SOT | Society of Toxicology

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2013 Annual Meeting Abstract Supplement

Late-Breaking Abstract Submissions

All Late-Breaking Abstracts will be presented on Thursday, March 14, from 8:30 am–12:00 noon.

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

52nd Annual Meeting and ToxExpo™

San Antonio, Texas

March 10–14, 2013

THURSDAY POSTER SESSION MAP

March 14—8:30 AM to 12:00 NOON—Exhibit Hall A

Poster Set Up—7:00 AM to 8:30 AM

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ENTRANCE

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

The abstract final ID# precedes the poster surface number that is in **bold**. Please display your poster only on the date and time assigned to you.

Thursday, March 14, Poster Session by Location

SESSION TITLE	ABSTRACT NUMBERS	POSTER BOARD #s
Late-Breaking Poster Session 1		
Alternatives to Mammalian Models and Animal Models	2514-2531	101-118
Chemical & Biological Weapons	2532-2535	119-122
Metals	2536-2542	123-129
Persistent Organic Pollutants (POPs)	2543-2544	130-131
Ecotoxicology, Natural Products, and Food Safety	2545-2559	133-147
Late-Breaking Poster Session 2		
Cardiovascular Toxicology/Hemodynamics, Inhalants, Cardiopulmonary	2560-2567	201-208
Liver and Kidney	2568-2579	209-220
Cell Death/Apoptosis	2580-2582	221-223
Epigenetics	2583-2585	224-226
Oxidative Injury and Redox Biology	2586-2588	227-229
Receptors	2589-2591	230-232
Carcinogenesis	2592-2595	233-236
Gene Regulation/Signal Transduction/Genotoxicity and DNA Repair	2596-2602	237-243
Inflammation and Disease, Methods and Mechanisms	2603-2604	245-246
Late-Breaking Poster Session 3		
Biological Modeling	2605-2606	301-302
Biotransformation/Cytochrome P450	2607-2608	303-304
Computational Toxicology	2609-2612	305-308
Disposition/Pharmacokinetics and Pharmacogenomics/Genetic Polymorphisms	2613-2614	309-310
Exposure Assessment/Biomonitoring	2615-2617	311-313
Risk Assessment	2618-2622	314-318
Safety Assessment: Drug Discovery and Development	2623-2630	319-326
Biomarkers	2631-2640	327-336
Clinical and Translational Toxicology	2641-2643	338-340
Systems Biology and Toxicology	2644	341
Stem Cell Biology and Toxicology	2645	342
Pesticides	2646-2650	343-347
Late-Breaking Poster Session 4		
Developmental Basis of Adult Disease, Developmental Toxicology and Reproductive Toxicology	2651-2661	401-411
Immunotoxicity	2662-2667	412-417
Endocrine Toxicology	2668-2670	418-420
Nanotoxicology	2671-2678	421-428
Neurotoxicity and Neurodegenerative Disease	2679-2697	429-447

Thursday, March 14, Poster Session by Topic

TOPIC	ABSTRACT NUMBERS	POSTER BOARD #s	SESSION TITLE
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Biological Modeling	2605-2606	301-302	Late-Breaking Poster Session 3
Biomarkers	2631-2640	327-336	Late-Breaking Poster Session 3
Biotransformation/Cytochrome P450	2607-2608	303-304	Late-Breaking Poster Session 3
Carcinogenesis	2592-2595	233-236	Late-Breaking Poster Session 2
Cardiovascular Toxicology/Hemodynamics, Inhalants, Cardiopulmonary	2560-2567	201-208	Late-Breaking Poster Session 2
Cell Death/Apoptosis	2580-2582	221-223	Late-Breaking Poster Session 2
Chemical & Biological Weapons	2532-2535	119-122	Late-Breaking Poster Session 1
Clinical and Translational Toxicology	2641-2643	338-340	Late-Breaking Poster Session 3
Computational Toxicology	2609-2612	305-308	Late-Breaking Poster Session 3
Developmental Basis of Adult Disease, Developmental Toxicology and Reproductive Toxicology	2651-2661	401-411	Late-Breaking Poster Session 4
Disposition/Pharmacokinetics and Pharmacogenomics/Genetic Polymorphisms	2613-2614	309-310	Late-Breaking Poster Session 3
Ecotoxicology, Natural Products, and Food Safety	2545-2559	133-147	Late-Breaking Poster Session 1
Endocrine Toxicology	2668-2670	418-420	Late-Breaking Poster Session 4
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Safety Assessment: Drug Discovery and Development	2623-2630	319-326	Late-Breaking Poster Session 3
Stem Cell Biology and Toxicology	2645	342	Late-Breaking Poster Session 3
Systems Biology and Toxicology	2644	341	Late-Breaking Poster Session 3

2013 Society of Toxicology Annual Meeting
Late-Breaking Abstracts

ABSTRACT FINAL ID: 2514 Poster Board -101
TITLE: Development of the Replacement Ocular Battery (ROBatt) Tiers 2 and 3: BCOP and PorCORA

