of Multiple Chemical Exposures on the Measure of Human Variability in Toxicokinetics

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KEYWORDS: Mixture, Risk Assessment, Interindividual Variability

ABSTRACT BODY: Vinyl chloride (VC), tri- and tetrachloroethylene (TCE and PERC) may appear together in the environment, but their risk assessment seldom considers their simultaneous occurrence. The objective of this study was to assess the impact of concomitant exposures on the magnitude of interindividual variability (IV) in internal dose metrics (IDM), using TCE and PERC as proofs-of-concept. PBPK models for adults (AD, 30 yrs) and infants (INF, 2-6 mo) were used to simulate inhalation exposure to "low" (ACGIH's TLV) or "high" (US EPA's AEGL1) concentrations of TCE or PERC alone, together, and in binary or ternary mixture with VC. Distributions of subpopulation-specific parameters were taken from the literature and Monte Carlo simulations allowed generating distributions of relevant IDM. Coefficients of variation (CV) of IDM were computed as proxy of intra-group variability. Besides, IV of a given IDM was assessed as the ratio of the 95th percentile value in INF over AD's median. For "low" exposure to single substances, IV ratios based on the area under the parent compound's arterial blood concentration vs time curve (AUC) of TCE and PERC were respectively 1.6 and 1.3. Corresponding numbers based on the amount of parent compound metabolized per liter of liver (AMET) were 0.74 and 1.53 whereas those based on the AUC of circulating metabolite (AUCTCA) where 0.96 and 1.96. These numbers barely changed in mixtures. For "high" exposure, IV ratios were 1.57 and 1.33 for respectively TCE and PERC alone and slightly decreased to 1.48 and 1.27 in ternary mixtures. Conversely, AMET-based IV ratios increased, from 0.77 to 0.83 for TCE and from 1.57 to 1.76 for PERC. In all cases, CV was ≈20% but AMET and AUCTCA from PERC exhibit CV values >40% in both AD and INF. Concluding, this study

analyzed the impact of multiple exposures on interindividual variability in toxicokinetics. Its results suggest that this impact depends of the chemicals' concentrations and biochemical properties as well as the IDM considered.

ABSTRACT FINAL ID: 2653 Poster Board -358
TITLE: Aerobic Bioremediation of a Contaminated Soil Changes PAH Composition and Toxicity

AUTHORS (FIRST INITIAL, LAST NAME): M. Geier[1], L. Chibwe[2], J. Nakamura[3], R. L. Tanguay[1], S. L. Massey Simonich[2, 1]
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KEYWORDS: Polycyclic Aromatic Hydrocarbon (PAH), Bioremediation, Complex Mixtures

ABSTRACT BODY: Soils contaminated with polycyclic aromatic hydrocarbons (PAH) pose a continuing health concern due to their persistence in the environment, toxicity, and mutagenic activity. The aerobic bioremediation of contaminated soils is an effective, cost-efficient method of reducing parent PAH concentrations, but this may not necessarily result in decreased soil toxicity. For example, there is the potential to produce more polar and reactive transformation products. Soils from a manufactured-gas plant contaminated with coal tar were treated with a laboratory-scale aerobic bioreactor. Extracts from soils pre- and post-bioremediation were prepared using pressurized liquid extraction prior to fractionation based on polarity. Fractions were quantitatively analyzed for 88 parent, oxy, nitro and heterocyclic PAHs. The soil extract fractions were tested for genotoxicity with the DT40 chicken lymphocyte bioassay, and developmental toxicity using the high throughput embryonic zebrafish (Danio rerio) bioassay. The DT40 bioassay identified 4 fractions with statistically significant increases in genotoxicity post-bioremediation. Additionally there was a significant increase in developmental toxicity in one of the fractions in the embryonic zebrafish bioassay, and morphological abnormalities including unusual caudal fin and swim bladder malformations were produced by exposures to these extracts. The increases in toxicity were in the more polar fractions of the soil extracts and are not attributable to the 88 PAHs analyzed, suggesting that the compounds responsible for toxicity are likely to include more polar transformation products or other classes of chemical in the complex mixture. This research was supported by the NIEHS; Grant P30 ES000210, T32 ES007060, P42 ES005948, and P42 ES016465.

ABSTRACT FINAL ID: 2654 Poster Board -359
TITLE: A Poison Information Centre Model Which Will Work in Africa

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KEYWORDS: Poisoning, Africa, funding

ABSTRACT BODY: Background: The burden of poisoning exposures in Africa is significant, however, only ten countries have Poison Information Centres (PICs). An 18 month observational descriptive cross-sectional study was done to find a means for improving the provision of poisons centre services in Africa. The project was funded by the Quick Start Programme of the Strategic Approach to International Chemicals Management. Methods: The study was carried out by an independent consultant under the guidance of a steering group. Sixteen countries in Eastern Africa were studied. Stakeholders were consulted through a series of international and national multi-stakeholder meetings and a survey questionnaire. An in-depth literature review was done. Results: The characteristics of poisoning in the Eastern Africa sub-region were documented. Possible models of poisons centre service and the requirements for their establishment were identified. Various models for a sub-regional poison centre were considered and the most favored was for countries to create and

maintain their own PIC service and then to be coordinated through a network hub. Other project outputs included proposals for four new national centres (in Ethiopia, Tanzania, Uganda and Zambia), a toolkit for poisons centre development and a document on sustainable funding. The main barriers to the development of this project would be the funding cost and lack of trained personnel in the countries. Conclusion: Poisons centres are needed to meet the current and future burden presented by poisoning. Different resources of funding should be explored. While this study focused on one sub-region, the findings could be applied in any other sub-region in Africa.

ABSTRACT FINAL ID: 2655 Poster Board -401
TITLE: Role of Oxidative Stress in the Pancreas of Pups Exposed to 2-Aminoanthracene *In Utero*

AUTHORS (FIRST INITIAL, LAST NAME): W. L. Jackson[1], R. D. Govan[1], E. W. Howerth[2], W. E. Gato[1]
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KEYWORDS: Oxidative Stress, 2-Aminoanthracene, Gene Expression

ABSTRACT BODY: The goal of this experiment is to understand the role oxidative stress plays in the pancreas of pups exposed 2-aminoanthracene (2AA) *in utero*. Environmental exposure to 2AA may increase the risk of developing diseases, such as diabetes. Oxidative stress has been linked with beta-cell dysfunction of the pancreas. Oxidative stress refers to the imbalance between production of reactive oxygen species (ROS) and antioxidant defenses. To examine oxidative stress response in pups exposed to 2AA *in utero*, specific gene expression of Duox1, Gpx1, Ncf2, Prdx1, Prkcg, Ptgs2 and Sod1 in the pancreas were quantified by qRT-PCR. Duox1, Gpx1, Ncf2, Pdx1, Prkcg and Ptgs2 were not expressed in the pancreas of pups. No significant expression differences in these genes were noted. PRDX6 was dose-dependently up-regulated in the pancreas of pups from dams that ingested 2AA during gestation. Sod1 was significantly up-regulated in pups exposed to 2AA *in utero*. Additional feeding study involving moderately high fat is being implemented after three months post-wean. Oxidative stress immunohistochemistry staining will also be performed. Results will demonstrate the role of oxidative stress in understanding vulnerability to diabetes in pups exposed to arylamine *in utero*.

ABSTRACT FINAL ID: 2656 Poster Board -402
TITLE: Investigating Pancreatic Response of Pups Exposed to 2-Aminoanthracene *In Utero*

AUTHORS (FIRST INITIAL, LAST NAME): R. D. Govan[1], W. L. Jackson[1], W. Yau[2], W. E. Gato[1]
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KEYWORDS: 2-Aminoanthracene, Diabetes, Beta Cell

ABSTRACT BODY: Environmental exposures to toxic chemicals increase the risk of developing diseases such as type 2 diabetes. 2-Aminoanthracene (2AA) is an aromatic amine commonly found in the environment including road tars, dyes, and cigarette smoke. Previous studies have found a link between 2AA and insulin-dependent gene expression changes. The goal of the present investigation is to examine the expression of pancreatic genes associated with metabolic syndrome in response to 2AA *in utero*. Timed Sprague Dawley pregnant rats were fed 0 mg/kg (control), 50 mg/kg (low dose), and 100 mg/kg (high dose) concentrations of 2AA diet. After sacrifice, a histological examination of the pancreas was performed to determine the microscopic anatomy of pancreatic cells followed by specific insulin staining and β-cell mass analysis. Quantification of insulin-dependent transcripts via qRT-PCR show that FTO, GCK, IGF2BP2, PPARG1 genes were not expressed. Diabetic related genes such as GLUT2 and INS1 were up-regulated in pancreas of pups whose mothers consumed various amounts of 2AA. Insulin IHC staining indicated that pancreatic islet of the low dose progeny had the highest total percentage of pancreatic tissue, followed by the control offspring and then the high dose exposed rats. This experiment is ongoing to examine how these parameters change with moderately high fat diet three months postwean. Quantification of the expression of genes associated with the regulation of insulin and obesity might suggest a link between diabetes and exposure to environmental chemicals such as an aromatic amine.

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ABSTRACT FINAL ID: 2657 Poster Board -403
TITLE: Inflammatory Effect of 2-Aminoanthracene (2AA) on Adipose Tissue Gene Expression in Pregnant Sprague-Dawley

AUTHORS (FIRST INITIAL, LAST NAME): S. L. Whitby[1], D. A. Hunter[1], W. Yau[2], E. W. Howerth[2], W. E. Gato[1]
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KEYWORDS: Inflammatory Response, Adipose Tissue (AT), TNFα

ABSTRACT BODY: The effect of 2-Aminoanthracene (2AA) on adipose tissue gene expression in pregnant Sprague Dawley rats was investigated. Adipocyte dysfunction may be a critical link between obesity and insulin resistance as a result of abnormal fat storage and mobilization. We have previously observed insulin-signaling related altered gene expression in animals exposed to 2AA. 2AA is an amino-substituted polycyclic aromatic hydrocarbon used in manufacturing dyes, chemical, inks, resins, and polyurethanes. 2AA is a known mutagen and carcinogen that occurs naturally and can be found in tobacco smoke and cooked foods. To examine insulin-dependent 2AA effects on the adipose tissue, nine timed pregnant dams were assigned into dose regimens of 0 mg/kg- (control-C), 50 mg/kg- (low dose-LD) and 100 mg/kg-diet (high dose-HD) 2AA. Dams were fed 2AA contaminated diet during the period of gestation and postpartum. Body weight gain during gestation and postnatal period indicated no significant differences in animals. Examination of the AT for microscopic changes suggests no alterations between control and low dose animals. However, AT of the high dose animals exhibited clusters of mononuclear cells and small numbers of eosinophils and mast cells. Inflammatory response was noted in dams fed 2AA. This is observed as phagocytic cells which are most likely macrophages as part of the inflammatory response. In addition, analysis of the mRNA expression of cytokines and adipokines demonstrate the importance of inflammation in ATs. For instance, TNFα, LEPTIN and IL-6 transcripts were relatively more expressed in the low dose animals than the high dose and control rats. It appears the effects of 2AA on pregnant dams were more pronounced in the low dose group than the high dose group. This means that rat offspring within this group might be more susceptible to diabetic-type conditions.

ABSTRACT FINAL ID: 2658 Poster Board -404
TITLE: Mercury Exposure and Its Health Effects on Children in China

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KEYWORDS: Mercury Exposure, Children, China

ABSTRACT BODY: INTRODUCTION According to the results of "The 16th Korea-China Joint Committee Meeting for Environmental Protection", the National Institute of Environmental Research, Korea and Peking University School of Public Health agreed to conduct a joint research project on "Mercury exposure and It's Health Effects on Children in China". This cooperative project has aimed to enhance the infrastructure for international joint research by building the Korea-China cooperation network and to protect children's health from environmental pollution between the two countries. METHODS This study has been conducted from January to December 2014 and the target areas were Tieling City, Liaoning Province, and Nanning City, Guangxi Province, which ranked the 1st and 6th, respectively, among the top 10 mercury emission provinces in China. 328 students of fourth grade from four elementary schools in both cities were recruited and urine and hair were collected as biomarker samples of mercury and analyzed by Gold-amalgam collection methods, along with a questionnaire survey, neurobehavioral test and balance test. RESULTS 1. Geometric mean(GM) of urine mercury of the total samples was 0.58 ug/g-cr, and that of boys was 0.54 ug/g-cr and girls 0.63 ug/g-cr. In Liaoning, GM was 0.83 ug/g-cr and in Guangxi it was 0.44 ug/g-cr (p=0.000). 2. GM of hair mercury was 0.42 ug/g, that of boys was 0.42 ug/g and girls 0.42 ug/g. In Liaoning the average value was 0.35 ug/g and in Guangxi it was 0.50 ug/g (p=0.000). 3. In the neurobehavioral test, it was found that students with higher mercury concentrations showed relatively low memory capacity ($p<0.05$). Mercury concentration showed some relationships with visual retention, memory scanning, simple visual reaction time, and aim tracing except line discrimination. 4. Postural reaction tests suggested that tremor and body sway are related with mercury

concentration in urine and hair. 5. In the relation with exposure factors, mercury in urine and hair showed relation with adjacency with factory, smoking by family, frequency of fish and shellfish intake, frequency of marine products.

ABSTRACT FINAL ID: 2659 Poster Board -405
TITLE: Particulate Air Pollution Is Associated with Increased Inflammatory and Allergic Symptoms in Adolescents

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KEYWORDS: Particulate Matter Air Pollution, Allergy, Biomonitoring

ABSTRACT BODY: The northern part of Belgium (Flanders) has among the highest annual concentrations of air pollutants in Europe. At the same time, the prevalence of allergic sensitization in children and adolescents keeps on rising. In the Flemish Environmental Health Surveillance program (FLEHS3), it was hypothesized that exposure to particulate matter (PM2.5 and PM10; ppb) at the home address was associated with respiratory and allergic symptoms and related biomarkers. pH of exhaled breath, exhaled nitric oxide (NO) and urinary 8-hydroxy-deoxyguanosine (8-OHdG) concentrations were determined in 408 14-15 year old adolescents. In a standardized questionnaire 608 participants supplied data on symptoms related to asthma, hay fever, eczema and infections. Regression models were used to calculate the change of effect for an increase of the exposure from P25 to P75. All models were adjusted for gender, age and a priori defined covariates. Multiple regression analysis showed 1.5% acidification of breath (95% CI=0.1-3.0%) with an increase of PM10 exposure. The urinary concentration of 8-OHdG increased by a factor 1.065 (95% CI=1.007-1.126) when PM2.5 changed. In addition, augmenting exposures to particulate matter (PM10 as well as PM2.5) were associated with more frequent reporting of allergies to household and personal care products (PM10: OR=1.665, 95% CI: 1.182-2.344; PM2.5: OR=1.834, 95% CI: 1.240-2.714), as well as allergies to pets (PM10: OR=1.952, 95% CI=1.284-2.967; PM2.5: OR=1.875, 95% CI=1.202-2.923). No significant associations were established between these air pollutants and exhaled NO. Prevalence of hay fever, eczema, asthma nor infections were associated with PM exposure. The increase of inflammatory and allergic symptoms confirms that the immune system is a target organ for particulate air pollutants.

ABSTRACT FINAL ID: 2660 Poster Board -406
TITLE: Maternal Tobacco Use As a Risk Factor for Small for Gestational Age (SGA) Is a Third-Trimester Effect

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KEYWORDS: Maternal Tobacco Use, Small for Gestational Age, Third Trimester

ABSTRACT BODY: Background: While small for gestational age (SGA) is a well-known consequence of maternal smoking, the magnitude of risk by week of gestational age has not been elucidated. To assess that risk, we analyzed the 1990-2009 birth certificate data for central Appalachian states. Methods: Live births (N = 3,032,928) with birth weight, gestational age (22-44 weeks), and maternal tobacco use history were categorized as SGA or not, based on 10th percentile gender-specific weights-for-age (Oken et al., 2003). SGA prevalence was analyzed for tobacco users and non-users, yielding relative risks and odds ratios. Gestational week-specific rates, rate differences, and multivariate logistic adjusted odds ratios were also analyzed. Results: SGA prevalences among tobacco users (19.5%) and non-users (9.1%) yielded significant SGA prevalence rate ratio and odds ratio of 2.1 and 2.4, respectively. The pattern for SGA by gestational week was similar for comparative rates, rate differences, and adjusted odds rates. The rate for tobacco non-users was steadily near 9% across the gestational

age range of 22 to 44 weeks. The rate for tobacco users was steady until week 33 when it rose monotonically through week 37 to about 20% at week 38 and remained high. Tobacco use was not seen to be an SGA risk factor for second-trimester births. Tobacco use as an SGA risk factor for third-trimester births grew during the period of premature birth and became fully evident with a two-fold risk for full term infants.Conclusion: We newly report the temporal pattern of tobacco-related SGA by week of gestational age. Tobacco-related SGA was only seen in the third trimester – increasing during weeks 33 through 37 with a doubling during weeks 38-44. This pattern is informative for issues of mechanism and demonstrates the benefit of maintaining tobacco cessation programs.

ABSTRACT FINAL ID: 2661 Poster Board -407
TITLE: Effects of Cadmium Exposure on Salivary Telomere Length in Adolescents in Terai, Nepal

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KEYWORDS: Telomeres, Metals, Children

ABSTRACT BODY: A growing body of research shows telomere length as a promising predictor of future disease risk, and may also be involved in the manifestation of heavy metal toxicity. In this study, the impact of cadmium on salivary telomere length was studied in male adolescents in Terai, Nepal. 159 male adolescents were recruited, and questionnaire interviews, saliva (for qPCR of telomere length), and urine collection (for ICP-MS of cadmium) took place. Multivariate linear regression was used to examine associations between urinary cadmium and salivary telomere length. To examine non-linear dose-response relationships, the participants were divided into two groups by their respective median urinary exposure measures to generate a high and low cadmium group and was re-examined with regression analyses. The geometric mean and standard deviation of urinary specific gravity-adjusted cadmium was 0.20 ± 0.20 μg/L. Urinary cadmium concentration was negatively associated with salivary telomere length (β = -0.18, 95% CI = -0.32 to -0.04) both before and after adjustment for confounders. When split into high and low urinary cadmium groups, the negative relationship was significant and stronger for the high cadmium group (β = -0.40, 95% CI = -0.76 to -0.04) compared to the low cadmium group (β = -0.16, 95% CI = -0.46 to 0.14). These findings demonstrate the detrimental impacts of cadmium exposure on adolescent telomere length even at fairly low exposure levels. These results agree with prior experimental studies showing this negative relationship in embryonic stem cells and epidemiological studies in adults and the placenta, and also help identify cadmium's DNA damaging mechanism of action that may have a role in its mutagenic effects. In conclusion, this study expanded current evidence on the harmful effects of cadmium exposure on telomere length even in adolescents.

ABSTRACT FINAL ID: 2662 Poster Board -408
TITLE: The Epigenetic Landscape of Repetitive Elements in Mice and Humans Prenatally Exposed to Bisphenol A

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KEYWORDS: Epigenetics, DNA Methylation, Bisphenol A

ABSTRACT BODY: Developmental exposure to bisphenol A (BPA) exhibits persistent effects on the epigenome. Next-generation sequencing has been used to detect DNA methylation changes associated with environmental exposures, however multi-mapped repetitive reads are typically ignored. Here we demonstrate the value in analyzing repetitive sequence data by developing and applying novel methodology to determine the extent to which BPA shifts epigenetic profiles of transposons and other repetitive elements. First, enzymatic enrichment for GC density and DNA methylation was done. Then, next-generation sequencing on Illumina IIx (mouse) and Hi-Seq (human) was performed, followed by computational analysis designed to categorize multi-mapped repetitive or hybrid reads containing unique and repetitive sequences. Next, bioinformatics analyses measured read counts to identify exposure dependent shifts in DNA methylation

overall and at individual transposons. We applied this methodology to human fetal liver samples with measured BPA levels from 35.4 to 96.8 ng/g (high), 3.5 to 5.8 ng/g (low), and <0.83 ng/g (non-detect). Similarly, liver DNA from 3 week mice gestationally exposed to 50 mg, 50 µg, or 0 BPA/kg diet were assessed. Transposons were grouped by Class, Family, and Subfamily for increasingly specific group comparisons. In humans, the Classes LINEs, LTRs, DNA elements, and Satellites exhibited non-monotonic responses, with reduced methylation in the low BPA samples. In contrast, mice lacked exposure-dependent DNA methylation differences by Class or Family. However, the repeat content of the mouse reads differed markedly from human, with the largest percentage in Satellites, LINEs, and LTRs, and the expected reduction of Alu/B1 elements, reflecting evolutionary differences. These findings suggest that the murine complement of transposons react differently to BPA and potentially to other environmental exposures. Since environmentally labile transposons have only been known in mouse, ongoing work will test for specific repeats in humans that are responsive to environmental exposures.

ABSTRACT FINAL ID: 2663 Poster Board -409
TITLE: Combined In Utero-Plus-Adult Secondhand Smoke Exposures Exacerbate Elastase-Induced Emphysema

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KEYWORDS: *In Utero* Exposure, Secondhand Smoke, Emphysema

ABSTRACT BODY: Protease-antiprotease imbalances are critical factors involved in the tissue remodeling associated with emphysema. Recently, we reported that *in utero* exposure of mice to second-hand smoke (SHS) alone alters both lung function and structure through 15 weeks of age, suggesting that in utero SHS may predispose the lungs to diseases in adulthood. Here, we hypothesized that *in utero*-plus-adult SHS exposures will exacerbate elastase-induced emphysema in mice. Pregnant Balb/c mice were exposed from gestation days 6-19 to 10 mg/m3 of SHS or air. At 11 weeks of age, female offspring were instilled intratracheally with 0.1 U/g of elastase or saline. Mice were also re-exposed to air or SHS from 11-15 weeks of age. At 15 weeks, lung function, gene expression, inflammatory responses, oxidative stress and airspace enlargement were assessed. Elastase treatment caused up-regulation of MMP12 and down-regulation of TIMP1 gene expression in the lungs. The lungs of mice treated with elastase and exposed *in utero* to SHS had an elevated MMP12/TIMP1 ratio and significant enlargement of airspace, with a mean linear intercept (MLI) value of 173 µm vs 66 µm for their respective controls. The lungs of mice treated with elastase and exposed *in utero* and as adult to SHS showed significant increases in airspace enlargement (203 µm), serum cotinine level, percentage of BALF lymphocytes and levels of 8-isoprostane, a biomarker of oxidative stress. For this same group, a significant increase in the dynamic airway resistance was also observed. Since MMP3 and 9 were not up-regulated in this mouse model of emphysema, these results imply that MMP12 may play a key role in the tissue destruction associated with elastase-induced emphysema. In addition, as emphysema pathogenesis involves lung resistance, inflammation, oxidative stress, and airspace enlargement, these findings suggest that both *in utero* and adult SHS exposures increase the lungs' susceptibility to develop emphysema-related responses following elastase treatment.

ABSTRACT FINAL ID: 2664 Poster Board -410
TITLE: A Novel Nonanimal Test for Developmental Toxicants Using *In Vitro* Morphogenesis of Mouse Stem Cells

AUTHORS (FIRST INITIAL, LAST NAME): Y. Marikawa[1], E. L. Warkus[1], A. A. Yuen[1], A. S. Li[1], C. G. Lau[1]
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KEYWORDS: Morphogenesis, Embryoid Body, *In Vitro*

ABSTRACT BODY: Certain chemicals, including therapeutic drugs, can impair embryo development, causing embryonic death or severe birth defects. Establishment of effective *in vitro* tests is crucial not only to identify developmental toxicants but also to reduce financial and ethical burdens of animal-based tests. Previously, our lab created the morphogenesis

system using mouse stem cells (P19C5), which mimics gastrulation and axial body elongation of embryos *in vitro*. Because many birth defects are caused by misregulation of cell behaviors rather than a lack of cell differentiation, the morphogenesis system may serve as a unique and sensitive tool to study developmental toxicants. The aim of the study is to evaluate the applicability and limitation of the system, using known therapeutic drugs that are contraindicated in pregnancy. These include fluorouracil, lovastatin, acitretin, dronedarone, thalidomide, and valproic acid (VPA). Dissociated P19C5 cells were aggregated to form embryoid bodies (EBs) in hanging drops of culture medium containing various concentrations of the drugs. After 4 days of culture, size and shape of individual EBs were measured using ImageJ program, which were then compared between drug-treated and control EBs. Control EBs steadily grew in size, and transformed from a spherical to elongated shape. In contract, EBs treated with the test drugs, except for thalidomide, exhibited significant deviations in size and/or shape in a manner unique to each drug. Notably, VPA at known therapeutic plasma concentrations inhibited axial elongation without compromising growth of EBs. The distinct effect of VPA on EB morphogenesis may represent the mechanistic link between impaired axial elongation and neural tube defects, one of the major anomalies caused by VPA. Although further studies are required using a larger number of chemicals with known developmental toxicity, the present study suggests that the *in vitro* morphogenesis may serve as an effective, fast, and convenient tool to detect possible developmental toxicants.

ABSTRACT FINAL ID: 2665 Poster Board -411
TITLE: Effects of Prepartum Ingestion of Ipomoea carnea on Neonate Behavior in Goats

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KEYWORDS: Neuroteratology, *I. carnea*, Goats

ABSTRACT BODY: *Ipomoea carnea*, is a toxic plant largely distributed throughout Brazil and others tropical countries and has been incriminated as responsible for several outbreaks of livestock poisoning, mainly in goats. *I. carnea* contains the indolizidine alkaloid, swainsonine. Related plants worldwide are *Astragalus* and *Oxytropis* species. Swainsonine cause cellular accumulation of oligosaccharides, due to inhibition of several important enzymes, resulting in cellular vacuolization and cell death in different organs, in goats mainly in CNS. The aim of this research is to evaluate the behavioral effects on kids whose mothers ingested the plant during gestation. Twenty-seven female goats were divided into 4 groups which received the following doses of *I. carnea* fresh leaves: 0, 1, 3 and 5 g/kg/day, since gestation day 35 until parturition. The parturition of all dams was assisted and mother-offspring behavior was examined during the two consecutive hours post partum. Kids were tested for ability to discriminate their own dam from an alien dam at 12 and 36hr after birth. These kids were also evaluated in relation to their ability to navigate a progressive maze with incrementally increasing difficulty at 2, 4, and 6 days after birth. Treated dams were less likely to stand for nursing. Kids from I.carnea-treated females were unable to stand, nurse and recognize their mothers. Animals from *I. carnea* treated mothers were also slower than controls kids to arrive at the mother in the progressive maze tests. The present study shows that *I. carnea* promotes behavioral alterations in neonates able to compromise their survival. In addition, the neurobehavioral tests employed here showed to be an important tool to monitorize the toxic effects promoted by toxicants during postnatal period. This research also suggests the inclusion of neurobehavioral evaluations in the ruminants teratology protocols.

ABSTRACT FINAL ID: 2666 Poster Board -412
TITLE: Oxidative Stress Affect Hypertrophic Process of Chondrocytes through Inhibiting Sox9 Phosphorylation in Chick Embryo

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KEYWORDS: Oxidative Stress, Bone Development, Hypertrophic Chondrocytes

ABSTRACT BODY: Oxidative stress has been closely associated with adverse embryo development. Based on previous studies, ROS inducer can cause serious malformation of the development of limb bud. Herein, the effect of oxidative stress on bone development is studied. Chick embryos were administrated with 0.5 μmol/egg of AAPH on the air sac at the 3rd, 5th, 7th, 11th and 13rd day of development to establish chronic oxidative stress model. Micro-CT imaging showed an adverse long bone ossification in AAPH-treated chick embryo at day 19 of development. Meanwhile, alcian blue & alizarin red staining showed that embryo under oxidative stress exhibited shortened femur and tibia length. The length of chondrocyte proliferating and hypertrophy zone was detected by H&E staining, which demonstrated that proliferating rate of chondrocytes and ossification rate of hypertrophic chondrocytes were decreased. Furthermore, the total number of chondrocytes in proliferating zone the number of chondrocyte in division stage were significantly decreased. RT-PCR results revealed that mRNA expressions of ossification-related Sox9, Runx2, type II collagen and Aggrecan were affected by chronic oxidative stress. Western blotting of p-Sox9 suggested that the down-regulation of p-Sox9 could be the reason for shortened limb length in chick embryo under chronic oxidative stress. In conclusion, AAPH-induced chronic oxidative stress affected the survival and metabolism of chondrocytes. The reduced protein level of p-Sox9 would cause an immature progression of pre-hypertrophic chondrocytes, leading to early terminal differentiation. These changes could induce adverse bone ossification. This research illuminated the effects of oxidative stress on embryo bone ossification and provided insight into the relationship between oxidative stress and bone development.

ABSTRACT FINAL ID: 2667 Poster Board -413
TITLE: SIK2 Expression Is Consistent throughout Zebrafish Embryonic Development and Is Not Altered by Exposure to Atrazine through 60 Hours Postfertilization

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KEYWORDS: Zebrafish, Atrazine, SIK2

ABSTRACT BODY: Atrazine is a widely used agricultural herbicide in the Midwestern United States. Although the US EPA has set the Maximum Contaminant Level (MCL) at 3 ppb in drinking water, it is suspected that even these levels can have adverse health effects. Atrazine is a suspected endocrine disruptor. Endocrine disrupting chemicals can change endocrine function and cause adverse effects at the level of the individual organism itself, its progeny, and subpopulations of the organism. In an ongoing study in our laboratory, transcriptomic analysis revealed expression alterations in genes associated with neuroendocrine and reproductive system development, function, and disease; cell cycle regulation; and carcinogenesis following a developmental atrazine exposure at 0.3, 3, or 30 ppb throughout embryogenesis in the zebrafish model system (1-72 hours post fertilization [hpf]). SIK2 is one of the genes that was shown to have increased expression, along with an increase in protein expression at 72 hpf following exposure. SIK2 localizes on the centrosome and plays a key role in regulating the onset of mitosis. SIK2 also mediates the expression of microphthalmia-associated transcription factor (MITF) which is a regulator of melanocyte development. The goal of this project was to first characterize the expression level of SIK2 throughout embryogenesis of the zebrafish using quantitative PCR. In addition, the quantitative level of SIK2 expression at additional embryonic time points after atrazine exposure was defined. Analysis of the developmental time course through 72 hpf showed expression of SIK2 was relatively stable with no statistical differences observed among the time points analyzed ($p=0.0711$; $n=6$). Moreover, no statistical differences were observed in expression at the additional embryonic time points following atrazine exposure (24 hpf: $p=0.0596$, 36 hpf: $p=0.4104$, 48 hpf: $p=0.2148$, 60 hpf: $p=0.5222$; $n=6$) indicating expression alterations associated with an embryonic atrazine exposure at the time points analyzed were specific to 72 hpf.

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Late-Breaking Abstracts

ABSTRACT FINAL ID: 2668 Poster Board -414
TITLE: Evaluation of 1066 ToxCast Chemicals in a Human Stem Cell Assay for Developmental Toxicity

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KEYWORDS: Developmental Toxicology, Computational Toxicology, Predictive Toxicology

ABSTRACT BODY: To increase the diversity of ToxCast assays used to assess potential developmental toxicity, we used the Stemina devTOX quickPREDICT assay. Human embryonic stem (hES) cells (undifferentiated H9 line) were screened to 1,066 ToxCast compounds for 72h; we screened 127 chemicals and 13 sample repeats in 8-point concentration series, and 939 chemicals at single concentration (maximum test concentration set at 1, 10 or 100 uM based on ToxCast cytotoxicity determinations). Each compound was tested in triplicate with a positive-control plate reference (1 uM methotrexate) and 0.1% DMSO control. Conditioned media from the final 24h period was analyzed using LC-MS based analysis to determine ornithine/cystine (o/c) ratio. Cell viability was assessed using CellTitre-Fluor. Preliminary analysis defined actives by o/c ratio at or below 0.88 and viable cell titers above 0.65 control values. These cutoffs flagged 165 compounds (15.5%) showing potential for developmental toxicity in a human system. Results in the concentration series indicate that strong teratogens are easily identified (LECs in parentheses) including trans-retinoic acid (3 nM), warfarin (3 nM), 5-fluorouracil (100 nM), methotrexate (100 nM), thalidomide (300 nM), and carbamazepine (3 uM). Many chemicals not yet classified were predicted positive, including TNP-470 (10 nM), pyridaben (30 nM), cladribine (300 nM), mirex (300 nM), maneb (3 uM), and 5HPP-33 (10 uM). Cross-referencing these with ToxCastDB and ToxRefDB databases provides novel opportunity to profile assay performance in predictive computational models for malformations, developmental delays, fetal weight reduction, resorptions and maternal developmental toxicity. [This abstract does not necessarily reflect US EPA policy]

ABSTRACT FINAL ID: 2669 Poster Board -415
TITLE: Combination Effects of FICZ or PCB126 with β-Catenin Effectors in Zebrafish Embryos: Implications for Physiological and Toxicological AhR Functions

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KEYWORDS: AhR, β-catenin, FICZ

ABSTRACT BODY: The aryl hydrocarbon receptor (AHR) and β-catenin are two important regulators of embryo development. While the AHR is well-known to mediate toxicity of persistent dioxin-like compounds (DLCs) an increasing number of studies show involvement of β-catenin in chemical toxicity. Recent studies indicate that AHR and β-catenin signaling are connected, yet, the complexities and mechanisms in the interaction are still clouded. We observed that zebrafish embryos exposed to the β-catenin inhibitor XAV939 display effects phenocopying those of the dioxin-like 3,3',4,4',5-pentachlorobiphenyl (PCB126). This led us to investigate AHR interaction with β-catenin during zebrafish development and ask whether DLC toxicity involves antagonism of β-catenin signaling. We examined phenotypes and transcriptional responses in zebrafish embryos exposed to XAV939 or to a β-catenin enhancer, 1-azakenpaullone (AZP), alone or in combination with PCB126 or the transient AHR agonist 6-formylindolo[3,2-b]carbazole (FICZ). We found that XAV and AZP are toxic to zebrafish embryos. In this context FICZ rescued the toxicity caused by AZP and increased the toxicity caused by XAV. The rescue occurred in the time window of Ahr2-mediated toxicity and was reversed by Ahr2-

knockdown by morpholinos. Regarding PCB126, addition of either AZP or XAV939 led to lower mortality than with PCB126 alone, but surviving embryos showed severe edemas and the survival was only temporary. Moreover, while AZP induced β-catenin-regulated genes this induction was blocked by FICZ or PCB126 and enhanced by Ahr2-knockdown. Overall, our results point to a stage-dependent direct repression of β-catenin activities by Ahr2 in the normally developing embryo. We hypothesize that this is one point of intersection linking toxicological and physiological processes governed by the AHR. (Formas; CTS; Superfund P42ES007381)

ABSTRACT FINAL ID: 2670 Poster Board -416
TITLE: Whole-Genome Expression Analysis of Endocrine Active ToxCast™ Compounds in Embryonic Zebrafish

AUTHORS (FIRST INITIAL, LAST NAME): D. E. Haggard[1], F. Tilton[1], P. D. Noyes[1], D. Thomas[2], K. M. Waters[2], R. L. Tanguay[1]
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KEYWORDS: Zebrafish, Endocrine Disruptors, Transcriptomics/Gene Expressing

ABSTRACT BODY: The EPA established the Endocrine Disruptor Screening Program (EDSP) to evaluate chemicals altering estrogen, androgen, or thyroid signaling across several *in vitro* and *in vivo* assays. Subsequently, ToxCast™ was initiated to develop methods for chemical testing prioritization to handle the overwhelming number of chemicals currently in use. By design, the ToxCast™ chemical library contains many known and suspect endocrine disrupting compounds (EDCs). We previously screened ~1060 ToxCast™ phase I and II chemicals using our high-throughput zebrafish assay. The current aim is to develop a predictive endocrine disruption framework from this dataset using our screening approach and whole-genome transcriptomics. Through mining positive 'hits' in our original dataset, quality filtering, and positive control addition, we selected 26 endocrine active compounds for in-depth study. In order to anchor gene expression to an observable phenotype, we re-evaluated developmental toxicity using our zebrafish assay and determined an EC_{80} for each compound. Embryos were exposed to these concentrations at 6 hpf and total RNA was collected at 48 hpf, after which transcriptomics was conducted using high-density ArrayXS Zebrafish microarrays. For the positive control compound, 17α-ethinyl estradiol, there were 980 misregulated transcripts. Functional analysis of this gene set implicated pathways involved in cholesterol biosynthesis, AhR signaling, WNT signaling, oxidative stress, cell viability, and others. Importantly, beta-estradiol was the most significantly predicted upstream regulator of this gene set, demonstrating the usefulness of this method to successfully predict the endocrine target of interest. The resulting analysis of the 26 compounds, their gene expression signatures, functional networks, and associations with the endocrine pathways of concern will be further explored. This research was supported by EPA #R835168, NIH P30 ES000210, and T32 ES007060.

ABSTRACT FINAL ID: 2671 Poster Board -417
TITLE: Consistent and Unaltered Expression of Glyoxylase 1 (GLO1) during Zebrafish Embryogenesis and following Exposure to the Agricultural Herbicide Atrazine

AUTHORS (FIRST INITIAL, LAST NAME): B. A. Qualizza[1], S. Wirbisky[1], J. L. Freeman[1]
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KEYWORDS: Atrazine, Zebrafish, Glyoxylase 1

ABSTRACT BODY: Atrazine (ATZ) is one of the most commonly used herbicides in the United States. It is a well-known endocrine disruptor. Endocrine disrupting chemicals (EDCs) such as ATZ are linked to adverse health effects including reproductive abnormalities and cancer. The U.S. EPA currently sets the Maximum Contaminant Level (MCL)of ATZ at 3 parts per billion(ppb) for drinking water; however, due to the ability of EDCs to elicit adverse health effects at low levels, it is suspected that detrimental effects can occur below the MCL. Previous studies in our laboratory have shown that the glyoxalase 1 (GLO1) gene and protein is overexpressed at the end of embryogenesis (72 hours post fertilization [hpf]) in the zebrafish model system as a result of ATZ exposure. The GLO1 enzyme functions primarily in cellular detoxification of the glycolysis by-product methylglyoxal (MG). It is suspected that the overexpression of GLO1 serves as an adaptation for

rapidly growing and multiplying cancer cells producing more MG as a result of increased glycolysis. The first aim of this project was to characterize expression of GLO1 during the developmental time course using the zebrafish model system with quantitative PCR (qPCR) at 24, 36, 48, 60, and 72 hpf. Expression of GLO1 was found to be consistent throughout these time points (p=0.25; n=6). The second aim of this project was to characterize the effects of ATZ exposure on GLO1 expression during embryogenesis at additional developmental time points. Zebrafish embryos were exposed to ATZ concentrations of 0.3, 3, or 30 ppb or a control treatment (aquaria water) through 24, 36, 48, or 60 hpf. Quantitative levels of gene expression were measured using qPCR. ATZ exposure was observed to not alter gene expression at 24 (p=0.43; n=6), 36 (p=0.32; n=6), 48 (p=0.39; n=6), or 60 hpf (p=0.49; n=3). Overall these results indicate that alterations in GLO1 expression are time-point specific at the developmental time points tested thus far with alterations only observed at the end of embryogenesis at 72 hpf.

ABSTRACT FINAL ID: 2672 Poster Board -418
TITLE: Improved Detection of Mitochondrial Toxicants Using Oxygen Consumption and Extracellular Acidification Rate in Comparison to the Glu/Gal Assay

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KEYWORDS: Mitochondrial Toxicity

ABSTRACT BODY: Mitochondrial dysfunction has been implicated in numerous drug induced adverse events, such as liver failure and cardiac toxicity. Potential of drugs to be mitochondrial toxicants can be determined by comparing the increase in cytotoxicity of compounds in media containing galactose compared to glucose. In galactose conditions (Glu/Gal assay), cells are more reliant on oxidative phosphorylation and therefore more sensitive to mitochondrial disruption. Alternatively mitochondrial toxicants can be predicted by determining cellular oxygen consumption rate (OCR), reserve capacity (RC) and extracellular acidification rate (ECAR). Both of these approaches were analysed for the predictivity of compounds known to result in mitochondrial toxicity by different mechanisms of action. Sixty compounds (know mitochondrial toxicants with varying mechanism of action and compounds with no mitochondrial effect) were screened through the Glu/Gal assay in HepG2 cells and comparative ECAR, RC and OCR data was also generated in three cell lines; HepG2, Huh7 and H9c2. Comparison of the cell line data for ECAR, RC and OCR data showed H9c2 cells were 10-fold more sensitive to the effects of mitochondrial toxins such as TTFA compared to the HepG2 and Huh7, whilst conversely these cell lines were approximately 6 fold more sensitive to the mitochondrial toxin etomoxir. Comparing the ECAR and OCR data with Glu/Gal data showed there were a number of toxins such as etomoxir and UK-5099 which were not detected in the Glu/Gal assay, however were flagged as being positive for mitochondrial toxicity based upon the ECAR, RC and OCR data. In summary, determining cellular OCR, reserve capacity and ECAR provides a more predictive, and sensitive measure of mitochondrial toxicity and in addition gives an understanding of potential mechanisms of action when compared to the Glu/Gal assay.

ABSTRACT FINAL ID: 2673 Poster Board -419
TITLE: Neurobehavioral and Metabolomic Signature of Developmental Arsenic Exposure

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KEYWORDS: Arsenic, Untargeted Metabolomics, Neurodevelopmental Toxicity

ABSTRACT BODY: Developmental arsenic (As) exposure is linked to cognitive deficits and an increased risk for chronic illnesses. The neurological impacts of As exposure are more pronounced and typically develop prior to other manifestations of toxicity; however, the mechanisms associated with As-induced neurological effects and increased susceptibility to other chronic illnesses has not been fully elucidated. To address this concern, 5D embryonic zebrafish (*Danio rerio*) were exposed to 10, 50 or 720 ppm As, as NaAsO2 at 6 hours post fertilization. Embryos were grown out to 5 days post fertilization and evaluated for mortality, morbidity, behavior abnormalities, and metabolomic changes relative to the unexposed controls.

Developmental malformations and mortality rates were not shown to be impacted by As exposure. The larval photomotor response assay revealed marginal hyperactivity at 10 ppm As, and significant hypoactivity in the 720 ppm As exposed embryos, both during the dark phase of the assay. Preliminary global LC-MS/MS metabolomic data analysis revealed a statistically significant ($P<0.05$ and 2-fold change) signature of 24 features (up to 18 unique small molecules) for developmental As exposure based upon student t-test analysis comparing the control group to all three arsenic exposures. Log non-Pareto (unsupervised) PCA-DA plots demonstrate small molecule signature similarity between the 10 and 50 ppm exposures, and greater variability and metabolic activation following 720 ppm As exposure. Future analysis of the metabolomic signature will elucidate the specific metabolites and pathways impacted by developmental inorganic As exposures. This research correlates to previous findings that link As exposure and abnormal behavior patterns. Additionally, metabolomic analysis may reveal mechanisms associated with developmental As exposure through small molecule end point signatures of impacted pathways. This research was supported by NIEHS Core Center Grant P30 ES000210 & NIEHS Training Grant T32 ES007060.

ABSTRACT FINAL ID: 2674 Poster Board -420
TITLE: Evaluation of Teratogenicity of Multiwall Carbon Nanotubes in Pregnant Mice after Repeated Intratracheal Instillation

AUTHORS (FIRST INITIAL, LAST NAME): N. Kobayashi[1], R. Kubota[1], R. Tanaka[2], H. Takehara[2], M. Naya[2], Y. Ikarashi[1], A. Hirose[1]
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KEYWORDS: Carbon Nanotube, Intratracheal Instillation, Teratogenicity

ABSTRACT BODY: Some studies have reported that maternal exposure to nanomaterials, including carbon nanotubes, may induce teratogenicity. Further information is needed to clarify the potential for chronic toxicity, and also reproductive and developmental toxicity of nanomaterials. In order to evaluate the developmental toxicity of multi-wall carbon nanotubes (MWCNTs) deposited in the lungs via inhalation, we conducted a repeated intratracheal instillation study of MWCNTs in pregnant mice. The MWCNTs (MWNT-7) were dispersed in 1% (w/v) of sodium carboxymethyl cellose (CMC-Na) solution by ultrasonication using an ultrasonic bath. The MWCNT dispersions were repeatedly administered to pregnant Crlj:CD1(ICR) mice on gestation day 6, 9, 12, and 15 at dosage of 0, 0.5, 1.0, and 2.0 mg/kg bw. The pregnant mice were dissected on gestation day 17. Ten pregnant mice per group were evaluated in the present study. The body weights of MWCNT exposed mice at dosage of 2.0 mg/kg decreased, although the changes were not statistically significant. Body weight of fetuses was significantly decreased in the 2.0 mg/kg MWCNT exposed group. However, external malformations of fetuses were not observed in all MWCNT exposed groups. No statistically significant difference was observed between the control group and all MWCNT exposed groups in the numbers of corpora lutea, number of implantations, and placental weights.

