methotrexate, (24-48 hours) AST and αGST were elevated but αGST was elevated to a greater degree (~5fold compared with ~2fold for AST). Other liver biomarkers were unchanged. αGST is more often elevated and to a greater degree than other biomarkers in subjects receiving chronic methotrexate therapy. Since subjects with normal transaminases would not normally be biopsied, αGST has the potential to identify those most at risk of hepatic injury.

836 BIOSENSOR DETECTION OF BLOOD NTE INHIBITION.

V. V. Malygin[1], G. F. Makhaeva[1], N. N. Strakhova[1], L. V. Sigolaeva[2], L. G. Sokolovskaya[2], A. V. Eremenko[2], I. N. Kurochkin[2] and R. J. Richardson[3]. *[1]Institute of Physiologically Active Compounds, RAS, Chernogolovka, Russian Federation, [2]Faculty of Chemistry, M.V. Lomonosov Moscow State University, Moscow, Russian Federation and [3]Toxicology Program, University of Michigan, Ann Arbor, MI.*

To enable NTE to be assayed in whole blood, an electrochemical method was developed, based on detection of phenol with a tyrosinase carbon paste electrode. The biosensor was used to establish correlations of NTE inhibition in blood with that in lymphocytes and brain 24 hr after dosing hens with di-1-propyl-2, 2-dichlorovinyl phosphate (PrDChVP). To improve sensitivity, the initial electrode was optimized to achieve a detection limit of 25 nM phenol in a flow cell, allowing NTE activity to be measured in blood diluted 1:500. The new electrode had improved operating stability and a working life >12 mo. Using the biosensor, the *in vitro* sensitivity of hen blood and brain NTE to OP compounds was compared. I50 values for inhibition of NTE with $RO(C_6H_5)P(O)ON{=}CCH_3Cl$ (R = Me, Et, n-Pr, n-Bu) were obtained amperometrically for blood and colorimetrically for brain. A good correlation was found between pI50 values for blood and brain NTE inhibition by the phenylphosphonates, as well as 3 other OP compounds (r = 0.988, n = 7). To assess the time dependence of blood NTE inhibition after OP compound exposure, NTE activity was measured in brain and blood 4 hr after dosing hens with PrDChVP (0.32-1.0 mg/kg, im), as well as 4, 24, 48, 72 and 96 hr after 1.0 mg/kg. Brain and blood NTE inhibition was dose related 4 hr after dosing, and highly correlated between brain and blood (r = 0.997). NTE activity in brain and blood of hens killed 4, 24, 48, 72, and 96 hr after dosing with 1 mg/kg PrDChVP differed significantly from respective control values. During all measured times, NTE inhibition relative to controls (mean ± SE, n = 5) was 72 ± 4% in brain and 75 ± 3% in blood. The results demonstrate that whole blood NTE is a reliable biomarker of exposure to neuropathic OP compounds during 96 hr between exposure and measurement. (Supported by CRDF grants #RB2-2035, #RB2-2488).

837 COMPARATIVE GENE EXPRESSION PROFILING OF EPOTHILONE B AND PACLITAXEL TO SEARCH FOR BIOMARKERS OF EFFICACY AND TOXICITY.

M. Saulnier, F. Staedtler, P. McSheehy, A. Mahl, J. Schaffner, D. Roman, P. Ulrich, M. Wartmann, S. Chibout, H. Firat and L. Mueller. *Novartis Pharmacology AG, Basel, Switzerland.* Sponsor: V. Nogues.