AUTHORS (FIRST INITIAL, LAST NAME): D. R. Cerven, D. Wolfinger, M. Piehl, M. Carathers, and G. L. DeGeorge.
INSTITUTIONS (ALL): MB Research Laboratories, Spinnerstown, PA.
KEYWORDS: Ocular Irritancy, In Vitro and Alternative Methods, BCOP

ABSTRACT BODY: The Replacement Ocular Battery (ROBatt) is being developed as a Tiered Testing Strategy to replace Draize *in vivo* ocular irritation testing with funding provided by an NIH/FDA Common Fund Grant. It comprises (1) Chorioallantoic Membrane Vascular Assay (CAMVA), mimicking the vascular reaction of the conjunctiva; (2) Bovine Cornea Opacity/Permeability Test (BCOP), to assess mild to moderate corneal damage; (3) Porcine Corneal Opacity/Reversibility Assay (PorCORA), to discriminate severe irritants from ocular corrosives; and (4) Porcine Confocal Assay (PorFocal), to separate nonirritants/slight irritants. In Tier 1, the Chorioallantoic Membrane Vascular Assay (CAMVA) was used to segregate 30 of 52 as "more than slight irritant" chemicals for testing in the Bovine Cornea Opacity Permeability test (BCOP). The BCOP measures changes in corneal light transmission following ocular insult, as well as corneal changes that may not be associated with opacity. A BCOP *in vitro* score of greater than 12 was chosen to further segregate 17 of the 30 chemicals as either Severe Irritants/Corrosive, and assign 13 chemicals (with scores less than 12) to the Moderate irritant category (HMIS 2, GHS 2A, EPA 3). The 17 severe/corrosive were then tested in the Porcine Cornea Opacity/Reversibility Assay (PorCORA), which uses cultured porcine corneas to determine reversibility (healing) of corneal insult. Following test chemical insult, the cultured corneas in PorCORA were evaluated for damage by fluorescein stain retention on days 1, 2, 3, 7, 10, 14 and 21 to determine reversibility of corneal damage. Corneas that retained fluorescein on Day 21 were considered to be corrosive (i.e., HMIS 3, GHS 1, EEC-R41, and EPA 1). Those corneas with damage that cleared prior to Day 21 were considered severe irritants (i.e., HMIS 2, GHS 2A, EEC-R36, EPA 2). Of the 17 chemicals tested in PorCORA, five reversed (cleared) by Day 21. The remaining12 had fluorescein stain retention on Day 21, indicating that corneal damage was permanent; these chemicals were assigned to the Corrosive category.

ABSTRACT FINAL ID: 2515 Poster Board -102
TITLE: Refinement of the Peroxidase Peptide Reactivity Assay and Prediction Model

AUTHORS (FIRST INITIAL, LAST NAME): J. A. Troutman, H. J. Dai, R. L. Dobson, M. Quijano, and G. F. Gerberick.
INSTITUTIONS (ALL): Procter & Gamble, Cincinnati, OH.
KEYWORDS: peptide reactivity, skin sensitization, alternatives

ABSTRACT BODY: We have previously developed a mechanistic in chemico peptide reactivity assay (with metabolism) that allows for quantitative analysis of a test chemical's reactivity for screening skin sensitization potential of chemical substances. A horseradish peroxidase-hydrogen peroxide (HRP/P) oxidation system has been incorporated into the assay for characterizing reactivity of hapten and pro-/prehapten sensitizers. Although a predictive accuracy of 83% (relative to the LLNA) was achieved, apparent false positives were attributed to cysteine depletion at high concentrations and for some chemicals expected to react with the -NH2 group of lysine, little/no depletion with lysine peptide was observed. To improve the PPRA, reactions with cysteine +/-HRP/P were modified by increasing the number of concentrations from 5 to 8 and the overall dose range was adjusted to 0.04 and 5 mM. Cysteine peptide depletion was determined for a total of 16 sensitizers and nonsensitizers following 24 h incubation. The prediction model for chemicals within the applicability domain for cysteine identified correctly 13/16 chemicals tested in the study. Two of the 3 misclassifications were false-negatives, all of which were weak sensitizers, and 1 was a false-positive. A series of experiments were subsequently conducted to compare and optimize reactivity of 24 test chemicals toward lysine peptide and to determine whether reactions with HRP/P enhance the characterization of contact allergens. Substantial increases in lysine depletion was observed in reactions containing 0.1 M phosphate buffer (pH 7.4) and 25% organic solvent compared to reactions with 0.1 M ammonium acetate buffer (pH 10.2) and 1% organic solvent. Depletion of lysine peptide in reactions with HRP/P and pre-/prohaptens ethylenediamine and lauryl gallate was negligible. We believe that recent refinements of the Peroxidase Peptide Reactivity Assay are

significant improvements and will help to meet the critical need of finding reliable nonanimal based methods for predictions of skin sensitization potential in the future.