ABSTRACT FINAL ID: 2675 Poster Board -421
TITLE: Developmental Toxicity of Ethanol As a Topical Antiseptic in an Occupational Setting: A Review

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KEYWORDS: Ethanol, Developmental, Topical

ABSTRACT BODY: In the U.S., topical antiseptics used on humans are regulated as drugs by the FDA and a Tentative Final Monograph. Based on the oral toxicity of alcoholic beverages, the FDA may require a warning for the repetitive, chronic use of alcohol-containing healthcare topical antiseptics by pregnant healthcare workers (HCW). To date, no safety issues have been reported for pregnant or nursing HCW using alcohol-based hand sanitizers (ABHS). High consumption of ethanol-containing beverages during gestation can cause adverse reproductive and developmental outcomes, including fetal mortality, congenital malformations, and mental retardation. However, the identification of a clear no observed adverse

effect level (NOAEL) from the epidemiology has remained elusive, in particular for more sensitive behavioral effects. Using a high-use scenario for ABHSs, we examined margins of exposure (MOE) for systemic ethanol doses based on NOAELs for developmental toxicity. Based on the dose-response patterns observed in numerous animal toxicity studies, and consistent with the existing epidemiology of alcoholic beverage consumption, peak blood alcohol concentration (BACpeak), rather than the cumulative dose, is the driver of these adverse effects. Repeated dermal applications of ABHS (55-95% ethanol) to volunteers resulted in a BACs of 0.06 to 0.21 mg/dL with no accumulation in the blood. The developmental NOAEL BACs in animal studies ranged from 2.7 to 50 mg/dL (a 10 to ~ 50-fold margin of exposure). The onset of developmental LOAELs begins at a BAC > 120 mg/dL (an MOE > 500-fold). This analysis of the available studies in humans and animals supports the conclusion that the repetitive, chronic use of ABHSs by pregnant or nursing health care workers does not present any safety concerns and does not support a warning statement for ABHSs used in the healthcare industry.

ABSTRACT FINAL ID: 2676 Poster Board -422
TITLE: XOMA 358, a Novel Allosteric Inhibitor of the Insulin Receptor, Is Well Tolerated in Cynomolgus Monkeys

AUTHORS (FIRST INITIAL, LAST NAME): J. Zhao[1], K. Der[1], L. Cao[1], A. Ahene[1], K. Johnson[1], P. Bezwada[1]
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KEYWORDS: Insulin Receptor, Hypoglycemia, Allosteric Inhibitor

ABSTRACT BODY: XOMA 358 is a fully human IgG_2 monoclonal antibody (mAb) that binds with high affinity to the insulin receptor (INSR) and acts as a negative allosteric modulator of insulin action on targeted cells. XOMA 358 is being developed as a novel therapy for unregulated insulin secretion that leads to profound hypoglycemia including congenital hyperinsulinemia. XOMA 358, when administered intravenously (IV), was efficacious in reversing hypoglycemia towards euglycemia in rodent models of hyperinsulinemic hypoglycemia at doses as low as 3 mg/kg. IV toxicity and toxicokinetic (TK) profiles of XOMA 358 were evaluated in cynomolgus monkeys via weekly dosing in both non-GLP 2-week and GLP 6-week studies at doses of 0, 30, 60, and 90 mg/kg. XOMA 358 was not associated with any test article-related toxicity in the monkeys including mortality, body weight, clinical observations, food consumption, clinical pathology, heart rate/blood pressure/electrocardiogram, ophthalmology, immunophenotyping, or gross pathology at doses as high as 90 mg/kg dosed weekly up to six weeks. Dose-related, reversible increases in serum insulin and C-peptide were observed in monkeys, consistent with XOMA 358's mode of action. No XOMA 358-related changes in fasting serum glucose were detected in monkeys up to 90 mg/kg. The only XOMA 358 anatomic pathology finding was noted in spleen of males given ≥ 60 mg/kg and females given 90 mg/kg, which was considered a typical antigenic response to high-dose exogenous mAb. Hence, a conservative repeat-dose NOAEL of 60 mg/kg could be assigned for the multi-week monkey studies. The TK indicated that XOMA 358 clearance was consistent with an INSR-mediated pathway. The half-life was nearly one week and modest accumulation following weekly repeat IV dosing in monkeys was observed. Minimal anti-drug antibody was identified and did not impact the interpretation of toxicity and TK findings. XOMA 358 is currently in clinical development for hyperinsulinemic hypoglycemia.

ABSTRACT FINAL ID: 2677 Poster Board -423
TITLE: Effects of Prenatal Exposure to DEHP on Hypothalamic Genes Expression Profile of Neonatal Rats

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KEYWORDS: Di (ethylhexyl) phthalate (DEHP), Hypothalamus, Neuroendocrine

ABSTRACT BODY: Studies showed that some kinds of endocrine disrupting chemicals may disrupt the development of hypothalamic neuroendocrine system, especially when the exposure occurs during critical development period. Our study focused on the effects of prenatal exposure to di(2-ethylhexyl) phthalate (DEHP) on hypothalamic neuroendocrine genes' change of neonatal rats. Pregnant SD rats were exposed daily to vehicle (corn oil) and 2, 10, 50 mg/kg DEHP from gestation

day 14 to 19 by gavage. 6 pups for each gender of each group (1 pup per litter) were euthanized on postnatal day 1 and hypothalami were collected for gene expression using a 48 gene Taqman low-density array. In male rats, compared with control, 18 target genes were significantly down-regulated by at least 50% at a dose of 10 mg/kg DEHP. These 18 genes include *Cyp19a1, Ar, Esr2, Oxtr, Grin1, Grin2b, Slc17a6, Kiss1r, Tacr3, Avpr1a, Crh, Arntl, Clock, Dbp, Mtnr1a, Per2, Hcrtr2* and *Trh*. Besides, 8 genes decreased significantly in 2 mg/kg DEHP group, including *Esr2, Cyp19a1, Grin1, Grin2b, Igf1r, Clock, Dbp* and *Per1*. In 50 mg/kg DEHP group, significant decreasing also was seen for *Arntl* and *Mtnr1a* genes. Moreover, *Mc3r* and *Drd1a* genes were up-regulated in 2 mg/kg and 10 mg/kg DEHP group respectively. In female rats, genes' changing pattern was not the same as that observed in male rats. In 2 mg/kg DEHP group, expression of *Mc3r* and *Drd1a* genes was up-regulated significantly. In 50 mg/kg DEHP group, increased expression of *Kiss1, Avpr1a, Ghrh* and *Trh* genes were found, but the changes were not observed in 10 mg/kg DEHP group. Taken together, our results indicated the sex-dependent and dose-dependent effects of prenatal DEHP exposure on hypothalamic gene expression of neonatal rats. Our data provide evidence that DEHP may interfere with neuroendocrine regulation processes of hypothalamus through multiple pathways.

ABSTRACT FINAL ID: 2678 Poster Board -424
TITLE: Specific Pharmacological Inhibition of Aldosterone Synthase (Cyp11b2) in the Cynomolgus Monkey Leads to Adaptive Hypertrophy of the Adrenal Zona Glomerulosa

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KEYWORDS: Aldosterone Synthase, Adrenal, Mechanism

ABSTRACT BODY: Aldosterone (ALD) is a member of the mineralocorticoid family of steroid hormones, produced in the zona glomerulosa of the adrenal cortex. Binding of ALD to its receptor in the kidney results in sodium and water retention, and urinary potassium secretion, thereby maintaining blood pressure. Pharmacologic inhibition of ALD synthesis (via inhibition of CYP11b2) may have therapeutic utility for the treatment of hypertension and other cardiovascular conditions related to hyperaldosteronism. In 4-week oral toxicology studies of a potent and highly selective Cyp11b2 inhibitor, reversable hypertrophy of the zona glomerulosa was the sole drug-related change observed. This change was seen in cynomolgus monkeys (a pharmacologically responsive species) but not in rats (a non-pharmacologically responsive species). Adrenal hypertrophy was considered to be a non-adverse adaptive response to inhibition of ALD production. Investigative studies were conducted in monkeys to test this hypothesis. Decreased plasma ALD levels, accompanied by increases in plasma renin activity and angiotensin II were observed, along with increased protein expression of Cyp11b2 in the zona glomerulosa. Dose-dependent increases in the immediate upstream precursors of ALD were observed in plasma of monkeys after 7 or 28 days of treatment. Importantly, plasma levels of precursor steroids did not accumulate over time. Similar observations were seen in monkey adrenal tissue homogenates. In conclusion, specific pharmacologic inhibition of ALD synthase (Cyp11b2) results in expected physiologic responses at the site of ALD production in the primate adrenal cortex.

ABSTRACT FINAL ID: 2679 Poster Board -425
TITLE: Aldosterone Secretion in a Human Adrenocortical Cell Line Is Increased by Brominated Diphenyl Ether-47

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KEYWORDS: Polybrominated Diphenyl Ethers, Aldosterone, Endocrine Disruptors

ABSTRACT BODY: There is growing evidence that polybrominated diphenyl ethers (PBDEs) and their metabolites alter various endocrine biosynthetic pathways including thyroid hormone, estrogens, and androgens. Additionally, PBDEs

accumulate in the adrenal gland, where a variety of steroid hormones are produced, including aldosterone. Elevated circulating aldosterone levels are associated with hypertension, thrombosis formation, cardiac hypertrophy, and congestive heart failure. Since the effect of PBDEs on aldosterone secretion is as of yet unknown, we characterized the effect of BDE-47 on aldosterone secretion in a human adrenocortical cell line. HAC15 cells were exposed to vehicle or various concentrations of BDE-47 (1 nM-100 μM). After 72 h, cell viability, aldosterone secretion, and gene expression of enzymes and cofactors involved in aldosterone synthesis was examined. 100 μM BDE-47 significantly decreased cell viability and was not further examined in all subsequent assays. Basal aldosterone secretion was significantly increased from 100 nM-10 μM BDE-47, with maximal secretion at 10 μM (in pg/mL: vehicle, 106.7±4.8; 10 μM BDE-47, 249.6±13.0; n=28). Ang II-stimulated aldosterone secretion was significantly increased from 10 nM-10 μM BDE-47, with maximal secretion at 1 μM (in pg/mL: vehicle, 5702.3±208.1; 1 μM BDE-47, 6863.3±300.0; n=12). ACTH-stimulated aldosterone secretion was significantly increased at 10 nM, 1 μM, and 10 μM BDE-47, with maximal secretion at 10 μM (in pg/mL: vehicle, 194.8±15.2; 10 μM BDE-47, 600.0±28.2; n=8). KCl-stimulated aldosterone secretion was significantly increased at 10 and 100 nM BDE-47, with maximal secretion at 10 nM (in pg/mL: vehicle, 1977.6±132.9; 10 nM BDE-47, 2936.7±198.5; n=8). Gene expression of most enzymes and cofactors involved in aldosterone synthesis were increased by 10 μM BDE-47. Aldosterone synthase (CYP11B2) had the greatest induction at 3.73±0.36 fold. These data indicate that BDE-47 disrupts the regulation of aldosterone secretion and provides further evidence that PBDEs are potential endocrine disruptors.

ABSTRACT FINAL ID: 2680 Poster Board -426
TITLE: A Hypothesis-Driven Weight-of-Evidence Analysis Evaluating Endocrine Disrupting Potential: Triclosan Case Study

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KEYWORDS: Triclosan, Weight of Evidence, Endocrine

ABSTRACT BODY: "Weight of Evidence" (WoE) is a common term, used often without a specific definition of what it entails. Frequently used only as a theoretical label for a process, WoE implies a more methodological approach for ensuring that all relevant data are collected, evaluated, and "weighted" according to the value of the information for testing specific scientific hypotheses. Testing specific hypotheses is particularly relevant for the data emerging from the US EPA Endocrine Disruptor Screening Program (EDSP) and related scientific information that focuses on identifying potential endocrine modes of action (MoA) of chemicals. Using a transparent and semi-quantitative WoE framework, a case study of the antimicrobial Triclosan (TCS) was conducted. The use of TCS was of particular interest as it is a data-rich compound that has been suggested to have endocrine activity. The WoE analysis employed evaluated the potential for TCS to interact as an agonist or antagonist within the estrogen, androgen and thyroid hormone pathways, and steroidogenesis. These eight hypotheses were tested by using the WoE procedure which involved systematic consideration of each endpoint observed in one or more study designs, focused on the screening level studies in the EDSP, as well as those in levels 1 through 4 of the OECD Conceptual Framework. A semi-quantitative weighting of relevance of each type of endpoint to a given hypothesis was conducted to reach scientifically justified conclusions based on the totality of the evidence. Maximal use of all existing relevant and reliable information and consistent observations in multiple studies increase support for or against a given MoA hypothesis. Using available data from more than 35 peer reviewed studies from multiple animal species and *in vitro* systems, this systematic and transparent WoE assessment indicated that TCS is not acting as an agonist or antagonist within the estrogen, androgen, thyroid or steroidogenesis pathways.

ABSTRACT FINAL ID: 2681 Poster Board -427
TITLE: Phthalate-Induced Methylation of Testisin Gene

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KEYWORDS: Phthalates, Testisin, Toxicity

ABSTRACT BODY: Scientific research in the mechanism of toxicity from exposure to endocrine disrupting chemicals has indicated that epigenetic mechanism may play a role in the mode of action of these chemicals. Among the epigenetic mechanism DNA methylation, microRNA expression and Chromatin restructuring have been explored. To investigate the mode of action of phthalate, we exposed in-vitro N-tera (NT2) cells immortalized from testicular tissues to mono-ethyl-n-hexyl phthalates (MEHP), under laboratory conditions. This study describes the expression and methylation status of the promoter region of Testisin gene in testicular cells exposed to phthalates. NT2 cells were exposed to MEHP at two concentrations (100µM and 10µM) at different time points (24hours, 48hours and 72hours). Additionally the cells were also exposed to 5-azacytidine (5-aza) at 10µM and combinations of 5-aza and MEHP at different time points. Control exposures were conducted using ethanol or media used in cell culture. Exposed and unexposed cells were monitored using iCELLIGENCE technology (ACEA Biosciences) and cell viability, proliferation and growth during the exposure was charted. DNA and RNA was extracted from the exposed and unexposed cells and locally designed expression and methylation primers were used to determine the expression and methylation status of the promoter region of the Testisin gene. Cell toxicity data indicates that exposure to MEHP showed a dose- and time-dependent cell cytotoxicity in the first eight hours after exposures; but the cells overcame the toxicity in the next 12 hours. Expression of Testisin was down-regulated in a dose- and time-dependent manner. The methylation status of the promoter region showed increased methylation but did not correspond to the exposure in a dose- nor time-dependent manner. The results indicate that phthalates are able to modify the expression of the Testisin gene in a dose- and time-dependent manner, possibly as a result of increased methylation of the promoter region rich in CpGs.

ABSTRACT FINAL ID: 2682 Poster Board -428
TITLE: Effects of Vinyl Chloride on Male Reproductive Endocrine System

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KEYWORDS: Vinyl Chloride, Reproductive Toxicity

ABSTRACT BODY: Objective To investigate the effect of vinyl chloride exposure on the damage of male reproductive endocrine system. Methods (1) Experimental survey: male rats were randomly divided into high-dose and low-dose treatment groups, and control group. 28 days after exposure, serum and testis samples were collected for testing testosterone (T), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2) and inhibin (InhB) levels. (2) Epidemiological studies: 22 TCE exposed workers and 22 non-exposed controlled subjects were enrolled in the study. The serum levels of FSH, LH, E2, T and InhB were analyzed using enzyme-linked-immunosorbent-assay (ELISA). Results (1) Experimental survey: Compared with the control group, after 14-day exposure serum levels of T and inhibin B were decreased ($P>0.05$), E2 and LH levels were increased ($P>0.05$), and testis level of T was decreased ($P>0.05$); after 28-day exposure, serum levels of T and inhibit B were decreased ($P<0.05$), FSH was increased ($P<0.05$), testis level of T and inhibit B were decreased ($P<0.05$). It was found that Leydig cell and Sertoli cell were damaged according to histopathological examinations. (2) Epidemiological studies: Adjusted by age, work age, gender, smoke and drink adjusted, serum E2 levels were decreased ($P<0.05$) in of VCM-exposed workers, whereas FSH and LH levels were increased ($P<0.05$) compared with unexposed workers. In addition, E2 level in exposed workers younger than 38 yr-old was lower than that of unexposed workers at the comparable age ($P<0.05$). FSH and LH levels of exposed workers older than 38 yr-old were higher than those of unexposed workers older than 38 yr-old ($P<0.05$). Conclusion Both results of experimental studies and occupational epidemiology survey indicated that VCM had reproductive and endocrine toxicity on male rats and male human subjects.

2015 Society of Toxicology Annual Meeting
Late-Breaking Abstracts

ABSTRACT FINAL ID: 2683 Poster Board -429
TITLE: Effects of Atrazine on the Quality Sperm in Adult Goat Semen

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KEYWORDS: Atrazine, Endocrine Disruptor, Goat

ABSTRACT BODY: Atrazine (ATZ) is an herbicide widely used worldwide in crops of maize and sugar cane. Experimental studies have been shown that ATR is an endocrine disrupter (ED). Thus, in adult male rodents ATZ causes a reduction in the number and motility of sperm, delayed sexual maturation, testicular atrophy and decreased testosterone levels. Considering the high risk of exposure to ED agrochemicals in farm animals the purpose of the present study was evaluate the effects of ATZ in adult male goats, a cheaper animal and physiologically similar to bovines and also may provide a new model species to evaluate other toxicants that could be potentially ED. Adult male goats were divided into two equal groups (n=5): that received daily 0 mg/kg/BW (C group) or 7.5mg/kg/BW (ATZ group) of ATZ for four months. On days 0, 30, 60, 90 and 120, the following parameters were performed: scrotal circumference, testicular consistency, sperm concentration and morphology. The sperm motility was assessed with computer-assisted sperm analysis and with flow cytometry was assessed plasma and acrosomal membranes integrity, lipidic membrane peroxidation and membrane lipid disorder. The animals did not showed any clinical changes or symptoms of intoxication by ATZ. The andrological evaluations did not revealed statistically significant changes between groups in all evaluated variables. It is known that the ATZ is an ED it causes sperm changes in rodents, thus, in this first study we extrapolate the ATZ dose from rats, used in previous studies which caused toxicity in those rodents. Thus, the present study shows clearly the great variability of effects between species and highlights the need to assess the effects of ED in livestock.

ABSTRACT FINAL ID: 2684 Poster Board -430
TITLE: High-Throughput (HTP) Toxicology Test System in 3-Dimensional (3D) Murine Ovarian Follicle Culture

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KEYWORDS: High-Throughput, 3D Culture, Ovarian Follicles

ABSTRACT BODY: Multiple toxicants, drugs and their metabolites are damaging to the functional unit of the ovary – the follicle. Ovarian follicles are susceptible to this damage at all stages of their development, yet assays to study reproductive toxicity are limited. Historically follicle growth and development was studied in 2D cultures. However 3-dimensional (3D) *in vitro* culture preserves the 3D architecture of the ovarian tissue and physiological structure-function relationship and allows detailed mechanistic studies of reproductive toxicology. Here we applied the novel 3D high-throughput (HTP) *in vitro* ovarian follicle culture system, based on fibrin alginate interpenetrating network (FA-IPN) to study the ovotoxic effects of an anti-cancer drug, Doxorobucin (DXR). The fibrin component in the system is degraded by plasmin and it appears as a clear circle around the encapsulated follicle. The follicle health strongly correlated with the degradation area around the follicle. For the purpose of the high throughput image analysis we wrote a MATLAB® custom code. We did not observe any significant difference between manual images processing using ImageJ to the MATLAB® methods, confirming that the automated program is suitable to measure fibrin degradation in attempt to evaluate follicle health. The cultured follicles were treated with DXR at concentrations ranging from 0.005 nM to 200 nM, corresponding to the therapeutic plasma levels of DXR in patients. Follicles treated with DXR demonstrated decreased survival rate in greater DXR concentrations. We observed partial follicle survival of 35.4% (n=80) in 0.01nM treatment and 47.6% (n=92) in 0.005nM, which we identified as

the IC50 for secondary follicles. In summary, we established a 3D *in vitro* ovarian follicle culture system that could be used in a HTP approach to measure toxic effects on female reproductive system.

ABSTRACT FINAL ID: 2685 Poster Board -431
TITLE: Monohaloacetic Acid Drinking Water Disinfection Byproducts Inhibit Mouse Ovarian Antral Follicle Growth and Alter Steroidogenesis

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KEYWORDS: Folliculogenesis, Disinfection Byproducts

ABSTRACT BODY: Disinfection by-products (DBPs) are formed when disinfectants react with organic or inorganic matter during the drinking water treatment process. The haloacetic acids (HAAs) are the most regulated DBP class by the U.S. EPA. HAAs are cytotoxic, genotoxic, mutagenic, and teratogenic. The general descending toxicity rank order of the monohaloacetic acids is iodoacetic acid (IAA) > bromoacetic acid (BAA) >> chloroacetic acid (CAA). Epidemiological studies have found associations between DBP exposure and increased risk of adverse pregnancy outcomes. However, the effects of the monoHAAs on the ovary are unknown. Thus, the present study tested the hypothesis that CAA, BAA, and IAA inhibit antral follicle growth and steroidogenesis in cultured mouse ovarian follicles. To test this hypothesis, antral follicles were isolated from adult CD1 mice (postnatal days 31-33) and cultured with either DMSO or different concentrations of the selected monoHAAs (0.025, 0.05, 0.1, and 0.25 mM of CAA; 2, 5, 10 and 15 μM of BAA; and 0.5, 2.5, and 5 μM of IAA) for 96h. Every 24 hours, the sizes of follicles were measured and after 96 hours, the media were collected and subjected to measurements of estradiol levels. The results show that all three monoHAAs significantly inhibit growth of antral follicles with a descending toxicity rank order of IAA > BAA > CAA. Furthermore, CAA (0.025, 0.05, 0.1, and 0.25 mM) and BAA treatment (5, 10, and 15 μM of BAA) significantly reduced estradiol levels compared to controls ($n=3$, $p<0.05$). Collectively, these data suggest that the monoHAAs may be ovarian toxicants.

ABSTRACT FINAL ID: 2686 Poster Board -432
TITLE: Sodium Fluoride and Sulfur Dioxide Impaired Male Reproduction by Disturbing Blood-Testis Barrier in Mice

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KEYWORDS: Fluoride and Sulfur Dioxide, Blood-Testis Barrier, Male Reproduction

ABSTRACT BODY: Blood-testis barrier (BTB), as a physical barrier of the testis to restrict the diffusion of various endogenous and exogenous toxic chemicals in mammal, dysfunction of which has be considered as one of the important pathways involved in chemicals-induced reproductive toxicity. Fluoride and sulfur dioxide, two well-known environmental toxicants, have been implicated to have adverse effects on male reproductive health in humans and animals. However, no sufficient data have been found on fluoride and sulfur dioxide inducing disruption of the BTB. Therefore, the objective of this study is to explore if the impairments of male reproduction induced by fluoride and sulfur dioxide are caused by disturbing BTB? Which gene or protein should be look as the target in this process? In this study, 48 healthy Kunming male mice were employed and divided randomly into four groups of twelve mice each, and then exposed to 100mg NaF/L in the drinking water, SO2 in ambient air (10ppm SO2, 3hr/day), or both NaF and SO2 together respectively for eight consecutive weeks. The results showed that the sperm quality, layers of spermatogenic cells in seminiferous tubule, and BTB normal ultrastructure in mice had been changed in all treatment groups. Meanwhile, fluoride treatment reduced the tight junction proteins (Occludin and ZO-1), basal ectoplasmic specialization proteins(N-cadherin and α-catenin) , gap junction protein (Connexin-43) genes mRNA expression. Fluoride, SO2 and their combination decreased significantly desmosome protein

(Desmoglein-2) gene mRNA expression levels. At the protein levels, NaF and SO2 treatments decreased the expression of N-cadherin, α-catenin, Connexin-43 and Desmoglein-2, whereas Occludin and ZO-1 unchanged statistically. In conclusion, fluoride and sulfur dioxide can disturb BTB integrity and function in he testis, and then interference spermatogenesis. Fluoride toxicity should be deriving more attention to male reproduction.

ABSTRACT FINAL ID: 2687 Poster Board -433
TITLE: The Study of Gonadotoxic Activity of Lambda-Cyhalothrin on Male and Female Wistar Han Rats

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KEYWORDS: λ-Cyhalothrin, Reproductive Toxicity, Gonadotoxicity

ABSTRACT BODY: Reproductive toxicity studies of technical λ-cyhalothrin 97% were conducted on 180 Wistar Han rats of both sexes. Functional indicators of gonad state and the animals' ability to reproduction were examined after the end of exposure period. The duration and the frequency of each stage of the estrous cycle in female rats and the number of motile sperm, the total amount of sperm and the number of abnormal forms of germ cells of the male rats were studied.The reproductive function state was evaluated on the 20-th pregnancy day in the exposed rats mated with intact rats. Thereby the number of corpora lutea in the ovaries, the number of alive, dead and resorbed fetuses and embryos, the fetus weight, the total weight of litters, the occurrence of malformations were registered. The reproductive indexes were taken into account. According to the result of the study it can be concluded that the test substance “technical λ-cyhalothrin 97%” in high dose of 3 mg/kg of body weight, according to the planned scheme of the experiment for evaluation of gonadotoxic activity on male and female Wistar Han rats, has sign of gonadotoxcitcity (antiandrogenic activity). It manifests in reduction of the motile sperm cells amount, the reduction of the motile sperm percentage and testicular weight). In the low dose of 0.3 mg/kg the test substance does not causes the general toxic effects and does not induce any negative effect on the reproductive function of male and female Wistar Han rats.

ABSTRACT FINAL ID: 2688 Poster Board -434
TITLE: MicroRNA Alterations Associated with the Testicular Toxicity Induced by Di(2-ethylhexyl)phthalate (DEHP) in Rats

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KEYWORDS: microRNA, Di(2-ethylhexyl)pthalate, Testis

ABSTRACT BODY: Di(2-ethylhexyl)phthalate (DEHP) is an industrial chemical used as a plasticizer in the production of flexible polyvinyl chloride products, such as toys, packaging film and sheets, medical tubing, and blood storage bags. The highest human exposure to DEHP occurs via parenteral exposure, specifically in neonates during medical treatments. Earlier studies from our laboratory showed that exposure to DEHP through intravenous (IV) administration for 21 days, starting on postnatal day 3, induced testicular toxicity in NCTR Sprague-Dawley rats. The effects were observed in rats treated with DEHP 300 and 600 mg/kg body weight (bw)/day, but not with DEHP 60 mg/kg bw/day, and included depletion of germ cell epithelium, vacuolization of Sertoli cells, and modulation of gene expression. To investigate further the effects of IV DEHP, the expression of 755 microRNAs was screened by quantitative real-time RT-PCR (qRT-PCR) in testes from the vehicle and DEHP 600 mg/kg bw/day dose groups. 72 microRNAs were significantly modulated (p-value < 0.05; fold-change > 1.5) in the DEHP-treated group compared to the vehicle control. Follow-up qRT-PCR was performed on vehicle and DEHP 60, 300, and 600 mg/kg bw/day dose groups to validate the expression of microRNAs of interest and characterize dose-response effects. The results confirmed the modulation of the microRNAs analyzed in the DEHP 600 mg/kg bw/day dose group and also showed their modulation in the DEHP 300 mg/kg bw/day dose group, but not in the DEHP 60 mg/kg bw/day dose group. The modulation of microRNAs parallels the effects observed at the testicular histopathology and gene

expression levels. The involvement of microRNAs in the induction of DEHP-induced testicular toxicity warrants further investigation.

ABSTRACT FINAL ID: 2689 Poster Board -435
TITLE: Timing of Rat Fetal Testis Seminiferous Cord Effects following Late Gestation Di-n-Butyl Phthalate Exposure

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KEYWORDS: Developmental and Reproductive Toxicology, Fetal Testis, Di-n-Butyl Phthalate

ABSTRACT BODY: Exposure to some phthalates, such as di-n-butyl phthalate (DBP), adversely impacts the development of the rat fetal testis through effects on both the Leydig cells and the seminiferous cords, which combine to produce a suite of early and later-life adverse male reproductive outcomes known as Phthalate Syndrome. While Leydig cell effects are well-characterized in the rat, the seminiferous cord effects of phthalates are not fully understood. DBP exposure results in an increase in multinucleated gonocytes (MNGs) within two days, but the mechanism by which these cells are formed and their impacts on testicular development are unknown. We exposed timed pregnant Sprague Dawley rats to 500 mg/kg/d DBP or corn oil vehicle by oral gavage beginning on gestational day 19, with concurrent exposure to 0.3 M bromodeoxycitidine (BrdC, converted to bromodeoxyuridine/BrdU *in vivo*) by subcutaneous osmotic pump. DBP caused a significant increase in terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) frequency in the seminiferous cords beginning at 6 h. MNGs were induced at 24 and 48 h. Concurrently, seminiferous cord diameter decreased in vehicle-treated testes, while remaining significantly greater in the testes of DBP-treated rats. The overall rate of cellular proliferation in the testis, as measured by BrdU labeling, was not affected by DBP treatment, and MNGs were very infrequently labeled with BrdU, suggesting formation by a non-proliferative mechanism. This is consistent with the hypothesis that MNGs form by collapse of intercellular bridges, rather than abnormal mitosis, and are a degenerative cell type. While several MNGs had TUNEL-labeled nuclei, these were not frequently observed. This indicates that while apoptosis is the likely mechanism of cell death for MNGs, most MNGs persist beyond 48 h after initiation of DBP exposure.

ABSTRACT FINAL ID: 2690 Poster Board -436
TITLE: An Environmentally Relevant Dose of Bisphenol A May Decrease Communication through GAP Junctions in the Cumulus Cells-Oocyte Complex

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KEYWORDS: Cumulus Cells, Expansion, Bisphenol A

ABSTRACT BODY: Prior to luteinizing hormone (LH) surge, molecules and nutrients transfer along the cumulus cells-oocyte complex (COC) via GAP junctions. Following LH surge, cumulus cells (CC) synthesize and assembly a viscoelastic extracellular matrix (VEM) which promotes detachment of COC and facilitates CC expansion, a crucial step for oocyte maturation. Bisphenol A (BPA), a monomer that leaches from plastics into food and water, interferes with CC expansion. Previously, we showed that BPA treated mice have more detached COCs as compared to control mice, which may alter GAP junctions. Thus, this study evaluated whether BPA alters COC expansion and GAP junctions intercellular communications. Female C57BL/6J mice (39 days old; n=5-8 per group) were orally exposed to BPA (50 µg/kg bw/day) or vehicle for three estrous cycles. After treatments, mice on estrus were *ip* injected with equine chorionic gonadotropin hormone (eCG; 5 IU) to promote formation of COCs in ovarian follicles, and 48 h post-eCG were euthanized to isolate COCs or *ip* injected with human chorionic gonadotropin hormone (hCG; 5 IU) to collect ovaries 10 h post-hCG. COCs were immediately used to evaluate GAP junctions intercellular communication by the Fluorescence Recovery After Photobleaching (FRAP) technique, whereas ovaries were processed for histology and then were evaluated for the CC expansion using a calibrated microscope and the Image Pro Premier© software. Expansion of CC or VEM was similar in BPA treated mice and control. In addition, the

distance between the oocyte and the closest CC, a parameter that indicates detachment of COCs, was similar in BPA treated mice and control. Recovery of calcein fluorescence in photobleached CC slightly decreased in BPA treated mice compared to control. These data suggest that BPA treatment may detach COCs by decreasing communication via GAP junctions, but not by altering CC expansion. Experiments are underway to evaluate the impact on CC apoptosis. CONACYT-México 167678.

ABSTRACT FINAL ID: 2691 Poster Board -437
TITLE: Visualization of 3-Dimensional Lung Microtissues for Assessment of Nanoparticle-Induced Toxicity

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KEYWORDS: 3D Microtissues, *In Vitro* Toxicology, Carbon Nanotubes

ABSTRACT BODY: The rapid advancement of nanotechnology poses an increasing risk of exposure that may compromise human health and safety. The potential adverse health impacts of nanomaterials emphasize the need for improved nanotoxicology testing platforms for high-throughput screening to enable design of safe nanomaterials. Three-dimensional (3D) *in vitro* platforms have been shown to closely recapitulate human physiology, compared to conventional two-dimensional (2D) *in vitro* or *in vivo* animal model systems, a substantial advantage in investigating disease mechanisms and in toxicity testing. As inhalation is a route typically associated with nanoparticle exposure, the use of pulmonary cell types to form scaffold-free 3D lung microtissues allows for a relevant, more efficient means of screening engineered nanomaterials. Despite the benefits of 3D cell culture, significant challenges remain due to limitations in visualization and imaging. To effectively visualize 3D lung microtissues, Clear^{T2}, a solvent free clearing method, was used to improve morphological visualization. Adaptation of this technique to 3D microtissues exhibits better visual 3D reconstructions, and application of immunohistochemistry to assess phenotypic cell markers. Further, confocal Raman microscopy was used to identify carbon nanotubes (CNT) within 3D lung microtissues, enabling monitoring of nanomaterial biodegradation through the signature D, G peak ratio. Correlation of dose delivered to the target tissue with adverse pulmonary outcomes and assessment of nanoparticle biodegradation are key endpoints in assessment of CNT-induced toxicity. These refined visualization techniques provide new and alternative methods for evaluating nanoparticle-induced pulmonary disease and subsequent delineation of toxicity pathways, enhancing the versatility of 3D *in vitro* testing platforms in toxicological applications. This research is supported by an NIEHS training grant (T32 ES07272), an NIEHS Superfund Research Program grant (P42 ES013660) and generous support by Donna McGraw Weiss ’89 and Jason Weiss.

ABSTRACT FINAL ID: 2692 Poster Board -438
TITLE: Silver Nanoparticle-Induced Oxidative Stress-Dependent Toxicity in Sprague-Dawley Rats

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KEYWORDS: Silver Nanoparticle, Hepatotoxic, Oxidative Stress

ABSTRACT BODY: Due to the intensive commercial application of silver nanoparticles (Ag-NPs), their health risk assessment is of great importance. For acute toxicity evaluation of orally administered Ag-NPs, induction of reactive oxygen species (ROS), activity of liver function enzymes [(alanine (ALT/GPT), aspartate (AST/GOT), alkaline phosphatase (ALP)], concentration of lipid hydro peroxide (LHP), comet assay, and histopathology of liver in the rat model were performed. Four groups of five male rats were orally administered Ag-NPs, once a day for five days with doses of 5, 25, 50, 100 mg/Kg, body weight. A control group was also made of five rats. Blood and liver was collected 24 h after the last treatment following standard protocols. Ag-NPs exposure increased the induction of ROS, activities of the liver enzymes (ALT, AST, ALP), concentration of lipid hydro peroxide (LHP), tail migration, and morphological alterations of the liver tissue in exposed groups compared to control. The highest two doses, 50 and 100 mg/kg showed statistically significant ($p < 0.05$) increases in ROS induction, ALT, AST, ALP activity, LHP concentration, DNA damage, and morphological alterations of liver compared to control. However, comparing the two highest doses, there was no statistically significant effect in elevating the ROS, DNA

damage, ALT, AST and ALP activity. Lipid hydro peroxide concentration and morphological alterations of liver showed dose-dependent response compared to control. Based on these results, it is suggested that short-term administration of high doses of Ag-NP may cause organ toxicity and oxidative stress in rats.

ABSTRACT FINAL ID: 2693 Poster Board -439
TITLE: Risk Assessment of Mycosynthesised Silver Nanoparticles on Rat Intestinal Cell Lines

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KEYWORDS: Nanotoxicity, Mycosynthesised Nanoparticles, Silver Nanoparticles

ABSTRACT BODY: The rapid growth of nanotechnology assures the great benefits for society. But with its growth some environmental concern are rising, which could have substantial societal implications. To study such concerns a new branch of nanotechnology called nanotoxicology has emerged. It include the study of possible hazardous impacts of nanomaterial in biological systems *in vitro* or *in vivo*. Now a days many nanoparticles are being used for various applications. Among those silver nanoparticles (AgNPs) are used frequently in nanomedicine. Past studies shown that silver nanoparticles are having antimicrobial effects on various pathogenic bacteria. Moreover, it is found that mycosynthesised AgNPs has the better antimicrobial activity as compared to the AgNPs synthesized by other methods. Recently, it has also been found to get accumulated in human and environment through various portals, causing a toxic effects. Nevertheless, the potential toxicity of silver nanoparticles especially which are synthesized by using fungi has not been thoroughly understood. Therefore, the present study was designed to assess the cytotoxicity of these particles against rat intestinal cell line (IEC-6) by using various cell viability assays like MTT, Neutral Red Utilization (NRU), Lactate Dehydrogenase Leakage and Reactive Oxygen Species (ROS) Assay. In result we found that these mycosynthesised AgNPs have IC50 of 8µg/ml. Furthermore, they were also observed to damage the cell membrane and induce the reactive oxidative species formation which plays the main role in their toxicity. This study probably is first of its kind which reports the toxicity of mycosynthesised AgNPs to animal model at the microscopic cellular level by applying suitable methods to reveal general mechanisms of toxicity and characterizing exposure to nanomaterials.

ABSTRACT FINAL ID: 2694 Poster Board -440
TITLE: Understanding the Transformation, Speciation, and Hazard Potential of Copper-Based Particles in a Model Septic Tank System Using a Zebrafish High-Throughput Screening Assay

AUTHORS (FIRST INITIAL, LAST NAME): A. Taylor[1], S. Lin[2], Z. Ji[2], C. Chang[2], N. Kinsinger[1], W. Ueng[2], S. L. Walker[1, 2], A. E. Nel[2]
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KEYWORDS: Copper Particles, Speciation, High-Throughput Screening

ABSTRACT BODY: It is important to understand the environmental hazard potential of commercially available copper-based particles that are currently used as fungicides, bactericides, and in consumer products. To study the implications of six Cu-based particles (nano-sized Cu and CuO, two nano $Cu(OH)_2$, and micron-sized Cu and CuO), a zebrafish high-throughput platform was used to compare the effects of the “as-received” particles and the “transformed” particles after introduction into a model septic tank system. While the nano-sized Cu materials were clearly more potent than the micron-scale particulates in embryo hatching interference, the transformation of the Cu materials added to the septic tank system demonstrated a decline in embryo toxicity, regardless of particle size and composition. The Cu species were identified as inorganic [e.g., $Cu(H_2PO_2)_2$] and organic compounds that were not bioavailable for embryo hatching interference. Moreover, it was demonstrated that the addition of humic acid could lead to a dose-dependent decrease in Cu toxicity in the embryo-screening assay. Thus, the use of zebrafish screening, in combination with treated wastewater samples from a

model septic tank, could provide a novel way to study the change in the hazard potential of Cu-based and the particle transformation and chemical speciation in the environment.

ABSTRACT FINAL ID: 2695 Poster Board -441
TITLE: Importance of Nanoparticle Agglomeration on Inflammation

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KEYWORDS: Nanotoxicology, Inflammasome Activation, Pulmonary Bioactivty

ABSTRACT BODY: Suspension of nanoparticles into biological fluids results in agglomeration, producing variability into the results, as the actual dose of nanoparticles no longer corresponds with the particle number. Therefore, it is important to determine how the agglomeration state of the nanoparticles affects their interaction with cells and animals. This study evaluates the effect of different dispersing media on the agglomeration state of nanoparticles, and in parallel measures their interactions *in vivo*, to better understand the importance of interactions when particles are well dispersed vs. agglomerated. The current study evaluated the role of NLRP3 inflammasome activation in the pulmonary bioactivity of nano-sized nickel-oxide (NiO) subjected to differing dispersion methods. Current evidence suggests that macrophage engulfed nanoparticles can cause lysosomal membrane permeability and release of cathepsin-B, thus activating the NLRP3 inflammasome. In order to test the role of agglomeration state in activation of the NLRP3 inflammasome, NiO was suspended in four different dispersion media (phosphate buffered saline, dispersion medium (DM), Survanta, and Pluronics). Well and poorly-dispersed (sonicated at 25W continuous output, 20 min or 5 min, respectively) suspensions were created. Mice (male, C57BL/6J, 7 weeks old) were given 0-80μg/mouse of NiO in the different states of dispersion via pharyngeal aspiration. At one day post-exposure, mice underwent whole lung lavage to collect samples for cytokine analysis. The results showed that pre-exposure dispersion status correlated with inflammasome activation. The sonication time/media combination that produced the smallest hydrodynamic particle size (NiO suspended in DM and sonicated for 20 minutes) produced a greatest increase in cathepsin-B release, as well as IL-1β, and IL-18 release, compared to other sonication/media combinations. These results indicate that a greater degree of pre-exposure dispersion increases cathepsin-B release and, thus, promotes inflammasome activation. This work was supported by NIH grant F32 ES021341.

ABSTRACT FINAL ID: 2696 Poster Board -442
TITLE: Comparison of the Toxicity of Aluminum Oxide Nanorods with Different Aspect Ratio

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KEYWORDS: Aluminum Oxide Nanoparticles, Nanorods, Aspect Ratio

ABSTRACT BODY: Aluminum oxide nanoparticles are listed among 14 high-priority nanomaterials published by the Organization for Economic Co-operation and Development, but limited information is available on their potential hazards. In this study, we compared the toxicity of two different aluminum oxide nanorods (AlNRs) commercially available *in vivo* and *in vitro*. Considering aspect ratio, one was 6.2 ± 0.6 (long-AlNRs) and the other was 2.1 ± 0.4 (short-AlNRs). In mice, long-AlNRs induced longer and stronger inflammatory responses than short-AlNRs, and the degree reached the maximum on day 7 for both types and decreased with time. In addition, *in vitro* tests were performed on six cell lines derived from potential target organs for AlNPs, HEK-293 (kidney), HACAT (skin), Chang (liver), BEAS-2B (lung), T98G (brain), and H9C2 (heart), using MTT assay, ATP assay, LDH release, and xCELLigence system. Long-AlNRs generally produced stronger toxicity than short-AlNRs, and HEK-293 cells were the most sensitive for both AlNRs, followed by BEAS-2B cells, although results from 4 kinds of toxicity tests conflicted among the cell lines. Based on these results, we suggest that toxicity of AlNRs may

be related to aspect ratio (and resultant surface area). Furthermore, novel *in vitro* toxicity testing methods are needed to resolve questionable results caused by the unique properties of nanoparticles.

ABSTRACT FINAL ID: 2697 Poster Board -443
TITLE: Biochemical Effects of Six TiO2 and Four CeO2 Nanomaterials in HepG2 Cells

AUTHORS (FIRST INITIAL, LAST NAME): K. T. Kitchin[1], B. L. Robinette[1], K. A. Wallace[1], J. Richards[2], N. Coates[2], B. T. Castellon[1]
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KEYWORDS: Nano-Bio Interactions, Cerium Nanoparticles, Nano Titanium Dioxide

ABSTRACT BODY: Because of their growing number of uses, nanoparticles composed of CeO2 (cosmetics, polishing materials and automotive fuel additives) and TiO2 (pigments, sunscreens and photocatalysts) are of particular toxicological interest. In dose-response and structure-activity studies, human hepatic HepG2 cells were exposed *in vitro* to between 10 and 1000 ug/ml of six different TiO2 and four CeO2 nanomaterials for 3 days. Various biochemical parameters were then evaluated to study cytotoxicity, cell growth, hepatic function and oxidative stress. Few indications of cytotoxicity were observed between 10 and 100 ug/ml. But between 300 to 1000 ug/ml a moderate to substantial degree of cytotoxicity was observed. The four major biochemical effects observed were decreased activities of glucose 6-phosphate dehydrogenase (G6PDH), superoxide dismutase, glutathione reductase and glutathione peroxidase. The two largest contributors to hepatic oxidative stress may be (a) GSH depletion (~80%) by free radical attack generated by the nanomaterials and (b) inhibition of G6PDH (~30% decrease) which will decrease the concentration of NADPH and the effectiveness of the GPX enzyme (~20% decrease observed). Nanomaterials with 99.9% chemical identity can be surprisingly different in their biological effects. Disclaimer: [This is an abstract or a proposed presentation and does not necessarily reflect EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.]