Epothilone B (EPO906) is a novel microtubule stabilizer developed for the treatment of solid tumors, including tumors insensitive and refractory to paclitaxel, a P-gp substrate. In preliminary clinical studies designed to define the optimal dose and schedule of the drug, diarrhea represents the most common dose-limiting side-effect of EPO906 so far. In preclinical models, diarrhea occurs in rats treated with 1.75mg/kg EPO906. In the present study, EPO906 was compared to paclitaxel to understand the mechanism of diarrhea after EPO906 treatment, and identify specific biomarkers. Both compounds were injected i.v. into groups of Lewis rats at roughly equipotent doses (EPO906: 1.75 mg/kg, paclitaxel: 10 mg/kg) with regard to suppression of tumor growth in a rat tumor model. Caecum samples were collected after 2, 24, and 48 hours post-injection; RNA was extracted and analyzed using rat DNA microarrays. Caecum transcript profiles showed that both compounds induced dose- and time-dependent changes related to their pharmacological effects on stabilization of microtubules, leading to cell-cycle arrest. With paclitaxel, most of the changes in terms of number and mRNA expression levels were weak and transient. An EPO906-specific expression pattern was observed, which included genes encoding acute phase proteins, coagulation cascades, tissue remodeling, angiogenesis, lipid metabolism and, particularly, the arachidonic acid pathway, indicating a strong inflammatory response. These data suggest that induction of diarrhea by EPO906 may be related to the expected antiproliferative and cytotoxic activity, which may, in turn, be exacerbated by the presence of a strong inflammatory response, leading to the loss of the intestinal electrolyte/water barrier function. In EPO906-treated animals, early, differentially expressed genes were defined as potential biomarkers for the prediction of EPO906-induced diarrhea.

838 ENVIRONMENTAL TOBACCO SMOKE INDUCED REACTIVE OXYGEN SPECIES GENERATION IN MICE BRAIN REGIONS.

K. C. Wise[1], T. Rangasamy[2], S. Biswal[2] and R. Govindarajan[1]. *[1]Department of Biology, Texas Southern University, Houston, TX and [2]Department of Environmental Health Sciences, Johns Hopkins University, Baltimore, MD.*

Environmental tobacco smoke (ETS) is a key culprit in indoor air pollution. Tobacco smoke contains a mixture of over 4700 chemical components many of which are toxic and have been implicated in the etiology of oxidative stress related diseases such as chronic obstructive pulmonary disease, Parkinsons disease, asthma, cancer and cardiovascular disease. However, the mechanism of action of cigarette smoke in the onset of these diseases is still largely unknown. Previous studies have revealed that the free radicals generated by cigarette smoke may contribute to many of these chronic health problems and this study sought to address the role of environmental tobacco smoke in oxidative stress related damage in different brain regions of a mouse model. In this study, male strain A/J mice were exposed for 7 h/day, 7 days/week, for six months to an atmosphere of ETS consisting of 89% side stream smoke and 11% mainstream smoke produced by burning reference cigarettes using a smoke machine. Chamber atmosphere was monitored for total suspended particulates (TSP) and carbon monoxide (CO). Our preliminary findings from this study indicates that exposure to tobacco smoke leads to increased generation of reactive oxygen species with a concomitant decrease in the level of glutathione, a natural antioxidant utilized to combat oxidative stress. Gel shift analysis also revealed the elevated level of the oxidative stress sensitive, proinflammatory transcription factor NF-kappa-B in different regions of the brain of cigarette smoke exposed AJ mice. Research is in progress to determine the cigarette smoke induced oxidative lesions in different regions of the brain of AJ mice. [This work was supported by grant: RCMI/NIH #G12RR03045-16].

839 IDENTIFICATION OF BIOMARKERS FOR OXYCHLORDANE-EXPOSURE IN RODENT LIVER USING MICROARRAYS.

I. Curran, A. Hierlihy, K. Smith, J. Green and G. Bondy. *Toxicology Research Division, Health Canada, Ottawa, ON, Canada.*