ABSTRACT FINAL ID: 2516 Poster Board -103
TITLE: *In Vitro* to *In Vivo* Dosimetry Predictions using a QSAR-Derived Generic PBPK Modeling Approach

AUTHORS (FIRST INITIAL, LAST NAME): J. A. Troutman and G. P. Daston.
INSTITUTIONS (ALL): Procter & Gamble, Cincinnati, OH.
KEYWORDS: PBPK, IVIVE, Alternatives

ABSTRACT BODY: As alternative approaches and technologies become available for identifying hazard, *in vitro* to *in vivo* extrapolation methods will be needed to identify safe exposure levels for human health risk assessments. The objective of the present study is to assess the utility of a QSAR-derived generic physiologically-based pharmacokinetic (PBPK) model to predict and compare simulated maximum concentrations in the liver to nominal chemical concentrations tested previously in an *in vitro* transcriptomic profiling study using rat hepatocytes. A combination of published equations to predict inputs on oral absorption and tissue:plasma partition coefficients were used to parameterize a generic PBPK rat model. Chemical-specific inputs were calculated using ChemSilico and ACDLabs for hepatotoxicants acetaminophen, β-naphthoflavone, chlorpromazine, clofibrate, methpyrilene, valproic acid, phenobarbital and WY14643. Model simulations were performed at oral rat NOAEL and MTD doses with bracketing hepatic metabolism assumptions using extraction ratios set to 0.3 and 0.7 and renal clearance set equal to glomerular filtration rate. The model-simulated maximum concentrations in the liver following MTD doses were equivalent to or higher than tested concentrations that produced significant changes in gene expression. There was a tendency for significant changes in gene expression even at doses that were lower than the calculated liver concentrations at the NOAEL. The overall PBPK modeling approach provides a quantitative format for extrapolating *in vitro* toxicity concentrations to *in vivo* dosages/exposures. With further development, we believe that the approach can be used to support human health risk assessment and will allow for subsequent studies of potential toxic mechanisms to be tested *in vitro*.

ABSTRACT FINAL ID: 2517 Poster Board -104
TITLE: A Micropatterned Culture with Human Hepatocytes and Kupffer Macrophages for Studying Inflammation-Drug Interactions

AUTHORS (FIRST INITIAL, LAST NAME): M. McVay, O. Ukairo, and C. Kanchagar.
INSTITUTIONS (ALL): Hepregen Corporation, Medford, MA.
KEYWORDS: Hepatotoxicity, Trovafloxacin, Co-cultures

ABSTRACT BODY: The appearance or relief of inflammation through drug therapy could differentially affect levels of enzymes involved in metabolism of co-administered drugs with potential pharmacological and toxicological consequences. An *in vitro* model that mimics liver inflammation may provide better predictive data in preclinical testing. We have developed a micropatterned co-culture of primary hepatocytes and embryonic fibroblasts (MPCCs) that retains high levels of phenotypic functions such as drug metabolism enzymes for 4 weeks *in vitro*. Here, we supplement the MPCC platform with primary Kupffer macrophages in order to mimic one component of inflammation. Species-matched Kupffer cells were added to human or rat MPCC at multiple ratios (to mimic both the normal and inflamed state of the liver) to generate a tri-culture with primary hepatocytes and embryonic fibroblasts. Recent evidence suggests that interaction between inflammatory stress and certain drugs may precipitate toxic responses. Here, we assess whether stimulation of the MPCC-Kupffer cell co-cultures with LPS sensitizes the cultures to trovafloxacin (TVX) toxicity. Rat or human MPCC- Kupffer cell co-cultures were treated with increasing concentrations of TVX (+/- LPS) and assessed for changes in hepatic ATP content. TVX caused a concentration-dependent toxicity in the MPCC-Kupffer cell co-cultures which was potentiated by addition of 50ng/mL LPS to the cultures (TC50= 87.29 vs 27.77 Cmax for the rat platform and 68.24 vs 30.26 Cmax for the human platform). This effect was not observed with the nontoxic analog, levofloxacin. Treatment with pentoxifylline (an inhibitor

of TNFα transcription) significantly decreased TVX/LPS- induced rat MPCC toxicity suggesting a synergistic effect between TNFα and trovafloxacin (TC50= 19.73 vs. 76.36 Cmax). In conclusion, rat or human MPCC- Kupffer cell co-cultures may be used to predict drug-induced liver injury mediated by inflammatory stress.