ABSTRACT FINAL ID: 2698 Poster Board -444
TITLE: Toxicity of Graphene Oxide Decorated with Silver Nanoparticles and Their Counterparts Graphene Oxide and Isolated Silver Nanoparticle to Macrophages

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KEYWORDS: Graphene, Silver, Macrophage

ABSTRACT BODY: Nanocomposites are promising materials to medical applications, but understand their toxicity is a challenge in nanotoxicology. Macrophages are professional phagocytes of body tissues and are responsible for destruction of invaders and internalized nanomaterials. Silver nanoparticles (AgNP) are known to be microbicide agents, but to improve their efficacy we developed a graphene-based nanomaterial functionalized with silver nanoparticles (AgNP). Graphene oxide (GO) was synthetized by exfoliation of natural graphite and the nanocomposite (GOAg) by reduction of silver ions in a GO aqueous dispersion. AgNP was synthetized from silver nitrate and stabilized by citrate. Transmission electron microscopy, UV-vis and inductively coupled plasma mass spectrometry were employed to nanomaterials characterization. In order to determine safe dosages of nanocomposite and understand its toxicity to immune cells, we evaluated the cytotoxicity of GO, AgNP and GOAg for a tumor lineage of murine macrophages (J774) after 24 and 48h, in microplates. Nanomaterials internalization was also determined. GO doses above 10 µg mL-1 were toxic to the macrophage. Both AgNP and GOAg were toxic in concentrations above 2.5 µg mL-1, but this effect was observed earlier for GOAg (24h). Also, after 24h the cells internalized about three times less GOAg than AgNP. The GOAg nanocomposite was more toxic than isolated silver nanoparticles, but less internalized by the cells, possibly because agglomeration of isolated AgNP reduces the silver

release to the cell media, and consequently the toxicity. In the other hand, GOAg nanocomposite avoid silver nanoparticles agglomeration.

ABSTRACT FINAL ID: 2699 Poster Board -445
TITLE: A Delphi Study in Brazilian Stakeholders about Nanotechnology, Nanomaterial, and Their Toxicological and Regulatory Implications

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KEYWORDS: NanoEHS, NanoELSI, Delphi

ABSTRACT BODY: We conducted an email pilot study using Delphi method 3 years ago. Last year we conducted the complete study, taking into account modifications in the approach of NGOs and legislators, as demonstrated at the pilot study. In the first phase, 1080 questionnaires (5 questions) were sent, 120 for each of the following groups: Researchers; Regulators, Personal from Funding Institutions (PFI), Public Health/Environmentalists, Producers, Workers/Unions Representatives, Consumers, Legislators, and NGOs. Two rounds were need. At the first, 501 (46%) questionnaires were completed. From the 501 sent, 402 (80%) were completed at the second round. Only four answers have changed, but similarly as in the pilot study it not modified the general results, which show: 55% of respondents considered the benefits equal or outweigh the risks, but recognize that population must better understand risks. More than 25% believe all research and production in nanotech should stop until more is known about nanorisk. Around 55% of the respondents believed Brazil is in a disadvantageous position in nanotech market products comparing to other developing countries, even though who agree that research is at a good level in the country. 90% consider inadequate and insufficient the legislation that deal with risks of nanotech, almost 100% understand consumers have insufficient information and more than 75% understand that even workers who deal with nanotechnology have little information on legislation and potential risks. It was used is to send emails, similar for all categories but NGOs and legislators. For they, after previous phone contact the questionnaires were sent to specific email addresses and their receipts were confirmed by e-mail.

ABSTRACT FINAL ID: 2700 Poster Board -446
TITLE: Effects of Poly (ε-Caprolactone) Lipid-Core Nanocapsules (LNC) on Melanoma Development

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KEYWORDS: Melanoma, Cell Toxicity, Lipid-Core Nanocapsules

ABSTRACT BODY: Evolution in nanomedicine has assumed an important role to improve therapy and diagnosis in the pharmaceutical field. Polymeric lipid-core nanocapsules have been used to prolonged drug release and to direct drugs toward their targeted therapeutic action site. But, recent literature has shown that LNC can be effective, by itself, to treat different diseases. Here the role of LNCs on *in vivo* melanoma model and their actions on *in vitro* melanoma and endothelial cell cultures were investigated. Murine melanoma cells (B16F10, $8X10^5$/100µL) were s.c. injected in the dorsal region of C57BL6 mice. Animals were treated with Saline or LNC ($1.1x10^{12}$ particles/day, i.p. or p.o, 7 days) in early or advanced melanoma development. *In vitro* human endothelial cells (HUVEC) and melanoma cells (SK-Mel-28) were incubated with RPMI or LNCs (3, 9, 18, 30 or $90x10^9$ particles) and cell viability, proliferation, cycle, adhesion, migration and nitric oxide (NO) levels were monitored. I.p. injection of LNC reduced melanoma growth, nevertheless they caused loss of weight, due to lower food intake and reduced the number of platelets. P.o administration of LNC reduced melanoma growth only in early development without change hematological and biochemical parameters. LNC treatment did not reduce the

endothelial and melanoma cell viability or cell migration. LNC treatment reduced the cell proliferation in both cell lines by induce arrest in S phase of cell cycle. In addition, LNC treatment inhibited only melanoma cells adherence and increases the NO production by both cell lines. Together, data obtained show that i.p route is not feasible to LNCs treatments, and the efficiency of LNCs on tumor cell growth detected by p.o. may be due to their higher activity on cell proliferation, adhesion and NO levels in melanoma cells. CNPq, FAPESP (2010/19802-1)

ABSTRACT FINAL ID: 2701 Poster Board -447
TITLE: Timecourse of Pulmonary Effects of Dry-Inhaled MWCNT

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KEYWORDS: Multiwalled Carbon Nanotubes (MWCNTs), Dry Dust Inhalation, Pulmonary

ABSTRACT BODY: Background: Multi-walled carbon nanotubes (MWCNT) are a nanomaterial that is growing in use and popularity. The health effects of occupational pulmonary exposure to MWCNTs are currently unknown. The goal of this study was to examine pulmonary effects of occupational levels of inhaled dry MWCNTs. Methods: We designed and built a dust generator capable of producing MWCNT concentrations in the occupational exposure range (25,000 to 50,000 particles/cm^3). C57BL/6J male mice were exposed to a daily average of approximately 36,000 and a daily peak of about 50,000 particles/cm^3 or air. Six mice/group were exposed for 4 h/d for 10 weekdays and sacrificed 1, 3, 7, 10, 14, and 28 days post-exposure. Bronchoalveolar lavage (BAL) fluid was collected to assess cellular profile and protein content. The right lung was used for collagen measurement. The left lung was used for histological evaluation. Results: Total protein, a measure of lung permeability, did not change significantly after MWCNT exposure compared to air controls at any time point. Likewise, eosinophil and neutrophil cell counts were not significantly different between air and MWCNT-exposed mice. However, compared to air controls, MWCNT-exposed mice had increased numbers of total cells and macrophages at 28d; increased epithelial cells from 3 to 10 days, and increased numbers of lymphocytes and monocytes from 10 to 14 days. The presence of MWCNT was visually noted in BAL cell pellets at all time points. Collagen levels were not different between exposure groups at any time point. Conclusions: Although short-term inhalational exposure to occupationally relevant levels of dry dusts of MWCNTs did not elicit significant increases in measures of lung injury or fibrogenesis, increases in mononuclear cells and lack of particle clearance may indicate an immunomodulatory effect which could lead to disease with further particle accumulation. Funded by NCBC.

ABSTRACT FINAL ID: 2702 Poster Board -448
TITLE: Development of a Robust Method for Measuring Engineered Nanoparticle Toxicity Using *Caenorhabditis elegans*

AUTHORS (FIRST INITIAL, LAST NAME): S. K. Hanna[1], M. E. Johnson[1], A. R. Montoro Bustos[1], S. Hosbas Coskun[1], B. C. Nelson[1], G. A. Cooksey[1], J. T. Elliott[1], E. J. Petersen[1]
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KEYWORDS: Nanoparticles, *C. elegans*, Ecotoxicity

ABSTRACT BODY: As uses for engineered nanoparticles (ENPs) are being realized, increasing masses of ENPs are being produced and released into the environment, sparking concern regarding their ecological consequences. However, there are a lack of standardized methods for determining the toxicity of ENPs within *in vivo* model systems. Our objective was to improve and adapt a current toxicity assay using *Caenorhabditis elegans*, ISO 10872, for use with ENPs. *C. elegans* provides a useful model for studying the impacts of ENPs on organisms in the environment because it is relatively short-lived and is ubiquitous in the environment. Briefly, the protocol examines the growth and reproduction of *C. elegans* over 96 hours and uses Benzylcetyldimethylammonium chloride as a positive control and Escherichia coli as feed. We began by developing a

semi-automated microscopic and image analysis procedure to allow for reliable measurements of nematode length and reproduction. We then tested the robustness of the protocol by testing the sources of variability in the assay. We then adapted the assay for ENPs by adding additional controls to account for potential artifacts and used this protocol to test amine stabilized polystyrene ENPs and citrate coated Au NIST standard reference material (SRM 8013). We found feed concentration and whether or not the plate was shaken during the assay to be the main factors contributing to variability in the assay. The EC50 for polystyrene ENPs was approximately 110 mg/L for growth and 65 mg/L for reproduction but no impacts were found for the Au NIST SRM. However, polystyrene ENPs caused E. coli aggregation, which we hypothesized contributed to growth and reproductive effects. To test this hypothesis we examined an amine coated Au ENP in our system and found similar impacts as the polystyrene ENPs, suggesting that the nematodes had difficulty feeding on the aggregated *E. coli*. We propose testing these ENPs in axenic media to avoid these complications.

ABSTRACT FINAL ID: 2703 Poster Board -449
TITLE: Thrombospondin-1 Is Elevated in Skeletal Muscle following MWCNT Exposure and May Mediate Nanomaterial-Induced Loss of Arteriolar Reactivity

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KEYWORDS: Arteriolar, Microvascular, Endothelial

ABSTRACT BODY: The extracellular matrix protein thrombospondin-1 (TSP-1), is an antagonist of nitric oxide-mediated vascular relaxation by preventing activation of guanylyl cyclase and other downstream targets. Nanomaterials, especially multi-walled carbon nanotubes (MWCNT) have been shown to decrease vessel reactivity to acetylcholine, a promoter of endothelium dependent vasodilation. Our group has previously demonstrated that TSP-1 knockout (KO) animals exhibit increased endothelium dependent vasodilation in response to acetylcholine. Our objective is to determine the effects of loss of TSP-1 on microvascular reactivity, and the role of TSP-1 in MWCNT-induced endothelial dysfunction. TSP-1 Protein levels were measured in C57B6 mouse skeletal muscle using standard western blot procedures 24hrs following exposure inhalation exposure to aerosolized MWCNT. For lung depositions of 2.5µg/kg, 10µg/kg, 40µg/kg MWCNT, TSP-1 was elevated by 274%, 327%, and 359%, respectively compared to filtered air control animals. Arteriolar responses to acetylcholine were measured in exteriorized gluteus maximus muscles of TSP-1 knockout (KO) mice using a microiontophoresis technique 24hrs following 50 µg MWCNT aspiration. TSP-1 KO mice demonstrated no detectable decrease in arteriolar reactivity to acetylcholine at ejection currents of 20, 40, or 80nA compared to unexposed TSP-1 animals, and were able to dilate 24.6%, 53.4%, 54.0% more than unexposed wild type C57B6 animals. These data indicate that TSP-1 is likely involved in previously observed MWCNT-induced loss of arteriolar endothelium dependent vasodilation. Understanding the mechanisms behind this process is important as many medical technologies incorporating MWCNT are being developed that interact directly with the cardiovascular system.

ABSTRACT FINAL ID: 2704 Poster Board -450
TITLE: Distribution and Biomarker of Carbon-14 Labeled Fullerene C60 ([14C(U)]C60) in Pregnant and Lactating Rats and their Offspring after Maternal Intravenous Exposure

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KEYWORDS: Nanomaterials

ABSTRACT BODY: The distribution of nanoparticles during reproductive states of the female has not been comprehensively studied. Effects due to nanoparticle exposure may be heightened during pregnancy and lactation. Therefore, we

investigated the distribution of [14C(U)]C60 in pregnant and lactating rats. Rats were administered [14C(U)]C60 formulated with polyvinylpyrrolidone (< 5% PVP) to result in a suspension in saline or with PVP-saline (vehicle). Pregnant rats were dosed via tail vein injection on gestational day (gd) 11, 15, or 18, and lactating rats on postnatal day (pnd) 8. Urine, feces, blood, and tissues were collected at determined timepoints after dosing. The percentage of recovered radioactivity was highest in liver and lung, and differences in distribution were seen between pregnant and lactating rats. The recovery of radioactivity in pregnant rat liver (~34%) and lung (~36%) was different than the recovery in lactating rat liver (~70%) and lung (~9%) highlighting the importance of investigating distribution in both pregnancy and lactation. Radioactivity was detected in placenta, fetus, milk, and pups demonstrating transfer from dam to offspring in both reproductive states. Elimination was low (<2%) across all timepoints. Plasma levels of select cytokines were minimally impacted with the administration of C60 with some differences noted in IL-6, MCP-1 in pups and PAI 1 pregnant dams. Metabolomic analysis of pregnant rat urine showed vitamin B6, lipid metabolism regulation, and aminoacyl-tRNA biosynthesis pathways as being impacted by C60 administration as well as the metabolites, choline and acetylcholine, involved in Rho-Rho kinase signaling. (Supported by NIEHS ES019525 and NIDDK 1U24DK097193-01)

ABSTRACT FINAL ID: 2705 Poster Board -451
TITLE: Toxicogenomic Analyses of Chemically Induced Liver Injuries

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KEYWORDS: Gene Modules, Toxicity Pathways, Biomarker Signatures

ABSTRACT BODY: Liver injuries caused by exposure to chemicals and industrial toxicants pose a serious health risk. Identifying and establishing the molecular-level toxicity pathways of liver injuries provides a way to identify mechanism-based biomarkers. Towards this end, we analyzed DrugMatrix, a toxicogenomics database with gene expression data from rats after exposure to diverse chemicals and drugs. We developed both transcriptional co-expression and integrated protein interaction network modules to capture the initiation and extent of chemical injuries in the liver. These modules exhibited different activation patterns characteristic of each injury, and the genes in the modules mapped to biochemical pathways associated with liver injuries. We validated the modules using external data from Gene Expression Omnibus. From these modules, we selected gene signatures associated with liver fibrosis and general liver injury. We evaluated nine genes predicted in our analysis to be associated with liver fibrosis using multiplex panel. We carried out a five-day oral gavage exposure studies using three model chemicals (4,4'-methylenedianiline, bromobenzene, and dexamethasone) at different doses in male Sprague Dawley rats. The genes in the signature were consistently above or below the 1.5-fold threshold for differential expression in animals with liver fibrosis. Five of these genes were differentially up-regulated in animals with liver fibrosis whereas none of these genes were up-regulated in fibrosis-negative cohort. Our results show that co-expression based analysis of toxicogenomics data enables us to identify new biomarker signatures of liver injuries.

ABSTRACT FINAL ID: 2706 Poster Board -452
TITLE: Mathematical Modelling of a Hollow Fibre Bioreactor for Systemic Toxicity Testing

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KEYWORDS: Mathematical Modelling, Bioreactor, IVIVE

ABSTRACT BODY: Current 2D *in vitro* test systems are poorly predictive of the toxicity of chemicals entering the systemic circulation. There is therefore an urgent need for models of systemic toxicity with improved predictivity across the pharmaceutical and chemical industry. We are currently developing a hollow fibre bioreactor (HFB) system for hepatotoxicity testing. Previously HFBs have shown promise for use as bioartificial livers and their use in hepatotoxicity testing is a natural extension to this work. To assist with the development of the HFB, the design has been mathematically modelled to inform its operating set up, interpret data from HFB outputs and aid in optimizing design to mimic certain hepatic physiological conditions. Additionally, the mathematical model has been used to identify the key HFB and compound parameters that will affect xenobiotic clearance. The analysis of this model has produced novel analytical results that allow the operating set up to be calculated and predictions of compound clearance generated efficiently and in a highly accessible form. The mathematical model predicts the inlet oxygen concentration and volumetric flow rate that gives a physiological oxygen gradient in the HFB to mimic a liver sinusoid. It has also been used to predict the concentration gradients and clearance of a test drug and paradigm hepatotoxin, paracetamol (APAP). The effect of altering the HFB dimensions and fibre properties on paracetamol clearance under the condition of a physiological oxygen gradient is analysed. These theoretical predictions can be used to help design the most appropriate 3D *in vitro* experimental set up and data analysis to quantitatively compare the functionality of cell types that are cultured within the HFB to those in other systems.

ABSTRACT FINAL ID: 2707 Poster Board -453
TITLE: Keeping an Eye on Molecular Imaging: Assessment of Drug Toxicity in Small Ocular Structure Using Mass Spectrometry Imaging

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KEYWORDS: Occular Toxicity, Mass Spectrometry Imaging, *Ex Vivo* Imaging

ABSTRACT BODY: Introduction: Mass spectrometry Imaging (MSI) applications to ophthalmic drug discovery have recently gained growing interest especially for pharmacological or toxicological studies. MSI was applied to assess the distribution of Benzalkonium chloride (BAK) compound (antiglaucoma eye drops preservative) in specific areas of the eye after instillation in animal model tissues. They have been reported to cause ocular surface disorders with tear film alteration, eye irritation and to promote dry eye. The distribution of BAK compound was investigated in small specific histological regions of the eye in order to estimate efficiency of action or adverse effects of the treatment. Experimental procedures: Eyes of New Zealand rabbits were instilled with different BAK solutions at 0.01% for 1 month or 5 months. After sacrifice, eyes were quickly enucleated and embedded in tragacanth gum and frozen. Serial sections were deposited on glass slides for H&E staining or immunohistochemistry (IHC) and conductive glass slide for mass spectrometry imaging. Results and novel Aspect: Local drug concentration differences were observed according to histological area and position on the eye section (anterior, posterior, temporal or nasal side). MSI and IHC results were put side by side to correlate inflammatory areas (degradation of CSE or apoptosis phenomena within cornea/conjonctiva region) with BAK localization. Moreover, a high accumulation of BAKs were observed at the sclerocorneal junction and near trabecular meshwork involved in aqueous humor outflow. Moreover, differential analysis was carried out to find disease state biomarkers of each ocular structure. Conclusions: MSI offers new insight in ocular therapeutic/pharmaceutical research, especially to give a better understanding of the drug candidate migration through the eye to assist drug efficiency or toxicity studies for specific tissue targeting eye diseases.

ABSTRACT FINAL ID: 2708 Poster Board -454
TITLE: Discovery of a Conserved Long Noncoding RNA Upregulated in Response to the Xenobiotic Activation of the Aryl Hydrocarbon Receptor

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KEYWORDS: AhR Signaling, Transcriptional Regulation

ABSTRACT BODY: The aryl hydrocarbon receptor (AHR) is necessary for proper vertebrate embryonic development but can be inappropriately activated by a diverse group of xenobiotics including chlorinated dioxins, biphenyls and polyaromatic hydrocarbons. To identify downstream AHR targets, RNA was isolated from 48hpf zebrafish embryos (exposed to 10uM 7,12-benz[a]anthracene quinone) and sequenced using paired-end Illumina sequencing. One of the most elevated transcripts was a novel long noncoding RNA (lncRNA), which we dubbed Sox9b-lncRNA due to its adjacency to the Sox9b gene (ortholog of human Sox9). AHR-dependent repression of Sox9, a conserved transcriptional regulator required for chondrocyte differentiation, is well established in both mammals and fish; however, the exact mechanism of repression is unknown. The predominant Sox9b-lncRNA transcript has been sequenced, contains 3 exons, and is 467nt long. The genomic architecture of this lncRNA is conserved in mammals, suggesting similar mechanisms of regulation. The (mammalian and fish) Sox9-lncRNA promoter contains putative AHREs and a potential lncRNA binding site has been identified in the Sox9b promoter. Developmental exposure to 1nM TCDD in AHR2-null (ortholog of human AHR) zebrafish lines showed Sox9b-lncRNA induction is AHR2 dependent. We hypothesize that the conserved Sox9-lncRNA is a direct AHR target gene that transcriptionally represses Sox9 upon induction by strong AHR ligands to produce target organ-specific toxicity. In support of this hypothesis, Sox9b-lncRNA is significantly upregulated during larval caudal fin regeneration at 2dpa in samples exposed to 1ng/mL of TCDD, while Sox9b is significantly downregulated. Furthermore, Sox9b-lncRNA morphants exposed to 1ng/mL TCDD, have a two-fold increase in Sox9b expression, suggesting a relief in repression. This research was supported by the NIEHS Core Center Grant P30 ES000210 and the NIEHS Training Grant T32 ES007060.

ABSTRACT FINAL ID: 2709 Poster Board -455
TITLE: Unraveling Genotoxic Stress Response Signaling Networks

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KEYWORDS: DNA Damage, Signaling, Systems Biology

ABSTRACT BODY: Tissue stem cells are important targets for genotoxic damage and cancer development. We have taken a systems biology approach to unravel signaling networks that mediate the DNA damage response (DDR) using a surrogate model, embryonic stem cells treated with the DNA cross linker cisplatin. Genome-wide RNAi screening, transcriptomics, phosphoproteomics (SILAC), and metabolomics datasets generated in this model were imported in bioinformatics tools, including Metacore, Cytoscape, Panther, and Ingenuity Pathway Analysis. Extracted information revealed known and novel integrated signaling networks that were experimentally probed for their role in the cellular response to genotoxic stress. We have already reported the identification of a novel adaptive branch of the DDR involving p53-independent activation of Wnt signaling (Science Signaling 6(259), ra5). In addition, we identified a group of metabolic pathways that were enriched following genotoxic stress and clustered around the metabolite S-adenosylmethionine, which is a hub for methylation and transsulfuration reactions and polyamine metabolism (PLoS ONE 8(10), e76476). Recently, using genome-wide ubiquitinase siRNAs several ubiquitinases were identified that regulate sensitivity to cisplatin and other genotoxic compounds. Many of these control the level or activity of p53. In addition, we discovered a novel signaling network by which genotoxic stress triggers mRNA translation arrest. We show that ATM activation in response to treatment with genotoxic compounds initiates recruitment of an E3 ubiquitin ligase not previously associated with DDR signaling to ribosomes. Here, formation of the initiation complex at the 5' mRNA CAP is abrogated and protein synthesis is attenuated. We show that this serves as an adaptive response in stem cells as well as other cell types, delaying the onset of cell death programs. These results identify novel DDR signaling networks that determine the outcome of exposure to genotoxic stress. They also serve as input for new 2D and 3D *in vitro* toxicity reporter models.

2015 Society of Toxicology Annual Meeting
Late-Breaking Abstracts

ABSTRACT FINAL ID: 2710 Poster Board -456
TITLE: Rapid Antibiotic Resistance Evaluation Using Microfluidic-Based Single Cell Reactor

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KEYWORDS: Microfluidics, Antibiotic, Resistance

ABSTRACT BODY: Microfluidic technology is a rapidly growing multidisciplinary field, and provides great potential in scientific detection and reaction applications, including advanced biological assays and biosensors. The biological studies and toxicological reactions can be performed on the chip, and readouts can be detected by integrated fluorescence, chemiluminescence, and bioluminescence to enable highly sensitive quantification. We utilized this state-of-art microfluidic technology to develop the novel antibiotic gradient generator with integrated single bacteria trapping reactor to monitor the antibiotics resistance. GFP expression Escherichia Coil (BL21 (DE3) Gold (pETck3) and Burkholderia were used as model bacteria to evaluate their resistance to antibiotics: ciprofloxacin and chloramphenicol, respectively. The fluorescence signals and phase contrast images of bacteria populations were captured every 5 min in 24 hrs by Automated Zeiss Axio Observer Z1 fluorescence microscope. Our study has shown that this microfluidic system can be used continuously monitoring the single bacteria growth in response to gradient contraction of antibiotics. The damaged bacteria cells were washed out by continuous antibiotic flow. The resistant bacteria reminded, and started to proliferate in the micro-trapping reactor in the microfluidic chamber under gradient contraction of antibiotics. By continuously monitoring and capturing 100 reactors array in the antibiotic gradient chamber, and interpreting the data using Image Pro Plus, the bacteria population/fluorescence intensity vs. antibiotic concentration vs. time relations were established. We observed that the resistant effects of these bacteria were developed, and the minimum inhibitory concentration (MIC) value can be measurements in 3 hrs without long wait times. We successfully demonstrate this novel sophisticated microfluidic-based antibiotic resistance evaluation platform which provide fast diagnosis of MIC and clinical phenotype-based evaluation.

ABSTRACT FINAL ID: 2711 Poster Board -457
TITLE: Systems-Based Approach for Prediction of Drug-Induced Liver Injury (DILI)

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KEYWORDS: Oxidative Stress, Cholestasis, Energy Metabolism

ABSTRACT BODY: A systems-based DILI prediction platform has been developed that integrates *in vitro* and modeling approaches to simulate pathways for hepatocellular homeostasis to predict alteration in energy metabolism, oxidative stress, steatotic and cholestatic liver injury. Several biochemical measurements including mitochondrial complex I/II, FAS, CPT1, γ-GCS, GR, MTP, ROS, and hepatic transporters, when available, are used as input for biosimulations. Five compounds (diclofenac, metformin, zomepirac, troglitazone and rosiglitazone) were evaluated in the model. Biochemical endpoints were determined by either direct treatment of extracts isolated from HepG2 cells or in extracts isolated from cells treated for up to 3 days to capture any adaptive responses. Cells were treated at 3 drug concentrations that were not cytotoxic (viability < 50%). Simulations were performed using both *in vitro* data and human exposures at 1× and 10× to span potential differences in disposition and to capture tissue drug accumulation. Simulations correctly predicted hepatocellular injury for troglitazone, while rosiglitazone and metformin were predicted to be safe at clinically-relevant doses. The cholestatic potential for troglitazone was simulated using transporter specific Ki values from the literature in a set of virtual patients with variations in BSEP activity to represent the polymorphic nature of the transporter. The model predicted cholestasis in some individuals based on troglitazone's inhibitory effect on mitochondria and BSEP transporter. The model was unable to predict hepatocellular injury by diclofenac or zomepirac, likely due to inability of HepG2 cells to metabolize either compound to acylglucuronides, the ultimate toxic metabolites. Prediction for diclofenac acylglucuronide was limited

to glutathione depletion. The prediction platform has utility for testing DILI potential for parent compounds or synthesized metabolites and can be improved in the next iteration by using a metabolically competent *in vitro* system.

ABSTRACT FINAL ID: 2712 Poster Board -501
TITLE: Glucose Metabolism and Adenosine Monophosphate-Activated Protein Kinase (AMPK)-Dependent Signaling Regulate the Toxicity of Paraquat (Environment) and Alpha-Synuclein (Gene) Interactions

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KEYWORDS: AMPK, Paraquat, Alpha-Synuclein

ABSTRACT BODY: Parkinson's disease (PD) is a multifactorial disorder with a complex etiology that includes genetic risk factors, environmental exposures, and aging. Energy failure and oxidative stress have been associated with the loss of dopaminergic cells in the substantia nigra and the toxicity induced by environmental toxins. However, little is known regarding the alterations in energy metabolism/signaling associated with mitochondrial dysfunction, and their causative role in cell death progression. We aimed to establish the role of glucose metabolism and metabolic stress/signaling in dopaminergic cell death induced by gene (alpha [α]-synuclein)-environment (paraquat) interactions. Glucose deprivation or inhibition of hexokinase with 2-deoxy-D-glucose (2-DG) protected N27 dopaminergic cells from paraquat. Paraquat increased the activation of the mammalian target of rapamycin (mTOR) / unc-51 like autophagy activating kinase 1 (ULK1) / adenosiene monophospnate-activated protein kinase (AMPK) signaling axis. Autophagy protein 5 (ATG5)/ULK1-dependent autophagy protected against paraquat toxicity. Paraquat induced an increase in glucose uptake and translocation of the glucose transporter type 4 (GLUT-4) to the plasma membrane. Inhibition of GLUT-like glucose transport with STF31 reduced paraquat-induced dopaminergic cell death. Sub-chronic exposure of C57Bl/6 mice to paraquat led to alterations in the midbrain metabolome and the activation of AMPK. Overexpression of wild type or A53T α-synuclein stimulated paraquat toxicity and metabolic dysfunction, and this effect was prevented by free radical scavengers (ascorbic acid) and inhibitors of glucose metabolism/transport and the pentose phosphate pathway (PPP) (6-aminonicotinamide). These results demonstrate for the first time that glucose metabolism and AMPK-dependent signaling regulate dopaminergic cell death induced by gene (α-synuclein)-environment (paraquat) interactions.

ABSTRACT FINAL ID: 2713 Poster Board -502
TITLE: A Longitudinal Study of Translocator Protein 18 KDA (TSPO) in Sandhoff Mice: An Early Preclinical Biomarker of Neurodegeneration in Sandhoff Disease

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KEYWORDS: Neurodegenerative Disease, Biomarkers, Inflammation and Disease

ABSTRACT BODY: TSPO is widely used as a biomarker of brain injury and neuroinflammation. Sandhoff disease is an autosomal recessive neurodegenerative disease in which a deficiency in the lysosomal enzyme, β-hexosaminidase, leads to accumulation of gangliosides and glycolipids in the brain resulting in progressive and widespread neurodegeneration. In this study, we examine the temporal expression of TSPO in the brain and its relationship to neuropathological and behavioral endpoints at asymptomatic and symptomatic stages of disease in Sandhoff mice. Using [3H]-DPA-713 quantitative autoradiography we show that at an early age when there is no behavioral expression of disease, there are significant increases in brain TSPO levels that are associated with the early aggregation of GM2 gangliosides in neurons. Increased TSPO expression is also associated with ongoing neurodegeneration and glial cell activation. Triple-label immunofluorescent confocal imaging confirms that TSPO colocalizes with both microglia and astrocytes and that this distribution changes as a function of disease progression. Further, we demonstrate for the first time that TSPO colocalizes with the gp91phox subunit of NADPH oxidase in microglia, but not in astrocytes, at an age when brain tissue is undergoing

neurodegeneration, suggesting that TSPO serves different functions in each cell type. Finally, microSPECT studies using the TSPO ligand [125I]-DPA-713 demonstrated the validity of this ligand to detect increased brain levels of TSPO in Sandhoff mice, an effect that could be blocked pharmacologically. Taken together, our findings provide strong evidence that TSPO can be used as an early biomarker of brain injury and inflammation, prior to clinical expression of disease and that TSPO likely serves different functions in microglia and astrocytes. [Supported by NIEHS grant ES007062 to TRG]

ABSTRACT FINAL ID: 2714 Poster Board -503
TITLE: Air Pollution and Parkinson's Disease: The Effects of Diesel Exhaust on Dopaminergic Neuron Toxicity and Disruption of Alpha-Synuclein Homeostasis

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KEYWORDS: alpha-Synuclein, Zebrafish, Diesel Exhaust

ABSTRACT BODY: Parkinson's disease (PD) is a debilitating neurodegenerative disorder characterized by loss of dopaminergic neurons in the substantia nigra. The disease is characterized by the pathologic accumulation of insoluble aggregates containing the protein alpha synuclein. Mutations in the human alpha synuclein promoter can lead to increased expression, which, along with gene duplication and triplication, lead to inherited forms of PD. Because the majority of cases of PD are not caused by known genetic mutations, it is thought that there remains a significant environmental component to disease risk. Recent epidemiological studies have uncovered potential links between long-term exposure to air pollutants and eventual development of PD. This project examines how diesel exhaust, a major component of urban air pollution, may induce dopaminergic neuron toxicity and whether this toxicity is caused by disruption in synuclein homeostasis. Exposure to diesel exhaust causes a 50% decrease in number of aminergic neurons in zebrafish embryos as seen by confocal microscopy at 5 days post fertilization. In order to determine if this neuronal toxicity is due to alpha synuclein, rat primary mescencephalic cultures were used as well as human neuroblastoma cell lines. In rat primary mescencephalic cultures, alpha synuclein protein levels increased within dopaminergic neurons after treatment with diesel exhaust for 24 hours as detected by immunohistochemistry. Additionally, in both rat primary cultures and human neuroblastoma cell lines, alpha synuclein gene expression was elevated via qPCR after exposure to diesel exhaust. From these results, it can be determined that diesel exhaust exposure alters alpha synuclein homeostasis and can induce dopaminergic neuron toxicity.

ABSTRACT FINAL ID: 2715 Poster Board -504
TITLE: Early Life Lead (Pb) Exposure and the Alteration of Epigenetic Regulators across the Lifespan of Wild-Type Mice: Implications for Alzheimer's Disease

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KEYWORDS: Alzheimer's Disease, Epigenetics, Lead

ABSTRACT BODY: Alzheimer's Disease (AD) is a progressive neurodegenerative disorder that usually (>90% of those diagnosed) presents as sporadic, or late onset AD (LOAD), with symptoms becoming apparent late in life (ages>65). The sporadic nature and delayed onset of LOAD suggest that epigenetic and/or environmental components may play a role in the etiology of the disorder. We have previously shown that developmental exposure to the environmental toxin Pb is correlated with a latent overexpression of AD-related genes such as the amyloid precursor protein (APP), and the microtubule associated protein tau (MAPT). It is well established that environmental toxins have the ability to alter the epigenome, and in turn gene expression. The objective of this study was to profile the changes that occur in epigenetic intermediates as a result of early-life Pb exposure. C57/BL6 mice were exposed to 0.2% Pb acetate through the drinking

water of respective dams from postnatal day (PND) 1 to PND 20. Tissue was collected at the following time points: PND 20, 180, 270, 540, and 700. DNA maintenance proteins and proteins involved in the regulation of the methylation cycle were examined. Pb induced a significant decrease, at all time points, in the protein levels for DNA methyltransferase 1 (DNMT1), DNMT3a, and methyl cytosine binding protein 2 (MECP2) relative to control. Pb increased both the protein expression and mRNA levels of methionine adenosyltransferase 2a (MAT2A) at PND 20, 180 and 540 relative to control. We posit that these alterations to the DNA methylation cycle following early-life exposure to Pb provide a potential explanation for changes in the expression of AD-related proteins observed across the lifespan of these mice.

ABSTRACT FINAL ID: 2716 Poster Board -505
TITLE: Developmental Pb Exposure Increases Human Tau Gene Expression in a Transgenic Animal Model: Relevance to Alzheimer's Disease and Tauopathies

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KEYWORDS: Lead Exposure, Alzheimer's Disease, Tau Hyperphosphorylation

ABSTRACT BODY: Alzheimer's Disease (AD), the 6th leading cause of death in the United States, is a neurodegenerative disease that is characterized by a decline in memory and cognitive function. Demographics show that more than 90% of patients are first diagnosed with AD after the age of 65, classified as Late Onset AD (LOAD). The remaining 10% are said to have Early Onset AD. Research has shown that no mutation is linked to LOAD. Thus we hypothesize that environmental and/or epigenetic factors may be playing a major role in LOAD. AD traits coincide with the presence of two pathological hallmarks found in the brain: amyloid-beta plaques and neurofibrillary tau tangles. The microtubule associated protein tau (MAPT) is primarily found in neuronal axons. A feature of AD is the hyperphosphorylation of tau, lowering its binding affinity to the microtubules and increasing the chance of toxic aggregates to entangle. This *in vivo* study uses a MAPT transgenic mouse model that has been knocked out for murine tau and knocked in for the human tau gene. Pups were exposed to lead from PND 0-20 with 0.2% Pb acetate through the drinking water of the dam. Mice were sacrificed at PND 20, 30, 40, 50 and 60. Protein levels of total tau, related kinases (CDK5) and phosphorylated tau were investigated. Mice developmentally exposed to lead exhibited elevated levels of tau, phosphorylated tau and CDK5 compared to controls. We can conclude thus far that developmental lead exposure increases the protein levels of genes related to the advancement of AD neurofibrillary tangles. These findings are the first of their kind to test the responsiveness of the human tau gene to an environmental toxin. In the future, the epigenetic basis for these changes of these animals will be studied.

ABSTRACT FINAL ID: 2717 Poster Board -506
TITLE: High Glucose Causes Neural Tube Defects in Chicken Embryos through Excessive O-GlcNAcylation of Pax3 Protein

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KEYWORDS: High Glucose, Neural Tube Defects

ABSTRACT BODY: Offspring involved in hyperglycemia gestation may have a higher risk of being born with congenital malformations, including cardiovascular defects, CNS abnormality and skeletal dysplasia. In this study, we investigated the effect of high glucose on chicken embryo neural system development and explored its underlying mechanism. Various doses of glucose were administrated into the air sac of chicken embryos after 24 h of incubation. Stillbirth, growth retardation and malformation of neural system were found in high-glucose-exposed embryos at embryo developmental day 5 (EDD 5). The incidence of neural defects increased with the dose of high glucose. Specifically, neural tube defects were found, as detected by stereomicroscopic examination and HE staining at EDD 5. On the other hand, blood and tissue

glucose levels were both increased by the exogenous glucose solution, as detected by kits and PET-CT scanning at EDD 3.5. Glucose transporter 1 and 3, which are identified as the major glucose transporters in the CNS and facilitate the transport of glucose across the plasma membrane of a cell, had a decreased gene expression after high glucose administration. This down-regulation was probably a cellular protective mechanism under the unfavorable high glucose environment. Moreover, we found that O-GlcNAcylation, a post-translational modification with a single N-acetylglucosamine at the serine or threonine residues of proteins, of the neural developmental marker paired box 3 (Pax3) was significantly increased in embryos after high glucose exposure. These results provided new insights in the understanding on how high glucose affected embryo developmental fate and offered a new target on the prevention of nutrient substance metabolic disturbance associated embryotoxicity and teratogenicity.

ABSTRACT FINAL ID: 2718 Poster Board -507
TITLE: Increased Activation in the Auditory Cortex of Developmentally PCB-Exposed Rats following $GABA_A$ Antagonism

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KEYWORDS: Polychlorinated Biphenyls, Endocrine Disruptor, GABA

ABSTRACT BODY: Previous work in our lab has shown changes in excitability in the inferior colliculus, a major nucleus in the auditory midbrain, of polychlorinated biphenyl (PCB) exposed rats. The current study investigated whether PCB exposure would alter auditory cortex (AC) activation in response to electrical stimulation of thalamocortical afferents. Long Evans dams were fed cookies that contained 0 or 6mg/kg of an environmental PCB mixture daily from 4 weeks prior to breeding through weaning. Living brain slices containing projections from the thalamus to the AC were collected from female adult offspring. Slices were bathed sequentially in artificial cerebrospinal fluid (aCSF), aCSF containing 200nM SR-95531, a $GABA_A$ receptor antagonist, and aCSF containing 50µM AP5, an NMDA receptor antagonist. During each condition, electrical stimulations ranging from 25-600µA were delivered to the thalamocortical afferents, and activation of the AC was measured using flavoprotein autofluorescence imaging. Statistical analysis revealed significant treatment x drug interactions at stimulations of 25, 50, 100, 150, 200, and 250µA in the upper layers of the auditory cortex and at 25 and 200µA in the lower layers when activation during aCSF was compared to the SR-95531 condition. Although there were no differences between groups in the aCSF condition, when bathed in SR-95531, the upper layers of the AC of PCB-exposed rats showed increased activation when compared to controls. No differences were noted between groups when slices were exposed to AP5. These data suggest that developmental PCB exposure leads to an increased sensitivity to the antagonism of $GABA_A$ receptors in the AC without a change in intrinsic excitability.

ABSTRACT FINAL ID: 2719 Poster Board -508
TITLE: Juvenile Rat Emotional Behavior and Social Play Are Altered by Preweanling Inhibitors of FAAH

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KEYWORDS: Chlorpyrifos, Endocannabinoid, Behavior

ABSTRACT BODY: There is a concern that chlorpyrifos (CPF), an organophosphorus insecticide, may cause developmental neurotoxicity in children leading to long term effects. Our laboratory has reported that developmental exposure of rat pups to low levels of CPF disrupts degradation of endocannabinoids in the brain through the inhibition of fatty acid amide hydrolase (FAAH). At juvenile ages, these exposed rats exhibit decreased emotional reactivity in an emergence test.

However, a single behavior only assesses a fraction of an animal's emotional profile. In this study, we further investigated the effects of developmental CPF exposure on emotionality using additional behavioral tests. In addition, it is also not clear if the inhibition of FAAH plays a role in the disruption of emotional behavior. This study compared the behavior of juvenile rats exposed developmentally to CPF with those exposed developmentally to PF-04457845, a specific inhibitor of FAAH. Ten day old rat pups were exposed orally to either 0.5, 0.75, or 1.0 mg/kg CPF or 0.02 mg/kg PF-04457845 daily for 7 days. In the open field (day 23), the high CPF and PF-04457845 groups exhibited increased motor activity but no differences in the time spent in the field's center. In the elevated plus maze (day 29), all three CPF dosage groups and the PF-04457845 group had increased % entries into and % time spent in the open arms. On day 36, social behavior was monitored and all three CPF dosage groups and the PF-04457845 group spent more time playing than did controls. The similarities in behavior between PF-04457845 and CPF suggest that developmental inhibition of FAAH could be responsible for the altered behavior induced by developmental CPF exposure.

ABSTRACT FINAL ID: 2720 Poster Board -509
TITLE: High-Content Imaging of Excitatory and Inhibitory Synapses in Primary Cultures of Rat Hippocampal and Cortical Neurons

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KEYWORDS: Neurotoxicology, Developmental

ABSTRACT BODY: Synaptogenesis is a critical neurodevelopmental process and many neurodevelopmental disorders are thought to reflect altered patterns of synaptic connectivity, including imbalances between excitatory (E) and inhibitory (I) synapses. Developing rapid throughput approaches for assessing synaptogenesis will facilitate toxicologic and drug screening studies of neurodevelopmental disorders. This study describes the use of high-content imaging to quantify the ontogeny of E and I synapses using *in vitro* models of neurodevelopment. The ontogenetic patterns of synapse formation were compared between hippocampal and cortical neurons over 28 days *in vitro*. Assessment of synaptophysin protein levels by ELISA showed a general increase in synapse formation in both cell types with increasing time in culture. In contrast, high-content imaging was able to delineate cell type-dependent differences in the formation of E versus I synapses. An overall greater number of synapses formed in hippocampal neurons relative to cortical neurons with marked differences in the pattern of inhibitory synapse development between these two neuronal cell types. Preliminary toxicologic screens using chlorpyrifos and its metabolites suggest that these compounds alter the E/I ratio in cultured neurons at concentrations that do not significantly inhibit AChE. These results suggest that high content imaging can be used to quantify synaptogenesis, which should provide a robust readout of toxicologic effects on this critical neurodevelopmental event. Support provided by NIEHS (grants ES011269 and ES024676) and the USEPA (grants 83543201 and 835550).

ABSTRACT FINAL ID: 2721 Poster Board -510
TITLE: Cholinergic Dysfunction and Muscarinic Receptor Uncoupling in Alzheimer's Disease

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KEYWORDS: Alzheimer's Disease, Cholinergic, Neurotoxicity

ABSTRACT BODY: The goal of this study was to characterize the mechanism underlying muscarinic receptor uncoupling in Alzheimer's disease (AD) using human brain tissue and a neuroblastoma cell model. Muscarinic receptor signaling is terminated by GRK phosphorylation, followed by β-arrestin binding, which begins the process of receptor uncoupling and internalization. We have demonstrated that muscarinic receptors were uncoupled from G-proteins in brains of patients

with Alzheimer's disease (AD) and non-demented controls with substantial β-amyloid deposition and plaque formation. We also found that as plaque levels and β-amyloid increased, levels of the G-protein coupled receptor kinase (GRK-2) were significantly decreased, and Gq/11 protein was shifted from the cytosol to the membrane fraction. Levels of β-arrestin, a protein involved in receptor recycling, were examined in four groups: patients diagnosed with Alzheimer's (AD), age matched controls with many amyloid plaques (MP), age matched controls with sparse plaques (SP), and age-matched controls with no plaques (NP). First, the extent of plaque formation was measured using an ELISA kit specific for β-amyloid and was positively correlated with loss of cholinergic neurons as assessed by choline acetyltransferase (ChAT) activity. Second, using western blot analysis, we demonstrated that β-arrestin, levels were decreased in both non-demented groups with neuritic plaques as well as in those with Alzheimer's disease, compared to the control group. Finally, preliminary data in SH-5YSY shows that exposure to β-amyloid for 24 hrs caused a both a decrease in GRK-2 and an increase in β-arrestin levels indicating alterations in the coupling of the receptor to its g-protein. It is likely that alterations in GRK, coupled with a decrease in β-arrestin, could impair muscarinic receptor and g-protein recycling and contribute to the cholinergic dysfunction associated with AD. Therapeutically, it may be of interest to attempt to circumvent impairment of signal transduction by addressing cholinergic dysfunction in the treatment of AD.