Oxychlordane (OXY) is the primary metabolite of the toxic mixture, technical chlordane. Such persistant organo-pollutants were widely used as pesticides until the 80s and thus have accumulated in the food chain. Recent reports indicate that the formation of this metabolite is more toxic and bioaccumulative than the parent contaminants. The goal of this study was to elucidate possible genetic markers that may be used in identifying toxic exposure before overt physiological effects are evident. Female and male rats were gavaged with 2.5mg/kg body weight/day OXY for 28 days; control animals were gavaged with corn-oil vehicle only. Total RNA was isolated from liver and pooled, followed by comparison of gene expression levels using microarray analysis. Three trials using Mergen chips (contain oligos of 1152 known rat genes and ESTs) were performed. Microarray data was verified using semi-quantitative RT-PCR on individual samples. Microarray analysis on female rat liver RNA showed that 31 targets were expressed at >2-fold increase in treated vs. control animals. Classification of these genes identified several membrane transport genes, as well as cytochrome p450 genes. RT-PCR confirmed increased levels of gene expression in treated female rats in such genes as sodium-dicarboxylate cotransporter (SDCT1), Na+, K+ ATPase-gamma subunit. Interestingly, sex-specific differences were detected in gene expression of 4 of the target genes. These included proton-coupled peptide transporter-2, Na+, K+ ATPase- gamma, SDCT1, and putative potassium channel. While female expression levels were shown to increase in these genes, transcript levels were observed to decrease in male OXY-treated livers. Results indicate that OXY elicits an effect on membrane proteins. This corresponds with previous studies that report depressed organic-ion transport to be an effect of chlordane, trans- and cis-nonachlor on membrane channel, ion transport and other membrane-related proteins. Further testing is currently underway to screen larger gene arrays at lower dose groups to identify additional gene targets.

840 MEETING GUIDELINES FOR CHOLINESTERASE MONITORING BY CLINICAL LABORATORIES IN CALIFORNIA.

B. W. Wilson[1], J. D. Henderson[1], D. E. Arrieta[1] and M. A. O'Malley[2, 3]. *[1]Environmental Toxicology, University of California, Davis, CA, [2]Employee Health, University of California, Davis, CA and [3]Department of Pesticide Regulation, California Environmental Protection Agency, Sacramento, CA.*

California (CA) is the only state with a long standing formal monitoring program for mixers, loaders and applicators of pesticides. When we found commercial clinical kits were not optimal for assaying blood cholinesterases (ChEs), CA regulations were revised to specify use of optimal Ellman ChE assay conditions. Alternate

methods were allowed if a correlation ($r^{2)}$ of 0.9 was achieved in a comparison with the specified method. We were enlisted to work with the clinical laboratories. Only 2 of 7 participating laboratories achieved an acceptable correlation for red blood cell acetylcholinesterase (AChE) and 4 of 5 laboratories for plasma ChE. When the CA Department of Pesticide Regulation (DPR) reiterated the need to meet this requirement we used bovine ghost RBC AChE as a standard to work with several of the clinical laboratories. Only 3 of 10 participating laboratories had acceptable correlations. Some said their instruments lacked the sensitivity to assay the bovine AChE samples. Next, we provided all interested laboratories with human blood and plasma samples to perform a comparison study outlined in the regulation (Section 6728f). Fourteen laboratories participated; 9 met the criterion for whole blood, 14 for plasma and 6 for RBC AChE. Based on such data, DPR notified the CA Agricultural Commissioners on July 8, 2003 that 9 clinical laboratories were approved for ChE testing. We continue to work with laboratories interested in being on the approved list. The current list may be seen at: www.cdpr.ca.gov/docs/whs/lablist.htm. Supported by NIOSH (#CDC U07/CCU906162-06) and NIEHS (#ESO5707).

841 BIOLOGICAL MONITORING OF BISPHENOL A IN CHILDREN.

S. Park[1], C. Shin[2], S. Kim[3] and M. Yang[1]. *[1]Preventive Medicine, Seoul National University College of Medicine, Seoul, South Korea, [2]Pediatric Endocrinology, Seoul National University Hospital, Seoul, South Korea and [3]Preventive Medicine, Eulji University School of Medicine, Taejon, South Korea.*