ABSTRACT FINAL ID: 2518 Poster Board -105
TITLE: Development of an *In Vitro* Reconstructed Human Epidermal Equivalent with Luciferase Reporter Activity for High-Throughput Evaluation of Skin Sensitizers

AUTHORS (FIRST INITIAL, LAST NAME): M. Miyazawa[1], C. Mankus[2], O. Takenouchi[1], Y. Kuroda[1], G. R. Jackson[2], H. Sakaguchi[1], and P. J. Hayden[2].
INSTITUTIONS (ALL): [1]Kao Corp., Tochigi, Japan; [2]MatTek Corp., Ashland, MA.
KEYWORDS: skin sensitization, epidermal equivalent, *in vitro* test

ABSTRACT BODY: Determination of skin sensitizing potential is an important concern for development of new consumer products and cosmetic ingredients. However, recent legislative and societal concerns against animal testing have necessitated development of *in vitro* alternative methods for predicting skin sensitization potential of chemicals. For testing of lipophilic ingredients and complex mixtures, an *in vitro* skin model that reproduces the 3D structure and function of native human skin is highly desirable. We have previously developed a PCR-based skin sensitization assay (EpiSensA), based on the expression of antioxidant response element (ARE)-regulated genes in an *in vitro* reconstructed human skin model (Miyazawa et al., 2011). We have also engineered the model to express a luciferase reporter gene under the control of promoter including ARE sequence (Hayden et al, 2011). The goal of the current work was to further develop a high throughput compatible assay with the ARE reporter model for prediction of skin sensitizers. A series of reference chemicals of known *in vivo* skin sensitizing potential were tested by topical application to the ARE model in a 96-well high throughput format for up to 48 hrs. Following chemical exposure, luciferase activity of ARE tissue lysates was evaluated in a microplate luminometer. Positive control sensitizers (e.g., 4-nitrobenzylbromide, dinitrochlorobenzene, and glyoxal) and lipophilic sensitizers (e.g., benzyl cinnamate and hexylcinnamic aldehyde) were correctly identified by the assay, while negative control nonsensitizers (e.g., glycerol and lactic acid) and lipophilic nonsensitizers (e.g., hexane) were correctly identified as nonsensitizers. These data suggested that the ARE reporter assay using an *in vitro* reconstructed human skin model may provide a fast, easy and reliable *in vitro* method for determination of skin sensitizing potential of chemicals including lipophilic ingredients.

ABSTRACT FINAL ID: 2519 Poster Board -106
TITLE: Role of Alcohol Dehydrogenase in Regulation of Angiotensinogen in Human Hepatocyte

AUTHORS (FIRST INITIAL, LAST NAME): R. A. Ansari, S. A. Rizvi, and M. A. Clark.
INSTITUTIONS (ALL): Pharmaceutical Sciences, Nova Southeastern University, Fort Lauderdale, FL.
KEYWORDS: Alcohol, Angiotensinogen, Hypertension

ABSTRACT BODY: Chronic alcohol usage is linked to hepatosteatosis, steatohepatitis, fibrosis, cirrhosis, and increased blood pressure. The alcohol-mediated increase in blood pressure is well documented. Octapeptide, angiotensin II (Ang II) is produced from its precursor, angiotensinogen (AGT), by sequential action of renin followed by angiotensin converting enzyme. Ang II is implicated in fibrogenesis of liver after the death of hepatocytes. The level of the precursor, AGT is less than the Michaelis-Menten constant (Km) of renin. Therefore, an increase in blood AGT levels would result in a corresponding increase in Ang II levels that might play crucial roles in blood pressure regulation and fibrogenic activity. Our initial studies with HepG2 and Hep3B cells have demonstrated an increase in secretion of AGT after ethanol treatment. It is known that hepatocytes, especially HepG2 loses the ability of alcohol metabolism as it is passaged. In the current study, a HepG2 cell line that stably expresses alcohol dehydrogenase, VA-13 (a kind gift from Dr. DL Clemens, VAMC, UNMC, Omaha, NE), was employed to study the effects of alcohol exposure on biosynthesis and secretion of AGT. The VA-13 cells were exposed to ethanol at 25, 50 and 100 mM for 4 hrs. The level of AGT protein in secreted media was analyzed by

Western blotting. It was observed that ethanol exposure to VA-13 resulted in increased secretion of AGT. The metabolic product(s) of ethanol activate(s) the transcription factors that are likely activating the human AGT gene.

ABSTRACT FINAL ID: 2520 Poster Board -107
TITLE: A Higher Throughput Microfluidic Chip for Quantifying Drug-Dependent Changes in Cardiac Contractile Function

AUTHORS (FIRST INITIAL, LAST NAME): A. Agarwal, J. A. Goss, A. Cho, M. L. McCain, and K. Parker.
INSTITUTIONS (ALL): Disease Biophysics Group, Wyss Institute for Biologically Inspired Engineering, School of Engineering and Applied Sciences, Harvard University, Cambridge, MA.
KEYWORDS: Cardiotoxicity, High-througput screening, microfluidic system