ABSTRACT FINAL ID: 2722 Poster Board -511
TITLE: A Dose Escalation Pilot Study of Physostigmine in Cynomolgus Monkeys

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KEYWORDS: CNS, Biomarkers, Physostigmine

ABSTRACT BODY: Physostigmine is a cholinesterase inhibitor commonly used in behavioral pharmacology research. Physostigmine reversibly binds to acetylcholinesterase, increasing acetycholine in the neuromuscular junction and stimulating nicotinic and muscarinic receptors. Various cholinesterease inhibitors have shown promise in treating Alzheimer's disease, various ocular disorders, Parkinson's and schizophrenia, and phystostigmine is a useful positive control in studies performed to assess these and other potential drugs. To characterize the effects of physostigmine (Trade name Eserine, Sigma-Aldrich®) in cynomolgus monkeys (*Macaca fascicularis*), a common primate research model, female monkeys (n=4 per group; 6 to 8 years of age and 3.3 to 5.7 kg) were intravenously (IV) dosed with either saline or physostigmine at doses of 0.02, 0.04 and 0.08 mg/kg, on Days 1, 3, and 5. Assessments included clinical observations taken 1 min, 15 min, and 30 min postdose; weekly body weights; urinalysis (Days -4, 8, and 11); clinical pathology parameters (Day -4; 0.5 hr. postdose on Days 1, 3, 5; Days 8 and 11); and standard necropsy/histopathology. Serum cholinesterase was assessed on Day -4; 0.25 and 0.5 hours postdose on Days 1, 3, and 5; and on Days 8 and 11. Transient clinical signs consistent with cholinesterase inhibition, including salivation, lacrimation, diarrhea, vomiting and shaking, were seen at all doses within 30 minutes postdose, and were most pronounced on Day 5 with 0.08 mg/kg IV. Cholinesterase decreased in a dose-dependent manner, peaking at the Day 5, 15 minutes postdose. Mean glucose levels increased on Days 1, 3, and 5, and may be a physiological response related to enhanced catecholamine release. Cholinesterase and glucose levels returned to baseline on Day 8. Collectively these data indicate that physostigmine has predictable, reversible effects in the monkeys, and was generally well tolerated under the escalating dose regimen employed. This provides data supporting the use of physostigmine as a control for CNS studies performed in a contract research organization setting.

ABSTRACT FINAL ID: 2723 Poster Board -512
TITLE: The Effect of 835 Mhz Radiofrequency Radiation Exposure to the Mice Brain in the Voluntary Exercise Model

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KEYWORDS: Radiofrequency, Tyrosine Hydroxylase, Voluntary Exercise

ABSTRACT BODY: Many environmental stimuli, including ionizing radiation and electromagnetic field, may trigger the intracellular and extracellular events. The prevailed use of mobile communication through the world has triggered public interest on the possible effects on the brain due to close proximity between a mobile phone and brain during usage. Many previous studies were focused on the evaluation the effect of radiofrequency (RF) exposure to the biological system. It has been suggested that the central nervous system may be adversely influenced by RF exposure. However, few studies were still conducted to diminish or overcome the RF radiation injury. In this study, voluntary exercise was chosen to investigate of reducing 835 Mhz RF injury in the mice brain. Adult mice were assigned randomly to four groups to study mRNA expression in the mice striatum using real-time RT-PCR for RNA quantification. The four groups comprised a sham control group, an 835 Mhz RF exposure group, an voluntary exercise group, and a 835 Mhz RF exposure/voluntary exercise group. Comparing with the sham group during 10 weeks, the decrease of total amount of exercise in RF exposure group for 6 hours per day was statistically significant. In the mice striatum, tyrosine hydroxylase (TH) mRNA expressions were also significantly reduced in RF exposure group. These results may indicate that radiation exposure can be attributed to a decrease TH mRNA expression in the striatum and subsequent alteration in TH metabolism may be attributed in locomotor activity due to the decrease of dopamine in the striatum as well.

ABSTRACT FINAL ID: 2724 Poster Board -513
TITLE: Acute Radiotoxicity on Fear Memory and Synaptic Proteins

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KEYWORDS: X-Radiation, Synaptic Plasticity, Learning and Memory

ABSTRACT BODY: Cranial irradiation causes acute cognitive deficits, but the underlying mechanism of the cognitive deficits is unknown. We have previously reported that X-irradiation decreases the density of dendritic spines and changes the spine morphology of neurons, and decreases the accumulation of drebrin, a marker for synaptic function, in dendritic spines (Shirai et al., 2013). To reveal the relationship between cognitive deficits and synaptic function, we used fear conditioning and synaptic protein localization analysis in this study. In *in vitro* study, we used primary hippocampal cultured neurons and analyzed synaptic proteins immunocytochemically. Drebrin was used as a marker of functional mature dendritic spines and Synapsin I was used as a presynaptic marker. The number of drebrin clusters was decreased at 8 hours after X-irradiation but it recovered to the former level by 24 hours after irradiation. On the other hand, the number of Synapsin I clusters were remain decreased at 8 hours and 24 hours after irradiation. For behavioral study, we performed fear conditioning 7 and 24 hours after irradiation. Contextual memory was tested 24 hours after the conditioning and the auditory memory was tested further 24 hours later. The animals irradiated 24 hours before conditioning showed no memory impairments whereas the animals irradiated 7 hours before conditioning showed both context and auditory memory deficits. In addition, we analyzed drebrin intensity immunohistochemically and found 2 and 8 hours after irradiation, the intensity of drebrin was decreased in irradiated side compared to the control side. These decreases of drebrin intensity were also recovered after 24 hours. Since we observed memory deficits and a decrease of drebrin clusters and intensities in parallel, radiation-dependent drebrin loss from dendritic spines is involved in the acute radiotoxicity on neuron that might elicit fear memory impairments.

ABSTRACT FINAL ID: 2725 Poster Board -514
TITLE: Pharmacology of Bezofurans Used As Novel Psychoactive Substances (Designer Drugs)

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KEYWORDS: Monoamine Transporter, Novel Psychoactive Substance, Benzofuran

ABSTRACT BODY: Background: Novel psychoactive substances are newly used designer drugs ("internet drugs", "research chemicals", "legal highs") potentially posing similar health risks to classic illicit substances. Benzofurans are newly used psychoactive substances, but their pharmacology is unknown. The aim of the present study was to pharmacologically characterize benzofurans *in vitro*. Methods: We assessed the effects of the benzofurans 5-APB, 5-APDB, 6-APB, 6-APDB, 4-APB, 7-APB, 5-EAPB, and 5-MAPDB and benzodifuran 2C-B-FLY on the human norepinephrine (NE), dopamine (DA), and serotonin (5-HT) uptake transporters using HEK 293 cells that express the respective transporters. We also investigated the release of NE, DA, and 5-HT from monoamine-preloaded cells, monoamine receptor binding affinity, and 5-HT2A and 5-HT2B receptor activation. Results: All of the benzofurans inhibited NE and 5-HT uptake more than DA uptake, similar to methylenedioxymethamphetamine (MDMA, ecstasy) and unlike methamphetamine. All of the benzofurans also released monoamines and interacted with trace amine-associated receptor 1 (TAAR1), similar to classic amphetamines. Most benzofurans were partial 5-HT2A agonists similar to MDMA, but also 5-HT2B receptor agonists, unlike MDMA and methamphetamine. The benzodifuran 2C-B-FLY very potently interacted with 5-HT2 receptors and also bound to TAAR1. Discussion: Despite very similar structures, differences were found in the pharmacological profiles of the benzofurans and compared with their amphetamine analogues. Benzofurans acted as indirect monoamine agonists that interact with transporters similarly to MDMA. The benzofurans also interacted with serotonergic receptors. This pharmacological profile likely results in MDMA-like empathogenic psychoactive properties. However, benzofurans produce 5-HT2B receptor activation associated with heart valve fibrosis. The pharmacology of 2C-B-FLY indicates predominant hallucinogenic properties and a risk for vasoconstriction.

ABSTRACT FINAL ID: 2726 Poster Board -515
TITLE: Neuronal Toxicity Due to Reactive Dopamine Metabolites and Fungicide Exposure

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KEYWORDS: Neurotoxicology, Dopamine Metabolism, ALDH Inhibition

ABSTRACT BODY: Parkinson's disease(PD) is a neurodegenerative disorder and is manifested by the rapid deterioration of dopaminergic cells. A reduction in levels of DOPAL is critical as this aldehyde has been shown to be toxic to dopaminergic cells and is a highly reactive electrophile. The protein-GAPDH(glyceraldehyde-3-phosphate dehydrogenase)is an enzyme known for its glycolytic activity and recent research has implicated its role in neuronal death. This work positively shows GAPDH as a target for DOPAL modification and for the first time, DOPAL as an inhibitor for GAPDH. LC-MS and chemical probes (thiol and amine modifiers) show that DOPAL modifies specific Lys and Cys residues of GAPDH. This modification is responsible for enzyme inhibition in the presence of DOPAL(EC50=5 uM). This enzyme inhibition is also time and DOPAL dose-dependent. It was determined that both the catechol and aldehyde groups of DOPAL are specific to binding with GAPDH and are necessary to achieve modification and enzyme inhibition. This work has also confirmed linking DOPAL levels to a fungicide associated with PD risk. The fungicide, benomyl was preliminarily shown to inhibit ALDH2 *in vitro* enzyme assays. The ratios of DOPAL and product of ALDH were measured by HPLC-ECD, and found that benomyl does inhibit ALDH2 in relevant cell models. The cytotoxicity of benomyl, DA, DOPAL and the combination of DA or DOPAL with benomyl was assessed by MTT assay. Surprisingly, the only toxic combination was the combination of DA or DOPAL with benomyl. This toxicity is synergistic as none of the single treatments are significantly toxic to the cells. This synergistic effect also affects GAPDH aggregation. Cells treated with the combined treatment, show greater intracellular GAPDH aggregation, highlighting further that GAPDH is a relevant protein targeted by DOPAL. Cell morphology is also drastically different in the presence of the combined treatments, compared to individual treatment of DA, DOPAL or benomyl; cells start to ebb and show apoptotic-like features at just 2h.

ABSTRACT FINAL ID: 2727 Poster Board -516
TITLE: Innovative Test to Predict Drug-Induced Neurotoxicity and Psychiatric Adverse Effects

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KEYWORDS: *In Vitro* Neurotoxicity, Biomarker of Neurotoxicity, Drug Safety Assessement

ABSTRACT BODY: Severe drug-induced psychiatric adverse effects as depression and suicide recently resulted in the market withdrawal of compounds such as rimonabant, emission of FDA alerts (Varenicline, Isotretinoine) or law suits (Paroxetine). Current non-clinical safety studies, whether safety pharmacology or toxicity studies cannot detect these severe adverse effects leading to human deaths and expensive late withdrawals. RNA editing of the serotonin 2C receptor (5-HT2cR) has been shown to be altered in post-mortem brains of depressed patients and suicide committers. Alcediag has characterized a specific RNA editing signature of the 5-HT2cR linked to depressed/suicide patients. By using next generation sequencing technology (Illumina), Alcediag determined the editing profiles of the serotonin 2C receptor in SH-SY5Y human neuroblastoma cell line, treated with 54 market-approved drugs at 3 concentrations. These compounds were selected from various therapeutic classes (including antidepressant, antipsychotic, anti-obesity, antiviral, anti-inflammatory, anti-fungal, antiepileptic, mood stabilizing agents) as potentially inducing suicidality (FDA warning label) or not (no psychiatric adverse effects reported). The screening could identify a specific 'at risk signature', similar to that found in post mortem brain of suicide patients. Clear dose-effect relationships were observed. An algorithm was generated to identify 'at risk' compounds with 97% sensitivity. Based on the current data set, this *in vitro* assay may rule out most of the drugs potentially triggering depression and/or suicide commitment in humans. This test, which is the first *in vitro* test able to characterize potential toxicity on the brain, may be included in the early selection of drug candidates and help limit market withdrawals and pharmacologically induced psychiatric adverse effects.

ABSTRACT FINAL ID: 2728 Poster Board -517
TITLE: Highly Sensitive Detection of SNAP-25 Cleaved by Specific Botulinum Serotypes A and E Using Stem Cell-Derived Neurons and Custom Antibodies

AUTHORS (FIRST INITIAL, LAST NAME): A. B. Bradford[1], J. Grynovicki[1], I. Gut[2], M. E. Lyman[1], P. H. Beske[1], K. Hoffman[1], P. McNutt[1]
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KEYWORDS: Stem Cell-Derived Neurons, Botulinum Toxin, Immunoassay

ABSTRACT BODY: Simple, rapid and specific tests are needed to detect the presence, serotypes and potencies of active botulinum toxins (BoNTs). We have developed and characterized a cell-based platform for BoNT testing based on central neurons (ESNs) derived from mouse embryonic stem cells. ESNs undergo the molecular and functional effects of intoxication with each BoNT serotype (A-G), exhibiting sensitivities similar to primary neurons. Immunoassays are less sensitive at detecting BoNT activity than electrophysiology, but are higher throughput. Current immunoblot detection of BoNT/A activity depends on identifying a small mobility shift of SNAP-25, and cleavage is not detectable by assays such as ICC. As a solution to these shortfalls, we have developed antibodies that exclusively detect BoNT/A-cleaved or BoNT/E-cleaved forms of SNAP-25. Mouse monoclonal antibodies were produced using synthetic peptides representing C-terminals of SNAP-25 cleaved by either BoNT/A or BoNT/E, and screened by ELISA. Selected clones were tested in BoNT/A- or /E-treated ESNs by immunoblot and fluorescent ICC and in rat muscle and mouse brain intoxicated *ex vivo*. Immunoblot results indicate that A-specific antibody detects cleaved SNAP-25 in lysates from ESNs treated with as little as 50 fM BoNT/A, a significant sensitivity improvement over existing SNAP-25 antibodies. ICC and tissue results clearly show the presence of cleaved products. The A-specific antibody works with ESNs intoxicated with therapeutic BOTOX formulation and in matrices such as milk and human serum. The /E-specific antibody is in development, but shows similar promise in immunoassays. By combining the sensitive ESN platform with these novel serotype-specific antibodies, we plan to develop a toolkit that offers

mutually reinforcing molecular and functional assays to rapidly, reliably and sensitively detect and characterize BoNT in many sample matrices.

ABSTRACT FINAL ID: 2729 Poster Board -518
TITLE: Alterations in the Metabolic Activity of SH-SY5Y Human Neuroblastoma Cells by Glucose and Insulin

AUTHORS (FIRST INITIAL, LAST NAME): A. Engin[1], R. Karakus[2]
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KEYWORDS: Neurotoxicity, Glucotoxicity, Cell Death

ABSTRACT BODY: Despite of the developments in the treatment strategies of diabetic complications, the complications related to hyperglycemia still remain as a problem. Several epidemiological studies showed that diabetic patients have higher incidence of neurological disorders such as Alzheimer's disease. Although glucose is the main enegry source of brain, uncontrolled levels of glucose may effect neuronal functions. The aim of our study was to assess the alteration in the metabolic activity of SH-SY5Y human neuroblastoma cell line by glucose and insulin. At various timepoints SH-SY5Y cells were incubated with or without insulin (10 µU/ml – 60 µU/ml) and with various glucose concentrations (120 mg/dl – 1200 mg/dl) that were determined by International Diabetes Federation as hyperglycemic conditions. The results reveal that the cell viability was significantly decreased at 72 hours of incubation. The metabolic activity of cells were significantly decreased after 24 hours and 48 hours of glucose exposure and insulin seems to contribute the toxicity of glucose by supporting the glucose uptake by human neuroblastoma cells.

ABSTRACT FINAL ID: 2730 Poster Board -519
TITLE: Mefloquine, a Long-Acting Antimalarial Agent, Produces Afferent Axonal Neurotoxicity in the Brainstem of Rats following a Single Oral High Dose

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KEYWORDS: Mefloquine, Neurotoxicity, Brainstem

ABSTRACT BODY: Mefloquine, a long-acting antimalarial agent, has been used extensively in the past for prophylaxis and treatment & is still used in endemic regions. Reported neurotoxic adverse effects include convulsions, nausea, dizziness, vertigo, tinnitus, loss of balance, sensory & motor neuropathies (paresthesia, tremor, ataxia), vestibular & hearing disorders as well as severe neuropsychiatric disorders including aggression, panic attacks, sleep disorders, hallucinations, psychotic/paranoia reactions & suicide. In preclinical studies in the rat, Dow et al. reported lesions in the brainstem as well as deficiencies in balance beam motor coordination. In order to verify & further investigate reported neurotoxicity, male rats were administered a single oral dose of mefloquine (765 mg/kg) in corn oil and the brain of the living rat imaged (MRI –T2 signals) several times over a 9 day period. Rats were sacrificed at various times throughout & a complete histopathologic study of 60 brain sections per rat was conducted on alternating sections stained with cupric-silver or H & E. Significant silver staining was seen in the nucleus gracilus & nucleus cuneatus of the brainstem. These lesions were in the synapsing axonal portion of the afferent fibers originating from the fasiculus gracilus and fasiculus cuneatus of the spinal cord that innervate the legs & the arms respectively. The corresponding H & E sections, however, revealed intact cell bodies in both brainstem nuclei; damage was limited to afferent axons. Lesions of this type in humans would produce irreversible proprioceptive problems involving loss of balance, altered vibration & joint position sense accompanied by compromised gait. There were no abnormal brain MRI signals, however an unusually large signal from facial muscles was detected. Future studies will examine spinal cord, sciatic & trigeminal nerves.

2015 Society of Toxicology Annual Meeting
Late-Breaking Abstracts

ABSTRACT FINAL ID: 2731 Poster Board -520
TITLE: Development of a Test System Based on Human Dorsal Root Ganglia Precursor Cells That Identifies Peripheral Neurotoxicants

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KEYWORDS: Peripheral Neurotoxicity, Human Stem Cell-Derived Neurons, *In Vitro* Neurotoxicity

ABSTRACT BODY: Human embryonic stem cell (hESC) technology provides a tool to recapitulate relevant aspects of early neurodevelopment, such as proliferation, differentiation, migration, axonogenesis and synaptogenesis. For the central nervous system, even small disruptions in these processes may result in a severe impairment of normal function and lead to neuropathogenesis and developmental disabilities. At present, little is known about how the developing peripheral nervous system is affected by toxicants. We intended to establish a human neurogenesis model based on hESCs to assess peripheral neurotoxicity by using neurite growth and calcium signaling as functional endpoints. Based on the combination of small molecule inhibitors that induced the differentiation of hESCs into sensory neurons, we generated a population of peripheral neuronal progenitor cells within 8 days of differentiation. After this stage, cells were cryopreserved. Freshly thawed cells developed neurites and formed a dense neurite network, which was quantified by live cell imaging. Moreover, hESC-derived sensory neurons showed strong calcium signaling upon depolarization. Quantification of neurite growth and of calcium responses on single cell level were optimized as endpoints to assess peripheral neurotoxicity. In this system we identified compounds with several biological activities that had an inhibiting as well as an accelerating effect on neurite growth. On this basis, we established a high content imaging test for sensory neuron structural integrity (SeNSI-test).

ABSTRACT FINAL ID: 2732 Poster Board -521
TITLE: Resource Deprivation: A Novel, Highly Translational Stress Paradigm

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KEYWORDS: Behavioral Toxicity, Stress, Resource Deprivation

ABSTRACT BODY: Humans are exposed to a variety of chemical and non-chemical stressors. Stress is ubiquitous and heightened in low socioeconomic status (SES) communities, findings hypothesized to underlie the increased incidence of many SES-related diseases. The hypothalamic-pituitary-adrenal (HPA) axis, the physiological system mediating the stress response, is a common target for toxicants, such as lead, and combined exposures have been shown to produce enhanced toxicity. As such, understanding the cumulative effects of co-occurring risk factors is critical for public health protection; however, researchers studying stress as a modifier of risk have been limited to physical stressors, cold, swimming or restraint stress, that do not translate to the psychological and physical stress of resource disparities associated with low SES. Therefore, we developed a highly translational stress paradigm using differential resource deprivation. Male mice were pair-housed, in side-by-side cages separated by a wire mesh. For one month, one mouse was given a highly preferred treat, a mealworm, while the other was not. After two weeks, resource deprived males showed significant increases in baseline corticosterone with diminished corticosterone responsivity to handling stress. Additionally, males underwent cognitive testing using spontaneous locomotor activity, Repeated Acquisition and Performance Chamber (RAPC) and Morse Water Maze (MWM). Although, there were no significant differences in MWM performance, resource deprived males significantly increased error rates compared to enriched males during RAPC performance. These differences were eliminated when males were additionally stressed, suggesting a modulating role for stress. Additionally, severe differences in locomotor behavior developed, with resource deprived males decreasing resting time and increasing ambulatory and stereotypic movement. Collectively, these findings show cognitive deficits with HPA axis shifts in rodents with differential access to resources, a much improved translational stress paradigm for studies focused on stress as a modifier of toxicological risk.

2015 Society of Toxicology Annual Meeting
Late-Breaking Abstracts

ABSTRACT FINAL ID: 2733 Poster Board -522
TITLE: Dopaminergic Cell Death Induced by Gene-Environment Interactions Involving Alpha-Synuclein Is Mediated by Nuclear Factor-Kappa B and p38 Signaling

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KEYWORDS: Cell Death, Synuclein, NF-kB

ABSTRACT BODY: The multifactorial etiology of Parkinson's disease (PD) involves genetic, environmental, and aging risk factors. Gene multiplications or point mutations in α-synuclein are associated with sporadic and familial PD. Several reports have demonstrated that the toxicity of α-synuclein is modulated by pesticides, metals, mitochondrial toxins and pro-oxidant conditions. However, there is little consensus regarding the molecular mechanisms involved. We found that overexpression of wild type (WT) and mutant A53T α-synuclein exerted a toxic synergism in N27 dopaminergic cells exposed to the structurally unrelated environmental toxicants paraquat and manganese (Mn). This toxic synergism was not replicated in lung-derived A549 cells or neuroblastoma cells with low levels of tyrosine hydroxylase (TH). α-synuclein and paraquat/manganese exposures synergistically activated the nuclear factor kappa light-chain-enhancer of activated B cells (NF-κB) and the mitogen/stress (MAPK/SAPK)-activated protein kinase p38 signaling. Inhibition of NF-κB (SN50) and p38 (SB203580) decreased the toxicity of paraquat and manganese and the toxic synergism induced by α-synuclein. Free radical formation and inducible nitric oxide synthase activity were primarily involved in the toxicity of α-synuclein and paraquat by positive feedback activation of NF-κB. Neither inhibition of the apoptosis signal-regulating kinase 1 (ASK1) or c-Jun N-terminal kinases (JNK) with NQDI-1 or SP600125, respectively, modulate the toxicity of α-synuclein and environmental agent exposures. These results demonstrate that NF-κB and p38 signaling are at the center stage of dopaminergic cell death induced by gene-environment interactions involving α-synuclein.

ABSTRACT FINAL ID: 2734 Poster Board -523
TITLE: Effects of Oral Lead Exposure on Neuroimmunological Characteristics of BTBR T+tf/J Mice Resembling Human Autism

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KEYWORDS: Autism, BTBR Mice, Neuroimmunology

ABSTRACT BODY: Autism spectrum disorder (ASD) is a neuro-developmental disorder which has been persistently increasing over the last decade and its etiology is uncertain. Neuroinflammation in brain is hypothetically suggested to play a role for autism induction. Using BTBR T+tf/J (BTBR) mice considered as an optimal laboratory animal for autism investigation, effect of metal lead (Pb) exposure on neuroimmunological characteristics was evaluated. FVB mice were used as a control for its positive social behaviors. Lead acetate (0.1 mM) or distilled water (DW) has been taken orally from gestational day 8 to postnatal day 21. Number of neonatal mice studied was 47 for BTBR (Pb:16, DW:31) and 78 for FVB (Pb:41, DW:37). Concerning IgG isotype deposited in brain perfused, the elevated IgG1 versus IgG2a ratio, an indicator for skewedness toward type-2 helper T cell (Th2) reactivities, was observed in the BTBR mice exposed to Pb compared to the control BTBR mice, which observation was not occurred in FVB strain. Levels of various cytokines expressed in whole brain were also evaluated. Enhancement of interleukin-4 versus interferon-gamma ratio was resulted in the Pb-exposed BTBR mice compared to the control BTBR mice, and the ratio was not different between the Pb-exposed FVB and the DW drinking control FVM mice. Levels of interleukin-18 and interleukin-33 were downregulated in the Pb-exposed BTBR compared with those of control BTBR mice, which was not observed in FVB strain. The level of brain-derived neutrophic factor (BDNF), a core protein for development of neuronal system, was lower in the brains of Pb-exposed BTBR than the DW drinking control BTBR mice, suggesting a probable neuronal damage. This alteration of BDNF was not observed in FVB strain. Overall, this study suggests that Pb exposure induce a detrimental effect on autistic prone BTBR mice, which could be

involved with occurrence of ASD-like phenotype in the strain. [supported by National Research Foundation of Korea 2010-0022169]

ABSTRACT FINAL ID: 2735 Poster Board -524
TITLE: Oxidative Stress and Proinflammatory Cytokines Gene Overexpression in Cortical Astrocytes Acutely Exposed *In Vitro* to the Monomethylated Arsenic Metabolite

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KEYWORDS: Arsenic, Inflammation, Neurodegenerative Diseases

ABSTRACT BODY: Human exposure to inorganic arsenic (iAs) has been related with cognitive, motor and learning impairment; therefore, it has been proposed as a risk factor for different neurodegenerative diseases; however, the associated molecular mechanisms have not been fully described. *In vivo* and*in vitro* exposure to iAs and to its methylated metabolites leads to inflammation-related to intracellular pathways activation and to pro-inflammatory cytokines over-production. Therefore, As-induced neurotoxicity, could involve its ability to induce neuroinflammation once it crosses the brain blood barrier. On the other hand, astrocytes are well recognized as important contributors to the pathophysiology of neurodegenerative diseases, since they modulate different brain functions and inflammatory responses. To determine the potential contribution of astrocytes-mediated inflammation in iAs-associated neurotoxicity, primary rat cortical astrocytes were exposed *in vitro* to the monomethylated arsenic metabolite (MMAIII). LDH release, MTS transformation and ROS production was evaluated in cultures after 24h exposure to different MMAIII concentrations to determine cellular toxicity, mitochondrial activity and oxidative stress. Total RNA was isolated from cells 6 h post-exposure to determine different pro-inflammatory genes expression. Exposure to 25-250 nM MMAIII led to increased mitochondrial metabolic activity in terms of MTS transformation ability and reactive oxygen species (ROS) production. Genes codifying for pro-inflammatory cytokines IL1-α, IL-6, TNF-α and COX2 were also over-expressed in cells exposed to these MMAIII concentrations. These results suggest that arsenic may induce neurotoxicity through astrocyte-mediated oxidative stress and neuroinflammation in exposed populations.

ABSTRACT FINAL ID: 2736 Poster Board -525
TITLE: Transport of α-Synuclein across the Blood-Cerebrospinal Fluid in Transwell Chamber and Rat Models: Effects of Manganese Exposure and Implications in Parkinson's Disease Pathology

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KEYWORDS: a-Synuclein, Manganese, Parkinson's Disease

ABSTRACT BODY: The choroid plexus (CP) maintains the homeostasis of critical molecules in the brain by regulating their transport between the blood and cerebrospinal fluid (CSF). Little is understood about how the blood-CSF barrier (BCB) in the choroid plexus regulates brain homeostasis of α-synuclein (a-Syn), a protein implicated in Parkinson's disease (PD) pathology. The current studies were designed to investigate the potential role of the BCB in a-Syn transport in the brain as affected by exposure to manganese (Mn), a toxic metal implicated in Parkinsonian disorders. It was hypothesized that the BCB regulates a-Syn brain homeostasis by clearing a-Syn from the CSF to the blood, and that toxic Mn exposure may alter this function, which could contribute to PD pathology. Transport of a-Syn across the BCB was studied using an *in vitro* Two-Chamber Transwell culture model of primary rat choroidal epithelial cells. Our data show that the barrier transported a-Syn

in both directions (i.e., from the CSF to the blood and from the blood to the CSF), and Mn exposure had no significant effect on a-Syn transport or intracellular a-Syn levels. However, in other studies, rats that received an intracerebroventricular injection of a-Syn into the right lateral ventricle showed a significant decrease in CSF a-Syn levels over time in both control and chronic Mn-treated groups. Further, rats chronically exposed to Mn prior to injection showed significantly higher a-Syn concentrations in the choroid plexus compared to controls at 3 hours post-injection ($p < 0.05$), presumably due to hampered clearance of a-Syn from the CSF. Overall, these findings suggest a-Syn can be cleared from the CSF by the BCB, and Mn exposure may alter a-Syn clearance by the CP *in situ*.

ABSTRACT FINAL ID: 2737 Poster Board -526
TITLE: Prenatal Stress and Perinatal Lead (Pb2+) Exposure: Effects on Adult Neurogenesis in the Subgranular Zone (SGZ) of the Dentate Gyrus in the Rat Hippocampus

AUTHORS (FIRST INITIAL, LAST NAME): J. L. McGlothan[1], K. Mancevska[1], K. H. Stansfield[1], D. Weston[2], D. A. Cory-Slechta[2], T. R. Guilarte[1]
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KEYWORDS: Lead (Pb2+), Stress, Neurogenesis

ABSTRACT BODY: Previous studies from our laboratory have shown that chronic developmental exposure to lead (Pb^{2+}) results in impairment of SGZ adult neurogenesis in young adult rats [Verina et al., 2007]. This work showed that Pb^{2+} exposure altered two aspects of adult granule cell neurogenesis, i.e., proliferation and survival. In the present study, we aimed to determine the effect of prenatal stress and perinatal Pb^{2+} exposure on neurogenesis, at a Pb^{2+} exposure level lower than in our previous study. Pregnant dams received three 45 minute sessions of restraint stress on both gestational days 16 and 17. For the Pb^{2+} exposure, 50 ppm PbAc was provided in the drinking water during gestation until weaning. This paradigm provided four different exposure groups: No Pb-No Stress (H20-NS), No Pb-Stress (H20-S), Pb-No Stress (Pb-NS), and Pb-Stress (Pb-S). Following these exposure conditions, we examined neurogenesis in the SGZ of the dentate gyrus in both adult male and female offspring using a BrdU injection protocol that allows the analysis of both granule cell proliferation and survival. Stereological cell counting of BrdU-labeled cells showed that none of the conditions of prenatal stress resulted in significant changes in adult neurogenesis of male or female offspring in the SGZ. On the other hand, we found a significant decrease in granule cell survival in the SGZ in only Pb-NS males relative to all other groups. Our studies did not find a Pb^{2+} and prenatal stress interaction or a prenatal stress effect on adult neurogenesis in the SGZ. However, the current study supports previous findings that developmental Pb^{2+} exposure either chronically throughout life (previous study) or perinatal exposure (present study) decreases granule cell survival in the SGZ of the dentate gyrus in male rats.
[This work is supported by NIEHS grant ES006189 to TRG]

ABSTRACT FINAL ID: 2738 Poster Board -527
TITLE: Effects of Chronic Manganese Exposure on *In Vivo* Dopamine Release in the Frontal Cortex of Nonhuman Primates Measured by Positron Emission Tomography (PET)

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KEYWORDS: Manganese, Positron Emission Tomography, Dopamine Release

ABSTRACT BODY: Previous studies from our laboratory have shown that non-human primates chronically exposed to manganese (Mn) express deficits in working memory [Schneider et al., 2009]. It is known that the dopaminergic system plays an important role in working memory performance [Guilarte, 2013] and we have previously shown that Mn exposure

inhibits *in vivo* dopamine release (DAR) in the corpus striatum measured by PET [Guilarte et al., 2008]. Based on this information, we aimed to determine if the working memory deficits in Mn-exposed non-human primates were related to disruption of *in vivo* DAR in the frontal cortex. [^{11}C]-FLB, 457 PET was used to measure DAR in six Cynomolgus macaques that were exposed to Mn (15mg/kg/week for 5 weeks and then 20mg/kg/week for the remainder of the study). Animals received PET prior to Mn administration (baseline) and at the end of the Mn exposure period. The results indicate that at the end of the Mn exposure period, four of the animals expressed significant reductions in %DAR in the frontal cortex relative to baseline. In one animal, there was no change in %DAR as a result of Mn exposure, and in one animal the %DAR actually increased from baseline. Previous studies have shown that DAR requires an optimal set point for optimal working memory performance, and decreases or increases from this set point can negatively modulate working memory performance. Taken together, these and previous studies from our laboratory suggest that Mn-induced alterations in %DAR measured by PET in the corpus striatum and now in the frontal cortex may be associated with the working memory deficits documented in these animals. [Supported by NIEHS grant ES010975 to TRG]

ABSTRACT FINAL ID: 2739 Poster Board -528
TITLE: Manganese Accumulations in Brain and in Toenails Reflect Different Time Periods of Exposure

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KEYWORDS: Manganese (Mn), MRI R1 Relaxation Rate, Toenails

ABSTRACT BODY: It is well established that occupational exposure to manganese (Mn) causes excess Mn deposition in several brain areas, including basal ganglia, motor cortex and frontal cortex. In this study, we investigated the relationship between Mn deposition in the brain as given by the R1 relaxation rate in magnetic resonance imaging (MRI) with Mn accumulation in toenails in welders occupationally exposed to Mn. MRI scans using a 3T GE Signa scanner were conducted on 31 welders and 19 non-exposed controls recruited from a U.S. truck-trailer manufacturer. Toenails were clipped at the time of the MRI exam and metal content was measured by ICP-MS. Individual Mn exposure was estimated with a model combining work histories and personal air sampling. A 3D spoiled gradient sequence with two echoes (3°, 17°) was used for R1 mapping of every subject. The R1 relaxation rates were extracted by automatic parcellation using SPM (UCL) from inferior frontal cortex, motor cortex, putamen, thalamus, hippocampus, and globus pallidus. The mean R1 values of these regions were then correlated with toenail Mn as well as Mn exposure integrated over different time periods by using an age-corrected Spearman correlation. A significant ($p<0.05$) increase in R1 relaxation rate was found in inferior frontal cortex, motor cortex, and basal ganglia. Toenail Mn was significantly increased in welders ($p<0.001$) and correlated with exposure 7-12 months ago ($p<0.0001$). Importantly, correlations were found between the R1 relaxation rate and Mn exposure over the past 3 months in inferior frontal cortex ($r=0.6$, $p<0.01$) and in motor cortex ($r=0.4$, $p=0.02$), areas responsible for behavioral and motor deficits respectively. No correlation is found between R1 with exposure 7-12 months ago, which agrees with a wash-out time of brain Mn of ~6 months. The lack of correlation between toenail Mn and R1 showed that while R1 in certain brain regions and toenail Mn may both serve as biomarkers of exposure to Mn, they reflect different time periods of exposure.

ABSTRACT FINAL ID: 2740 Poster Board -529
TITLE: Effects of Lead and Cadmium Exposure on the Adult CBA/CAJ Mouse Vestibular System

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KEYWORDS: Vestibular System, Heavy Metals

ABSTRACT BODY: The vestibular organs of the inner ear provide vertebrates an awareness of their position and movement within their environment. Epidemiological literature suggests that lead (Pb) and cadmium (Cd) exposure can lead to vestibular dysfunction in children and adults. A controlled experimental study in adult rats demonstrated chronic low-dose lead exposure may cause a decline in the vestibuloocular reflex, commonly used to assess vestibular function. The study presented here applied a general measure of motor function, the rotarod test, to assess the vestibular function of CBA/CaJ mice before and after a 10-week water-based exposure to Pb (3 mM) or Cd (300 μM). Additionally, a novel method for measuring the vestibulocollic reflex based on head motion was used. Damage to hair cells and vestibular nerve fibers was assessed via immuno-staining for myosin VIIa and neurofilaments, respectively. No changes were detected in the vestibulocolic reflex or the histological analyses, and the rotarod testing yielded variable results. In conclusion, the study demonstrated a successful mouse model for studying the effects of Pb and Cd, but did not identify any Pb- or Cd-related effects on the adult vestibular system.

ABSTRACT FINAL ID: 2741 Poster Board -530
TITLE: A Novel Event Analysis for Early Detection of Methylmercury Toxicity and Nimodipine Neuroprotection

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KEYWORDS: Methylmercury, Nimodipine, Behavioral Toxicity

ABSTRACT BODY: INTRODUCTION. Methylmercury's (MeHg) sensorimotor toxicity is thought to be mediated by its disruption of Ca2+ homeostasis in nerve terminals. We generated high-rate operant behavior in two age cohorts to assess MeHg neurotoxicity and neuroprotection by the L-type Ca2+ channel blocker, nimodipine (NIM). Log survivor analysis partitioned lever-pressing into motor- and motivational-driven components. METHODS. Adult (PND 72) and retired breeder (RB; PND 296) BALB/c mice were exposed to 0 or 10ppm MeHg, in drinking water, and 0 or 200ppm dietary NIM in a 2 X 2 X 2 factorial design with 12-16 mice per group. To produce high-rate responding, mice nose-poked under a multiple percentile (PCNT) differential reinforcer of high rate (DRH) procedure, producing robust responding, with RBs responding at higher rates than adults. Each animals' daily average component performance was standardized using the mean and SD of its age-matched control group (0ppm MeHg, 0ppm). An animal was considered impaired when performance on a given measure fell and remained 1 SD below the age-matched mean. Event analysis quantified the latency to impairment for all measures. RESULTS. Response rate, reinforcer rate, and 3 parameters from log-survivor analysis were differentially sensitive to MeHg. Within-bout rate (a marker of sensorimotor disruption) was the most sensitive parameter for all MeHg-exposed animals, regardless of NIM. Bout initiation rate (a marker of motivation) and bout length decreased mush later. NIM's neuroprotection was age-dependent, with greater protection occurring in younger mice. CONCLUSION. The microstructure of high-rate responding revealed MeHg insult and selective NIM neuroprotection. Motivation to run was unaffected even as the ability to run was impaired. These data reveal age-dependent neuroprotection by NIM against MeHg toxicity in mice. Further studies should seek to extend the utility of this analytical technique in developing early detection models. [Funded by NIH ES003299]

ABSTRACT FINAL ID: 2742 Poster Board -531
TITLE: Chronic Methylmercury Exposure Affects mRNA Expression of Voltage Gated Calcium Channels and Glutamate Receptors in Mouse Cerebellum and Spinal Cord

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KEYWORDS: Methylmercury, Cerebellum, Glutamate Receptors

ABSTRACT BODY: Methylmercury (MeHg) is an environmental toxicant that targets the central nervous system. MeHg neurotoxicity has cell specificity and cerebellar granule cells (CGC's) are the most susceptible targets. Chronic exposure to MeHg can lead to disturbances in sensation, hearing, speech, balance and movement. MeHg toxicity results in dysregulation of Ca^{2+} concentrations in CGC's and motor neurons. The objective of our study was to compare effects of chronic MeHg exposure in mRNA levels of voltage gated calcium channels (VGCCs) and glutamate receptor alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) in mouse cerebellum (CB) and spinal cord (SC). Comparing the effects of MeHg in receptors that contribute to Ca^{2+} dysregulation in neurons can help us elucidate susceptibility differences, after MeHg exposure, in these two regions. AMPAR GluA1 - 4 and VGCC α1A, α1B, α1C and α1E subunit expression were compared between CB and SC. We hypothesized that chronic MeHg exposure would cause more prominent effects in the CB due to the susceptibility of this region to MeHg. 90 day old Balbc mice were exposed to 5ppm MeHg *ad lib* in drinking water for 6 months. Isolated RNA from 10mg of CB or SC tissue was reverse transcribed and real time qPCR was used to determine the mRNA expression levels of the AMPAR and VGCC subunits. Chronic MeHg exposure decreased expression of all the VGCC subunits studied in CB and SC of mice. AMPAR subunits decreased in CB of mice after chronic MeHg exposure. In SC mRNA levels of AMPAR subunits were unaffected. These results suggest that alterations in mRNA expression of VGCCs and AMPA receptors, after chronic MeHg exposure, may not be one of the underlying factors contributing to the observed increases in intracellular Ca^{2+} in the CB and SC of mice.

ABSTRACT FINAL ID: 2743 Poster Board -532
TITLE: Tyrosine Hydroxylase Immunohistochemistry in the Substantia Nigra and Ventral Tegmental Area of Nonhuman Primates Exposed to Manganese: Preliminary Findings

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KEYWORDS: Basal Ganglia, Movement Disorders, Dopamine

ABSTRACT BODY: Basal ganglia dysfunction has been implicated in the movement and cognitive disturbances associated with manganese (Mn) overexposure. Previous clinical and pre-clinical data from our laboratory and others support the notion that intact but dysfunctional midbrain dopaminergic neurons underlie these Mn-induced abnormalities (Guilarte, 2006, 2008). Serial sections (1:8) from the entire rostrocaudal extent of the dopaminergic midbrain were collected for optical density analyses and stereological cell counts of tyrosine hydroxylase (TH) immunoreactive fibers and neurons. In the rostral substantia nigra pars compacta (SNc) and the midcentral SNc and ventral tegmental area (VTA), we performed semiquantitative immunohistochemical TH staining in normal and Mn-exposed monkeys (n=6) by quantifying the relative optical densities of TH-labeled fibers. Preliminary findings from our control and experimental groups show that no significant differences exist in the fiber densities between the dorsal and ventral tiers of the SNc or the VTA. Follow-up studies will further investigate TH-positive fiber densities in three additional monkeys and other functional regions of the SNc. In addition, the absolute number of TH-labeled neurons will be quantified using stereological cell counting in each dopaminergic midbrain region. [Supported by NIEHS grant ES010975 to TRG and Provost fellowship to KKG].

ABSTRACT FINAL ID: 2744 Poster Board -533
TITLE: Twice-Weekly Administration of Paraquat for Three Weeks Does Not Result in a Loss of Dopaminergic Neurons in the Substantia Nigra of C57BL/6 Male Mice

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KEYWORDS: Neurotoxicity, Paraquat, MPTP

ABSTRACT BODY: Multiple studies were conducted in mice using a paraquat dichloride (PQ) dosing paradigm (twice-weekly dosing for 3 weeks at 10 mg/kg/dose i.p.) previously shown by some investigators to result in a reduction of dopaminergic neurons in the substantia nigra pars compacta (SNpc). Variables examined in these studies included: age of mice at initiation of treatment (9 vs. 16 weeks), stereological methodology and assessment (3D analysis at one laboratory vs. 2D analysis at another laboratory), source of mice (C57BL/6J vs. C57BL/6NHsd), source of PQ (Syngenta vs. Sigma), and housing conditions (single animal housing at one facility vs. multiple animal housing at another facility). Separate groups of mice were administered 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) as positive controls. One week after the final dose (either PQ or MPTP) the mice were subjected to in situ perfusion fixation while under anesthesia. The collected brains were coded and block-randomized prior to tissue processing and immunostaining. Stereological assessments of tyrosine hydroxylase positive (TH+; dopaminergic) neurons in the SNpc were performed at two different laboratories by investigators who were 'blinded' to treatment group. The mean numbers of TH+ neurons in the SNpc of all groups of PQ-treated mice were statistically comparable to mean control values ($p > 0.05$), whereas the MPTP-treated groups exhibited reductions ($p < 0.01$) in the number of dopaminergic neurons ranging from 32 to 46%. It is concluded that treatment of C57BL/6 male mice with PQ twice weekly for 3 weeks (10 mg/kg/dose; 20 mg/kg/week) does not result in a reduction in the number of dopaminergic neurons in the SNpc. These results are consistent with a previous study (Breckenridge, 2013) in which treatment with PQ was not associated with neuronal degeneration/loss, glial activation, or changes in neurochemistry in the SNpc and/or striatum.

ABSTRACT FINAL ID: 2745 Poster Board -534
TITLE: Dietary Administration of Diquat for 13 Weeks Does Not Result in a Loss of Dopaminergic Neurons in the Substantia Nigra Pars Compacta (SNpc) of C57BL/6J Mice

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KEYWORDS: Neurotoxicity, Diquat, MPTP

ABSTRACT BODY: We previously showed that dietary exposure of mice to paraquat for 13 weeks does not result in nigrostriatal toxicity, despite some reports suggesting its role in Parkinson's disease. In the current study we assessed the potential nigrostriatal toxicity of a similar non-selective herbicide, diquat dibromide (DQ). Male and female C57BL/6J mice were administered DQ in the diet at concentrations of 0, 12.5 ppm (~1.5 mg DQ ion/kg/day) or 62.5 ppm (~7.5 mg DQ ion/kg/day). Separate groups of mice were administered 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) i.p. as positive controls. After 13 weeks of DQ exposure, the concentrations of dopamine (DA) and its metabolites (DOPAC, HVA) in the striatum, and the number of tyrosine hydroxylase positive (TH+; dopaminergic) neurons in the SNpc were determined. The SNpc and/or striatum were examined histopathologically after 4, 8 and 13 weeks using a variety of tissue stains/immunostains (AmCuAg for neurodegeneration, TH antibody/DAB for DA neurons/terminals, IBA1 for microglia, GFAP for astrocytes, TUNEL for apoptosis, caspase 3 for apoptosis, and thionine for general morphology). Morphological and 3D stereological assessments were performed by investigators who were 'blinded' to treatment group. Dietary exposure to DQ did not significantly reduce mean striatal dopamine concentrations or alter dopamine turnover. No evidence of neuronal degeneration or astrocyte/microglial activation was observed in the SNpc and/or striatum. The mean number of TH+ neurons in the SNpc was not significantly reduced in DQ-treated mice. In contrast, MPTP caused significant decreases in mean striatal DA concentrations, reductions in mean number of TH+ neurons and pathological changes (neuronal degeneration and astrocytic/microglial activation). Similar to paraquat, 13 weeks of continuous exposure of mice to DQ did not result in nigrostriatal toxicity.