Health risk of bisphenol A (BPA) among potential endocrine disrupters (EDs) is not clear, yet. In addition, children are thought to be more sensitive to BPA than adults. Therefore, we studied exposure levels of BPA and association between the exposure levels and endocrine disorders in Korean children (N= 168; 9.1 ± 2.2 yrs; male, 41.1 %) to provide safe exposure levels of BPA. Cases of endocrine disorders included short stature, precocious puberty, hyperthyroidism, etc. Controls were 94 healthy children. Using our established analysis method of BPA in urine (HPLC/FD), we measured conjugated BPA as a biomarker for BPA exposure. As results, range of BPA was 0.04- 83.5 μg/L in detectable urine samples (N=149). When we gave half of the lowest level, 0.02, to the other 19 non-detectible samples, the median of BPA levels was 5.85 μg/L. The level of BPA was higher in cases (N=65) than controls (N=94): geometric mean (geometric standard de*via*tion) in cases and controls, 3.9 μg/L (5.8) and 1.9 μg/L (12.0), respectively: p = 0.13. When we considered age and sex for BPA levels, the odd ratio for case was 4.7 (95% CI, 1.3-19.4). Even though our results should be confirmed in enlarged populations, our results suggest that exposure to BPA may affect endocrine disorder in children. In addition, approximate 3 μg of BPA is thought to be daily exposed to the children. Our results will be useful for establishment of future safe levels of BPA.

842 METABOLOMIC ANALYSIS OF THE MECHANISMS OF ACETAMINOPHEN LIVER TOXICITY IN RATS.

A. J. Higgins, T. J. Colatsky, B. R. Bullard and S. S. Sumner. *Paradigm Genetics, Inc., Research Triangle Park, NC.*

Acetaminophen (APAP) overdose remains one of the most common causes of hospital admissions for acute liver toxicity. The initiating mechanism is believed to involve hepatic metabolism of APAP to a reactive oxidative species, NAPQI, which subjects the liver to oxidative stress, resulting in depletion of glutathione. Oxidative stress is also believed to play a pivotal role in the hepatotoxicities of a wide range of chemical and pharmaceutical agents, and APAP may therefore serve as a useful model agent. Single oral doses of APAP, ranging from 50 mg/kg (no histopathological changes) to 2000 mg/kg (frank necrosis) were administered to rats. Groups of 6 rats were serial sacrificed up to 48h and livers were snap frozen. Samples were extracted and analyzed by LC/MS (ToF) with an ESI source in either positive or negative mode. Mass spectra at each retention time were matched to a library of around 500 known standards using proprietary software and linked to metabolic pathways. Data were reduced and visualized by principle component analysis. As early as 6h after the acute administration, changes in biochemical profiles could be clearly observed with doses of 1500 mg/kg and above. The time-related trajectories were similar for the 1500 and 2000 mg/kg groups. A similar qualitative trend was also apparent at 150 mg/kg, a dose that produced only minimal histopathological changes. Analysis of individual metabolites revealed major perturbations in pathways associated with known injury and repair mechanisms - e.g. depletion of glutathione and cystathionine (oxidative stress), decreases in NAD and various nucleotides (nucleic acid repair), and a decrease in CDP-choline (phospholipid turnover). Several of these decreases in liver metabolites were also reflected in urine, suggesting that they might serve as useful biomarkers for early detection of liver disease. Time and dose-related changes were also observed in various other biochemicals that were not predicted by current knowledge. Samples for this study were kindly provided by NIEHS.