ABSTRACT BODY: High rates of attrition due to unforeseen cardiac toxicity are the primary reason for drug development delays and market withdrawal, leading to falling productivity of pharmaceutical industry. Hence, there is a compelling need for creative preclinical screening approaches to predict drug efficacy and cardiotoxicity *in vitro*. We sought to design a higher throughput "heart on a chip" which utilizes a commercial laser engraver as an engineering tool to fabricate sub millimeter sized thin film cantilevers in soft elastomer of standard and reproducible dimensions, and in a batch process which is amenable to an assembly line type fabrication. We are able to produce up to 50 technical replicates from a chip with a cell requirement of 1 million cardiac myocytes and as a result this design significantly advances the throughput of our Muscular Thin Film (MTF) technology for building a heart on a chip. We also present the design of a one channel fluidic device completely built out of autoclaveable and nonabsorbent materials which incorporates various features required for an optical cardiac contractility assay: metallic base which fits on a heating element for temperature control, transparent top for recording cantilever deformation and embedded electrodes for electrical field stimulation of the tissue. We employed our fluidic higher throughput tool to test the effect of isoproterenol (a positive inotropic agent) on cardiac contractility at dosages ranging from 0.1nM to 100µM. We find a close match of pD2 values obtained from our *in vitro* tool (=7.15) and from *ex vivo* muscle strip experiments (=6.77). The higher throughput chip has applications in testing of cardiac tissues built from rare/expensive healthy and diseased cell sources (such as primary human cardiomyocytes and stem cells) and the fluidic device enables drug testing studies for both chronic and acute exposure, and for integration with other organ mimics.

ABSTRACT FINAL ID: 2521 Poster Board -108
TITLE: The 1000 Genomes Toxicity Screening Project: Utilizing the Power of Human Genome Variation for Population-Scale *In Vitro* Testing

AUTHORS (FIRST INITIAL, LAST NAME): N. Abdo[1], M. Xia[2], O. Kosyk[1], R. Huang[2], S. Sakamuru[2], C. Brown[4], J. Jack[4], P. Gallins[1], Y. Zhou[1], A. Motsinger-Reif[4], C. Austin[2], R. Tice[3], F. A. Wright[1], and I. Rusyn[1].
INSTITUTIONS (ALL): [1]University of North Carolina at Chapel Hill, Chapel Hill, NC; [2]NCGC/NCATS, Rockville, MD; [3]DTNP/NIEHS, Durham, NC; [4]North Carolina State University, Raleigh, NC.
KEYWORDS: qHTS, *in vitro* model, human genome

ABSTRACT BODY: The HapMap and 1000 Genomes projects have established human lymphoblast cell lines from racially and geographically diverse populations. We screened 1086 cell lines, representing 9 populations from 5 continents, in a cell viability (CellTiter-Glo®) assay with 179 chemicals at 8 concentrations (0.3 nM-92 µM). Each chemical was screened twice in 1536-well plates for ~70% of the cell lines, revealing excellent reproducibility (r=0.93 for EC10 values). Similarly, EC10 values for 9 duplicate chemicals showed excellent concordance in both median and range across cell lines. The extent of interindividual variability was less than 10 fold for about 2/3 of the compounds; however, some compounds showed more than a 100-fold range. Across all compounds, clustering of EC10 profiles revealed similarity of responses in cell lines according to the genetic ancestry. Population differences were evident for about 40% of the compounds. Using populations with parent-child trios we observed that cytotoxicity of 22 chemicals was heritable. Analyses of genome-wide association and association pathway analyses were performed to identify associations between variants/genes/pathways and

cytotoxicity. Across the 179 compounds, 142 polymorphisms were identified as suggestive of the genetic association (p-values from 10^-6 to 10^-10). Basal gene expression (RNAsequencing) data is publicly available for 344 of 1086 cell lines and we performed correlation analysis with cytotoxicity across the 179 chemicals to identify additional genes and pathways that may also be associated with interindividual differences in toxicity. We conclude that genomic-anchored *in vitro* screening offers unique advantages for identifying variations in toxicity responses at the DNA sequence level and for defining experimentally based confidence intervals for population variability.

ABSTRACT FINAL ID: 2522 Poster Board -109
TITLE: Distinct Developmental Anomalies in Zebrafish Embryos Exposed to Selected Flame Retardant Chemicals

AUTHORS (FIRST INITIAL, LAST NAME): B. Feldman[2], B. L. Goodwin[3], B. J. Collins[1], and C. P. Weis[1].
INSTITUTIONS (ALL): [1]National Institute for Environmental Health Science, Bethesda, MD; [2]National Institute of Child Health and Human Development, Bethesda, MD; [3]National Center for Advancing Translational Sciences, Bethesda, MD.
KEYWORDS: zebrafish, fire retardants, developmental toxicity