ABSTRACT FINAL ID: 2746 Poster Board -535
TITLE: An Investigation into the Therapeutic Properties of the Neuroendocrine System following Soman (GD) Exposure in Mice

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KEYWORDS: Soman, Neuroendocrine

ABSTRACT BODY: Few studies have been performed to elucidate the effects of the neuroendocrine system on status epilepticus, fewer still on seizures propagated by organophosphorous agent exposure. Though limited, there is promising data regarding the role of androgens, specifically testosterone and its metabolites dihydrotestosterone and 3α-androstanediol (3α-diol), in attenuating seizures and reducing inflammation markers, helping to reduce damage if a seizure is not completely prevented. 17β-estradiol, a metabolite of testosterone, can exacerbate the damage inflicted by the onset of status epilepticus in various brain regions. We found that available testosterone in C57BL/6J mice was markedly decreased after exposure to soman (GD) nerve agent. The lost testosterone may be metabolized to 17β-estradiol, agitating seizures and resulting in a loss of its anti-inflammatory properties. Here, we applied testosterone, 3α-diol, and the aromatase inhibitor letrozole as possible treatments for agent exposure. Replacing the lost testosterone enables it to be used as both an anti-inflammatory and as an anti-convulsant through its metabolism to 3α-diol. Treating mice with letrozole prevents the added testosterone from being metabolized into 17β-estradiol, thereby avoiding damage from treatment. The views expressed in this talk are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. These studies were funded by the Defense Threat Reduction Agency (DTRA).

ABSTRACT FINAL ID: 2747 Poster Board -536
TITLE: Transport of Glyphosate in *Caenorhabditis elegans* Is Not Mediated via Pre-Synaptic Neurotransmitter Transporters

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KEYWORDS: Glyphosate, *C. elegans*, Parkinson's Disease

ABSTRACT BODY: Previous work in *Caenorhabditis elegans* (*C. elegans*) treated with the glyphosate-containing herbicide TouchDown® (TD) showed evidence of neurodegeneration similar to that observed in Parkinson's disease. The purpose of these studies was to determine if TD is transported into neurons via pre-synaptic neurotransmitter transporters. In order to test this hypothesis, *C. elegans* were pre-treated with neurotransmitter transporter antagonists for dopamine (DAT), serotonin (SERT), or GABA (GAT) transporters to determine if this ameliorated TD-induced neurodegeneration. Following incubation with the respective antagonist, three green fluorescent protein (GFP) worm strains were exposed to various concentrations of TD for 30 min (acute) or 24 h (chronic). Data from DAergic::GFP worms pre-treated with a DAT antagonist indicated statistically significant decreases (*$p < 0.05$) in fluorescence at the lowest TD concentration compared to non-pre-treated worms. Data from pan-neuronal::GFP worms pre-treated with a SERT antagonist in an acute TD paradigm also demonstrated a statistically significant decrease (*$p < 0.05$) at the lowest TD concentration compared to worms not receiving the antagonist. When the studies were repeated with the chronic TD dosing paradigm in pan-neuronal::GFP worms, statistically significant decreases (*$p < 0.05$) in fluorescence were observed in all treatment groups relative to controls. Finally, GABA::GFP worms pre-exposed to a GAT antagonist prior to an acute TD exposure indicated statistically significant increases (*$p < 0.05$) in fluorescence at the mid-range concentration when compared to worms not treated with the GAT antagonist. In chronic TD studies, however, pre-treated worms showed a statistically significant decrease (*$p < 0.05$) in fluorescence at the lowest TD concentration compared to controls. Since an increase in fluorescence would be

interpreted as protection, these data indicate that DAT, SERT, and GAT do not play a role in the transport of TD into pre-synaptic neuronal populations examined here.

ABSTRACT FINAL ID: 2748 Poster Board -537
TITLE: Imidacloprid & Fipronil Insecticides: Comparison of *In Vivo* Toxicity Endpoints & ToxCast Profiles

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KEYWORDS: Pesticides, ToxCast, Risk Assessment

ABSTRACT BODY: Imidacloprid (IM) and fipronil (FP) are insecticides with more selective toxicity to insects vs. humans for control of pests/parasites on crops and pets. IM is a nicotinic acetylcholine receptor (nAChR) agonist at the neuronal and neuromuscular junctions. FP (CNS toxin) blocks γ-aminobutyric acid (GABA)-gated Cl- channels. To investigate potential use of USEPA's ToxCast high-throughput screening assays (HTS including zebrafish, ZF) in risk assessment we first reviewed available *in vivo* animal toxicity studies to establish toxic endpoints and no-observed-effect-levels. We then examined ToxCast data for indications of pathway disruptions that could lead to effects manifested as overt toxicity. *In vivo* toxicities: Both showed neurotoxicity, developmental neurotoxicity (DNT) and thyroid, liver and kidney pathology and were potent inducers of the hepatic CYP enzymes. In chronic rodent bioassays FP caused thyroid and liver neoplasia. USEPA considers IM an unlikely carcinogen (Group E), whereas FP is classified as a Group C (possible human) carcinogen based on thyroid follicular cell tumors in rat. Positive ToxCast data: IM: Brain nAChR ion-channels, liver cell PXR activation associated with liver CYP induction and ZF (mortality). FP: Numerous pathways for thyroid and liver cancer metastasis, chronic inflammation, oxidative stress, mitochondrial alterations, indicators of tissue-damage, regrowth and repair (angiogenesis, growth signaling, tissue remodeling). FP had positive associations between the rat thyroid lesions and assays for human genes (CXCL10 & TP53) linked to thyroid cancer hallmark processes. FP was positive in ZF assays for malformations (axis, yolk sac & pericardial edema) and DNT.ToxCast assays were predictive of targets and activities relative to *in vivo* IM and FP endpoints. Pesticides like IM and FP, with complete *in vivo* databases, can be used for validation of ToxCast predictive toxicity models. HTS profiles may also be used to support modes of action and adverse outcome pathways for human risk assessment.

ABSTRACT FINAL ID: 2749 Poster Board -538
TITLE: Exposure to Chlorpyrifos Induces Lipid Accumulation, Fatty Acid Uptake, and Increased Levels of Lipogenic Proteins in McArdle-RH7777 Hepatoma Cells

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KEYWORDS: Chlorpyrifos, Steatosis, *In Vitro*

ABSTRACT BODY: The prevalence of the metabolic syndrome and the associated disease states including type II diabetes mellitus and obesity are increasing worldwide at an alarming rate. The diagnosis of having the metabolic syndrome is significant due to the associated risk of cardiovascular disease. While not part of the diagnosis, hepatic steatosis or non-alcoholic fatty liver disease is said to be the hepatic manifestation of the metabolic syndrome and is associated with hepatic insulin resistance as well as increased triglyceride secretion leading to hypertriglyceridemia. The current study was designed to determine if direct exposure to an organophosphate insecticide, chlorpyrifos (CPS), could promote hepatic steatosis and identify putative mechanisms of CPS-induced steatosis. To determine if CPS exposure increased intracellular lipid accumulation, McA-RH7777 cells were incubated with CPS (0, 1.25, 5, 20, or 80 μM) for 48 hours then lipid accumulation was determined by Oil Red O staining. Exposure to CPS (5, 20, and 80 μM) significantly increased lipid accumulation in a concentration-dependent manner. To determine if this CPS-induced lipid accumulation was preceded by

an increase in fatty acid uptake or an increase in *de novo* lipogenesis, fatty acid uptake assays and production of fatty acid synthase were determined following exposure to CPS for 24 hours. Exposure to CPS (20 µM) increased both uptake of Bodipy-labelled fatty acid and protein levels of fatty acid synthase. In addition, exposure to CPS (20 µM) for 24 hours increased oleic acid/palmitic acid-induced intracellular lipid accumulation. In summary, the present studies indicate direct exposure to CPS can increase intracellular lipid accumulation that may be due, at least in part, to increased fatty acid uptake and increased lipogenesis.

ABSTRACT FINAL ID: 2750 Poster Board -539
TITLE: Evaluation of the Possible Immunotoxic Effects of *Uncaria tomentosa* (Cat's Claw) on the Humoral Immune Response of Rats

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KEYWORDS: *Uncaria tomentosa*, Natural Products, Immunotoxicity

ABSTRACT BODY: *Uncaria tomentosa* (UT) (Cat's claw) is a phytotherapic employed worldwide for the treatment of many immunomodulated diseases. It is known that either enhanced immune response or decrease could be deleterious; thus, this study verified if UT administration for 90 days impaired the humoral immune response of rats. Forty male rats were divided into 1 control (Co) and 3 experimental (EUT) groups and the immunotoxic protocol employed is the Plaque-Forming Cell assay (PFC) proposed by international agencies of risk assessment. Rats were orally treated with 0, 15, 75 or 150mg/kg of UT, for 90 days. On experimental day (ED) 83 all animals were intraperitoneally challenged with $2x10^9$ sheep red blood cells (SRBC). On ED 90 all animals were killed for spleen collection to the PFC assay; briefly, $3x10^5$ splenocytes were added to SRBC (10%) and 70µL of guinea pig serum (source of complement) in 0.5mL of a Bacto Agar solution (0.5%) and plated on glasses slides. After 3 hours of incubation at 37°C, the slides were observed at a light microscope and the halos formed by lysed SRBC were counted and represented as PFC/10^5 splenocytes. The results obtained were 37.1±19.0; 114.5±31.9; 160.1±35.8 and 164.0±38.4 respectively to Co and 15, 75 and 150EUT groups. Despite no statistical significance ($P<0.06$), it could be suggested that UT can promote a dose-dependent increasing in the specific antibodies production. This result here obtained could be a beneficial effect to immunosuppressed subjects; however, other studies must be performed in order to verify if this improvement in the T-dependent antibodies production could lead to harmful effect on subjects with diseases related to autoantibody production as hypersensitivities and autoimmune diseases. Conclusion: UT exerts immunestimulation activity on the humoral immune response of rats. Financial support: FAPESP (proc. 2012/09565-8) and CNPq (proc. 476572/2013-4).

ABSTRACT FINAL ID: 2751 Poster Board -540
TITLE: Genotoxic Responses of Monocytes and Macrophages to Alkylating Agents

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KEYWORDS: DNA Repair, Blood Cells, Drug Sensitivity

ABSTRACT BODY: Acute hematotoxicity and subsequent immunodeficiency is a severe and therapy limiting side effect of DNA damaging anticancer therapy. In previous studies we analysed human monocytes and compared them with macrophages, which were derived from them, as to DNA repair and sensitivity to the methylating anticancer drug temozolomide (TMZ). We observed that monocytes were more sensitive than macrophages to the killing effect of TMZ. We also showed that the expression of the base excision repair (BER) proteins XRCC1, LigIII3, PARP-1 and DNA-PK is lacking in monocytes and becomes upregulated during their differentiation into macrophages [1, 2]. Here, we translated this study to

mouse where we investigated monocytes following treatment *in vivo*. We compared mouse monocytes isolated from bone marrow (BM) with T lymphocytes collected from spleen by Western blot analysis. Similar to human, mouse monocytes lack the expression of XRCC1, LigIII, PARP-1 and DNA-PK on protein level. We also investigated the mRNA expression in monocytes, macrophages and T lymphocytes by qPCR and observed a reduced XRCC1 expression in monocytes compared to macrophages and T lymphocytes. We also determined the sensitivity of mouse monocytes and compared them with macrophages after TMZ treatment. Following drug injection in C57BL/6 mice, apoptosis of monocytes isolated from bone marrow and macrophages collected from the peritoneum was measured by annexin V staining and flow cytometry. The data revealed that monocytes were more sensitive than macrophages to TMZ. Additionally, peripheral blood was analyzed after TMZ treatment and a depletion of different cell populations, including monocytes was observed. In summary, we show that mouse monocytes are more sensitive than macrophages to TMZ, which is likely the result of downregulation of base excision and DNA double-strand repair in this blood cell population. References: Bauer et al., *PNAS*, 2011, 108(52):21105-21110; Bauer et al., *PloS ONE*, 2012, 7(6):e39956.

ABSTRACT FINAL ID: 2752 Poster Board -541
TITLE: Crack Cocaine: A Preliminary Immunotoxic Study in Rats Exposed to Its Smoke for 28 Days

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KEYWORDS: Crack Cocaine, Immunotoxicity

ABSTRACT BODY: Many epidemiological studies revealed the devastating effects of crack cocaine; however, nonexistent are the studies in human relating crack with its possible immunotoxic effect due to the interference of other drugs abuse like alcohol, marijuana and others. Thus, the aim of this study was to evaluate the immunotoxic effects of crack in rats exposed to its smoke twice a day for 28 days. Thirty male rats were equally divided into 1 control group (Co), 1 crack cocaine group (CC) and 1 pair-fed group (PF). Rats from CC groups were exposed during 10min twice a day to the smoke resulted from the burning of 250 mg of crack (cocaine blood level = 175 ng/mL) confined into an acrylic device developed in our lab for 28 consecutive days. Rats from Co group are only confined into the acrylic device for the same time and period and the PF group only received the same amount of food ingested by the CC group. Food consumption (FC) and body weight gain (BWG) were recorded every day. In the end of the experiment, all animals were anesthetized to blood collection (haemogram) and after death, immune organs (thymus and spleen) and bone marrow cells are harvest to relative weight and cellularity evaluation. As expected, CC group revealed a statistical reduction on the FC when compared to Co group; interestingly, the lowest weight gain was only observed in the PF group. Moreover, PF groups showed decreased RBC, WBC, Hg and Ht values when compared to Co and CC groups. Finally, only PF group showed a reduction in the thymus relative weight. The results here obtained revealed that crack did not impair the haemogram and the immune organs here evaluated. On the other hand, we found that the stress of the forced food restriction to the PF group caused immunotoxic effects, leading us to conclude that neither the crack exposure nor the central anorexia caused by the drug promoted toxic effects on the parameters here evaluated. Fapesp: proc n. 2012/24550-7

ABSTRACT FINAL ID: 2753 Poster Board -542
TITLE: Characterization of the Role of Cannabinoid Receptors on Dendritic Cell Antigen Presenting Function

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KEYWORDS: Dendritic Cells, Cannabinoid Receptor

ABSTRACT BODY: Dendritic cells (DCs) are professional antigen presenting cells essential in the adaptive immune response. They present exogenous antigens on MHC class I molecules to initiate CD8+ T cell response in a process known as cross-presentation. Δ9-Tetrahydrocannabinol has robust immunostimulatory effects, largely attributed to cannabinoid receptor 1 (CB1) and 2 (CB2) ligation. Simultaneous deletion of CB1 and CB2 resulted in exacerbated immune reactivity and DCs were identified to have a large role in influenza induced immunopathology of these mice lacking CB receptors. The role of the CB receptors on DC development and function has not been characterized. The objective of the present study was to characterize the role of CB receptors on the development and function of DC subsets from mouse bone marrow. Bone marrow was extracted from femurs and tibias of WT (C57Bl/6) and CB KO mice were stimulated with LPS and stained for DC markers. Our results indicate that the percent of DC populations, determined by CD11c expression, composes ~38% of freshly isolated bone marrow cells in WT mice and ~48% in CB KO mice. We also found that bone marrow from CB KO mice had a higher percent of MHCI+ cells. Bone marrow cells isolated from CB KO mice elicited a CD8+ T cell response in the absence of LPS stimulation. Then we assessed the expression of antigen-bound MHC I complexes on the surface of DCs (CD11c+ CD11b+). After 24 hours of incubation in the presence or absence of LPS, cells were washed thoroughly then pulsed with SIINFEKL peptide for two hours and subsequently washed and stained with an MHC I-SIINFEKL complex antibody. We found a significant increase in antigen-MHC I complexes in the surface of DCs from CB KO (55%) mice in comparison with WT (35%). Our findings suggest a possible role of CB receptors in regulating antigen cross-presentation in DCs. (This work was supported by DA007908)

ABSTRACT FINAL ID: 2754 Poster Board -543
TITLE: Absorption of PCB126 by Upper Airways Impairs G-Protein-Coupled-Receptor-Mediated Immune Response

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KEYWORDS: Biphenyl Polychlorinated, Chemotaxis, Dioxin-Like

ABSTRACT BODY: PCB126 is a dioxin-like polychlorinated biphenyl (PCB) environmental pollutant which toxic mechanisms have not been fully elucidated. We here investigated the toxicity of nasal instillation exposure to PCB126 on mechanisms of the innate immune defence of blood cells. Male Wistar rats were exposed to PCB126 (0.1; 1 or 10 μg/kg; during 15 days; once a day) or vehicle (0.5% DMSO saline solution). PCB126 exposure was characterized by enhanced expression of the aryl hydrocarbon receptor (AhR) in the liver, lung, kidney, spleen and adipose tissues (by western blot), and by elevated PCB126 levels in the lung and liver (gas chromatography/mass spectrometry analysis). PCB126 exposure reduced basal expression of L-selectin adhesion molecules on blood leukocytes, and the effect was strengthened if leukocytes were *ex vivo* activated by N-formyl-methionyl-leucyl-phenylalanine (fMLP), a G-protein coupled receptor (GPCR) agonist, as shown by impaired β2 integrin and platelet endothelial cell adhesion molecule-1 (PECAM-1; flow cytometry) expressions, neutrophil chemotaxis (Boyden chamber) and oxidative burst activation (flow cytometry). The specificity of PCB126's actions on the GPCR pathway was demonstrated by the normal oxidative burst activation in neutrophils after phorbol-12-myristate-13-acetate stimulation, a lipossoluble activator of intracellular kinases. Therefore, we highlight that nasal PCB126 exposure impairs pivotal inflammatory pathway to the host defence against infections.

ABSTRACT FINAL ID: 2755 Poster Board -544
TITLE: The AhR Ligand, 10-Cl-BBQ, Prevents Insulitis Independently of Foxp3+ Regulatory T Cells

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KEYWORDS: Aryl Hydrocarbon Receptor , Regulatory T cell, Type 1 Diabetes

ABSTRACT BODY: The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that has been associated with potent immunosuppression. We have previously reported that suppression of type 1 diabetes by TCDD, a potent AhR ligand, is associated with an increased percentage of Foxp3+ regulatory T cells. Similar associations between AhR activation and increased Foxp3+ cells have been reported for suppression of other autoimmune diseases. Based on the ability of AhR ligands to induce Tregs, AhR is a potential target for the treatment of autoimmune disease. We have recently discovered a novel, rapidly metabolized AhR ligand, 10-chloro-7H-benzimidazo[2,1-a]benzo[de]Iso-quinolin-7-one (10-Cl-BBQ), that activates AhR in CD4+ T cells in a similar manner to TCDD. Here we show oral treatment with 10-Cl-BBQ, like TCDD, resulted in almost complete suppression of pancreatic islet infiltration throughout 20 weeks of age in the NOD mouse model. Furthermore 10-Cl-BBQ suppressed insulitis in the absence of any changes in clinical measures of toxicity. When we looked at changes in Foxp3+ cells, a small yet significant increase in the frequency, but not total number, of Foxp3+ cells was observed. This raised the yet unanswered question, is the increase in Foxp3+ Tregs driving AhR-mediated immune suppression? To directly assess the requirement of Foxp3+ cells in AhR-induced suppression of islet infiltration, transgenic NOD.Foxp3DTR (human diphtheria toxin receptor under the control of the Foxp3 locus) mice were employed. In the vehicle control group, Foxp3 depletion led to rapid pancreatic islet infiltration. However, treatment with 10-Cl-BBQ suppressed infiltration even in the absence of Foxp3+ cells. These results suggest that AhR functions as a Foxp3-independent transcription factor driving suppression of T cell dependent immune responses.

ABSTRACT FINAL ID: 2756 Poster Board -545
TITLE: Broad-Spectrum Oral Antibiotics Induce Changes in Regulatory T Cells in the Cecum and Colon in Mice with Uveitis

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KEYWORDS: Gut Microbiota, Autoimmune Uveitis

ABSTRACT BODY: The ability of changes in the gut microbiota to induce alteration of immune cell populations has been investigated in various immune-mediated diseases such as in animal models of multiple sclerosis, but not in uveitis. We previously reported that the severity of experimental autoimmune uveitis (EAU) in mice was ameliorated with broad-spectrum antibiotics. The purpose of this study was to investigate how altering the gut microbiota with antibiotics specifically affects the regulatory T lymphocyte (Treg) population in lymphoid tissues in EAU. Interphotoreceptor retinoid-binding protein peptide 161-180 was used to induce uveitis in B10.RIII mice. Mice were treated with oral antibiotics starting 1 week prior to antigen challenge. Spleen, cervical lymph nodes (CLN), mesenteric lymph nodes (MLN), and lamina propria lymphocytes (LPL) from the cecum and colon were collected 7, 14, 21, and 28 days after immunization. Flow cytometry analysis was performed to quantify Treg populations. There were higher proportions of inducible Treg (CD4+, FoxP3+) cells in the LPL of oral antibiotic-fed mice on day 7, 14, and 28, compared to water-fed mice (day 7: 41.7% vs. 25.1%, $p<0.05$; day 14: 45.8% vs. 31.6%, $p<0.05$; day 28: 32.7% vs. 14%, $p<0.01$). Higher frequencies of Treg were observed in the MLN of oral antibiotic-fed mice on day 21 and day 28, compared to the controls (day 21: 17.7% vs. 13.2%, $p<0.05$; day 28: 12.2% vs. 9.9 %, $p<0.05$). No differences in thymic-driven Treg (Helios+, FoxP3+) cells were found between the two groups. Our results suggest that alteration of the gut microbiota with oral antibiotic administration modulates the development of EAU, in part, by regulating the inducible (non-thymic) Treg population. Further investigation of the gut bacteria and their metabolites associated with the gut microbial change is warranted to determine the underlying detailed mechanism.

ABSTRACT FINAL ID: 2757 Poster Board -546
TITLE: Oleo-Gum-Resin (Myrrh) Attenuates Inflammatory Progression in Colon

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KEYWORDS: Antioxidant

ABSTRACT BODY: Background and Aims: The prevalence of inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) has increased in the last decades. The pathogenesis of UC is associated with weakened antioxidant capacity and increases inflammatory process. Myrrh is used traditionally for inflammations and stomach disorders and also proven its antioxidative and anti-inflammatory properties. Thus the present study was designed to evaluate the Myrrh effect on experimental model of UC in rats. Methods: UC were induced by acetic acid (AA) in rats previously treated with Myrrh (125, 250 and 500 mg/kg/day) and mesalazine (300 mg/kg/day) for seven days. Levels of inflammatory cytokines were assayed in colon tissues. Colonic levels of thiobarbituric acid reactive substances (TBARS) and sulfhydryl's groups (NP-SH) as well as superoxide dismutase (SOD) and catalase (CAT) activities were estimated. Total proteins (TP) and nucleic acids including DNA and RNA levels were measured and histopathological changes were determined in colonic tissues. Results: Pro-inflammatory cytokines and TBARS levels were markedly increased, while NP-SH, TP, nucleic acids and the enzymatic activities of SOD and CAT were significantly dimensioned in AA administered group. Pretreatment with Myrrh and mesalazine attenuated the impaired oxidative stress and inflammatory biomarkers. The enzymatic activities were found near to normal in Myrrh and mesalazine pretreated groups. The apparent UC protection was further confirmed by the histopathological screening and found high dose of Myrrh is in comparable to mesalazine. Conclusion: Myrrh is of potent therapeutic value in the amelioration of experimental colitis in laboratory animals by inhibiting the proinflammatory mediators and improving the antioxidative enzymatic activities.

ABSTRACT FINAL ID: 2758 Poster Board -547
TITLE: NLRP12 Regulates Both Inflammasome Activation and T Cell Differentiation during Cigarette Smoke-Induced Pulmonary Inflammation

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KEYWORDS: COPD, NLRP12, T Cells

ABSTRACT BODY: Chronic airway inflammation is central to the pathogenesis of parenchymal destruction in Chronic Obstructive Pulmonary Disease (COPD), the fourth leading cause of death worldwide. At this time little is known about the impact of NOD-Like Receptors (NLRs) including NACHT, leucine-rich repeat, pyrin domain protein 12 (NLRP12) on pulmonary inflammatory responses following cigarette smoke (CS) exposure. Here, we investigated the role of NLRP12 in CS-induced inflammation with regard to T cell activation and IL-17 production. NLRP12 KO and C57Bl/6 (WT) mice were exposed to CS for 6 weeks. CS-exposure induced both IL-17A and IL-17F proteins in the lungs of WT, but not of KO mice. CS exposure also induced activation of receptor interacting protein 2 (RIP2), NF-κB and MAPKs in lungs of WT, but not in KO mice. We observed reduced caspase-1 activation and IL-1β and IL-18 levels in the lungs and spleens of CS-exposed KO mice. Furthermore, we isolated naïve T-cells from the spleen of KO and WT mice for Th1, Th2 and Th17 differentiation. Naïve T cells isolated from the spleen of KO mice showed reduced *in vitro* differentiation into Th17 subset when compared to naïve T-cells obtained from WT mice. In a similar manner, treatment with CS-condensate induced Th17 differentiation of Naïve T-cells from C57Bl/6 mice, but not from KO mice. By contrast, Th1 and Th2 differentiation was not different between two (KO and WT) groups. These results show that NLRP12 regulates both inflammasome activation and Th17 cell differentiation to modulate lung inflammation in response to CS exposure.

ABSTRACT FINAL ID: 2759 Poster Board -548
TITLE: Investigating Diabetic-Like Effects in Adipose Tissues of Progeny Exposed to 2-Aminoanthracene *In Utero*

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KEYWORDS: Interleukin-6, Adiponectin, Gene Expression

ABSTRACT BODY: In recent times, there has been an increase in the incidence of type-2 diabetes (T2D) particularly in children. T2D is when the body does not produce enough of insulin, or when the body cannot use insulin to its full potential. Understanding the etiology and biology of this disease is important for early detection and treatment. It is thought that, environmental chemical exposure during early years of life might be a significant contributing factor to the increase in the incidence of T2D. This study tests the idea that exposure to environmental contaminants (2-aminonanthracene-2AA) *in utero* will show effects in the adipose tissue that signify T2D vulnerability. One specific PAH, 2AA has been detected in broiled food and tobacco smoke as well as in a wide range of products including coal, tar, crude oil, cereals, grains, flour, vegetables, and pickled foods. To accomplish the study objective, pregnant dams were fed various amounts of 2AA adulterated diets (0 mg/kg-, 50 mg/kg-, and 100 mg/kg-2AA diet) from gestation through postnatal period. Adipose tissues of pups were analyzed for specific diabetes related gene expression using qRT-PCR; ADIPONECTIN, TNF-α, IL-6, LEPTIN, CD14 and CD68. Specific staining for CD68 positive cells was greater in the low dose group though not significantly different compared with the control. In contrast, CD68 positive cells were significantly reduced in the high dose group relative to control rats. It is interesting to note the over-expression of gene transcripts in the low dose (50 mg/kg-2AA diet) group compared to both control and high dose (100 mg/kg-2AA diet) animals. ADIPONECTIN, IL-6, CD14 and TNFα were up-regulated in the animals that ingested 50 mg/kg-2AA diet. Similarly, ADIPONECTIN was up-regulated in the progeny of dams that ingested to 100 mg/kg-2AA diet. Further study is ongoing to assess the response of offspring to moderate high fat diet.

ABSTRACT FINAL ID: 2760 Poster Board -549
TITLE: Involvement of Treg and Th17 Shift in Inflammatory Responses Induced by Fine Ambient Particulate Matter in Mice

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KEYWORDS: PM2.5, Th17, Treg

ABSTRACT BODY: Epidemiologic studies have reported the association between fine particles (aerodynamic diameter ≤ 2.5 μm; PM2.5) and health effects, but the immunological mechanisms are not clear. To investigate the dose and time-dependent role of Treg cells and Th17 shift in local and systemic inflammation induced by PM2.5, mice were subjected to intratracheal instillation of 2.5, 5, or 10 mg/kg PM2.5 in this study. After 24 h, 72 h, 7 days, and 14 days, mice were sacrificed to measure Treg and Th17 expressions and Th17 related cytokines in bronchoalveolar lavage fluid (BALF) and peripheral blood. Histopathological changes in lung were also examined. Inflammatory infiltration and macrophages with engulfed particles were found by lung histopathology after PM2.5 exposure. Treg positive cells decreased in BALF but increased in blood at 24 h after the exposure. The low percentage of Treg positive cells continued to day 14 in BALF, but recovered at day 7 and decreased further to lower than the control value at day 14 in blood. Treg positive cell changed similar to Treg in BALF on the dose effects. In BALF at 24 h after the exposure, the Th17 related cytokines IL-17 increased dose-dependently; and in blood. These results suggest that acute exposure of PM2.5 leads to acute inflammatory responses locally and systemically in mice. Treg are involved in this process and PM2.5 can drive a Th17 related immune response.

ABSTRACT FINAL ID: 2761 Poster Board -550
TITLE: Modulation of Inflammation in Synovial Fibroblasts

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KEYWORDS: Rheumatoid Arthritis, Synovial Fibroblasts, C-DIMS

ABSTRACT BODY: The release of pro-inflammatory mediators by activated immune cells plays a pivotal role in autoimmune-related disorders such as Rheumatoid arthritis (RA). However, several lines of evidence have indicated that synovial fibroblasts can also become activated under disease conditions, and can contribute to inflammatory damage at the joint. Anti-inflammatory compounds that can specifically target molecular pathways within non-immune cells could be a useful treatment for RA, possibly reducing the need for global immunosuppressive drugs that have undesirable side effects. A series novel, para-substituted diindolylmethane compounds (C-DIMs) have shown to modify selective inflammatory pathways in pancreatic, bladder, and colon cancer studies, as well as models of neuroinflammation. In this study, primary synovial fibroblasts were isolated from murine ankle joints and characterized utilizing immunofluorescence and flow cytometry. Synovial fibroblasts were treated with 10 ng/ml of Tumor necrosis factor- alpha (TNF-α) for 24 hours to mimic RA conditions. Immunofluorescence confirmed positive Vimentin expression. Flow cytometric analysis of primary culture revealed > 80-90% CD90.2 positive and < 1% Mac-1 positive. Vascular cell adhesion molecule 1 (VCAM-1) and intracellular adhesion molecule 1 (ICAM-1) are constitutively expression on synovial fibroblasts and surface expression is further increased after exposure to TNF-α. Current data supports successful characterization of synovial fibroblasts and suggests a significant shift in the expression of inflammatory markers. Therefore, the use of the C-DIMS in this model of RA can be useful as an alternative therapeutic investigation for treatment of RA and other arthritic conditions.

ABSTRACT FINAL ID: 2762 Poster Board -551
TITLE: Screening Assay for Environmental Obesogens Acting via PPARs

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KEYWORDS: Screaning Methods, Peroxisome Proliferator, Obesogen

ABSTRACT BODY: The U.S. population is exposed to a number of environmental chemicals termed endocrine disruptors that may be associated with the increasing incidence of metabolic disorders and obesity, particularly in children. A number of these chemicals are believed to act as peroxisome proliferator activated receptor (PPAR) agonists and affect homeostatic pathways associated with glucose metabolism, lipid metabolism and inflammatory responses. The health consequences of aberrant PPAR activation are potentially significant and include increased risk for obesity, type-2 diabetes, cardiovascular disease and chronic inflammatory diseases. Studies were conducted to establish a high throughput *in vitro* cell culture screening assay to identify gene expression changes in key biological pathways that occur from putative environmental PPAR agonists using a low-density focused array card followed by RNA silencing studies of the PPARa and PPARg genes using selected human cell lines including THP1 cells, a human monocyte cell line. In our hands we found this approach to lack high sensitivity due to the multiple cell manipulations required. However, some of the more potent responses associated with treatment with positive controls (fibrate and glitazone) and test chemicals (such as tributyltin chloride and perfluorooctanoate) indicated that PPARa, PPARg or both were associated with the regulation of genes associated with several pathways including cholesterol homeostasis and acute phase proteins. Our results should be helpful particularly in epidemiological studies of children exposed to environmental obesogens as it provides potentially biologically relevant markers that can be examined. Supported by NIEHS RO3 Grant.

ABSTRACT FINAL ID: 2763 Poster Board -552
TITLE: A Single Method for the Quantitation of Sirolimus in Multiple Species and Matrices

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KEYWORDS: Rapamycin, LC-MS/MS, Immunosuppressant

ABSTRACT BODY: Sirolimus (rapamycin) was approved for human use as an immunosuppressant in 1999. It has also seen extensive use as a stent coating, and has shown promising results in studies regarding cancer, autism and Alzheimer's. As interest grows, the need for more robust analysis in multiple and varied matrices has increased. Sirolimus analysis in porcine matrices was previously validated using liquid/liquid extraction with column switching. This method required significant equilibration time, and was prone to carryover. We describe validation of a dual extraction technique using protein precipitation and supported liquid extraction with a simple and more reliable LC gradient. Methods: Matrix containing sirolimus is treated with a zinc sulfate precipitating solution with tacrolimus added as an internal standard. Supernatant is then loaded on a supported liquid extraction (SLE+) plate, washed, and eluted using methyl-tert-butyl ether and evaporated to dryness. Residues are reconstituted with methanol/ammonium acetate/formic acid/water and injected into an LC-MS/MS system using a Polar-RP column with a gradient ammonium acetate/formic acid/water/acetonitrile mobile phase. Concentration is monitored using either the sodium or ammonium adduct. Data/Conclusion: Reliable analysis of sirolimus was demonstrated in whole blood from pig, dog, rat, monkey, and rabbit, in rat urine feces homogenate, and porcine homogenates of artery, liver, lung, kidney and heart tissue. The method performed reliably, with minimal carryover, at analytical ranges of 0.1 to 100 mg/mL in whole blood and urine, 0.5 to 500 ng/g in feces, liver, lung, kidney, and heart tissue homogenate, and 100 to 10,000 ng/g in arterial tissue homogenate. Representative statistics for canine whole blood validation are: calibrator % bias: -4.5% to +2.3%, %CV: 3.4 to 9.7%, R-squared values > 0.99; intraday % bias: -6.7% to +17% and %CV: ≤12.4%, interday %bias: ≤ 6.0% and %CV ≤ 11.8%; extraction efficiency of 57.7% for analyte and 104.9% for internal standard; matrix factor was 1.14±0.01. This assay allows for quantitation of sirolimus in a wide variety of samples.

ABSTRACT FINAL ID: 2764 Poster Board -553
TITLE: Diepoxybutane Induces the Expression of an Apoptosis Mediating Novel p53-Target Gene XCL1 in Exposed Human Lymphoblasts

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KEYWORDS: Diepoxybutane, p53, Apoptosis

ABSTRACT BODY: Diepoxybutane (DEB) is the most potent active metabolite of the environmental chemical 1, 3-butadiene (BD). BD is a mutagen and human carcinogen, and possesses multi-organ systems toxicity. The XCL1 gene was found to be up-regulated 10-fold in a p53-dependent manner in TK6 lymphoblasts undergoing DEB-induced apoptosis. XCL1, a chemokine, plays an active role in inflammation, and potentially apoptosis. The purpose of this study was to determine whether XCL1 is a novel direct p53 target gene in human lymphoblasts exposed to DEB, and deduce its role in DEB-induced toxicity. Chromatin-immunoprecipitation-quantitative PCR assays, *in silico* studies using p53 scan algorithm, and reporter gene assays in the presence/absence of endogenous/exogenous wild type p53 were utilized to determine the status of XCL1 as a p53 target gene. The role of p53 in DEB toxicity was tested through XCL1 siRNA knockdown experiments. Transfection of XCL1 promoter nanoluciferase reporter construct resulted in a 5-fold increase in reporter activity in the p53-proficient TK6 cells exposed to DEB as compared to un-exposed control cells. Co-transfection of wild type p53 and XCL1 promoter reporter constructs resulted in a 3-fold increase in luciferase activity in the DEB-exposed p53-deficient NH32 cells as compared to control un-exposed cells. This transactivation was reduced by 32% when mutant p53 was used. Inactivation of a p53 binding site within the XCL1 promoter region also prevented transactivation by 40%. XCL1 siRNA knock-down reduced DEB-induced apoptosis by 50% in exposed cells, as compared to cells with control non specific siRNA. Collectively our results show that XCL1 is a novel direct p53 target gene in DEB-exposed human lymphoblasts, and mediates DEB-induced apoptosis in these cells. These findings have important implications towards the understanding of p53 signaling and action in DEB toxicity, thus contributing to the understanding of molecular mechanisms of DEB and BD toxicity.

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Late-Breaking Abstracts

ABSTRACT FINAL ID: 2765 Poster Board -554
TITLE: Association between GSTA1, GSTM3 Gene Polymorphyisms, and Attention Deficit Hyperactvity Disorder in Korean Children

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KEYWORDS: Genetic Polymorphism, ADHD, Mercury

ABSTRACT BODY: Background: Recent studies have suggested that environmental exposure to mercury (Hg) have associated with Attention Deficit Hyperactivity (ADHD). Objectives: We hypothesized that some single-nucleotide polymorphisms (SNPs) in toxicokinetics genes are influencing individual difference in mercury biomarker levels, and the allele frequency of those SNPs are different in the ADHD and control groups. Methods: We selected samples of 1,553 children from “Korean Environmental Health Survey for Children” cohort. This cohort measured blood Hg level, and ADHD symptoms. ADHD symptoms were evaluated with Dupaul rating scale. Through literature survey, we selected candidate SNPs. We surveyed SNPS of samples with a Vera Code Golden Gate assay. We compared the genotype frequency in the ADHD and control groups. Results: The GSTA1 (rs3957356) genotype of the 1,360 subjects in the control group and the 191 subjects in the ADHD group were T/T (3.38%; 2.09%), T/C (27.1%; 19.4%), and C/C (69.6%; 78.5%), and there was a significant difference in the frequency between the two groups(X2=6.47, P=0.01). The GSTM3 (rs7483) genotype of the 1,361 subjects in the control group and the 192 subjects in the ADHD group were G/G (5.73%; 4.17%), G/A (38.1%; 31.8%), and A/A (56.1%; 64.1%), and there was a significant difference in the frequency between the two groups(X2=4.13, P=0.04). When we adjusted age, and sex, odds ratio of the genotype with genetic polymorphism in the control group and the ADHD group were significant at 0.65(p=0.01) for the GSTA1 (rs3957356), and for the GSTM3 (rs7483), the odds ratio was significant at 0.73(p=0.02). Conclusion: Our findings suggest that some hg toxicokinetics related genetic polymorphism frequency may different in ADHD and control group.

ABSTRACT FINAL ID: 2766 Poster Board -555
TITLE: Genomic Analysis of Exposure of Human Primary Lung Epithelial Cells for 24-Hour to Arsenic and Its Methylated Metabolites and to Arsenic Trioxide

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KEYWORDS: Gene Expression, Arsenic, Mode of Action

ABSTRACT BODY: Gene expression changes were evaluated in human primary lung epithelial cells exposed either to arsenic trioxide or to a mixture of arsenic trioxide and its methylated metabolites. Concentrations from 0.06 µM to 6 µM total arsenic were chosen to correspond with a previous study of human primary bladder epithelial cells, and are in the range of highly exposed human populations. Biochemical assays did not show evidence of cytotoxicity from any exposure; however, a concentration-dependent increase in cellular heme oxygenase was observed. This increase was later confirmed by gene expression changes. Pathway enrichment analysis showed concentration-related responses in pathways related to cell adhesion, cytoskeleton remodeling, morphogenesis, cell cycle control, and inflammatory response. These cellular responses were similar to those observed in the previous human bladder cell study. Benchmark dose analysis indicated similar potency of arsenic across primary cells from the two different tissues as well as between the two arsenic treatments. Multiple genes showing concentration-dependent changes across all individuals were identified, including heme oxygennase 1, thioredoxin reductase, DNA damage binding protein 2, and thrombomodulin. Together, the similarity of

response to the arsenic mixture and arsenic trioxide, and between the human lung epithelial and urinary cell lines, supports a conclusion that biological responses to arsenic are unlikely to occur below a concentration of 0.1 µM. These *in vitro* data, together with PBPK modeling for extrapolation to equivalent *in vivo* exposures, can provide a point of departure for arsenic risk assessments.

ABSTRACT FINAL ID: 2767 Poster Board -556
TITLE: Evaluation of *In Vitro* Biotransformation Using HepaRG Cells to Improve High-Throughput Chemical Hazard Prediction: A Toxicogenomics Analysis

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KEYWORDS: Toxicogenomics, High-Throughput Screening, ToxCast

ABSTRACT BODY: An inherent criticism of many *in vitro*-based toxicity testing strategies is the inability of assays to detect *in vivo*-relevant activities in cells lacking biotransformation capacity. To address this potential limitation, we used an *in vitro* liver toxicogenomics approach to explore gene-specific perturbations elicited by 1060 environmental chemicals from the US EPA ToxCast library in metabolically competent HepaRG cells treated in 8-point concentration-response for 48 hours. The expression of 96 genes, including numerous Phase (Ph) I and II metabolizing enzymes, was evaluated by qPCR using Fluidigm 96.96 dynamic arrays. Data show 38% of the library (n = 404 chemicals) significantly increase the expression of at least one cytochrome P450 gene (CYP1A1/A2/2B6/2C19/2C8/2C9/2E1/3A4/3A5) regulated by xenobiotic-sensing nuclear receptors (CAR, PXR) and transcription factors (AhR) at half-maximal activity (AC50) values < 25 µM. Results indicate that 40% (n = 194) of the chemicals that induce Ph I enzymes also elicit increased expression of Ph II enzymes within a similar activity range. For example, comparisons of chemicals that activate AhR responsive genes (cyp1a1, cyp1a2, ugt1a1) expression in HepaRG cells with those in AhR reporter gene assays (HepG2) support the identification of true positive, classical (benz(a)anthracene) and non-classical (2,3-diaminotoluene) AhR ligands. This method provides a unique toxicogenomics-based dataset for the expression of metabolic enzymes for over 1000 chemicals. These data will improve chemical hazard predictions by allowing integration of potential effects of biotransformation on existing bioactivity data in the ToxCast library. This abstract does not necessarily reflect EPA policy.

ABSTRACT FINAL ID: 2768 Poster Board -557
TITLE: 8-Oxoguanine DNA Glycosylase-1 DNA Repair-Signaling Induces Gene Expression Associated to Airway Remodeling

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KEYWORDS: OGG1-BER, DNA Damage/Repair, Airway Remodeling

ABSTRACT BODY: Chronic airway inflammation is a common feature of asthma and other airway diseases. It is normally accompanied by tissue injury inflicted by reactive oxygen species (ROS) and other molecules. Tissue remodeling includes structural changes such as epithelial alterations, subepithelial fibrosis, increased airway smooth muscle mass and collagen deposition, whose basic mechanism remains unknown. All these changes are linked to ROS but the role of oxidatively modified DNA-signaling in these processes has not been established. One the most abundant DNA base lesion is the mutagenic 8-oxoguanine (8-oxoG), which is continuously repaired by 8-oxoguanine DNA glycosylase-1-initiated base excision repair pathway (OGG1-BER). Recent studies showed that OGG1-BER induced a rapid activation of small GTPases

and downstream signaling and gene expression in lungs. Thus we asked whether chronic OGG1-BER-signaling leads gene expression associated to structural changes in the airways. We mimicked chronic DNA damage/repair by repeatedly challenging lungs with OGG1-BER product 8-oxoG. RNAs were isolated and analyzed by RNA-sequencing and datasets were evaluated by gene ontology (GO) and statistical tools. Results showed gene expression related to various biological processes, and signaling pathways involved in airway remodeling. Importantly, these data are supported by histological observations showing epithelial-alterations, subepithelial fibrosis and collagen deposition. These results imply that OGG1-BER-signaling induce gene expression consistent with structural changes in lungs. Supported by: NIEHS T32ES007254, NCATS 1UL1TR000071 and NIEHS RO1 ES18948

ABSTRACT FINAL ID: 2769 Poster Board -558
TITLE: Effect of CCM3 Gene Defect on Lead-Induced Cell Genotoxicity in Mouse Embryonic Fibroblasts

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KEYWORDS: CCM3 Gene, Lead, Genotoxicity

ABSTRACT BODY: [Objective] To study the effect of CCM3 gene defect on lead induced cell genotoxicity in mouse embryonic fibroblasts. [Methods] Primary mouse embryonic fibroblasts were isolated from C57 female mice mated with CCM3 gene heterozygous male mice. After genotyping, wild type and heterozygous cells were treated with 6.25, 12.5, 25, 50, 100, 200 μmol/L of lead acetate. Cell viability, genotoxicity and protein expression were detected by MTS assay, CB micronucleus test and Western blot respectively. [Results] After 24 hours and 48 hours treatment, cell activity of wild type cells in all treatment groups significantly decreased ($P<0.05$) compared with that of the control group. After 24 hours treatment, cell activity of heterozygous cells in 25-200 μmol/L groups were significantly reduced ($P<0.05$) compared with that of the control group. After 24 hours treatment, the cell activity in 25 μmol/L - 200 μmol/L groups was significant different ($P<0.05$) between two kinds of genotype cells. After 48 hours treatment, the cell activity in all treatment groups was significant different ($P<0.05$) between two genotypes cells. In wild-type cells, the micronucleus cell rates (‰) of the control, 6.25, 25, 100 μmol/L group were 29.6 ± 2.2, 47.3 ± 6.6, 55.5 ± 9.1, 66.8 ± 3.5, respectively. In heterozygous cells, micronucleus cell rates (‰) of the control group, 6.25, 25, 100μmol/L group were 35.3 ± 5.6, 50 ± 8.3, 57 ± 8.5, 58.8 ± 2.1, respectively. Micronucleus cell rates (‰) in 100 μmol/L groups was significant different ($P<0.05$) between two genotypes. Western blot analysis showed that with increased exposure doses, gene expression of CCM3 and γ-H2AX increased. Conclusion CCM3 gene may play an important role in lead-induced genetic toxicity of mouse embryonic fibroblasts. CCM3 gene-lead interactions effects on mouse embryonic fibroblasts cell toxicity.