843 METABOLOMICS: URINE AND SERUM BIOMARKERS FOR ACETAMINOPHEN HEPATOTOXICITY IN RATS.

T. J. Colatsky, A. J. Higgins, B. R. Bullard and S. C. Sumner. *Paradigm Genetics, Inc., Research Triangle Park, NC.*

Acetaminophen (APAP) is a common cause of liver toxicity in humans. Standard markers of liver toxicity are relatively insensitive to the effects of APAP and have limited value in predicting outcomes for patients who have overdosed with the drug. Biochemical profiling (metabolomics) is a zero-based diagnostic approach (i.e. requiring no prior assumptions) that has the potential to simultaneously map changes in multiple biochemical processes that serve as novel biomarkers of liver disease. APAP was administered to groups of 6 rats in a single oral dose of either 50 or 1500 mg/kg. Urine was collected during various periods up to 48h and a single serum sample was also obtained at 48h. Biochemical profiles were determined in urine and serum by LC/MS (ToF) with an ESI source in either positive or negative mode. Mass spectra at each retention time were matched to a library of around 500 known standards using proprietary software. Data were reduced and visualized by principle component analysis (PCA). Results from urine showed separation of the 1500 mg/kg group from the control and low dose groups within the first 6h collection period. Progressively greater separation occurred from 6-24h and 24-48h. PCA analysis of serum showed similar classifications at 48h. The mechanisms underlying the changes in urine included decreases in homocysteine and cystathione, reflecting protection against oxidative stress. Amongst the other changes observed, some were representative of effects on phospholipid and nucleic acid turnover. Others mainly involved various pathways of amino acid metabolism. The most striking alteration in urine was a large, rapid increase in cAMP at the high dose and a smaller, transient increase at the low dose. A large increase in cAMP was also observed with the high dose in serum. These results suggest a basis for development of a novel panel of biomarkers for early detection and monitoring of liver toxicity. Samples for this study were kindly provided by NIEHS.

844 GENE EXPRESSION PROFILING IN A RAT CARDIAC ISCHEMIA MODEL.

P. H. Koza-Taylor[1], B. Lu[1], M. Wenfang[3], S. Eustis[2], X. Li[2] and M. Lawton[1]. *[1]Molecular and Investigative Toxicology, Pfizer, Groton, CT, [2]Pathology, Pfizer, Groton, CT and [3]Comparative Medicine, Pfizer, Groton, CT.*

Cardiotoxicity in preclinical animal species is a common finding during development of vasoactive or cardiotonic pharmaceutical agents. There are multiple mechanisms of cardiotoxicity, with cardiac ischemia being the most common cause for cardiac injury. We have utilized a rodent model of cardiac ischemia to search for biomarkers for the early prediction and/or identification of cardiac injury. In this model, male Sprague-Dawley rats were anesthetized with isoflurane, and a left thoracotomy was performed aseptically to expose the heart and the left coronary artery. For ligation, the left anterior coronary artery was tied completely to occlude the blood flow. For sham control, the needle/suture were passed through under the artery, but was not tied. The heart and whole blood samples were collected for gene expression profiling at 3, 6, 9, 24 and 48 hours after coronary artery ligation or sham surgery. Moderate acute cardiomyocyte necrosis at 3-9 hours and necrosis with mixed inflammatory cell infiltration at 24 -48 hours were observed in the left free ventricular wall after ligation. Principal component analysis (PCA) of the global gene expression data showed clear separation between the sham and treated animal groups. An immediate-early stress response was observed starting the early time points and increasing at the later time points. The genes differentially expressed in the ischemia-induced animals fell into distinct clusters: the down-regulated genes included metabolism, glycolysis and catalase pathway members whilst up-regulated genes included acute phase and inflammatory response, heme oxidation pathway members, heat shock proteins, matrix metalloproteinases and fibronectin. Using both hierarchical clustering and Gene Logic's linear discriminant analysis (LDA)-based predictive models, the transcriptional profile in the hearts of ischemic rats was similar to catecholamine- and minoxidil-like responses, suggesting common mechanisms of ischemic cardiac injury.

845 METABOLISM AND HEMOGLOBIN ADDUCTS OF [1, 2, 3-$^{13}C_3$] ACRYLAMIDE IN HUMANS.

T. Fennell[1], R. Snyder[1], J. P. Burgess[1] and M. A. Friedman[2]. *[1]RTI International, Research Triangle Park, NC and [2]UMDNJ, Newark, NJ.*

Acrylamide (AM), which is used in the manufacture of polyacrylamide and grouting agents, is produced during the cooking of foods. Exposure to AM in the workplace can occur through dermal and inhalation exposure. The objectives of this study were to evaluate the metabolism of AM in humans on oral administration, and to compare hemoglobin adduct formation on oral and dermal administration. The study protocol was reviewed and approved by IRBs at both the laboratory performing the analysis of samples, and the clinical research center conducting the

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, workshop, roundtable, platform and poster sessions of the 43rd Annual Meeting of the Society of Toxicology, held at the Baltimore Convention Center, Baltimore, Maryland, March 21-25, 2004.

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