ABSTRACT BODY: A central goal of toxicology is to estimate the impact on human health of chemicals to which humans are exposed. But many potentially harmful chemicals are also known to have practical applications, creating a challenge for the assessment of potential interventions. Chemical flame-retardants comprise such a group. Included in a range of household items, some flame-retardants are suspected of disrupting hormone signaling and disturbing neurological development following exposure. Zebrafish represent a popular approach for minimizing rodent-based assays while providing a convenient model for ontogenological investigation. Here we report on a zebrafish-based screen of nine flame-retardants identified as potential toxins through cell-based assays. Treatment of zebrafish embryos from 24 hours postfertilization at high dose (80 to 100 micromolar) causes developmental abnormalities for each compound, and three groups of compounds stand out for their ability to induce acute and reproducible phenotypes. These are: (1) tert-butylphenyl diphenyl phosphate and isopropylated phenol phosphate, which cause shortening of the anterior-posterior axis and cardiac defects within 24 hours of exposure; (2) tri-o-cresyl phosphate and chloropyrifos which after 48 hours of exposure cause an abnormal “curly-up” phenotype that has previously been associated with zebrafish organ laterality defects; and (3) triphenyl phosphate and tricresyl phosphate which lead to diminished spontaneous movement and touch-response after 48 hours of exposure. Our future plans include transcriptome profiling of embryos displaying these three flame-retardant-induced phenotypes, with an eye to identifying affected molecular pathways. For the present, we conclude that a zebrafish embryo-based screen provides an observable and straightforward means of classifying candidate toxic flame-retardants into distinct functional groups.

ABSTRACT FINAL ID: 2523 Poster Board -110
TITLE: A 3D Metabolically Competent HepG2 Cell Culture Model for Toxicity Screening

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KEYWORDS: *in vitro* screening, hepatotoxicity, 3D cell culture

ABSTRACT BODY: When primary hepatocytes are placed into culture on tissue culture plastic their hepatic gene expression profile and metabolic competence declines rapidly. Similarly, hepatocyte cell lines, such as HepG2, show a de-differentiated gene expression profile, with low expression of hepatic markers and metabolic enzymes, reducing their value for toxicological studies. The culturing of hepatocytes in three-dimensional (3D) culture improves their tissue-specific properties although the available technologies are generally not suited to high throughput screening. We have developed a simple, reproducible, 3D *in vitro* model for culturing metabolically competent HepG2 spheroids in a 384 well format. In this model, HepG2 cells differentiate, undergo apico-basal polarization with bile canaliculi formation and show enhanced

metabolic competence compared to 2D cultures. Cells maintain their metabolic competence for up to 4 weeks making chronic exposures feasible. The basal expression level of key xenobiotic receptor-transcription factors AhR, CAR and PXR are up-regulated and their agonists induce expression of phase 1 and 2 metabolic enzymes. Consistent with these findings, RP-HPLC/LC-MS analysis demonstrated a pronounced increase in xenobiotic metabolite formation by HepG2 spheroids compared to HepG2 cells in 2D culture. With its improved metabolic and tissue-like properties, our 3D model may represent a more practical alternative to existing *in vitro* hepatotoxicity models for higher throughput toxicity testing.

ABSTRACT FINAL ID: 2524 Poster Board -111
TITLE: Development of a New Model of Intestinal Inflammation to Test Nanotoxicity *In Vitro*

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KEYWORDS: food-related nanoparticles, alternative *in vitro* method, gastro-intestinal barrier

ABSTRACT BODY: The use of engineered nanoparticles (NP) in food and related products is rapidly growing, whereas knowledge of their impacts on intestinal health is still poor. NP are characterised by a size range between 1 and 100 nm and an increased biological activity, compared to their larger counterparts. Therefore, they may induce inflammation and may be more toxic in the inflamed intestine. To study the impact of ingested NP hereon, a new *in vitro* model was established. Polarized human intestinal epithelial Caco-2 cells were co-cultured with differentially activated primary human neutrophils (PMN), prior to apical treatment with various food-relevant NP. The impact of NP on proinflammatory effects, barrier integrity and cytotoxicity was evaluated. Using the transwell system, PMN were coated in a matrix on the basal side of the cells, giving them the opportunity to actively migrate towards and into the epithelial cell layer. The PMN were activated directly, by using PMA, or indirectly by pretreating the Caco-2 cells with a cytokine cocktail. The former induced a reduction in epithelial barrier integrity, as determined by measuring the transepithelial electrical resistance, whereas the latter induced a higher increase in the production of IL-8 as a marker of proinflammatory effects. The three different NP used in this model, SiO2, TiO2 and ZnO, acted differentially on the cells, with ZnO leading to a significant increase in cytotoxicity after PMA-activated PMN treatment. These data prove the relevance of the new cell model, which offers an enhanced tool to mimic the inflamed epithelium *in vitro*.