ABSTRACT FINAL ID: 2770 Poster Board -559
TITLE: 6-Well Bacterial Reverse Mutation Assay: Retrospective Evaluation of Its Appropriateness for Assessing Mutagenicity of Impurities

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KEYWORDS: Ames Assay, Genotoxic Impurities, ICH M7

ABSTRACT BODY: The bacterial reverse mutation (Ames) assay has been used for decades as a primary *in vitro* test for mutagenicity. In the pharmaceutical industry, the design used for Regulatory purposes follows the methodology originally described by Dr. Bruce Ames and colleagues with a number of modifications. During large scale drug synthesis, reactive chemicals are often used, and many of these may require an evaluation for mutagenicity due to structural alerts, as outlined in the ICHM7 guideline. To support drug substance manufacturing campaigns, Ames testing is often needed early, when it may be challenging to synthesize sufficient quantities of an alerting impurity. To address this issue, our laboratory has modified the Petri plate test by adapting it to a 6-well format. With this modification, the test can be completed with

1/10th the compound amount and using 20-25% of the typical Ames test recipe. The 6-well assay has a 1 mg/well limit compared to the 5 mg/plate limit dose in Petri plates, but in over 75% of the tests, either insoluble test item or toxicity (or both) limits the highest effective concentration to the bacteria. The objective of this study was to confirm, using historical data, that negative results in the 6-well format at a limit of 1 mg are similar to those in the Petri plate format at 5 mg. Internal data covering more than a decade of testing showed that negative results in the 6-well test are perfectly concordant with those of the standard plate assay (68 compounds tested in both assay formats). Mutagenic findings in the assay typically lead to discontinuation (for most non-oncology drug candidates) or control strategies (for impurities). Adding previously published data, 222 compounds were tested in both the 6-well and Petri plate formats with the same bacterial strains, with concordant results for 221 of them. In conclusion, these data indicate that the 6-well Ames test is an appropriate platform for testing potentially mutagenic impurities to support clinical development and marketing applications as outlined in the ICHM7 guideline.

ABSTRACT FINAL ID: 2771 Poster Board -560
TITLE: Evaluation of cII Gene Mutations in the Brain of Big Blue Mouse Exposed to Acrylamide and Glycidamide in Drinking Water for up to Four Weeks

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KEYWORDS: Acrylamide, cII Mutant Assay, Glycidamide

ABSTRACT BODY: Potential health risks for humans from exposure to acrylamide (AA) and its reactive epoxide metabolite, glycidamide (GA), exist because substantial amounts of AA are found in a variety of fried and baked starchy foods. AA is tumorigenic in rodents, and a large number of *in vitro* and *in vivo* studies indicate that AA is genotoxic in multiple organs from male and female mice and rats. Although AA is neurotoxic, there are no reports on AA-induced gene mutations in the mouse brain. Therefore, to investigate if gene mutation can be induced by AA, or its metabolite GA, we screened brains for cII mutant frequency (MF) and types of mutations in previously treated male and female Big Blue (BB) mice with 0, 1.4 mM and 7.0 mM AA or GA in drinking water for up to 4 weeks. High doses of AA and GA induced significant increases in the cII MFs only in males and the induced MFs were 2.5-fold higher than the corresponding male controls ($P \leq 0.05$; control MFs = 11.4 ± 2.1 ×10-6). Molecular analysis of the mutants from males showed that AA and GA induced up to a 3-fold increase in the incidence of G:C → T:A, A:T→ T:A, and A:T → C:G transversions compared to the vehicle controls whereas the mutational spectra between AA and GA were similar. These results suggest that the MFs and types of mutations induced by AA and GA in the brain are consistent with AA exerting its genotoxicity via metabolism to GA. Further, the significant induction of mutations in the mouse brain warrants further studies on health risks associated with AA exposure in humans.

ABSTRACT FINAL ID: 2772 Poster Board -561
TITLE: Positive Ames Test Results for Boronic Acids Do Not Represent a General Eukaryotic Liability

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KEYWORDS: Boronic Acids, Genotoxicity, GADD45a

ABSTRACT BODY: O'Donovan et al reported positve Ames test results for 12 of 13 Boronic acids: they are often used as intermediates in compound synthesis. The aim of this study was to discover whether there was a corresponding eukaryotic genotoxicity hazard. We chose the GADD45a reporter assays (GreenScreenHC and BlueScreen HC) with and without S9 metabolic activation for this study because they identify all mechanstic classes of genotoxin. Nine compounds were readily available for study. Positive results were produced for one compound in GreenScreen and four compounds in BlueScreen. Only negative results were produced when tested with S9 metabolic activation. There is not a general genotoxic liability in

eukaryotes, within this chemical domain. Positive results were only produced only at concentrations between 1 mM and 10 mM.

ABSTRACT FINAL ID: 2773 Poster Board -562
TITLE: Study on the Capacity of Pesticides to Produce Double-Strand Breaks

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KEYWORDS: Pesticide, H2AX, Lymphocytes

ABSTRACT BODY: Introduction: Several studies have found an association between the exposure to pesticides with chronic pathologies including neurological and reproductive effects or developmental problems and cancer. In the last years, these compounds have been proposed as leukemogenic agents, probably by producing reciprocal translocations. However, this idea hasn't been explored yet, having thus an information gap. Objective: The aim of this study is to generate knowledge about the role of some pesticides and their metabolites in the formation of reciprocal translocations. We will evaluate the principal steps that lead to the formation of this type of damage: the production of double strand breaks's (DSB's) in DNA and their subsequent recombination repair, either via HR or NHEJ. Methodology: We selected eight pesticides. In a first phase, 250 µL of whole blood was treated with four concentrations of each pesticide during 1.5 hour. Lymphocytes were harvested and fixed, and DBS's were evaluated as foci of phosphorylated histone H2AX by immunofluorescence (γ-H2AX). 50 cells in triplicate were scored per treatment. The damage was classified in three categories: 1 – without foci, 2 – with a few foci and 3 – abundant foci. Results. We found a significant increase in foci with Permetrin, Glyphosate, Paraoxon, Pentaclorophenol and Endosulfan lactone, the latter in a dose dependent manner, being the last three the metabolites of Parathione, Lindane and Endosulfan. Not effect was found with Propoxur, Endosulfan and AMPA. Conclusion. Pesticides are capable of producing DSB's in lymphocytes.

ABSTRACT FINAL ID: 2774 Poster Board -563
TITLE: Characterization of MUS81-EME1 As a Novel Anticancer Therapeutic Target

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KEYWORDS: DNA Recombination, Anticancer Therapy, *In Vitro* Pharmacology

ABSTRACT BODY: Traditional DNA damage-based anti-cancer therapies target the enhanced rates of replication of cancer cells, but are accompanied by adverse affects to normal cells. A novel therapeutic approach is to exploit the genetic differences between cancer cells and normal cells. A potential target is MUS81-EME1, an enzyme involved in DNA damage repair by homologous recombination (HR). Importantly, siRNA inhibition of MUS81 caused cancer cells to be more sensitive to 5-fluorouracil (5-FU), an anti-cancer drug used in the clinic, highlighting the dependence of cancer cells on MUS81-EME1 activity for survival. In order to develop small molecule inhibitors that are feasible for clinical use, for the first time reported, milligrams full-length human MUS81-EME1 has been purified from Sf9 cells that over-express MBP-MUS81/GST-EME1. The enzyme is catalytically active with similar kinetics as the well-studied yeast homolog Mus81-Mms4, with a K_M of ~25 nM and a k_{cat} of ~2 min^{-1} for all DNA substrates tested that represent intermediates that arise in HR. A Förster Resonance Energy Transfer (FRET)-based nuclease assay utilizing fluorescently-labeled DNA has a 3.5-fold window to monitor inhibition of MUS81-EME1. An initial pilot screen of the ~2,000 compounds from the MicroSource library reveals ~10% hit rate. An additional ~150,000 compounds from the Diversity Library will be tested in this FRET-based assay as well as the established cell-based assay using MCF-7 breast cancer cells and 5-fluorouracil or Mitomycin C treatment to verify if MUS81-EME1 inhibition renders cancer cells even more sensitive to DNA damage-based cancer therapy. Lead inhibitors may serve as adjuvant therapy with existing chemotherapy or as potential anti-cancer drugs in telomerase

negative/Alternative Lengthening of Telomeres (ALT) positive cancers where *MUS81* is essential. This work represents a study of targeted drug discovery that exploits tumor dependence on homologous recombination.

ABSTRACT FINAL ID: 2775 Poster Board -564
TITLE: Development of a Novel *In Vitro* Skin Sensitization Assay with Reconstructed Human Epidermis: Analysis of Expression Mechanism of Marker Genes and Assessment of Predictivity to Animal Testing

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KEYWORDS: Skin Sensitization, Alternative Method, 3D Model

ABSTRACT BODY: <Backgrounds and purposes> To evaluate lipophilic chemicals inapplicable to existing *in vitro* skin sensitization tests, we have developed a novel *in vitro* assay, focused on multiple marker genes relevant to keratinocyte responses (inflammatory response and cytoprotective response) in reconstructed human epidermis (3D model). In this study, we assessed the followings. 1. Whether up-regulation mechanisms of marker genes in keratinocyte reflect those in *in vivo*, 2. Predictive performance of the 3D-based assay on various chemicals including lipophilic chemicals. <Materials and methods> 1. As key molecules regulating the keratinocyte responses *in vivo*, we focused on the ATP receptor $P2X_7$, (inflammatory response) and the transcription factor Nrf2 (cytoprotective response). KN-62, an antagonist of $P2X_7$, or siRNA of Nrf2 was pre-treated to normal human epidermal keratinocyte, followed by the exposure of a sensitizer DNCB. After 6hrs exposure, the expression of marker genes (inflammatory response; ATF3 and IL-8, cytoprotective response; DNAJB4 and GCLM) were quantified. 2. A total of 36 chemicals (30 sensitizers and 6 non-sensitizers) including 21 lipophilic chemicals were exposed to 3D model. After 6hrs exposure, the expression levels of marker genes were quantified. Test chemicals were judged as positive when at least one out of four marker genes met positive criteria. <Results and discussions> The pre-treatment of KN-62 or Nrf2 siRNA significantly suppressed the up-regulation of marker genes (ATF3 and IL-8, or DNAJB4, GCLM, respectively) induced by DNCB, suggesting that the up-regulation mechanism of each marker gene in keratinocyte was similar to that in *in vivo*. Also, the sensitivity, specificity and accuracy of the 3D-based assay for 36 test chemicals were 90%, 89% and 89%, when compared with the *in vivo* test (LLNA). Taken together, the 3D-based assay could be a mechanism-based test which can overcome the limitation in the existing *in vitro* tests.

ABSTRACT FINAL ID: 2776 Poster Board -565
TITLE: Penetration of Para-Phenylenediamine in Human Epidermis and Its Spatial N-Acetylation by Primary Keratinocytes

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KEYWORDS: Skin Penetration, N-Acetylation, Para-Phenylenediamine

ABSTRACT BODY: The hair dye molecule and strong skin sensitizer para-phenylenediamine (PPD) is known to undergo several conversions during skin penetration. The molecule is instable due to auto-oxidation and during skin penetration acetylated PPD derivatives are generated by N-acetyltransferase 1 (NAT1), predominantly from keratinocytes as major epidermal cell type. In the present study, we analyzed the fate of PPD in different human skin layers *in vivo* using Raman spectroscopic real-time profiling of the epidermis, and *in vitro* using primary keratinocytes in varying states of differentiation. Shortly after PPD application (1% in petrolatum, application time 0.5-23 hours), PPD penetrated into the stratum corneum as well as in the living epidermis until 20 µm depth. No indication for PPD accumulation in certain layers was found. In contrast, PPD content decreased over time yielding a half-life of approximately 3 hours. In order to study whether the decreased PPD content in the outer epidermis is accompanied by N-acetylation of differentiated keratinocytes we treated primary keratinocytes with calcium for up to 5 days to model keratinocyte differentiation, and compared their N-acetylation with non-differentiated, proliferating keratinocytes. NAT1 activity, NAT1 mRNA levels and concentrations of

acetyl-PPD in cell culture supernatants of differentiated and proliferating keratinocytes were comparable. These data show skin penetration kinetics of PPD for the first time *in vivo*, demonstrating fast uptake and short half-live of PPD in the skin. *In vitro* differentiation data indicate N-acetylation capacity for penetrated PPD in both differentiated and non-differentiated viable epidermal layers.

ABSTRACT FINAL ID: 2777 Poster Board -566
TITLE: Automated Assessment of the Barrier Function of *In Vitro* Epidermal Models Using a Dual-Arm Robotic System

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KEYWORDS: Alternatives to Animal Testing, Automation, Epidermal Model

ABSTRACT BODY: Reconstructed human epidermis (RHE) closely resembles human epidermis, and thus, can be used as an alternative to animal models. An essential quality criterion of RHE is a high barrier function, which is determined by ET-50 tests. Currently, these assays are conducted manually, which is labor intensive and binds trained personnel. Thus, an automation of the ET-50 would be preferable. Additionally, automation increases reproducibility and accuracy of measurements and reduces the error rate in comparison to manual processes. Here the barrier function of RHE was assessed by transferring the manual process to a dual-arm robotic system. ET-50 assay was performed manually and automatically under same experimental conditions. After a topical application of Triton X-100 for 1.5, 3, 4.5 and 6 hours, viability was calculated for every exposure period. An exponential decay curve was determined for each process and ET-50 values were calculated from the respective regression equations. For the manual test, an ET-50 value of 5.58 hours was obtained. The automated process resulted in an ET-50 value of 5.29 hours. To determine the discrepancy between data and regression equation, the residual sum of squares (RSS) was calculated. With a RSS value of 382.3 for the manual process, approximation of measured data by an exponential function was better than for the automated procedure (RSS: 1049.2). The results show that, the dual-arm robotic system can perform ET-50 tests. In addition, the automated process was established time and cost effectively as most process steps were conducted using standard lab equipment. Taken together, the results of this study demonstrates that dual-arm robotic systems are a promising tool for the automation of bioengineering processes, which is a vital perquisite for future high throughput testing.

ABSTRACT FINAL ID: 2778 Poster Board -567
TITLE: Development of an *In Vitro* Skin Sensitization Screening Strategy for Agrochemical Formulations and Pesticide Active Ingredients

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KEYWORDS: Skin, Sensitization, Alternative

ABSTRACT BODY: In response to the 7th Amendment to the Cosmetics Directive, much effort has been made in researching alternative approaches to the evaluation and assessment of skin sensitization potential. To date, 3 assays are currently in the advanced stages of the validation process at ECVAM, the Direct Peptide Reactivity Assay (DPRA), the KeratinoSens™ assay, and the Human Cell Line Activation Test (h-CLAT). Each of these assays measure key events within the Adverse Outcome Pathway (AOP) for skin sensitization as published by the OECD in 2012. As such, none of these assays are intended to be used as standalone replacements for the *in vivo* tests but should form a part of an integrated testing and assessment approach (IATA). The combination of these test methods as part of an integrated testing strategy (ITS) could serve as a cost-effective screening tool to predict sensitization hazard for DuPont agrochemicals without the use of animals. DuPont submitted 10 agrochemical co-formulants or formulation blanks, and 2 pesticide active ingredients (12 total) for *in vitro*

testing in these assays to compare results to the existing *in vivo* data. The set of co-formulants and formulation blanks consisted mainly of vehicle solvents such as methylated soyate that had produced positive results in either the Local Lymph Node Assay (LLNA) or Guinea Pig Maximization Test (GPMT). The two active ingredients had tested positive in GPMT studies only, these were indoxacarb and tribenuron methyl. The *in vitro* assays were aligned with the sensitization potential as determined *in vivo* (GPMT and/or LLNA) for 8 of 10 sensitizing formulations and 1 of 2 sensitizing pesticide active ingredients when tested individually. Taken together, these results provide evidence to support the potential application of a combination of the DPRA, KeratinoSens™, and h-CLAT assays for the evaluation and/or prioritization of *in vivo* testing regarding the skin sensitization potential for DuPont crop protection active ingredients and as well as corresponding agrochemical formulations.

ABSTRACT FINAL ID: 2779 Poster Board -568
TITLE: Testing Framework for Predicting Ocular Irritation of Contact Lens Multipurpose Solutions Using *In Vitro* Colony Formation and alamarBlue Assays

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KEYWORDS: Biocompatibility, Ocular, Cytotoxicity

ABSTRACT BODY: Contact Lens Multipurpose Solutions (MPS) represent the majority of lens care systems used for the care of soft contact lenses. The ability of these solutions to achieve effective lens cleaning, maintain ocular surface health, and enhance lens-wearing comfort is fundamental to safe long-term contact lens use. MPS contain various components including disinfecting agents, surfactants, viscosity-enhancers, and chelators. Sixteen MPS were tested *in vitro* using Colony Formation (ISO 10993-5) in fibroblasts (V79) and alamarBlue Cell Viability Assays in simian virus-40 transformed human corneal epithelial cells (HCEC). The effect of MPS on cell viability and its correlation with the levels of disinfectants, surfactants, pH and osmolarity of each MPS were evaluated. These results confirm the correlation in 13 of 16 MPS evaluated (81.25% correlation rate) among *in vitro* models and provide an ocular irritation analysis model for the predictions of non-ocular irritants based on MPS composition.

ABSTRACT FINAL ID: 2780 Poster Board -569
TITLE: Biological Safety Evaluation of a Complex Surgical Stapler Used in Robotic-Assisted Surgery

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KEYWORDS: Medical Device, Biocompatibility, Biological Safety

ABSTRACT BODY: Here we present the method for applying the ISO 10993 guidelines to the biological safety evaluation of an advanced surgical stapler instrument used for minimally invasive surgery. The patient contact regions or components of the stapler include the instrument's distal tip, stapler reloads, and staples. Patient contact materials include 18 metals/alloys, 10 polymers and 15 additives, including colorants, adhesives and lubricants. To assess biological safety, the devices were characterized with respect to material composition; a literature review was then performed to identify any potential hazards associated with each component and in lieu of chronic animal testing. Lastly, biocompatibility testing was performed on the final sterilized devices. The overall evaluation was based on a toxicological assessment of the risk of adverse health effects considering the nature and duration of exposure. The instrument and stapler reloads are externally communicating devices, limited duration (≤ 24 hour), indirect blood path; staples are implant devices, blood, permanent contact. For the stapler instrument, biocompatibility tests performed were ISO MEM elution cytotoxicity, ASTM direct contact and extract hemolysis, rabbit intracutaneous reactivity, guinea pig maximization sensitization, acute systemic toxicity and pyrogenicity. Additionally, staples were evaluated for *in vitro* and *in vivo* mutagenicity and intramuscular implantation in rabbits. Extractable substances from staples were identified by GC-MS and ICP-MS and then assessed for potential health risks. Ethylene oxide sterilization residuals were determined for the stapler reloads. The complexity of the

patient contacting portions of this surgical device is well illustrated by the diversity of materials, the different sterilization methods, and the distinct classifications under ISO 10993. Thus, a multi-faceted evaluation methodology is required to ensure biological safety of this sophisticated medical device.

ABSTRACT FINAL ID: 2781 Poster Board -570
TITLE: Development and Characterization of an *In Vivo* Inhalation Exposure System for Testing an Electronic Device That Heats Tobacco

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KEYWORDS: Tobacco, E-Cigarettes, Cigarettes

ABSTRACT BODY: The purpose of this project was to design, construct, and characterize a nose-only inhalation exposure system that could be used for the evaluation of an aerosol produced by a battery powered electronic device that heats tobacco. A cigarette smoking machine that is typically used for smoking combustible cigarettes to create a tobacco smoke aerosol was modified to hold the electronic device (Test) and used a modified Canadian intense puffing regimen to generate the aerosol. The Test product was manually turned on and loaded to the machine, the generated aerosol was delivered to a Cannon style nose-only exposure tower. 3R4F cigarettes were used as Reference product. The aerosol delivered to the nose-only exposure port was characterized over one hour by measuring temperature, humidity, wet total particulate matter (WTPM), and particle size. Selected chemical constituents were measured at the nose-port, including propylene glycol, nicotine, and selected carbonyls. Spatial and temporal stability among exposure ports were also monitored. The aerosol concentrations generated using this system were reproducible within the target range of 200 to 1200 µg/L WTPM. Aerodynamic particle sizes at the nose-port were approximately 2.1 (Test) and 0.7 µm (Reference). For chemical constituents, the test product delivered no measurable carbon monoxide (CO), substantially lower nicotine and carbonyl yields, but higher propylene glycol levels compared to the Reference product. In summary, we have developed and characterized an *in vivo* exposure system to evaluate aerosols produced by a battery powered electronic device that heats tobacco to generate an aerosol. This inhalation exposure system may also be adapted for exposures generated from electronic cigarette devices that use e-liquids rather than tobacco.

ABSTRACT FINAL ID: 2782 Poster Board -601
TITLE: Analytical Performance of ACCESS 2 Immunoassay System in Detecting Cardiac Troponin I in Animals' Serum

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KEYWORDS: ACCESS 2 Immunoassay System, Analytical Performance, Troponin I

ABSTRACT BODY: OBJECTIVE To validate the main performance of Access 2 immunoassay system in detecting Cardiac Troponin I (cTnI) in animals' serum from rats, dogs, and monkeys. METHODS The precision was evaluated by within-subject biological variation (CVwithin%) and between-subject biological variation (CVtotal%). The target values range of BIO-RAD immunoassay plus control samples at three levels were calculated by precision data. The accuracy was assessed by Relative Bias (Bias%) and target values. Carryover circumstance was evaluated by carryover rates. Linear was evaluated by regression equation. RESULT The within-subject biological variation (CVwithin%) of BIO-RAD immunoassay plus control samples at three levels were less than 8%, the between-subject biological variation (CVtotal%) of BIO-RAD immunoassay plus control samples were less than 63.75%; The within-subject biological variation (CVwithin%) of serum cTnI from rats, dog, and monkey were less than 8%, respectively. The target value range of BIO-RAD immunoassay plus control samples at three levels were at 0.3638 -0.4735 ng/mL, 1.8986 - 2.8193 ng/mL and 7.9503 -9.3676 ng/mL, respectively. The Bias% of cTnI was less than 16.32%, and the measured results were all within the respective target value range. The carryover rates of cTnI was <10% within the requirements. The equation of linearity range (0.0073-82.8433 ng/mL) of the theoretical value

and detected value was got after serial dilution, in which a value was within the range 0.95-1.05 (r>0.975). The low limit of detection (LLD) was 0.0076 ng/mL. CONCLUSION The precision, accuracy, carryover rates, and the linearity have a good performance, and the linearity range and the low limit of detection (LLD) of serum cTnI from rats, dogs and monkeys were established. In conclusion, Access 2 immunoassay system meets the requirements of the laboratories in non-clinical diagnosis.

ABSTRACT FINAL ID: 2783 Poster Board -602
TITLE: Effect of Cyclosporine A Treatment on Plasma Bile Acid Profiles and 7α-Hydroxy-4-Cholesten-3-One Levels in Rats

AUTHORS (FIRST INITIAL, LAST NAME): H. Schadt[1], D. Stiehl[1], M. Schwald[1], C. Vicente Vieira[1], A. Dietz[1], S. Chibout[1], P. Moulin[1], F. Pognan[1], A. Wolf[1]
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KEYWORDS: Bile Acids, Individual Bile Acids, Transporter

ABSTRACT BODY: Cyclosporine A (CsA) is an immunosuppressant drug that can cause cholestasis in few transplant patients. The underlying mechanisms to induce cholestasis are poorly understood, but inhibition of bile acid (BA) transport seems to play a role. In various publications an inhibitory effect of CsA on the hepatic BA transporters NTCP, OATP1B1/1B3, MRP2 and BSEP has been demonstrated *in vitro*. The *in vivo* relevance of these findings however remained questionable. In order to evaluate BA transporter inhibition *in vivo*, rats were treated by CsA and circulating BA components were investigated as potential biomarker candidates. Male Wistar rats were treated once daily with 20 mg/kg CsA for 1 week. Plasma BA profiles and 7α-hydroxy-4-cholesten-3-one (C4), a peripheral marker for Cyp7A1 activity and *de novo* BA synthesis, were analyzed by multiplexing LC-MS/MS and gene expression profiling was conducted in liver. Treatment with CsA resulted in a significant increase in the primary BA cholic acid, chenodeoxycholic acid and the muricholic acids in plasma with a trend towards decreased levels of secondary BA. No transcriptional changes in hepatic BA transporters or biosynthesis enzymes were observed by gene expression analysis. Despite the absence of transcriptional up-regulation of hepatic Cyp7A1, plasma C4 levels were slightly increased. Our results suggest that the observed changes in plasma BA profiles are likely the result of functional BA transporter inhibition and might serve as potential biomarker for BA transporter activity *in vivo*. C4 as a marker for *de novo* BA synthesis was also increased which suggests a role of hepatic transporters in C4 influx/efflux processes.

ABSTRACT FINAL ID: 2784 Poster Board -603
TITLE: Translational Qualification of Drug-Induced Vascular Injury Biomarkers

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KEYWORDS: Biomarker Qualification, Drug-Induced Vascular Injury, Clinical Translation

ABSTRACT BODY: Drug-induced vascular injury in animals (preclinical DIVI) is a toxicity characterized by hemorrhage, vascular endothelial and smooth muscle damage and inflammation. Because preclinical DIVI is of unknown relevance to humans, and because non-invasive biomarkers are not available for monitoring, DIVI findings often result in delays or termination of drug candidates. The European Union-funded SAFE-T Consortium and the Predictive Safety Testing Consortium (PSTC) are both supporting industry-wide efforts to achieve regulatory qualification of DIVI biomarkers. The two consortia have identified relevant preclinical and clinical biomarkers for evaluation in preclinical animal models and in clinical conditions that have morphologic similarities to preclinical DIVI. Serum samples were collected from vasculitis patients (active, n=91; remission, n=147), healthy volunteers (n=266), and patients undergoing coronary angiography (n=98) or balloon angioplasty (n=120 over 3 time points). Biomarker assays were developed and validated by Firalis SAS for a series of soluble markers (IP10, SAA, IL6, GROa, thrombomodulin, sICAM3, MIP-1a, p-selectin, VEGF, IL-8, E-selectin, VCAM-1, ICAM-1, MCP-1, CRP, I-TAC, and MIG) that were measured in clinical samples. Univariate statistical analysis resulted in poor performance for the ability of individual biomarkers to discriminate disease samples from healthy volunteers. Several

multivariate statistical methods were then used to identify combinations of biomarkers with improved performance. In one example, AUROC (Area under the Receiver Operating Characteristic) of the 5 best combinations was greater than 0.8. The SAFE-T Consortium is currently launching additional studies to confirm these findings in independent patient populations. The best-performing biomarker candidates will also be evaluated in animal models by the PSTC to enable the translational qualification of these biomarkers.

ABSTRACT FINAL ID: 2785 Poster Board -604
TITLE: Evaluating Studies Associating NHANES Exposure Biomarker Data with Disease for Use in Risk Assessment

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KEYWORDS: Biomarkers, Exposure/Biomonitoring, NHANES

ABSTRACT BODY: Studies associating NHANES exposure biomarker data (NHANES 1975-2010) with health outcomes were evaluated for their potential use in risk assessment. These studies model the statistical relationships between biomarkers of exposure and health outcomes of interest. Targeted studies look for associations between a specific marker (e.g., blood lead levels) with a specific disease (e.g., hypertension). Semi-targeted studies restrict one end of the relationship (typically the health outcome) while allowing the other end (number or chemical class of biomarkers) open to many possibilities. The majority of the studies identified were targeted studies (116 out of 121; 102 of which were cross-sectional). The majority of targeted studies evaluated "persistent or bioaccumulative compounds" including metals (49/116; primarily lead, cadmium, mercury) and persistent organic compounds (15/116; PAHs, dioxins, OCs); or toxic compounds including plasticizers (20/116; e.g., bisphenol a, phthalates). The top three adverse effects of interest were cardiovascular disease (24/116; hypertension, peripheral artery disease, heart failure), endocrine disruption (14/116; thyroid and reproductive hormones), and impaired lipid or glucose metabolism (17/116; diabetes, obesity). Some compound-disease associations were sufficiently consistent to suggest a possible causal relationship, however, many studies did not report consistent single- or similar compound-disease associations prompting the need for further prospective or experimental studies. General limitations of associative studies include: difficulty evaluating cumulative (mixture) effects on health or a compound's potential for multiple effects; limited standards for analysis and reporting; spot biomarkers that may be poor surrogates for exposure; and difficulty in comparing different subsets of populations and datasets from the domain of extant NHANES data. Further studies are being designed to address some of these challenges. [The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.]

ABSTRACT FINAL ID: 2786 Poster Board -605
TITLE: Role of Ethanol Stem Bark Extract of *Khaya senegalensis* in the Remediation of Sodium Arsenite-Induced Toxicities

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KEYWORDS: *Khaya senegalensis*, Arsenic, Micronucleus

ABSTRACT BODY: Occupational and domestic exposures to arsenic have been associated with cancer, organ injury and immunotoxicity in man and domestic animals. Consequently, therefore, notable medicinal plants are being screened in our laboratory and other laboratories for their protective effects against arsenic-induced toxicities. The use of *Khaya senegalensis* (the African mahogany) in traditional medicine as anti-diarrhea, anti-malarial and anti-microbial agent is well documented. We report here the effect of ethanol stem bark extract of *K. senegalensis* (ESKS) in the presence and absence of sodium arsenite (SA). Thirty male Wistar rats were randomly divided into six groups of five animals each. Group I rats served as negative control and were given distilled water only. Group II had SA at 2.5 mg/kg body weight. Groups III and IV

received ESKS at 50 and 100 mg/kg body weight respectively, and Groups V and VI received ESKS at 50 and 100 mg/kg body weight as well as SA at 2.5 mg/kg body weight respectively. All treatments were for fourteen days. SA significantly ($p<0.05$) increased the activities of the liver biomarker enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT), and number of micronucleated polychromatic erythrocytes (nMPCEs) in the bone marrow. Enzyme activities and nMPCEs were significantly ($p < 0.05$) reduced in the presence of ESKS (Groups V and VI) as compared with group II. Findings from this study suggest that ESKS can protect against SA-induced toxicities.

ABSTRACT FINAL ID: 2787 Poster Board -606
TITLE: Amphotericin B-Induced Candidate Renal Tubular Injury Biomarker Changes in Male Cynomolgus Monkeys

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KEYWORDS: Amphotericin B, Monkey, Nephrotoxicity

ABSTRACT BODY: Serum creatinine (sCr) and blood urea nitrogen (BUN) are nonspecific traditional biomarkers for monitoring drug induced kidney injury (DIKI). Most investigations of novel DIKI biomarkers have been carried out in rodents. In this study, we evaluated progressive changes in novel DIKI biomarkers in male cynomolgus monkeys following intravenous infusion of the nephrotoxicant, Amphotericin B (AmpB). Monkeys (5/group) were intravenously dosed at 0 and 0.4 mg/kg/day for 10 days. All animals were necropsied on Day 11. AmpB related kidney histopathology findings were characterized primarily as increased intra-tubular crystal deposition [interstitial], increased intra tubular casts and moderate to mild increased cortical tubular dilatation and basophilia. Amongst the traditional biomarkers examined, AmpB induced significant increase in sCr was apparent on Day 2, while significant increase in BUN appeared on Day 3; these parameters remained increased through termination. AmpB induced significant urinary N-acetyl β-glucosaminidase (uNAG) was evident on Day 2 and persisted throughout the duration of the study. AmpB induced significant increase in serum cystatin C (sCys C), urinary total protein (uTP), and urinary albumin (uAlb) was evident at termination only. Increases in kidney injury molecule 1 (KIM-1) and neutrophil gelatinase associated lipoprotein (NGAL) immunolabeling were localized in the damaged cortex medullary proximal tubular epithelial cells concurrent to moderate increases in urinary KIM-1 and urinary NGAL. AmpB induced acute changes in uNAG were indicative of proximal tubular alterations and appeared prior to increases in sCr, BUN, sCys C, uTP and uAlb. Concurrent changes in urinary and renal KIM-1 and NGAL appeared as candidate selective biomarkers for monitoring progressive AmpB induced tubular injury in monkeys.

ABSTRACT FINAL ID: 2788 Poster Board -607
TITLE: Association of Endotoxin Level in Dusts from Indoor Animal Husbandry Buildings with Chicken or Pig Immunity

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KEYWORDS: Endotoxin, Industrial Animals, Cell-Mediated Immunity

ABSTRACT BODY: Endotoxin, a lipopolysaccharide component of gram negative bacteria which is inherent in husbandry indoor environment, has been associated with various pulmonary illnesses. We investigated the relationship of endotoxin level in dust with the various immunological markers of chicken or pig from four and seven different farms, respectively, in Korea. Peripheral bloods from wing vein were obtained from 20 broiler chickens and 20 laying hens. Level of interferon-γ production from peripheral blood mononuclear cells (PBMC) stimulated with concanavalin A was significantly lower in the broilers or layers from the farms with higher endotoxin concentration than the chickens from the farms with lower endotoxin level. Regarding plasma cortisol level, the opposite trend was exhibited, in that higher cortisol level in the

chickens from the farms with higher endotoxin level. Among peripheral lymphocyte, percentage of CD3-Ia+ B cell was lower in the layers from the higher endotoxin farm than those from the lower endotoxin farm. Peripheral blood samples were also obtained from pigs' jugular veins. Significantly lowered interferon-γ production from PBMC stimulated with concanavalin A was resulted in the pigs from the farms with higher endotoxin concentration than the pigs from the farms with lower endotoxin level. In addition, CD4+-CD8+ double positive T cell population was also lowered in the pigs from the higher endotoxin farm. But, high endotoxin level group showed increased percentage and numbers of phagocytosing cells. Collectively, our results suggest the probable negative association of dust endotoxin level and cell-mediated immunity in chickens and pigs. [supported by Korean Rural Development Agency #PJ00867806]

ABSTRACT FINAL ID: 2789 Poster Board -608
TITLE: Assessment of Prenatal Exposure to Pyrethroid Pesticides and Metabolites in Umbilical Cord Blood

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KEYWORDS: Pyrethroid Pesticides, Cord Blood, Metabolites

ABSTRACT BODY: Regulatory changes banning the residential use of organophosphate (OP) pesticides during the past decade have led to the increased application of pyrethroid insecticides in commercial and residential environments. Pyrethroid insecticides are rapidly metabolized and excreted from the body, but recent epidemiological studies have highlighted the susceptibility of developing systems to chronic insecticide exposure. It is therefore important to assess the potential threat the use of these compounds in the home may pose to the developing fetus. We have developed a GC-MS/MS method for quantifying the presence of pyrethroid metabolites including: cis- and trans-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane carboxylic acid (cis/trans-DCCA), cis- and trans-chrysanthemum dicarboxylic acid (cis/trans-CDCA), cis-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid (cis-DBCA), 4-fluoro-3-phenoxybenzoic acid (4-F-PBA), and 3-phenoxybenzoic acid (3-PBA), in human serum from cord blood for the measurement of metabolites as a biomarker of fetal exposure. Sample preparation included liquid-liquid extraction followed by derivatization using hexafluoro-2-propanol and N,N'-diisopropylcarbodiimide. Five different solvents with or without acid were evaluated for recovery efficiencies: methyl-tertiary butyl ether (MTBE), acidified MTBE, and acidified dichloromethane (DCM), hexane, ethyl acetate, and acetone. Among the solvents tested, non-acidified MTBE and acidified DCM gave the greatest recoveries, which ranged from 0-79.2% for MTBE, 13.8-36.2% for acidified MTBE, and 21.5-65.17% for acidified DCM (cis/trans-CDCA were not recovered in acidified MTBE). This method is being used to determine pyrethroid metabolite concentrations in umbilical cord blood to estimate fetal exposure during the late third trimester of pregnancy. Supported by NIEHS P30ES005022, T32ES019854 and T32ES007148.

ABSTRACT FINAL ID: 2790 Poster Board -609
TITLE: Quantitative Multiplex Immunoassay Is a Novel Approach for Simultaneous Monitoring of Multiple Protein Biomarkers in Preclinical Rat Models of Drug-Induced Liver Injury

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KEYWORDS: Quantitative Multiplex Immunoassay

ABSTRACT BODY: The liver is a multifunctional organ. As drugs may cause liver injury through various mechanisms, a panel of biomarkers is required to ensure the potential for hepatotoxicity is identified early in drug development. To meet this need, we developed a Luminex® bead-based multiplex immunoassay (MILLIPLEX® Rat Liver Injury Magnetic Bead Panel) can simultaneously measure Predictive Safety Testing Consortium-endorsed hepatotoxicity biomarker ARG1, GOT1, GST-α, 5NT and SDH protein levels in the blood. These biomarkers are hepatocellular enzymes that are released into circulation upon

hepatotoxicity. Performance evaluation of the Panel revealed the robustness of assay sensitivity (MinDC at 12 pg/ml), specificity (no cross-activity), accuracy (80~100% recovery and linearity), precision (<10% intra-assay CV and <15% inter-assay CV) and wide linear dynamic range (3 logs). We stablished acute acetaminophen and thioacetamide-induced rat hepatotoxicity models in which centrilobular hepatocyte necrosis was present to biologically validate the panel. Significant blood protein dynamic changes were noticed in both models. Baseline protein levels are barely detectable; the hepatocellular enzymes were released into circulation massively upon hepatotoxicity. The peak increase (up to 250 folds) was observed at 24 hours post-dosing. Strongly correlated with the protein concentration data, robust increases in enzyme activities were detected. The protein levels gradually declined and returned to normal levels in approximately four days. In this study, we also measured 27 prominent cytokines by MILLIPLEX® Rat Cytokine/Chemokine Magnetic Bead Panel and revealed the dynamic change of 8 cytokines is paralleled with hepatocellular enzyme biomarkers indicating they are involved in the hepatotoxicity pathogenesis. The results of this study demonstrated the robustness and ease of multiplex immunoassays for multiple biomarker quantification. The MILLIPLEX® platform provides an ideal approach for investigators to monitor biomarkers in translational drug-induced liver injury studies.

ABSTRACT FINAL ID: 2791 Poster Board -610
TITLE: Assessment of Novel Multiplex Biomarkers of Nephrotoxicity in Canine Urine Samples

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KEYWORDS: Kidney Toxicity Biomarkers, Canine Kidney, Multiplex Assay

ABSTRACT BODY: Biomarkers of kidney damage are valuable for preclinical drug evaluation; however, existing markers such as blood urea nitrogen (BUN) and serum creatinine lack the sensitivity to detect nephrotoxicity before renal function is compromised. Recently identified urinary biomarkers of nephrotoxicity offer greater predictive value for preclinical assessment of drug safety. In this study, we developed a novel multiplex assay to measure four kidney toxicity biomarkers [Albumin, Beta 2-microglobulin (B2M), Trefoil factor 3 (TFF3), and Retinol-binding protein 4 (RBP4)] in the urine of dogs treated with the nephrotoxicant, gentamicin sulfate. Briefly, adult female beagles were housed in metabolic cages and urine was collected at the start of the study to establish baseline measurements. Dogs were given daily intramuscular injections of gentamicin sulfate (n=3) or saline (control; n=3) for a total of 7 days. Following the injections, dogs were allowed to recover for an additional period of 7 days. Urine samples were collected on days 1, 4, 8, 11, and 14 of the study. Protein biomarkers were measured from urine samples and compared to baseline concentrations. Albumin, B2M, TFF3, and RBP4 were elevated in the urine of gentamicin-treated dogs beginning 24 hours post-treatment. During the recovery phase, mean biomarker concentrations declined, but did not return to baseline levels. Interestingly, saline-injected dogs also exhibited biomarker induction, generally featuring a later onset relative to gentamicin, with mean concentrations often peaking during the recovery phase. In contrast to the multiplex biomarkers, serum creatinine and BUN concentrations were insensitive to gentamicin treatment. This new multiplex assay provides a practical resource for researchers evaluating nephrotoxicity in non-rodent models. Further, this study illustrates the importance of evaluating multiple biomarkers of nephrotoxicity to more precisely ascertain the preclinical effects of a drug in a canine model.

ABSTRACT FINAL ID: 2792 Poster Board -611
TITLE: Refinement of a Poly(ADP-Ribose) Polymerase Activity Assay

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KEYWORDS: Polymerase Activity, Assay, PARP

ABSTRACT BODY: Poly(ADP-Ribose) Polymerase (PARP), a ubiquitous nuclear repair signaling protein, senses DNA strand aberration and responds by catalyzing the transfer of ribose to histones, utilizing the oxidized nicotinamide adenine dinucleotide as the ribose donor. As a result, nicotinamide is produced whose α-alkyl-substituted amide moiety has served

as the active structural backbone for a growing class of experimental and pre-clinical compounds known as PARP inhibitors. As PARP-1, the most ubiquitous isoform, is post-translationally modified or cleaved, depending on cascades from several signaling pathways, the activity of this PARP isoform under instances of cellular injury, especially oxidative stress and death, is of functional importance. This investigation has refined a method currently available for measuring the activity of PARP-1 in cellular nuclear fraction extracts. Fraction V histones were plated on a high binding plate overnight, followed by an overnight blocking in 3% BSA. Either purified PARP enzyme or whole cell lysates were then assayed with a streptavidin-conjugated horseradish peroxidase-biotinylated NAD+ system with oxidized 3,3',5,5'-tetramethylbenzidine as the reporter; PARP activity was induced by incubation with 100 ng of sheared DNA. Exposure of commercially available purified PARP enzyme to varying concentrations of a known PARP inhibitor, 3-aminobenzamide, resulted in a Ki value of 2.4 µM, similar to published values. At 1.5 mM of biotinylated NAD+, the assay achieved sensitivity between 1 and 10 mU of PARP. Nuclear extracts of mouse liver (5 µg) were utilized to confirm PARP activity in lysates; co-administration of lysate to 3 mM 3-AB returned the colorimetric reporter to assay baseline, ensuring specificity.

ABSTRACT FINAL ID: 2793 Poster Board -612
TITLE: Comparison of Serum miRNAs, Serum Creatinine, and Blood Urea Nitrogen to Assess Nephrotoxicity in Male Rats Coexposed to Melamine and Cyanuric Acid

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KEYWORDS: Melamine, Kidney, Serum miRNAs

ABSTRACT BODY: The kidney failure outbreak that occurred in cats and dogs in the United States in 2007, due to the adulteration of pet food with melamine (MEL) and cyanuric acid (CYA) prompted the FDA to conduct a series of studies to characterize better the risks of nephrotoxicity due to co-exposure to MEL & CYA. In a previous study, we found a 28-day combined dietary exposure to MEL & CYA induced histological changes in the kidneys of male NCTR F344 rats dosed with 180 ppm or higher of MEL & CYA; however, the nephrotoxicity markers assessed originally [blood urea nitrogen (BUN) and serum creatinine] failed to detect the effects of treatment in the 180 ppm MEL & CYA dose group. BUN and serum creatinine levels were significantly increased only in rats fed doses of 240 ppm or higher. Serum microRNAs (miRNAs) have been proposed as useful markers of tissue injury or disease, potentially more sensitive than traditional biomarkers BUN and serum creatinine. We reported previously that rno-miR-144-3p levels were down-regulated in serum of male rats fed 240 ppm MEL & CYA. In the current study, we expanded our studies to screen the male rat serum miRNome (755 miRNAs) using quantitative real-time RT-PCR (qRT-PCR, n = 4/dose group). Eighteen miRNAs were significantly down-regulated (p-value < 0.05; fold-change > 1.5) in the group treated with 240 ppm MEL & CYA, with rno-miR-128-3p also being significantly down-regulated in serum of rats exposed to the 180 ppm dose. These data were validated by follow-up confirmatory qRT-PCR (n = 10-12/dose group). These miRNAs might be potential biomarkers of nephrotoxicity in male rats co-exposed to MEL & CYA, and rno-miR-128-3p may be more sensitive that BUN and serum creatinine. Future studies will investigate the temporal dynamics of these biomarkers and their profile upon a recovery period after a long-term period of exposure. IAG FDA 224-12-0003/NIEHS AES12013.

ABSTRACT FINAL ID: 2794 Poster Board -613
TITLE: Citrulline—A Potential Translational Safety Biomarker for the Small Intestine

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KEYWORDS: Citrulline, Small Intestine, Translational Biomarker

ABSTRACT BODY: Introduction: Plasma citrulline is an intermediary metabolic amino acid produced mainly by enterocytes of the small intestine. At present, citrulline is the only biomarker used in a clinical setting to quantify the enterocyte integrity in various small intestinal diseases. The present study investigated the value of plasma citrulline as a translational safety biomarker for unmonitorable intestinal toxicity in beagle dogs treated with a reversible and selective peptidase inhibitor, MS-229. Method: Dogs received oral doses of 0.75, 1.5 and 3 mg/kg/day over 4 weeks (5 males/5 females per group) or Methocel as vehicle control (3 males/3 females), followed by a 4-week recovery period. Clinical observations, food consumption, body weight determinations, toxicokinetics and pathology were performed in all dogs. Blood samples for investigations of citrulline were taken from all dogs during the pretreatment-, treatment- and recovery period. Results: Repeated administrations of 0.75 mg/kg/d were clinically tolerated and did not induce morphological alterations. At 1.5 mg/kg/d food consumption and body weight was moderately decreased and pathology revealed a reversible, mild to moderate mucosa degeneration, crypt necrosis and villi atrophy of the small intestine. The dose of 3 mg/kg/d was clinically not tolerated and moderate to marked mucosa degeneration, crypt necrosis and villi atrophy of the small intestine were observed. Further target organs were bone marrow and lymphatic system. Plasma citrulline concentrations decreased dose-dependently. Toxicokinetic data showed an almost dose-proportional increase in exposure to MSC2492280A. Conclusion: A dose- and exposure- dependent decrease in plasma citrulline was seen after repeated oral administration of MSC2492280A. This decrease in plasma citrulline correlated with pathological findings in the small intestine. The study results demonstrate that plasma citrulline is a potential translational safety biomarker for small intestinal toxicity in the dog.

ABSTRACT FINAL ID: 2795 Poster Board -614
TITLE: Induced Interstitial Pulmonary Fibrosis (IPF) Model: Unlabeled Bleomycin Distribution and Early Markers Identification by MALDI Imaging

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KEYWORDS: Pulmonary Fibrosis, MALDI Imaging Mass Spectrometry, Biomarkers

ABSTRACT BODY: Introduction: Interstitial Pulmonary Fibrosis (IPF) is a chronic and progressive lung disease. It is currently believed that fibrosis is caused by aberrant alveolar epithelial cell activation and repair leading to fibroblastic/myofibroblastic foci, accumulation of extracellular matrix and irreversible destruction of the lung tissue. Combined with classical histological staining, Mass Spectrometry Imaging (MSI) was used to improve the understanding of the Bleomycin IPF rat model and to identify several potential early biomarkers of this pathology. Experimental procedures: Rats were administered seven doses of Bleomycin delivered to the lungs at 1 mg/kg. Control animals received seven doses of saline. Both Bleomycin and saline were administered by oropharyngeal aspiration route. Three treated animals were followed for 7 days; three control and three treated animals were followed for 22 days. Lung sections were analyzed by MSI. Results: We describe a process which combines MSI and classical staining approach to follow the distribution of molecules implicated in fibrosis, and allows a better understanding of lung damage and repair. Indeed most of the identified biomarkers were located in extracellular medium and in the plasma membrane, as some upregulated lipids (included LPA18:0 and LPA16:0) and novel lipids not previously described. These lipids are known to be involved in cell signaling, chemotaxis or membrane stability, which might be associated with disregulated alveolar epithelial repair and/or fibrosis. Conclusions: Combination of MSI and histological staining provides information regarding molecules distribution and identification in the tissue by studying their co-distribution and by comparing their relative abundance at active sites of fibrosis. Here we identified a number of biomarkers which may be useful diagnostic and/or therapeutic targets and therefore useful tools to help understand the efficacy and safety of novel treatments in IPF.

ABSTRACT FINAL ID: 2796 Poster Board -615
TITLE: Classification of Hepatotoxicity Due to Cholestasis and Necrosis Using Transcriptomics on Human Precision-Cut Liver Slices

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Late-Breaking Abstracts

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KEYWORDS: Biomarker, Precision-Cut Liver Slice, Hepatotoxicity

ABSTRACT BODY: Human toxicity screening is an important stage in the development of safe drug candidates. Hepatotoxicity is one of the major reasons for withdrawal of drugs from the market due to the fact that the liver is the major organ involved in drug metabolism and it can generate toxic metabolites. There is a need to screen the molecules for drug-induced hepatotoxicity in human at an earlier stage. Transcriptomics is a technique widely used to screen molecules for toxicity and to unravel toxicity mechanisms. The aim of this study was to classify known hepatotoxicants on their phenotype of toxicity using gene expression profiles *ex vivo* in human precision-cut liver slices (PCLS). Hepatotoxicants which are known to induce either necrosis (n=5) or cholestasis (n=5) were used at concentrations inducing low (<20%) and medium (30-50%) toxicity, based on ATP or LDH. Random Forest and Support Vector Machine algorithms were used to classify hepatotoxicants using leave-one-compound-out cross-validation method. Optimized biomarkers sets for each of the cross-validation steps were used to derive a consensus list of markers. In this approach, the classification correctly predicts the phenotype of toxicity with an accuracy of 70-80%. The classification is slightly better for the low than for the medium toxicity. The consensus list of markers includes bile acid transporter (SLC10A7), cholesterol and lipid metabolism and transport (HACL1 and SORL1), heat shock and other stress response genes. This study shows that human PCLS are a useful model to predict the phenotype of drug-induced hepatotoxicity. Additional compounds should be included to confirm the consensus list of markers, which could then be used to develop a biomarker PCR-array for hepatotoxicity screening.

ABSTRACT FINAL ID: 2797 Poster Board -616
TITLE: Profiling of an AhR Response in Whole Blood

AUTHORS (FIRST INITIAL, LAST NAME): J. Yee[1], A. Vollrath[1], P. Geiger[2], G. Kennedy[2], Z. Zhang[2], J. Mezrich[2], C. A. Bradfield[1], K. Malecki[3]
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KEYWORDS: Aryl Hydrocarbon Receptor , Gene Expression Profiles

ABSTRACT BODY: The responsome is the collection of all physiological responses that can be influenced by the multitude of chemicals and environmental stimuli to which humans are exposed. Understanding the responsome could be used to help identify chemical exposures and understand their cumulative and aggregate effects on human health. In an initial effort to define the human responsome, we began examining changes in gene expression caused by activation of the aryl hydrocarbon receptor (AHR) pathway in an easily sampleable tissue, whole blood. To determine if blood provided a responsive system, we first exposed mice to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), collected blood, isolated its RNA and used quantitative PCR (qPCR) to look for the known biomarkers of AHR activation, Ahrr and Cyp1a1. Our results demonstrated a significant increase in Ahrr (p=0.0001) and Cyp1a1 (p=0.015). Next, we examined a human model to see if "blood" biomarkers of Ahr activation could be measured. We chose smokers based on the prediction that they are chronically exposed to high levels of polycyclic aromatic hydrocarbons (PAHs), known antagonists of the AHR pathway. Blood was collected from 19 smokers and 19 nonsmokers. RNA was isolated from the whole blood and was examined by qRT-PCR and microarray. Our results show that smokers had possible AHR activation, as seen in higher level of AHRR(p=0.013) expression. We were also able to identify novel biomarkers of smoking with the microarray. The genes we identified were GPR15, LRRN3, IGHD, IGLV3-1 and IGHA1. Improving the sensitivity of the model presented will allow us learn more about the chemical exposures of various individuals.

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ABSTRACT FINAL ID: 2798 Poster Board -617
TITLE: Using Targeted Metabolomics to Predict Drug Hepatotoxicity in Diversity-Outbred Mice

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KEYWORDS: Targeted Metabolomics, Drug Hepatotoxicity

ABSTRACT BODY: Quantifiable changes in endogenous serum metabolite patterns offer potentially sensitive and predictive biomarkers of drug hepatotoxicity, yet these changes during idiosyncratic drug-induced liver injury have not been studied owing to a lack of experimental models. Diversity Outbred (DO) mice, comprising genetic diversity comparable to humans, provide a model to identify such biomarkers and elucidate mechanisms underlying clinical idiosyncratic hepatotoxicity. In this study, changes in endogenous liver and serum metabolite patterns were characterized in DO mice in response to zileuton (asthma drug) treatment. Young adult female DO mice were orally dosed daily with either 300 mg/kg zileuton or vehicle control for 7 days (N=50/group). Pre- and post-dose serum Alanine aminotransferase (ALT) and microRNA-122 levels indicated presence of zileuton-induced liver injury. Concentrations of 216 metabolites including amino acids, biogenic amines, glycerophospholipids, acylcarnitines, sphingolipids, fatty acids, bile acids, and ∑hexose in post-necropsy liver and serum samples were measured using HPLC/Flow injection-MS/MS. PLS-DA and one-way ANOVA tests (FDR $p<0.05$) indicated significant differences in metabolite concentrations between the control and zileuton-treated groups in both serum (12 metabolites) and liver (27 metabolites). Post-sacrifice ALT concentrations were correlated with select metabolites ($p<0.05$). Ornithine and spermine were elevated in mice classified as sensitive to zileuton-induced hepatotoxicity by ALT fold-change and arginine levels decreased. These results indicate possible disruption of a common polyamine synthesis pathway (i.e. arginine -> ornithine -> putrescine -> spermidine -> spermine). These preliminary experiments suggest that a novel mouse population model of idiosyncratic hepatotoxicity, coupled with sensitive metabolomic detection, may offer insights into potential biomarkers that may inform safety assessment of potentially hepatotoxic drugs.

ABSTRACT FINAL ID: 2799 Poster Board -618
TITLE: Analyses of Urinary Biomarkers in Nonhuman Primate Kidney Transplant Recipients Dosed with a Blocking Anti-CD40 Antibody

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KEYWORDS: Biomarkers, Kidney

ABSTRACT BODY: The anti-CD40 antibody CFZ533 prolongs renal allograft survival in cynomolgus monkeys. To investigate correlations of urinary biomarkers from these transplanted animals to serum chemistry, histological parameters and gene signature, β2-microglobulin (β2M), Clusterin, Cystatin C, KIM-1 and NGAL were measured in urine collected on days 1, 3, 7, 10 and 14, followed by every 7 days thereafter until the end of the study. Peak urine biomarker increases were noted days 3-7 post-transplant relative to pre-operative levels, and were approximately 10-fold for Clusterin, 23- to 35-fold for Cystatin C, NGAL and KIM-1, and 832-fold for β2M. Increases in tubular and glomerular injury biomarkers followed a similar kinetic pattern with gradual resolution of acute increases up to day 14 followed by a variable pattern of values decreasing from peak after day 14. One animal demonstrated a discernible second peak for the tubular injury biomarkers, KIM1, and NGAL from day 35, with values that exceeded those in the acute phase. At necropsy (day 76), this animal had enlarged kidney allograft with microscopic tubular dilation and degenerative/regenerative changes. Allografts of the other animals showed no remarkable gross or histologic pathology or notable late-onset increase in the urine kidney biomarkers. The early post-operative kinetics of these biomarkers in all animals was likely due to cell injury during transplantation, in particular

ischemic reperfusion injury. The second peak in the single animal correlated with histologic findings and demonstrates the value of these biomarkers for detecting kidney injury including allograft dysfunction, demonstrating their value in a NHP transplantation model.

ABSTRACT FINAL ID: 2800 Poster Board -619
TITLE: Immunohistochemical Characterization of Candidate Biomarkers of Drug-Induced Vascular Injury in Rat

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KEYWORDS: Biomarkers, Immunohistochemistry, Vascular Injury

ABSTRACT BODY: Drug induced vascular injury (DIVI) in nonclinical species is a major hurdle to drug development due to the lack of sensitive and specific methods to non-invasively detect and/or monitor DIVI. The PSTC VIWG identified a panel of nonclinical circulating candidate biomarkers based on three primary features of vascular injury: endothelial cell (EC) changes, vascular smooth muscle (VSM) damage, and inflammation.The biomarkers were regulated in circulation following administration of vascular toxicants to rats and were validated using the PSTC guideline for advanced validation of nonclinical assays for safety biomarker qualification. Immunohistochemical (IHC) methods were developed in a variety of tissues expected to be both positive and negative for expression. These IHC studies in normal rat tissues were intended to aid in nonclinical qualification by assessing tissue specificity and distribution of selected candidates associated with VSM damage (SM actin(Acta2), transgelin (Tagln), caldesmon (Cald1), calponin 1 (Cnn1)), EC changes (lipocalin 2 (LCN2)) and inflammation (tissue inhibitor of metalloproteinase-1 (Timp1)). Acta2, Tagln, Cald1, Cnn1 were expressed in VSM and/or SM of multiple tissues, including mesentery, heart, pancreas, skin, spleen, stomach and lung. Expression of LCN2 was observed in vascular EC of multiple tissues, including mesentery which is a primary vascular bed affected in rats following administration of vascular toxicants., Timp1 expression was observed in inflammatory cells in multiple tissues. However, Timp1 expression also was observed in vascular ECs of multiple tissues, including mesentery. In addition, Acta2, Cald1 and LCN2 were also expressed by cells other than VSMC or vascular EC. The methods provide a framework that can be utilized in our nonclinical qualification strategy of the PSTC VIWG to support the biology of proposed candidate biomarkers and the pathology interpretation.

ABSTRACT FINAL ID: 2801 Poster Board -620
TITLE: Integrated Profiling Framework for Investigating PAH-Induced Metabolic Alterations

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KEYWORDS: Metabolomics, Biomarkers, Exposome

ABSTRACT BODY: Developing uniform chemical profiling is a critical component of environmental surveillance and sequencing the exposome. In this study, integrated metabolic profiling was applied to thirty non-identified serum samples obtained from the Department of Defense Serum Repository. High performance metabolomics were completed in tandem with quantification of 16 priority polycyclic aromatic hydrocarbons. Naphthalene, anthracene and benzo(a)pyrene were detected in all serum samples, with 50th percentile concentrations of 1.62, 1.63 and 20.9 ng/mL, respectively. Untargeted

metabolomics resulted in 6,010 unique chemical matches, with 625 present in the human metabolic map. Sparse partial least squares regression analysis identified dose dependent alterations in core biochemicals, including histamine, threonine and linoleate. Metabolome wide association of these metabolites included enrichment in linoleate and cysteine/methionine pathways. Comparison of intensity profiles between low and high exposure indicated increased expression of oxidative products, including 9(10)-EpOME and hypotaurine. Acetyl-methionine, which has been observed to protect against liver toxicity, was down regulated in higher exposed individuals. Due to BaP association with histamine, a panel of 33 inflammatory biomarkers were assayed, including IgE, cytokines and chemokines to identify PAH induced immune activation. With this approach, identification of relevant chemical exposures and dose-response characteristic could explicitly show whether pathophysiologic processes are likely to have been initiated by environmental factors.

ABSTRACT FINAL ID: 2802 Poster Board -621
TITLE: Exposure Assessment of BPA by Total "Backward" and Dietary "Forward" Intake Methods

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KEYWORDS: BPA, Exposure Assessment, BPA Dietary Intake

ABSTRACT BODY: Food consumption is believed to be a primary exposure pathway for BPA. Exposures such as thermal paper, dust and air may also contribute as sources of BPA total intake. The purpose of this study was to estimate adult BPA intakes using two different approaches that compare dietary only (i.e. forward) and total (i.e. backward) intake pathways. We measured BPA food concentrations in our own convenience food sample collection from supermarkets in Dallas, Texas and used published USEPA food intake estimates to calculate dietary BPA intakes. We used NHANES urinary BPA concentration data from the US general population to calculate total BPA intake estimates, which integrates all possible BPA exposure pathways. We calculated using the forward method a dietary BPA intake of 12.6 ng/kg-day of which 12.4 ng/kg-day came from canned foods. The backward approach yielded a central tendency of 30 to 70 ng/kg-day of total BPA intake. Our canned food BPA measurements were lower than reported in other studies including three from the US, but our findings of higher levels of BPA in canned foods relative to other foodstuffs was consistent with other studies. There are limitations that may explain our discrepancy in intake estimates with other studies. Our convenience food sampling may neither be extensive enough nor representative of all foods. Alternative BPA exposure pathways may also play a larger role in adult exposures. Our findings using two novel intake estimate methods suggest dietary exposures, especially canned foods, are substantial sources of BPA exposure; however, more integrated approaches that include multiple BPA exposure pathways are warranted. Larger, more representative sampling of food and other sources of BPA intake are also indicated.
Study was funded by NCI/NIH.

ABSTRACT FINAL ID: 2803 Poster Board -622
TITLE: World Trade Center-Derived Oxidative Stress and Inflammation in Nasal, Neural, and Pulmonary Tissues

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KEYWORDS: World Trade Center, Oxidative Stress, PM Exposure

ABSTRACT BODY: Currently, there is an overall lack of scientific data with regard to effects of World Trade Center dust (WTCPM) exposure and its inflammatory/oxidative potential. This study evaluated intranasally administered WTCPM<53µm derived oxidative stress in primary and secondary targets (nasal, neural, and pulmonary tissues). Male 12-15 week old C57Bl/6 mice were acutely exposed to 1mg/50µl WTC via intranasal instillation, with significantly observed

polymorphonuclear neutrophil (PMN) responses ranging from 20-30% in both nasal and bronchoalveolar lavage fluids. Repeated acute intranasal exposures at 1mg, 0.5mg, and 0.25mg/ 50µl WTC resulted in significant PMN influx (12-30%) in the lower and upper respiratory tracts. Most significantly, expression in APP (3.8-fold) and antioxidant genes Prdx2 (5.8-fold) and Txnrd6 (5.9-fold) were found to be upregulated in the olfactory bulbs of acutely exposed mice 24 hrs and 7 days post exposure. SOD-2 was found to be significantly downregulated in the olfactory epithelium, while HO-1 and SOD-2 were equally downregulated in pulmonary tissues after acute repeat exposures at varying doses of WTCPM. In a co-exposure scenario, mice were intranasally treated with 10ng/10µl LPS, 1mg/50µl WTC, or 10ng/10 µl LPS+1mg/50µl WTC, whereby WTCPM was found to significantly potentiate LPS exposure in the upper and lower airways (30-35% PMN increase), as well as SOD-2 downregulation in pulmonary tissues. These data suggest WTCPM exposure propagates intracellular antioxidant proteins in neural tissues, while concurrently depleting antioxidant responses in the nose and lung. These novel findings may be due to PM compositional effects (i.e. pH, metals, silica) or potential particle translocation. More importantly, biological cascades within the upper and lower respiratory system could be highly exploited and augmented in combination with other exposures resulting in co-exposure scenarios, further potentiating environmentally induced injuries/ diseases brought about by repetitive insult from ambient pollutants.

ABSTRACT FINAL ID: 2804 Poster Board -623
TITLE: Ratio of γ-H2AX Level in Lymphocytes to That in Granulocytes Detected Using Flow Cytometry As a Potential Biodosimeter for Radiation Exposure

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KEYWORDS: RL/G of γ-H2AX, Radiation, Biodosimeter

ABSTRACT BODY: This study aims to assess the use of ratio of γ-H2AX in lymphocytes to that in granulocytes (RL/G of γ-H2AX) in blood as a rapid method for population triage and dose estimation during large-scale radiation emergencies. After obtaining written informed consent from volunteers and ethical approval from the Subcommittee on Human Investigation of the Beijing Institute of Radiation Medicine (2012–0128), blood samples are collected from healthy volunteers and the blood samples are exposed to 0 Gy to 10 Gy of 60Co irradiation. The blood samples irradiated were cultured for 0 h to 24 h and then analysed using flow cytometry to measure the levels of γ-H2AX in lymphocytes and granulocytes. The basal levels of RL/G of γ-H2AX in healthy human blood, the response of RL/G of γ-H2AX to ionising radiation and its relationship to doses, time intervals after exposure and individual differences were also analysed. The level of γ-H2AX in lymphocytes increased in a dose-dependent manner after irradiation, whereas that in granulocytes was not affected. A linear dose–effect relationship with low inter-experimental and inter-individual variations was observed. Our results indicated the RL/G of γ-H2AX may be used as a biomarker for population triage and dose estimation during large-scale radiation emergencies if blood samples can be collected within 24 h.

ABSTRACT FINAL ID: 2805 Poster Board -624
TITLE: A Modified Molisch Test for Bisphenol A and Crystal Violet Lactone in Cash Receipts

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KEYWORDS: Bisphenols, Cash Receipts, Ring Test

ABSTRACT BODY: Bisphenol A (BPA) ranks among the top 2% of high volume chemicals produced in the U.S. It is used in the manufacture of printing materials, polycarbonate plastics and resins, all of which can become portals for human exposure. Among others, exposure to BPA is associated with endocrine disruption, birth defects and metabolic syndrome. The impact of BPA on human health has been debated with toxic effects becoming evident at doses below 0.05 mg/kg (currently permitted daily intake). The deleterious health impact of BPA is compounded further by reports showing that contact with

thermal paper (typically used at cash registers) can be a significant source of BPA exposure and absorption through the skin. In the present work, a simple test for the detection of BPA was attempted with minor modifications of the well-known Molisch test, extensively used for the detection of sugars, particularly monosaccharides and disaccharides. Cash receipts (CR) collected from different vendors in Baton Rouge, LA and approximately 1 sq. cm; *ca.* 100 mg were cut and extracted with 1 mL of isopropyl alcohol at room temperature. About 5 drops of this extract (*ca.* 0.1 mL) was mixed with 2 mL of 2% sugar solution (glucose, sucrose or galactose) in a clean test tube and mixed thoroughly. Following this, using a dropper, concentrated sulfuric acid (*ca.* 2 mL) was slowly added along the sides keeping the test tube slightly slanted but steady (to prevent mixing). A rapid development of pink, purple or dark brown ring at the interface of the two solutions was considered positive for BPA. Crystal violet lactone (CVL) which is also used in the production of thermal paper forms a similar ring at the interface. We observed that almost all cash receipts were positive for Molisch test (described here) indicating the presence of BPA, CVL, or both. The Molisch test described here can be a learning tool to introduce students to concepts in toxicology and increase awareness of exposure to hazardous pollutants.

ABSTRACT FINAL ID: 2806 Poster Board -625
TITLE: The Production of S-Phenylmercapturic Acid (PMA) Antisera and the Development of Benzene Biomonitoring Immunoassays for Laboratory and Point-of-Use Testing

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KEYWORDS: Biomonitoring of Exposure, Biomarker and Biomonitoring, Benzene

ABSTRACT BODY: Objectives: Benzene is an important industrial chemical, a ubiquitous pollutant and known carcinogen. PMA is a specific urinary metabolite of benzene and its measurement has facilitated benzene biomonitoring. However, current methods of analysis are laboratory based, laborious and provide slow sample turnaround. Immunoassays overcome these limitations and enable the development of point of use tests. Methods: Using PMA-protein conjugates biomarker specific antisera were induced in sheep. A urinary ELISA was developed, validated and used to detect PMA in workers exposed to benzene. A lateral flow test, which will allow "in the field screening," is being considered. Results: Test kits have been used to detect elevated levels of PMA in the urine of workers exposed to benzene. Occupational samples (n=39) determined by ELISA and GC-MS (range 0-1130µg/l) were in good agreement (Corr. Coeff. = 0.9). Optimisation of a lateral flow test is underway. For point of use testing careful consideration was given to urine hydration. The target is for a test cut-off which will distinguish between concentrated urines containing background levels and dilute urines containing elevated levels of PMA. Analysis of a UK database of occupational samples (n=2000) shows that a target cut-off of 7.5µg/l would result in 12% of samples screening positive. 7.5µg/l is roughly a third of the current US (ACGIH) guidance value and seems a reasonable level for requiring laboratory confirmation and possible workplace intervention. Conclusion: Immunoassays enable the production of cost-effective benzene biomonitoring tests. An ELISA kit has been produced which will allow laboratories to provide a routine test service. A point of use test is being optimised. This will enable on-site testing and provide immediate confirmation of good working practice. These developments will increase the utility of benzene biomonitoring and improve the protection of workers health.

ABSTRACT FINAL ID: 2807 Poster Board -626
TITLE: Genotoxic Effects of Benzene and Xylene on *Allium cepa* (Onion)

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KEYWORDS: Monocyclic Aromatic Hydrocarbons, Genotoxicity, Exposure Assessment

ABSTRACT BODY: Humans are exposed to many chemicals as a result of the air they breathe, food and water they ingest, or occupational contacts with these chemicals. There is a need then to determine the genotoxic effects of these chemicals on living systems. Genotoxic effects of two monocyclic aromatic hydrocarbons (MAHs), benzene and xylene, were evaluated in the root tip meristem cells of *Allium cepa*. Mitotic index and chromosomal aberration were used as indicators of genetoxicity. The effective concentration (EC50) values were found to be 1.39 ml/l and 0.074ml/l for benzene and xylene respectively. Reduction in mitotic index and chromosome aberration were significant ($p<0.05$) in cells exposed to different concentrations (0.05, 0.1, 0.5 and 1.0) of benzene and xylene. Chromosomal aberrations noticed in *Allium cepa* root tips include binucleated, vagrant, fragmented, abnormal anaphase, abnormal metaphase and abnormal telophase chromosomes. The suppression percentage in *Allium cepa* was significant in 1ml of the test chemicals. These studies demonstrated the usefulness of the *Allium cepa* root chromosomal aberration assay for assessing the genotoxicity of benzene, xylene and potentially other environmental chemicals.

ABSTRACT FINAL ID: 2808 Poster Board -627
TITLE: A Tool to Reduce Uncertainty in Risk Characterization: Combining Bioaccessibility of Metals in Soils with *In Vitro* Cell-Based Bioassays for Toxicity

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KEYWORDS: Bioavailability, Metals, HepG2

ABSTRACT BODY: While exposure to harmful contaminants such as asbestos, lead, and other heavy metals remain of great concern, events such as 9/11 and its subsequent aftermath and human health impacts caused by the explosion debris have taught us that potential contaminants can come in many forms. Some risk assessment tools for oral bioavailability are limited in their scope, and some, like *in vivo* animal models, are costly and time consuming, thereby limiting their widespread use. As a result, simulated human gastrointestinal fluids have emerged as an *in vitro* surrogate and have become a well-established tool for bioaccessibility estimates and a basis for *in vivo* bioavailability calculations. A human liver hepatocellular carcinoma cell line (HepG2) is employed to asses risk by evaluating the relationship between bioaccessible metal exposure levels, an estimate of bioavailability, and subsequent cell uptake and cytotoxicity. Additionally, a quantitative link between metal bioaccessibility to *in vitro* toxicological response would further validate and complement results of oral bioaccessibility and bioavailability measurements. Heavy metal contaminated soils from different U.S. regions were evaluated and in several cases these results highlight the potential value and utility for the use of a human cellular model in risk assessment. By using simulated human body fluids and human cells instead of animal model surrogates to evaluate the transport/transformation of heavy metals in dusts and soils, a clearer picture of what happens inside the human body post ingestion may be established.

ABSTRACT FINAL ID: 2809 Poster Board -628
TITLE: Comparisms of Haematological Profiles of Culture (Hormonal Sex Reversed) and Wild (Lagos Lagoon) Tilapia, *Sarotherodon melanotheron*

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KEYWORDS: Haematology, Sex Reverse, *Sarotherodon melanotheron*

ABSTRACT BODY: The present study compared the haematological profiles of sex reversed tilapia *Sarotherodon melanotheron* with wild tilapia from Lagos Lagoon. *S. melanotheron* fry were reared in six (6) plastic tanks for three (3) months, of which three (3) tanks served as treatment tanks while the other three (3) served as the control. The fry were fed with 17α-methyl testosterone enzyme, which functions as a sex reversal hormone. The fry were administered this hormone

for 30 days, to ensure complete sex reversal. All the *S. melanotheron* fry were reared to table size for duration of three (3) months. Wild specimens of *S. melanotheron* were also captured from the Lagos lagoon, and their blood samples were analyzed. The results showed that blood parameters like White Blood Cell (WBC), Urea and Creatainine, Plasma enzyme activities (alkaline phosphate, alanine transaminase, and aspartate transaminase) were highest in tilapia from the wild specimens compared with the sex reversed specimens. The Red Blood Cell (RBC), Haemoglobin Concentration (HGB), Mean Corpuscular Valve (MCV) were highest in sex reversed tilapia culture with the following values 0.67 × 109, 2.7g/dL, 14.7fL respectively and lowest in the wild specimens (RBC, 0.14 × 109, HGB, 1.7g/dL, MCV, 10.2fL) from Lagos Lagoon. In this study, the results showed that sex reversal in tilapia culture is not harmful to the metabolism of the fishes, and in fact, results in larger healthier fishes, than those caught from the heavily polluted waters of the Lagos lagoon.

ABSTRACT FINAL ID: 2810 Poster Board -629
TITLE: Characterization of Diacetyl Exposures Associated with the Preparation and Consumption of Unflavored Coffee

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KEYWORDS: Diacetyl, Bronchiolitis Obliterans, Popcorn Lung

ABSTRACT BODY: Diacetyl, a suspected cause of respiratory disorders in some food/flavorings workers, is also a natural component of many consumer goods, including roasted coffee. A study was conducted to characterize potential diacetyl exposures occurring in a coffee shop during the preparation and consumption of unflavored coffee. Long-term (3 hr) personal and area air samples were collected while one study participant, the barista, used commercial equipment to grind whole beans, and brew and pour over 20 pots of coffee into cups. Simultaneously, long-term (3 hr) personal samples were collected as two participants, the consumers, drank 1 cup of coffee each per hr. Short-term (15 min) samples were also collected in the breathing zone of the barista during which time the barista ground whole beans, brewed two pots and poured 16 cups of coffee. The simulation was conducted in duplicate in a 40.3 m3 room, and air sampling and analysis were conducted using the OSHA 1012 method. The long-term concentrations for the barista and area samples were similar, and ranged from 0.013-0.016 ppm with a mean of 0.015 ppm; long-term concentrations measured for the consumers were slightly lower and ranged from 0.010-0.014 ppm, with a mean of 0.013 ppm. All long-term measurements were at least two-fold higher than the Recommended Exposure Limit of 0.005 ppm (8-hr TWA) proposed by NIOSH, and all long-term samples exceeded the ACGIH Threshold Limit Value of 0.01 ppm (8-hr TWA) for diacetyl. Short-term concentrations ranged from below the limit of detection (<0.0047 ppm) to 0.016 ppm and increased throughout the course of each simulation. All short-term samples were slightly below the proposed NIOSH and ACGIH 15-min short-term exposure limits of 0.025 ppm and 0.02 ppm, respectively. Given that workers and consumers may be routinely exposed to natural sources of diacetyl at levels that exceed recommended occupational exposure limits, the practicality and scientific basis of the proposed and adopted occupational exposure limits for diacetyl merits further consideration.

ABSTRACT FINAL ID: 2811 Poster Board -630
TITLE: Indoor Air Chemistry: Comparative Study between Conventional Cigarette and Heat-Not-Burn Technology

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KEYWORDS: Indoor Air Quality, Risk Assessment

ABSTRACT BODY: Philip Morris International (PMI) is developing products with the potential to reduce the risk associated with smoking. The Tobacco Heating System (THS 2.2) operates by heating tobacco rather than burning it and results in an aerosol with substantially lower levels of harmful or potentially harmful constituents when compared to combustible cigarette (CC) smoke. Additionally, THS 2.2 does not produce sidestream aerosol in the same manner as CC, since aerosol is only generated when puffs are taken. Thus, the impact on air quality of using THS 2.2 indoors is expected to be very

different to CC. To verify this hypothesis, PMI built an environmentally controlled, furnished room and developed analytical methods to measure air pollutants under diverse simulated indoor environments focusing on: (i) ISO measurement standards for Environmental Tobacco Smoke and, (ii) selected carbonyls and volatile organic compounds. A study was conducted with 3 simulated conditions (office, hospitality, residential) with conditions defined according to CEN standard EN 15251:2007. Three test items were compared: CC (Marlboro Gold 6 mg), THS 2.2 and background. Each study was duplicated, resulting in 18 separate sessions in total, each with a duration of 5 hours, with 4 hours of sample collection. In case of statistical equivalence, no impact on air quality is reported. When levels are statistically above background, the levels are adjusted by subtraction of the background and reported (in mass per cubic meters). For CC, all analytes for the 3 conditions were above background. For THS 2.2, no difference was detected between background and THS 2.2 for fifteen of the eighteen analytes investigated, irrespective of the environmental conditions applied. For the 3 analytes that were demonstrated to be statistically increased between THS 2.2 and background (nicotine, acetaldehyde and nitric oxides), the levels measured for THS 2.2 were only slightly increased compared to the background, 1 or 2 orders of magnitude lower than levels for CC.

ABSTRACT FINAL ID: 2812 Poster Board -631
TITLE: Polybrominated Diphenyl Ethers (PBDE) and Their Hydroxlyated and Methoxylated Derivatives in Blood from E-Waste Recyclers, Commercial Fisherman, and Office Workers in the Puget Sound, WA, Region

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KEYWORDS: Occupational, Contaminants, Emerging

ABSTRACT BODY: The most important routes of human exposure to PBDEs are from contaminated food and contact with dust found in households and workplaces. Structurally related derivatives of PBDEs are the hydroxylated (OH-PBDEs) and methoxylated forms (MeO-PBDEs). Humans can metabolize some PBDEs into the hydroxylated forms, which is a concern due to greater health risks associated with OH-PBDEs. However, certain OH-PBDEs and MeO-PBDEs are also marine natural products and it is unclear although likely, that marine fish and shellfish, which bioaccumulate these compounds serve as a vector for human exposures. In this study, we are measuring approximately 50 different PBDE, OH-PBDEs and MeO-PBDEs in household / workplace dust and blood plasma samples provided by volunteers living in the Puget Sound region of Washington State and working in the commercial fishing, electronic recycling or non-specific office occupations. Prior to blood sampling, a two-week food consumption diary is obtained from each volunteer. The commercial fishing occupation is largely an outdoor activity that promotes above average seafood consumption while electronic recycling may expose workers to dust with higher than average levels of PBDEs. Results from analysis of 60 plasma samples (1/2 of the planned 120 volunteer enrollment) indicate the sum PBDE levels varied between 23 and 2740 ng/ml ww. The OH-PBDE and MeO-PBDE levels varied between 5 – 706 ng/ml ww and 0 – 930 ng/ml respectively. For 90% of the volunteers, the sum PBDE levels were greater than either the OH-PBDE and MeO-PBDEs. However, a few individuals had either OH-PBDE and/or MeO-PBDE levels that exceeded the sum PBDE concentrations. These individuals tended to have consumed the highest amounts of seafood (more than 5 and up to 18 servings/week). Electronic waste recyclers generally consumed low amounts of seafood and had PBDE, OH-PBDE and MeO-PBDE blood levels that were below average. Supported by NIOSH Grant 1R21OH010259-01A1.

ABSTRACT FINAL ID: 2813 Poster Board -632
TITLE: Lipidomics in Blood and Organs of Rats Administered Marijuana Extract and Its Association with Cardiovascular Risk Index

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KEYWORDS: Marijuana Extract, Lipidomics, Cardiovascular Risk

ABSTRACT BODY: Adverse effects have been reported concerning the acute and chronic use of marijuana. While its effect has been investigated in blood and related to cardiovascular diseases, its effects on lipid level in organs remain sparse. To investigate these effects and their association with a cardiovascular risk index, male rats were administered marijuana extract (12.5, 25 and 50mg/kg body weight) and the control rats were given olive oil daily for seven weeks. Blood and organs lipid profiles were determined while anthropogenic index (AI) was estimated. A significant increase was observed in cholesterol and phospholipid levels in the plasma and VLDL-LDL (dose-dependently). Significant decreases were observed in phospholipid levels in erythrocytes and HDL. Only the 12.5mg/kg dose caused an increase of 11 and 59% in plasma and HDL triaclyglycerol (TAG) respectively. AI increased as dose increased compared with control but was not dose dependent. Cholesterol levels decreased in the testes and brain and increased in heart and liver, while a non-dose dependent increase was observed in phospholipid levels compared with control. AI positively associated with phospholipid in brain, liver, testes, plasma and VLDL-LDL. AI also positively associated with cardiac, hepatic and VLDL-LDL cholesterol and with TAG levels in the brain and VLDL-LDL. AI was however negatively associated with pulmonary, splenic, testicular, HDL and brain cholesterol and with HDL and erythrocytes phospholipid. Furthermore, AI negatively associated with pulmonary, splenic and erythrocytes TAG. These results may explain, in part, the cardiovascular risk observed in marijuana users.

ABSTRACT FINAL ID: 2814 Poster Board -633
TITLE: The Exposome and Its Role in Chronic Human Disease

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KEYWORDS: Esposome, Biomarkers, EWAS

ABSTRACT BODY: It may be surprising to learn that the genetic heritability of respiratory disease, cardiovascular disease and cancer is very low: estimated to be 10% - 20%. The lack of empirical data supporting the notion of genes dictate disease has caused a shift towards whole genome sequencing in search of 'missing heritability' and 'genetic dark matter.' In contrast to the idea that genes and genes alone determine health, epidemiology posits a person's risk of succumbing to chronic disease is linked to his or her genome (G) and personal exposures. The exposome (E) has been defined to represent these lifetime exposures from both internal and external sources. Accordingly, 80% - 90% of chronic human disease is determined by E and GxE (including epigenetics). Comprehensive, unified instrumentation was developed and utilized to elaborate the human genome through the activities of the Human Genome Project. In contrast, causal exposure assessment still relies on de-facto self-reported and geographic information. However in the exposome model, sophisticated "omics" technologies are used to measure the breadth of exposures in the human system. These technologies include mass spectrometry and bioinformatics. The implementation of these technologies in exposome-wide association studies (EWAS) that elaborate the E-matrix in diseased and healthy populations, can be used to identify new biomarkers of both exposure and disease. Herein, analytical strategies to measure the exposome are defined and proof of principle data are presented.

ABSTRACT FINAL ID: 2815 Poster Board -634
TITLE: Concentrations of Polybrominated Diphenyl Ethers (PBDEs) in Residential Dust and Children's Blood Samples from Monterrey, Nuevo León, México

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KEYWORDS: Polybrominated Diphenyl Ethers, México, Children

ABSTRACT BODY: In México have been demonstrated exposure to Polybrominated Diphenyl ethers (PBDEs) in people living in different sites through the country. Then, the purpose of this study was to measure exposure levels of polybrominated diphenyl ethers (PBDEs) in the blood serum of children living in an industrial site in México; Monterrey, Nuevo León (n=45). We analyzed five PBDE congeners (BDE 47, BDE 99, BDE 100, BDE 153 and BDE 154) by gas chromatography–mass spectrometry. BDE 77 was used as internal quality control in each sample. In serum samples from Monterrey, Nuevo León, the level of total PBDEs was 25.9 ng/g lipid and BDE 154 was found as dominant congener, followed by BDE 100, BDE 99, BDE 153 and BDE 47. Moreover, we quantified the level of PBDEs in residential dust in different areas at the city of Monterrey (n=50). In this case, the mean total PBDEs levels was 13.0 ng per g of dust and BDE 153 as dominant congener (6.7 ng per g of dust). Total PBDE levels in serum and dust samples obtained in this study are in some cases higher than those reported in other parts of the world. Our data indicate that children living in industrial areas studied in this work are exposed to high levels of PBDEs.

ABSTRACT FINAL ID: 2816 Poster Board -635
TITLE: Ozone-Mediated Oxidation of Acesulfame Potassium: An Option for Waste-Water Treatment

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KEYWORDS: Acesulfame Potassium, Artificial Sweetener , Ozone-Mediated Oxidation

ABSTRACT BODY: Acesulfame potassium (ACK) is an artificial sweetener present in over 4,000 food products, and is marketed under the trade names *Sunnet* and *Sweet One*. It is about 200 times sweeter than sucrose, but like saccharine, has a bitter aftertaste. Due to its high thermal stability, ACK is often blended with other sweeteners to give a more sugar-like taste and mask aftertaste and possibly camouflaging for consumer acceptance. The daily intake of ACK is around 1g in a Western diet and is claimed to be excreted unmetabolized. Although approved by FDA, the long term toxicological effects of ACK are not well understood. Importantly, ACK is present in waste water effluent (>100 ng/L) and thus likely to seep into the ground water. In the present investigation, a method of ozone-mediated destruction of ACK was undertaken. The second order rate constants for the reaction of ACK-ozone is about 7-fold smaller than that of the indigo carmine-ozone reaction (indigo carmine is a known agent used for measurement of ozone). ACK-ozone reactions performed between pH 2.0 and 7.0 have nearly identical changes in the absorption spectrum as those performed in deionized water. This and the presence of a single isosbestic point for reactions of ACK-ozone performed at various pH suggest that the reaction is simple process wherein the initial step involves the formation of a primary ozonide (1,2,3-trioxolane) between ozone and the olefinic group of ACK. From that stage, there is normal breakdown of the primary ozonide to carbonyl oxide and aldehydic primary/secondary products.The reversed-phase HPLC analysis of the ozonation mixtures showed little or no evidence for the formation of a secondary ozonide(s) of ACK resulting from the retroaddition of carbonyl oxide and aldehydic products. Unlike certain other oxidant systems reported in the literature, ozone appears to remove ACK by >99% to innocuous products. Based on our results ozonation to oxidize trace amounts of ACK present in waste and runoff waters seems to be a strong possibility.

ABSTRACT FINAL ID: 2817 Poster Board -636
TITLE: Detection of Methyl Radicals in the Gas-Phase Chemistry of Phenoxyl Radical Cations of Bisphenol A (BPA) and Its Metabolites: A Missing Link in BPA-Induced Epigenetic C Imprinting

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KEYWORDS: DNA and Protein Methylation, Bisphenol Phenoxyl Radicals, Epigenetic Changes

ABSTRACT BODY: Bisphenol-A (BPA) is a semi-persistent organic pollutant and a known endocrine disruptor. The US National Toxicology Program considers BPA as "a chemical of some concern" and associates with in utero toxicity. Methylation plays a major role in epigenetic changes regulating various genes and protein expression. Low dose exposures of BPA in mouse alters methylation process in the brain especially in cytosine-guanosine islands. These changes in methylation patterns are believed to cause developmental adult onset hormonal carcinogenesis (e.g., prostate cancer). There has been a growing evidence from both animal and human studies supporting BPA-induced changes in global methylation patterns involving DNA as well as histone proteins. Previously, we have reported the synthesis of some putative chlorinated (*BBRC* 426, 215-220, 2012) and nitrated (*Acta Crystallogr Ser E* 67, o2556-2557) metabolites of BPA that could be formed in reactions of BPA with peroxynitrite ($\pm$ CO_2) and $HOCl/^{-}OCl$. In all our chemical characterizations using Gas Chromatography (GC) coupled with Electron Ionization-Mass Spectrometry (EI-MS) detection, we have consistently observed a base peak (major daughter fragment) with an *m/z* value of $[M-15]^{+}$ (indicates loss of methyl radicals from the molecular ion, $[M]^{+}$). A similar loss of methyl radicals from the $[M]^{+}$ was also observed when chlorinated and nitrated derivatives of BPA are subjected GC-EI-MS analysis. Based on these observations and accumulating evidence of BPA in epigenetic changes, we reason that the oxidative biotransformation(s) BPA and its metabolites could be a significant source of 'methyl radicals' and play a key role in DNA and histone protein methylation. Being phenolic compounds, we envision different scenarios where BPA and its metabolites can undergo 1-e^{-}-oxidation in biologicals systems. We present a strong case for the direct role of BPA in epigenetic imprinting and eventual developmental toxicity.