ABSTRACT FINAL ID: 2525 Poster Board -112
TITLE: Understanding the Function of LXR Activation During Zebrafish Development

AUTHORS (FIRST INITIAL, LAST NAME): C. L. Pinto, P. Jonsson, M. Bondesson, and J. Gustafsson.
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KEYWORDS: zebrafish development, liver x receptor

ABSTRACT BODY: Liver X receptor (LXR), a ligand-activated transcription factor, regulates important metabolic pathways in mammals, including lipid, cholesterol and carbohydrate metabolism. While there are two LXR isoforms in mammals, LXRα (NR1H3) and LXRβ (NR1H2), only one LXR gene with higher sequence similarity to mammalian LXRα has been reported in zebrafish (zf). LXR has been shown to be expressed at early developmental stages in zf, suggesting a primordial role of LXR. To assess the effects of LXR activation during zf development, zf larvae were exposed to the LXR agonists T0901317 (2μM) and GW3965 (1μM) at dpf 4, with treatment renewal after 24h. RNA was extracted from 6-day-old larvae for microarray and qRT-PCR analyses. Microarray data demonstrated several conserved effects of the LXR ligands with mammalian models, including upregulation of LXR target genes, such as ATP binding cassette transporters (abca1a, abcg1, abcg5, abcg8), sterol regulatory element binding factor1 (srebf1), fatty acid synthase (fasn), acetyl-coenzyme A carboxylase alpha (acaca). Genes involved in carbohydrate metabolism, such as glucose-6-phosphatase (g6pca.1) and pyruvate dehydrogenase kinase (pdk4)

were downregulated by treatment with the LXR agonists. Moreover, the most significantly enriched biological pathways modulated by the LXR ligands were associated with lipid and cholesterol metabolic/homeostatic processes. Our data suggests that LXR plays an important role in the network that regulates metabolic processes during zf development and support zebrafish as an alternative model for LXR-related studies.

ABSTRACT FINAL ID: 2526 Poster Board -113
TITLE: Approach to the Photoallergenicity Assay for Cosmetic Ingredients Using Nonanimal Methods

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KEYWORDS: Photoallergenicity, Nonanimal methods, Cosmetic ingredients

ABSTRACT BODY: [Background] Photoallergenicity is the essential endpoint in safety evaluation for cosmetic ingredients. However, few nonanimal methods of photoallergenicity have been previously reported. This study aimed to develop photoallergenicity tests *in vitro* for cosmetic ingredients focusing on the cellular response stage. [Methods] As test substances, 22 substances including photo- or nonphoto-allergens from *in vivo* test were selected and applied on modified SH/NH2, ARE (Antioxidant Response Element) and photo-h-CLAT tests. Photo-SH/NH2 test took advantage of changing cell-surface thiols and amines on THP-1 cells. Photo-ARE assay could detect luciferase activity on AREc32 cells as oxidation in cells. And, photo-h-CLAT measured changing cell surface CD54 and CD 86 of THP-1 cells. [Results & Discussion] In photo-SH/NH2 test, it was positive when change of cell-surface thiols or amines on UV irradiated on treated THP-1 cells increased over 15%. Results of 88% photoallergens showed positive response while 67% nonphotoallergens were negative. In photo-ARE test, reaction was regarded as positive when luciferase activity of UV irradiated on treated AREc32 cells was over 150%. Results of 69% photoallergens showed positive while 83% nonphotoallergens were negative. Adding on those, in photo-h-CLAT test, it was positive when at least one of change of CD86 or CD54 on UV irradiated to treated THP-1 cells is over 150% or 200%. Positive results were observed in 69% of photoallergens while negative results were 75% nonphotoallergens. Photo-SH/NH2 test would become a good tool for detecting photo-allergens, and photo-ARE test would accurately reflect negative results of nonphoto allergens. These results suggested that photoallergenicity can be detected by combining those *in vitro* tests.

ABSTRACT FINAL ID: 2527 Poster Board -114
TITLE: Characterization of Cryopreserved Skeletal Muscle Cells to Build a Model for Skeletal Muscle Toxicity *In Vitro*

AUTHORS (FIRST INITIAL, LAST NAME): S. Kustermann, C. Zihlmann, K. Dernick, E. Pietilae, T. Singer, T. Weiser, F. Boess, and A. Roth.
INSTITUTIONS (ALL): F. Hoffmann-La Roche Ltd, Basel, Switzerland.
KEYWORDS: skeletal muscle toxicology, *in vitro* model

ABSTRACT BODY: The goal of this study was the establishment and characterization of a test system for *in vitro* safety assessment of drug candidates with regards to skeletal muscle toxicity. To be able to investigate potential species differences and to assess the human relevance of observed effects, we set up a battery of cell culture models including cryopreserved primary myoblasts of dog, rat and human as well as a rat L6 myoblast cell line. Skeletal myoblasts were differentiated *in vitro* for one week to resemble as closely as possible muscle fibers *in vivo* and differentiation status was assessed using markers of differentiated muscle, (i.e. Myosine Heavy Chain [MHC] and muscle Creatine Kinase [CKM]). We could demonstrate that after one week of differentiation, rat L6 myoblasts and human skeletal myoblasts did express high levels of MHC as shown by immunocytochemistry, concomitant with an up-regulation of CKM mRNA. In contrast to this, we