ABSTRACT FINAL ID: 2818 Poster Board -637
TITLE: Characterizing the Toxicity of MINA Using *In Vitro* and *In Vivo* Pharmacokinetic Approaches

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KEYWORDS: Oxime, Monoisonitrosoacetone, CYP450

ABSTRACT BODY: The current limitation of available oximes is their inability to readily cross the blood-brain barrier (BBB) and reactivate acetylcholinesterase (AChE). However, the tertiary oxime, monoisonitrosoacetone (MINA) readily enters the brain and has been reported to be effective against lethal nerve agent exposure in rodents and shown to prevent or terminate the seizures associated with agent exposure. This is in contrast to traditional oxime countermeasures which don't exhibit anti-convulsant properties, presumably because of their inability to cross the BBB and reactivate brain AChE. MINA has been observed to produce behavioral toxicity in various species at higher doses, consistent with cyanide poisoning. Previous studies suggest that cyanide formation may be directly responsible for the observed toxicity of MINA at high doses. Since the liver is the principal organ for drug metabolism, the metabolism of MINA by the cytochrome P450 enzyme family, using liver microsomes, was investigated. A method was developed to determine the concentration of MINA in microsomes and plasma using liquid chromatography tandem mass spectrometry. This method was applied to a microsomal stability assay using human, monkey and mouse microsomes and cDNA-expressed individual P450 enzymes. MINA was observed to have a relatively slow half-life (~60min) indicative of a stable compound which is not rapidly metabolized by the cytochrome P450 enzyme family. Furthermore, MINA metabolism was only observed in one of the CYP450 enzymes (CYP2C9). The *in vivo* clearance of MINA was also investigated in non-human primates where a half-life of ~20 min was observed. These data indicate that MINA may be only partially metabolized by liver microsomes in *in vivo* systems.

ABSTRACT FINAL ID: 2819 Poster Board -638
TITLE: Age-Dependent Human Hepatic CYP2C8 and 1A2 Expression

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KEYWORDS: CYP2C8 ontogeny, CYP1A2 ontogeny, Pyrethroid metabolism

ABSTRACT BODY: Predicting age-specific metabolism of pyrethroids is important in evaluating age-related sensitivity. Our goal is to use an *in vitro* to *in vivo* extrapolation (IVIVE) approach to predict pyrethroid metabolism for different ages incorporating enzyme ontogeny and expressed enzyme kinetic data. Multiple cytochrome P450s and carboxylesterase enzymes are responsible for metabolism of pyrethroids in humans. This study aimed to determine age-dependent expression levels of human hepatic CYP2C8 and 1A2, for which only limited ontogeny data were available, to support IVIVE. Liver microsomal fractions were prepared from 224 subjects with ages ranging from 8 weeks gestation to 17 years after birth. The CYP2C8 and 1A2 protein levels were measured by quantitative western blotting. The median CYP2C8 expression was significantly greater in samples after 35 postnatal days (n=122) than in fetal and neonatal samples (fetal to 35 days postnatal, n=102) (0 vs. 13.78 pmol/mg microsomal protein; $p<0.0001$). In contrast, the median CYP1A2 expression was significantly greater in samples after 4 months postnatal age (n=79) than in fetal and younger postnatal samples (fetal to 3 months postnatal, n=145) (0.0095 vs. 1.909 pmol/mg microsomal protein; $p<0.0001$). Both CYP2C8 and 1A2 expressions reach adult level within 6 months of age. CYP2C8 protein levels significantly correlated with those of CYP2C9, CYP2C19, and CYP3A4 ($p<0.05$). This study provides key data for IVIVE modeling of age-dependent pyrethroid metabolism and indicates that CYP2C8 and CYP1A2 ontogeny appear to be controlled by different mechanisms. (supported by the Council for Advancement of Pyrethroid Human Risk Assessment, LLC)

ABSTRACT FINAL ID: 2820 Poster Board -639
TITLE: Metabolism and Bioactivation of Sunitinib *In Vitro*

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KEYWORDS: Bioactivation, Biotransformation, Hepatotoxicity

ABSTRACT BODY: Sunitinib is an oral, multi-targeted receptor tyrosine kinase inhibitor used in anti-cancer therapy; however, its use is associated with severe liver toxicity. The purpose of this study was to characterize the metabolic pathways of sunitinib *in vitro* and evaluate the ability of sunitinib to undergo bioactivation as a potential underlying mechanism of drug toxicity. Sunitinib was incubated with human liver microsomal preparations or recombinantly expressed enzymes, and LC-MS/MS analysis was utilized for metabolite identification and structure elucidation. The primary metabolites identified from liver microsomal incubations were N-desethyl-sunitinib, hydroxy-sunitinib and glucuronide conjugates. Recombinant human UDP-glucuronosyltransferases (UGT) 1A9 was the major enzyme involved in formation of the sunitinib glucuronide metabolite. In addition, glutathione (GSH) conjugates of sunitinib were detected from glutathione trappings studies in human liver microsomes, and the GSH conjugates were formed in an NADPH-dependent manner. The proposed bioactivation mechanism involves epoxidation, followed by oxidative defluorination to form a reactive quinone imine. These findings provide direct evidence for the formation of reactive intermediates from sunitinib and offer novel insights into the potential role of drug metabolism in sunitinib-induced hepatotoxicity.

ABSTRACT FINAL ID: 2821 Poster Board -640
TITLE: The Role of Peroxisome Proliferator-Activated Receptor-Alpha in the Relationship between Trichloroethylene Toxicokinetics and Toxicodynamics

2015 Society of Toxicology Annual Meeting
Late-Breaking Abstracts

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KEYWORDS: Superfund, PPAR, Toxicokinetics

ABSTRACT BODY: Exposure to the ubiquitous environmental contaminant trichloroethylene (TCE) is associated with cancer and non-cancer toxicity. Peroxisome proliferator-activated receptor-alpha (PPARα) is thought to be a critical element in toxicodynamics of TCE; however, the role of PPARα in TCE toxicokinetics has not been elucidated. We hypothesized that TCE toxicokinetics would be independent of PPARα status. To this end, male and female wild-type (WT), Pparα-null, and humanized PPARα (hPPARα) mice were exposed to 400 mg/kg TCE (i.g.) in single- (time-course) and repeat-dose (5d/wk, 4 wk) studies. Interestingly, following a single- or repeat-dose exposure to TCE, levels of trichloroacetic acid (TCA) were greater in liver and kidney of male WT mice compared to other strains. Levels of trichloroethanol (TCOH) were similar in all strains. Males consistently had higher levels of TCA and TCOH in all tissues compared to females. In single- and repeat-dose studies, a similar degree of induction of PPARα-responsive genes was observed in liver and kidney of hPPARα and WT mice, despite the difference in hepatic and renal TCA levels. Additional sex- and strain-dependent effects were observed in the liver, including hepatocyte proliferation and oxidative stress, which were not dependent on TCA or TCOH levels. In summary, these data refute the hypothesis and provide evidence that PPARα status does affect the relationship between TCE toxicokinetics and toxicodynamics in liver and kidney, which may be an important consideration for health assessments of TCE.

ABSTRACT FINAL ID: 2822 Poster Board -641
TITLE: Evaluation of Empirically Based PBPK Modeling Approaches for Modeling Ante- and Postmortem Ethanol Concentrations in Biological Matrices

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KEYWORDS: Ethanol, PBPK Model, Alcohol Neoformation

ABSTRACT BODY: Ethanol concentrations in biological matrices offer information regarding intoxication of an individual at the time of death. Antemortem levels of ethanol are typically calculated retrospectively from postmortem findings with little consideration regarding the change in ethanol concentration with time after death. Uncertainties such as environmental conditions surrounding the body, biological matrices, storage and analytical methodology are associated with retrospective calculations. The objectives of this study were to: 1) evaluate the typical relationships between ethanol concentrations in various biological matrices, 2) compare and contrast existing PBPK modeling approaches for determining ethanol concentrations under normal living physiological conditions, 3) present an empirical modeling approach for correlating postmortem ethanol concentrations with PBPK modeled antemortem concentrations up until the time of death, and 4) describe best practices for determining ante- and post-mortem ethanol concentrations with a focus on potential sources of error. In order to generate an empirical modeling approach, we evaluated existing parameters including ADME, body type, the Widmark factor as well as novel factors including alcohol tolerance and ADH level. The PBPK modeling approach also includes a novel method for superimposition of multiple drinks consumed at various times, and suggestions for adjusting the elimination rate based on body weight. We analyzed available data on postmortem alcohol production and identified conditions and potential markers for ethanol production through decomposition and putrefaction. Several examples and case studies are provided. These data indicate that this novel empirical model provided an accurate

estimation of ethanol concentration while minimizing potential sources of error. This study provides further data to help standardize the process of determining ethanol concentration in the field of forensic toxicology while minimizing uncertainties in real world cases.

ABSTRACT FINAL ID: 2823 Poster Board -642
TITLE: LC-MS/MS Method Development and Validation for the Quantification of Propofol Hemisuccinate and Propofol Concentrations in Mouse Blood

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KEYWORDS: LC-MS/MS, Propofol Hemisuccinate, Prodrug

ABSTRACT BODY: Propofol hemisuccinate (PHS) is a water soluble prodrug of propofol being developed for intrapulmonary administration as an antiseizure and antimigraine agent. When deposited into the lung, PHS confers protection against seizures in rodent models. PHS is converted to propofol by nonspecific esterases. To determine the site and kinetics of the conversion of PHS to propofol following intrapulmonary delivery we developed an LC-MS/MS method that can simultaneously determine PHS and propofol concentrations in mouse blood. We first diluted the blood samples with water and then precipitated plasma proteins with acetonitrile to stop esterase reaction. The internal standard, thymol (m/z=149.2), was included in the acetonitrile. Phospholipids and proteins remaining in the supernatant were removed with a Biotage PLD+ filter. The filtrate was further diluted with water and injected into the LC-MS/MS system. PHS and propofol were separated on a Waters BEH C18 column and PHS was first eluted and fragmented in-source to remove the hemisuccinate group, forming propofol (m/z=177.1). Propofol in the mouse blood was then eluted after the propofol generated from PHS as a second propofol peak. Both peaks were monitored at APCI (atmospheric pressure chemical ionization) negative SIM (single ion monitoring) mode. Extraction yields were all above 100% and the matrix effect were -9.89% for PHS, -0.07% for propofol, and -14.58% for thymol. The detection range is from 5 ng/ml to 2000 ng/ml, with an LLOQ at 5 ng/ml for both compounds.

ABSTRACT FINAL ID: 2824 Poster Board -643
TITLE: Targeting M2a Polarized Rat Macrophages Using Mannosylated Nanocarriers

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KEYWORDS: Nanocarrier, Macrophage Polarization, Nanotechnology

ABSTRACT BODY: Upon trauma, infection, or tissue stress, resting macrophages are activated (polarized) into two main phenotypes, M1 and M2a. Polarized macrophages are involved in a number of pathological conditions including fibrosis, tuberculosis, atherosclerosis and tumor development. The mannose receptor (MR), a marker of the M2a phenotype, is over-expressed in M2a but not in M1 macrophages. The feasibility of targeting M2a phenotypic macrophages with optimized mannosylated nanocarriers (NC) was investigated. Rat primary peritoneal macrophages (PMs) were polarized into M1 and M2a cells with IFN- γ and IL-4/IL-13, respectively. Expression of the M1 marker iNOs, M2 marker Arg-1 and M2a marker at both the mRNA and protein levels showed that PMs were well polarized. FITC-labeled mannosylated NCs were synthesized using solid phase synthesis and confirmed by MALDI-TOF and HPLC. The M1 and M2a PMs were treated with the FITC-labeled NC in culture, examined by confocal microscopy and their intracellular fluorescence was quantified.

The results showed that uptake of mannosylated NC in M2a PMs was 2.4-fold and 11.8-fold higher than in resting PMs and M1 PMs, respectively, in agreement with the marker expression levels. Mannan, a mannose competitive inhibitor, abrogated NC uptake. In addition, NCs colocalized with a fluid phase endocytosis marker, suggesting that uptake was mediated by MR-mediated endocytosis. In conclusion, the current study demonstrates that a specific polarized population of M2a phenotypic macrophages could be selectively targeted using mannosylated NCs potentially providing a foundation for nanocarrier-based immunotherapy. Support: NIH AI051214 and the CounterACT Program through the National Institute of Arthritis and Musculoskeletal and Skin Diseases (Award U54AR055073).

ABSTRACT FINAL ID: 2825 Poster Board -644
TITLE: Urban PM2.5 Extracts from Puerto Rico Induces ABCC1 and ABCC3 Gene Expression and Proinflammatory Mediators in BEAS-2B

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KEYWORDS: BEAS-2B, Particulate Matter, ABCC

ABSTRACT BODY: Incidence of Asthma in Puerto Rican is 113% higher than in the non-Hispanic white and 50% higher than non-Hispanic black population. Asthma exacerbation in children has been reported to increase during seasonal events of particular matter (PM). Several pulmonary drugs are substrates for ABC transporters and therefore, the delivery of these drugs to the site of action may be highly dependent on the presence and activity of many ABC transporters in several cell types. The role of altered function of ABC transporters in highly prevalent pulmonary diseases such as asthma or chronic obstructive pulmonary disease (COPD) has hardly been investigated so far. We have initiated the study on expression of ATP-Binding Cassette genes upon exposure to airborne PM 2.5 using the human bronchial epithelial cell line (BEAS-2B) as model. PM2.5 was collected in an urban industrial site of Guayama, Puerto Rico. Filters were extracted with microwave assisted extraction system (MAE). Extract toxicity was determined using the neutral red bioassay in BEAS-2B cells. citotoxicity for this extract was determined at 50 µg/ml. Subsequent cell exposure were performed at 25µg/ml (a non-toxic concentration) of organic extract and mRNA isolated at different times intervals (5, 6, and 7 hr). ABCC 1 and 3 cDNAs were obtained from transcribed mRNA samples and amplified with using Taq-man gene expression assay from Applied Bio-system. A significant increase in ABCC1 and ABCC3 mRNA levels was observed at 6 and 7hrs. IL-6, IL-8, and IL-10 were measured using the multi-analyte profiling kit from R & D Systems on a Luminex 200. IL-6 and IL-8.were significantly induced at 5 hrs at 25ug/ml. ABCC1, and ABCC3 follow IL6 and IL8 induction after PM2.5 organic extract exposure. Insight in the function of ABC transporters in the lung may open new avenues to facilitate treatment of lung disease.

ABSTRACT FINAL ID: 2826 Poster Board -645
TITLE: Refining Margin of Exposures for High-Throughput Chemical Risk Assessments: Benfluralin—A Case Study

AUTHORS (FIRST INITIAL, LAST NAME): S. Chaudhuri[1], F. Zhang[1], M. J. Bartels[1], S. Papineni[2], D. Wilson[1], S. Marty[1]
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KEYWORDS: IVIVE, Biological Modeling, Risk Assessment

ABSTRACT BODY: The purpose of this study was to develop a method for high-throughput Margin of Exposure (MOE) assessments for chemicals. Earlier initiatives by Wetmore et al (2010) included a well-designed reverse dosimetry approach utilizing data from *in vitro* assays (metabolic clearance, plasma protein binding) and integrating them into a PK model of steady state exposure. Our initiative defines a process for refining these default assessments utilizing additional exposure information as well as improved modeling tools. A case study example of this process is presented for the agricultural product, Benfluralin. As opposed to the prior reverse-dosimetry approach (blood levels modeled at 1 mg/kg exposure, then scaled to dietary uptake levels), a forward dosimetry approach is utilized to best model blood levels at relevant dietary

concentrations (US EPA). Measured values of plasma protein binding and hepatic clearance were incorporated into a PBPK model (GastroPlus) to predict systemic exposure in a physiologically relevant design, which included QSAR-based estimates of fractional absorption in the gut vs. a default assumption of 100% uptake. Additionally, age based physiology was modeled, vs the prior default of adult age only. MOE values were calculated utilizing three approaches in adults and children- the default PK approach, the PK approach corrected for fractional absorption, and the PBPK approach. Comparisons were made with the most sensitive endocrine (E) and non endocrine (NE) assays from the ToxCast datasets. MOE values in adults were 52957 (E) and 68845 (NE). These values improved 3-fold (higher) after including the predicted fraction absorbed in the gut, and greater than 100x (higher) via PBPK modeling. In summary, the refined process for deriving MOE values from *in vitro* bioprofiling data affords more realistic exposure assessments, as well as providing the ability to model both steady-state and acute exposures.

ABSTRACT FINAL ID: 2827 Poster Board -646
TITLE: Inhalation Dosimetry of the Flavoring Agents Diacetyl, 2, 3-Pentanedione and Acetoin in the Rat and Human Respiratory Tract

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KEYWORDS: Respiratory Dosimetry, Computational Fluid Dynamics, Dose Response

ABSTRACT BODY: Anatomically accurate computational fluid dynamics (CFD) models of the upper respiratory tracts of the rat and human were used to simulate inhaled airflow and vapor uptake of the flavoring agents diacetyl, 2, 3-pentanedione, and acetoin. The Multiple-Path Particle Dosimetry (MPPD) model was used to simulate vapor uptake in each airway generation of the lungs. Absorption rates were calculated from air:tissue partitioning, diffusivity, and saturable metabolism. At an exposure concentration of 1 ppm, predicted nasal uptake of diacetyl, 2, 3-pentanedione, and acetoin was 37, 30, and 74% in the rat and 9, 10, and 33% in the human, respectively. Total respiratory tract uptake was predicted to be between 90 and 98% for each compound in the rat and human, although human lung uptake predictions were higher due to lower nasal extraction. Wall mass flux predictions in the upper lung airways were higher in the rat but MPPD predictions showed greater penetration of each vapor into lower tracheobronchial airways in the human. Dosimetry results from the CFD and MPPD models were used in conjunction with pathology data from nose-only inhalation exposure studies (animals were exposed 6 hours/day, 5 days/week for two weeks at target concentrations of 8.8, 17.5, and 35 ppm) to develop dose-response relationships for each compound. At inhalation exposure concentrations equal to the NOAEL (17.5 and 8.8 ppm for diacetyl and 2, 3-pentanedione, respectively) or NOEL (35 ppm for acetoin), human nasal and lung flux values were less than the rat nasal flux values for all three flavoring agents. This work demonstrates that nasal and lung dosimetry predictions may be used to precisely relate exposures to site-specific doses throughout the respiratory tract for risk assessment of inhaled vapors. This study was funded by the Research Institute for Fragrance Materials, Inc.

ABSTRACT FINAL ID: 2828 Poster Board -647
TITLE: Physiologically Based Pharmacokinetic (PBPK) Model for Assessing Dermal Exposures to Ethanol

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KEYWORDS: PBPK Modeling, Dermal Exposure, Hand Sanitizer

ABSTRACT BODY: The use of alcohol-based hand sanitizers (ABHSs) in the healthcare industry is widespread. It is, thus, desirable to evaluate the exposure of healthcare workers to ethanol in ABHSs. To support the assessment of potential hazards, the published PBPK model of Martin et al. (2014) was modified to include a skin compartment to allow for simulation of complex dermal exposures consistent with hand sanitizer use. In addition, the model was expanded to include

the hepatic formation and urinary excretion of ethyl glucuronide, since this metabolite is frequently used as a biomarker for ethanol exposures. Data sets from the published literature were used to parameterize and validate the updated PBPK model. Dermal absorption of ethanol from ABHSs is predicted to be low (less than 1% of the applied dose), even in healthcare workers.. The PBPK model was used to support the toxicity, exposure, and risk characterization components of a human health risk assessment for repetitive, chronic use of ABHSs by healthcare workers. The PBPK model was used to estimate the blood alcohol concentration of ethanol for key animal toxicity and epidemiology studies. For the exposure assessment, blood alcohol levels were modeled for several exposure scenarios, including "maximum use scenarios" as defined by FDA. For the risk characterization, comparative risk scenarios (e.g., non-alcoholic beverages) were evaluated. Even under hypothetical maximum use scenarios, peak blood concentrations of ethanol remain below 0.01%. The relative importance of route of exposure is discussed. Data needs for the dermal component of the model are identified.

ABSTRACT FINAL ID: 2829 Poster Board -648
TITLE: Toward a Unified Computational Model for Predicting Nanomaterial Disposition

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KEYWORDS: Nanotoxicology, Computational Toxicology, Biological Modeling

ABSTRACT BODY: Physiologically-based pharmacokinetic (PBPK) models are an important tool for extrapolation of chemical risk across exposure routes and toxicity test systems. But current PBPK techniques which work well for chemicals are of limited use for nanomaterials (NMs) due to the complex dependence of kinetics on particle properties such as shape, size and surface chemistry. As a result, most PK models for NMs are partly empirical, requiring some parameters to be estimated from *in vivo* data, thus yielding models of limited predictive capability. The purpose of this research was to construct a first-of-kind PBPK model that includes explicit representation of fundamental processes related NM biodistribution. Objectives included: 1) support for diverse NM types, 2) prediction of organ/cellular quantities of NMs, and 3) no need for calibration of model parameters from *in vivo* data. To this end, we have developed a PBPK model requiring only input data that can be determined from easily measured NM properties, or from high-throughput *in vitro* tests. The resulting model includes representation of: transport of particles across the vascular membrane through pores and endosomes, and clearance from interstitia via lymphatic circulation; NM-cell interactions such as phagocytosis and endocytotic processes (adsorption/desorption/internalization); binding site occupancy on tissue cells; and NM-protein interactions which influence disposition. A set of novel *in vitro* techniques capable of determining necessary model input quantities was additionally defined. Tissue-specific cellular level concentration profiles predicted by the initial version of the model were compared against *in vivo* data for PAA-PEG and PLGA-mPEG nanoparticles in rats. Predictions agreed with measured data to within a factor of two for most tissues. Future model refinements will include effects of protein corona and target-mediated disposition, development of additional *in vitro* assays for identifying specific input quantities, and computation of model parameters from *ab initio* methods (molecular dynamics simulation).

ABSTRACT FINAL ID: 2830 Poster Board -649
TITLE: Secondary Methylation of Arsenic Is Modified by Folate, Cobalamin, and Homocysteine in a Population of US Adults

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KEYWORDS: Arsenic, Folate, Methylation

ABSTRACT BODY: Arsenic (As) is an environmental contaminant known to increase risk for the development of several diseases. Inorganic As is methylated via the one-carbon metabolism pathway, facilitating urinary As elimination. Monomethlyarsenic (MMA) is more toxic than dimethylarsenic (DMA), and higher proportions of MMA are associated with disease. Though several studies have shown folate status to be an important factor in determining methylation capacity,

they have typically been conducted in nutrient-deplete populations with high As exposure. The objective of this study was to characterize the relationships between As methylation capacity and several nutrients in a folate-fortified US population where As exposure occurs at lower levels. A cross-sectional study was conducted in 678 adults from the National Health and Nutrition Examination Survey (NHANES) conducted in 2003-2004 with measures of urinary As metabolites, plasma and red blood cell folate, cobalamin, vitamin B6 and homocysteine. Secondary methylation index (SMI) is the ratio of DMA:MMA, and %DMA was calculated as a function of (total DMA/ total urinary As). The population exhibited deficiencies in serum folate (8.11%), red cell folate (14.45%), cobalamin (20.50%), and B6 (26.84%). In linear regression analyses adjusted for sex, BMI, smoking, race and urinary creatinine, SMI was positively associated with cobalamin (β = 0.114, [0.009, 0.219], p = 0.032). Percent DMA was not associated with cobalamin, though it was negatively associated with vitamin B6 (β = -0.096, [-0.145, -0.046], p = 0.0002). Percent DMA was positively associated with serum folate (β = 0.086, [-0.018,0.191], p = 0.10) and negatively associated with homocysteine (β= -0.123, [-0.271, 0.025], p = 0.10). These findings indicate that in a largely nutrient-replete population, folate and cobalamin influence the metabolism of As. Our unexpected finding that vitamin B6 is negatively associated with %DMA warrants further study.

ABSTRACT FINAL ID: 2831 Poster Board -650
TITLE: 2,4-Dichlorophenoxyacetic Acid and Non-Hodgkin's Lymphoma, Gastric Cancer, and Prostate Cancer: Meta-Analyses of the Published Literature

AUTHORS (FIRST INITIAL, LAST NAME): C. Loftus[1], J. E. Goodman[1], K. Zu[1]
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KEYWORDS: 2,4-D, Cancer, Meta-Analysis

ABSTRACT BODY: Despite evidence from experimental studies indicating that the herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D), is not carcinogenic, several epidemiology studies have evaluated links between 2,4-D and cancer. Some studies suggest that 2,4-D is associated with non-Hodgkin's lymphoma (NHL), gastric cancer, and prostate cancer, but results have been inconsistent. We conducted meta-analyses to evaluate the weight of epidemiologic evidence for these cancers. We identified articles from PubMed, Scopus, and TOXLINE databases and review article citations. We evaluated study quality and calculated summary risk estimates using random-effects models. We conducted subgroup and sensitivity analyses data permitting. We identified studies evaluating NHL (9), gastric cancer (3), and prostate cancer (2) for inclusion in our meta-analysis. Exposure to 2,4-D was not associated with increased risk of NHL (RR = 0.97, 95% CI = 0.77-1.22, I-squared = 28.8%). This null association was robust to subgroup analyses by study design, type of exposure, geographic location, and sex of the participants and was generally insensitive to variations in study selection. In addition, 2,4-D exposure was not associated with increased risk of gastric (RR = 1.14, 95% CI = 0.62-2.10, I-squared = 54.9%) or prostate cancer (RR = 1.32, 95% CI = 0.37-4.69, I-squared = 87.0%). In conclusion, the epidemiologic evidence does not support an association between 2,4-D and NHL, gastric cancer, or prostate cancer risk.

ABSTRACT FINAL ID: 2832 Poster Board -651
TITLE: Comparative Sensitivity of Human iPS Cell-Derived Neurons and Neuron/Astrocyte Cocultures to Neurotoxicants Using High-Content Analysis

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KEYWORDS: Neurons, Astrocytes, Neurotoxicants

ABSTRACT BODY: This study evaluated the feasibility of using automated high content analysis (HCA) to detect chemical inhibition of neurite outgrowth on human iPS cell (hiPSC) derived neurons, alone and in co-culture with hiPSC-derived astrocytes. To date, limited data has been published on the sensitivity of hiPSC-derived neurons to neurotoxicants and nothing has been published describing co-culture with astrocytes, which might more closely mimic the complexity of human

brain. After 2 h post plating neurons in single culture or in co-culture with astrocytes plated 48 h prior, neurons were exposed to acetaminophen (neg. control), Bis-1, rotenone, diazinon, and U0126 (pos. controls) at 0.1 to 100 μM, and stained for beta-III-tubulin 24 h post chemical exposure for morphological (neurite length, cell body area, number of neurites per cell body) and cytotoxicity (neurons per field, area nuclei) endpoints. The number of neurites per cell body was the most sensitive toxicity endpoint in both single and co-culture. Bis-1 exposure to neurons resulted in decreases in neurites at ≥10 μM, area of cell body at ≥30 μM, neurite length at 100 μM, and cell viability at 100 μM; whereas co-culture attenuated both the decreases in neurites at ≥10 μM and in cell body area at ≥30 μM. Rotenone exposure to neurons alone resulted in decreased neurite length at ≥1 μM and neurites at ≥1 μM, with no change in cell viability at ≤100 μM; whereas co-culture had increased cytotoxicity at ≥10 μM. Diazinon and U0126 toxicity were similar between single and co-cultures. Acetaminophen had no neurotoxicity in single or co-culture. With the exception of Bis-1, co-culture with astrocytes had minimal impact on the responses and/or sensitivity to the chemicals. These results show that HCA can be used to quantify neurotoxicity parameters in single or co-culture with astrocytes. Future studies may help demonstrate the benefit of a co-culture system in predicting neurotoxicity.

ABSTRACT FINAL ID: 2833 Poster Board -652
TITLE: The Use of Human iPS-Derived Cardiac Myocytes in Preclinical Assessment of Cardiotoxicity

AUTHORS (FIRST INITIAL, LAST NAME): A. Easter[1], A. Bruening-Wright[2], C. A. Obejero-Paz[2], N. N. Kim[1]
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KEYWORDS: Stem Cell, Cardiac Ion Channel, QT Prolongation

ABSTRACT BODY: Cardiotoxicity is assessed in preclinical discovery by assessing compound effects in cell lines expressing cardiac ion channels, including hERG, $Cav_{1.2}$ and $Nav_{1.5}$. These channels underlie the formation of the cardiac action potential, a critical component of cardiac function. However, these assays cannot measure the net effect of activity at multiple channels, including arrhythmic events. This can be assessed *in vitro* using primary myocytes or *in vivo* in telemetry studies. These models are resource intensive and low throughout, limiting use in drug screening. The aim of this study was to evaluate multi-electrode array (MEA) recording from human induced pluripotent stem cell (iPS)-derived cardiac myocytes as a higher throughput method for assessing potential effects on cardiac function. We assessed the effects of 18 Biogen Idec small molecules, selected to cover a range of hERG/$Cav_{1.2}$/$Nav_{1.5}$ activities. MEA field potential recordings were made using 48-well plates, compounds were tested over the range 0.3-100 μM (Chantest, a Charles River Company, OH, USA). Three endpoints were measured: field potential duration (FPD), Na^+ spike amplitude and beat period. Data were normalized to vehicle control (0.1% DMSO) and expressed as % change from baseline. Two selective hERG channel blockers were associated with the largest changes in FPD and arrhythmic activity. Compounds with mixed ion channel activity evoked a variety of different effects including 9/13 with arrhythmic activity. In contrast, 3 ion channel inactive compounds had minimal effects on field potential parameters and were not associated with arrhythmia. These data indicate that MEA recording can differentiate compounds based on cardiac ion channel activity and is of sufficient throughput for use in drug screening. In conclusion, MEA recording from iPS-derived myocytes is of potential value in preclinical screening of cardiac risk prior to the *in vivo* cardiovascular study.

ABSTRACT FINAL ID: 2834 Poster Board -653
TITLE: Arsenic Dysregulates Human Prostate Stem-Progenitor Cell Homeostasis, Perturbs Autophagy, and Drives Transformation through p62 Accumulation

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KEYWORDS: Arsenic, Stem Cells, Prostate

ABSTRACT BODY: Inorganic arsenic (iAs) is an environmental toxin that increases cancer risk, including prostate cancer, for chronically exposed populations worldwide. The underlying biological mechanism for iAs-induced prostate carcinogenesis is presently unclear. Herein, we examined whether iAs can target and transform normal human prostate stem and progenitor cells. Primary prostate epithelial cells (PrEC) of young, disease-free donors were utilized and stem-progenitor cells isolated using FACS and 3D prostasphere (PS) culture. Treatment with 1 µM iAs increased stem-like cell numbers in 2D PrEC cultures and PS formation in 7-day 3D cultures whereas 5 µM iAs reduced stem cell numbers. Further, iAs at either dose hindered differentiation of both PS and *in vitro* derived prostate organoids. Autophagy is a cell self-protective mechanism that plays a critical role in stem cell survival and tumor suppression. iAs exposure markedly increased LC3B-II and p62 protein levels in day-7 PS in a dose-dependent manner suggesting autophagy flux blockade. PS serial passage showed continued p62 elevation through passage 5 due to p62 mRNA induction with prolonged iAs exposure. To test whether p62 can transform normal prostate epithelial cells, we stably overexpressed p62 in RWPE-1 cells and xenografted them in nude mice. Overexpression of p62 transformed the RWPE1 cells which formed high Gleason grade tumors with faster growth and size vs empty vector cell grafts. Immunohistology and qRT-PCR revealed EMT (elevated vimentin, Twist, Snail, reduced E-cadherin), YAP activation and YAP and βcatenin downstream gene induction. In summary, iAs perturbs prostate stem-progenitor cell homeostasis and blocks autophagy flux leading to chronic p62 accumulation which transforms normal human prostate epithelial cells. We propose that chronic iAs exposure may drive human prostate carcinogenesis through chronic p62 accumulation in prostate stem-progenitor cells that maintain the prostate epithelium. (Support: NIH ES022071).

ABSTRACT FINAL ID: 2835 Poster Board -654
TITLE: Chronic Low-Dose Bisphenol A (BPA) Exposure Alters Rat Prostate Stem Cell Homeostasis

AUTHORS (FIRST INITIAL, LAST NAME): W. Hu[1], G. Shi[1], L. Xie[1], L. A. Birch[1], D. Hu[1], S. Majumdar[1], G. S. Prins[1]
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KEYWORDS: Bisphenol A, Stem Cell, Prostate

ABSTRACT BODY: Previous work found that early-life, low-dose BPA exposure increased rat prostate susceptibility to hormonal carcinogenesis with aging. Herein we tested whether chronic BPA exposure might target prostate epithelial stem and progenitor cells as part of the larger CLARITY-BPA consortium (Schug et al, *Repro Tox* 40:35, 2013). NCTR Sprague-Dawley rats were gavaged daily with vehicle, ethinyl estradiol (EE; 0.5 µg/kg BW), 2.5, 25 or 250 µg BPA/kg BW from gestation day 6 to 6 months. At 6 months, prostates were removed at rapid autopsy (at FDA's NCTR) and shipped overnight to UIC. Dorsal lobe epithelial stem cells were isolated by prostasphere (PS) 3-D culture (N=3-5/treatment). PS were passaged 3 times to enhance stem cell isolation, with final data derived from day-7 passage 3 PS. Sample identification (treatment groups) remained de-identified until all data was uploaded to a data repository (NIEHS-CEBS). *In vivo* exposure to EE and 2.5 µg BPA doubled the total PS number, reflecting an increased stem cell quantity (ANOVA P=0.059). PS size, a marker of progenitor cell proliferation in the cultured PS, was increased steeply by EE and 25 µg/kg BW BPA (P<0.01). To characterize the PS phenotype, stem and progenitor lineage markers were quantitated by q-RT-PCR. Tightly paralleling PS size effects, exposure to EE and 25 µg/kg BW BPA significantly increased CK5, Sox2 and HoxB13 expression while EE, 25 and 250 µg/kg BW BPA suppressed CK8, Trop2 and Tbx3 mRNA. These data suggest that chronic BPA exposures modify lineage hierarchy of the prostate stem cell progeny, increasing basal progenitors and suppressing luminal progenitor cells. Together, the present findings show that chronic low-dose BPA exposure alters adult prostate stem cell homeostasis in a dose-dependent manner, increasing stem cell numbers, progenitor cell proliferation and shifting lineage commitment to favor basal progenitor cells. We propose that these alterations may underpin increased carcinogenic risk with aging. (ES U01-020886; AES12013001-1-0-4)

ABSTRACT FINAL ID: 2836 Poster Board -655
TITLE: Prediction of Teratogens Using Human Induced Pluripotent Stem Cells

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KEYWORDS: Stem Cell, Assay

ABSTRACT BODY: Within the past decade, human induced pluripotent stem cells (hiPSCs) have become the center of concentrated research efforts in pharmaceutical and toxicological assessments. As hiPSCs are highly similar to human embryonic stem cells (hESCs) in genetic and epigenetic regulation of gene expression, proliferation ability, and differentiation capacity, hiPSCs have been identified as a promising alternative to hESCs in *in vitro* toxicity evaluations such as the embryonic stem cell test (EST). Originally murine-based, the EST is a well-established assay that evaluates embryotoxicity of compounds by measuring cell viability and capacity of ESCs to differentiate into functional cardiomyocytes following treatment. At present, EST teratogenicity screens featuring hiPSC-derived cardiac and non-cardiac tissue endpoints have not yet been reported though numerous screens with hESCs have been successfully carried out. This study investigated the robustness and predictive capacity of the hiPSC-based EST featuring cardiac and bone tissue endpoints. hiPSCs were treated with compounds possessing established toxicity assessments: 5-fluorouracil (5FU), all-trans retinoic acid (RA), and penicillin G (non-teratogenic; negative control). Differentiation inhibition was measured via beating and calcium assays for cardiac and bone endpoints, respectively. Cell viability was measured by MTT assays for both tissue endpoints. Day 10 changes in cardiac and osteogenic gene expression were assessed by qPCR analysis. Dose-dependent cell death, differentiation inhibition, and down-regulation of gene expression were observed in 5FU and RA in both tissue endpoints. Results demonstrate that cardiac and bone endpoints in the hiPSC–based EST are comparable to the hESC-based EST in their predictive capacity for toxicological analysis.

ABSTRACT FINAL ID: 2837 Poster Board -656
TITLE: Aryl Hydrocarbon Receptor Signaling and Immunomodulation by Mesenchymal Stem Cells

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KEYWORDS: Cellular Immunity, Immune Mechanisms, Immunotoxicology

ABSTRACT BODY: Clinical trials occurring worldwide by our group and others are exploring bone marrow-derived mesenchymal stem cells (MSCs) as a cell therapy for a variety of immune-mediated diseases. We hypothesized that environmental and endogenous aryl hydrocarbons (such as byproducts of Trp catabolism) may share signaling modalities as observed in the microstroma of human tissues. The catabolism of Trp by indoleamine 2,3-dioxygenase (IDO) is a key correlate of the immunomodulation by human MSCs. AHR is a cytosolic protein expressed by a variety of cells, that upon activation, initiates transcription at aryl hydrocarbon response elements (AHREs). We show MSCs express AHR basally, and hypothesized that the catalytic activity of IDO and signal transduction via AHR are linked, as intracellular signals which deploy MSC suppressive properties. To test this, we treated MSCs with TCDD or FICZ, observing 100-fold induction of Cyp1a1/1b1 mRNA, reflective of AHRE activation. We next examined the response of MSCs after 48h treatment with 1MT, Trp, kynurenine (Kyn) or kynurenic acid (KynAc), the latter two being IDO-catalyzed Trp metabolites. We observed 1MT and KynAc both induced Cyp1a1/1b1 expression by 60-fold. These data support the theory that IDO-catabolism of Trp generates endogenous ligands (i.e.KynAc) which can directly activate AHR. We performed T cell suppression assays, and observed Trp catabolites augment the immunosuppressive function of MSCs (independently of PDL1 or IDO). Our findings suggest endogenous AHR signals from IDO catalysis may play a role in MSC immunomodulation. This observation supports a novel paradigm for intracellular signaling from catabolic conversion of Trp to AHR ligands and induction of immunomodulatory properties.

2015 Society of Toxicology Annual Meeting
Late-Breaking Abstracts

ABSTRACT FINAL ID: 2838 Poster Board -657
TITLE: Human iPSC-Derived Hepatocytes and Cardiomyocytes for Drug Toxicity Testing

AUTHORS (FIRST INITIAL, LAST NAME): R. Annand[2], R. Vardaro [2], B. Hamilton[2], R. Akahira[1], K. Tamura[1], S. Yoshida[1], Y. C. Lin[1], D. Toyoda[1], H. Kogami[1], Y. Okuda[1], T. Watanabe[1], M. Inamura[1]
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KEYWORDS: iPS Cells, Hepatotoxicity, Cardiotoxicity

ABSTRACT BODY: Pharmaceutical candidate compounds require an extended period of time and high development costs before reaching the market. However, for various reasons, a high number of the candidate compounds are eliminated in the development process. The candidate compound elimination is frequently based on hepatotoxicity and cardiotoxicity. Although human primary cells are widely used for drug toxicity testing, they have posed issues such as lot-to-lot variation. Moreover, it is difficult to perform long-term tests with the same donor when using human primary cells due to the limited supply. Human iPSC-derived hepatocytes ReproHepatoTM cells were cultivated on 96-well plates, and the cells were exposed to five representative toxic compounds: acetaminophen, amiodarone, cyclophosphamide, diclophenac, and flutamide. Using conventional assays to measure ATP and LDH, we observed dose-dependent toxicity, and each compound showed IC50 values similar to the cytotoxicity observed using primary human hepatocytes (PHHs). Furthermore, we also observed toxicity using high content analysis, which simultaneously measures cell number, reduction in glutathione level, active oxygen, and mitochondrial membrane potential. These results show that ReproHepato can be used as an alternative to PHHs for routine cytotoxicity testing. We cultivated Human iPSC-derived cardiomyocytes ReproCardio 2TM cells on 96-well plates, and exposed the cells to five representative toxic compounds: aspirin, verapamil, isoproterenol, E-4031 and flecainide. Upon the addition of chemical compounds, the prolongation or reduction of the field potential duration was observed in a Multi-Electrode Array (MEA) experiment, with small lot-to-lot variation. These results demonstrate that ReproCardio 2 is a suitable model system for *in vitro* cardiotoxicity screening for QT prolongation and arrhythmia potential. In summary, human iPSC-derived hepatocytes and cardiomyocytes can be used for drug toxicity testing, as alternative sources of human primary cells.

ABSTRACT FINAL ID: 2839 Poster Board -658
TITLE: Development of a Human Pluripotent Stem Cell-Derived Osteoblast Model for Toxicity Testing

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KEYWORDS: Developmental Toxicity, Human Pluripotent Stem Cells, Alternative Testing Method

ABSTRACT BODY: Human pluripotent stem cell (hPSC) derived osteoblasts hold promise to be utilized in the discovery of chemical-induced osteotoxicity, however the applicability of hPSC-derived osteoblasts in this context have not been elucidated. Our laboratory has uncovered a promising approach to study skeletal teratogenicity *in vitro* that involves osteogenically differentiating human embryonic stem cells (hESCs). Though versatile, hESCs bring forth ethical concerns. To improve our *in vitro* osteotoxicity screening, we propose that utilization of human induced pluripotent stem cells (hiPSCs) will replace the challenges of hESCs and limit reliance on animal models. Here we explored a hPSC based model to evaluate the toxicity of 5-fluorouracil (5FU), all-trans retinoic acid (atRA), 13-cis-retinoic acid (13cisRA), and penicillin G (penG) during skeletal development. Human ESCs of the H9 line were directed towards an osteogenic lineage using 1,25(OH)2 vitamin D3 (VD3), β-glycerophosphate (βGP), and ascorbic acid (AA). In an effort to overcome the ethical challenges hESCs possess, hiPSCs underwent the same osteogenic differentiation and drug testing in comparison to the hESCs. Treatment of 5FU, atRA, and 13cisRA on osteogenically differentiating hESCs and hiPSCs inhibited osteoblast production and cell viability. Within 10 days, quantitative PCR analysis demonstrated a dose dependent down regulation of markers associated with osteoblast differentiation suggesting altered differentiation. MTT and calcium assays revealed no statistical difference in

sensitivity between the two hPSC lines when treated with test compounds. Our approach holds promise as a robust and simplistic alternative to predict toxicity.

ABSTRACT FINAL ID: 2840 Poster Board -659
TITLE: Transmembrane Potential Measurements in Cardiac Micro Tissues Derived from Human Stem Cells

AUTHORS (FIRST INITIAL, LAST NAME): V. Zamora-Rodriguez[1], M. Hortigon-Vinagre[1], V. Alageswaran[1], D. Craig[1], D. Fluri[2], I. Agarkova[2], J. M. Kelm[2], G. Smith[1]
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KEYWORDS: Cardiomyocyte

ABSTRACT BODY: The electrical characteristics of mammalian adult myocardium depend critically on the geometry of the tissue. Production of large quantities of human cardiac myocytes differentiated from induced pluripotent sten cells (iPSC-CMs) is now available commercially. These cells, normally cultured on 2D surfaces, are increasingly used to assess the electrophysiological effects of drugs. This study examined the electrical activity of a commercially available iPSC-CM cell line in both 2D culture and in 3D culture of micro-tissues. iCell Cardiomyocytes were purchased from Cellular Dynamics Inc (USA) and either (i) seeded onto wells of a 96well plate (25,000 cells/well) and cultured for 10 days or (ii) formed into micro tissues by creating hanging drops of approximately 0.3mm diameter in a 96 well format using the a patented automated hanging drop platform (Insphero AG). In both cases the cells were transferred to serum free media and transiently exposed to the voltage sensitive dye (Di-4-ANEPPS 3μM). The Di-4-ANEPPS fluorescence was recorded at 10KHz from regions 2D and 3D cultures for periods up to 30s in the 96 well plates on the CellOPTIQ electrophysiology platform (Clyde Biosciences Ltd). The average time between spontaneous action potential (AP) firing in the two culture formats was similar (1.5±0.47s vs. 1.12±0.14s; 2D vs. 3D), as was the rate of depolarisation of the AP (5.9±1.3ms vs. 5.1±0.6ms; 2D vs. 3D). But the action potential duration (APD) in 3D micro tissues was significantly shorter than 2D format. At 90% repolarisation APD was 428±50ms vs. 222±25ms (2D vs. 3D $P<0.01$). Similarly, APD at 30% repolarisation was shorter (188±36ms vs. 128±16ms 2D vs. 3D $P<0.01$). In conclusion, micro tissues of human iPSC-CMs exhibit a significantly shorter APD than comparable 2D cultures, the difference may arise from increased electrotonic interactions within micro-tissues.