were not able to detect a signal for MHC or CKM mRNA upon differentiation of dog and rat primary myoblasts even though morphological changes similar to those seen after differentiation of L6 and human primary skeletal myoblasts were observed. We aimed to improve differentiation of the primary rat cells by using different media compositions without success. To test response upon xenobiotic challenge we treated cells with increasing concentrations of Cerivastatin, a drug known to induce muscle toxicity *in vivo*. Rat, dog and L6 skeletal cells did show signs of cytotoxicity upon 3 days of treatment as shown by increased LDH release and a decrease in ATP level. Human skeletal muscle cells showed a decrease in ATP level as well but no increased LDH release. Taken together, by using cryopreserved skeletal muscle cells we could successfully establish an *in vitro* model for skeletal muscle for human and rat which closely resembles mature skeletal myotubes *in vivo* including a cytotoxic response to xenobiotic challenge with a known muscle toxicant.

ABSTRACT FINAL ID: 2528 Poster Board -115
TITLE: Human Primary Hepatocytes under Controlled Hemodynamics Elicit Induction Responses to Drugs at Clinical Cmax Concentrations

AUTHORS (FIRST INITIAL, LAST NAME): A. Dash[1], T. Deering[1], S. Marukian[1], J. Thomas[1], C. Desbans[2], E. Alexandre[2], L. Richert[2], B. Blackman[1], and B. Wamhoff[1].
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KEYWORDS: hepatocyte, Cmax, *in vivo*

ABSTRACT BODY: Background: *In vitro* hepatocyte systems typically elicit drug induction/toxicity responses at concentrations much higher than corresponding clinical plasma Cmax levels, contributing to poor *in vitro-in vivo* correlations. We previously described a system that uses controlled hemodynamics to stably retain metabolic function wile restoring polarized morphology and phenotype in rat hepatocytes relative to static cultures. We tested the hypothesis that restoring these key parameters in human hepatocytes could elicit drug responses closer to clinically relevant concentrations. Methods: Plateable cryopreserved primary human hepatocytes, precharacterized for their high response to prototypical inducers according to standard protocols of FDA and EMA guidelines, were cultured under controlled hemodynamics and compared to static controls overlaid with Matrigel for 1 or 7 days, before treating with phenobarbital (50 or 500 µM), rifampicin (2.5 or 25µM), B-napthoflavone (3 or 30µM) for 72 hrs. CYP450 expression and activity were assessed by qRTPCR and *ex situ* metabolite analysis respectively. Results: Under controlled hemodynamics, hepatocytes restore polarized morphology and efflux transporter localization. Basal activity of CYP1A2, 2B6, 2C9, 3A4 and 2D6 remained stable between 4 & 10 days under controlled hemodynamics and at 2-20 fold higher levels than static. Induction responses of hepatocytes cultured in the system to all drugs were elicited at 10-fold lower concentrations than static and matched reported clinical Cmax ranges. CYP450 activity correlated with gene expression from the same conditions, assessed independently. Conclusions: We demonstrate that compared to Matrigel overlay, human primary hepatocytes under controlled hemodynamics restore polarized morphology, bile cananiculi, sustain albumin/urea production, retain CYPP450 expression/activity and induction responses at clinical drug levels achieved in humans, concurrently. Funding: EU-IMI MIP-DILI 115336-2.

ABSTRACT FINAL ID: 2529 Poster Board -116
TITLE: Predictive High-Content/High-Throughput Assays for Hepatotoxicity Using Induced Pluripotent Stem Cell (iPSC)-Derived Hepatocytes

AUTHORS (FIRST INITIAL, LAST NAME): E. F. Cromwell[1], J. Hesley[1], S. Einhorn[2], I. Rusyn[3], V. Ott[2], and O. Sirenko[1].
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KEYWORDS: pluripotent stem cells, high-content screening, liver toxicity

ABSTRACT BODY: Human iPSC-derived hepatocytes have been developed as a replacement for primary cells and show promise with respect to liver-like phenotype, unlimited availability, and a potential to establish cells from individuals who

are prone/resistant to adverse drug reactions. Accordingly, there is great interest in using iPSC-derived hepatocytes as tools for screening in drug development. While unlimited supply of such cells from multiple donors addresses one common bottleneck (i.e., availability of cells), it is yet to be shown that iPSC-derived hepatocytes are amenable to high-throughput and high-content screening analyses. In this project we tested several automated screening approaches for assessing general and mechanism-specific hepatotoxicity using iPSC-derived hepatocytes. Specifically, we used a library of 240 compounds (0.3-100uM range) and high-content imaging via automated multiparametric image analysis on a cell-by-cell basis. The endpoints assessed were cell viability, nuclear shape, average and integrated cell area, mitochondrial potential, accumulation of phospholipids, cytoskeleton integrity, and apoptosis. We found that multiparametric automated image analysis greatly increases assay sensitivity while also providing important information about possible toxicity mechanisms. Specifically, we found that multiparameter assessment increased sensitivity of the assay to 60% (with 93% specificity) which was superior to evaluation of cell viability endpoint only. In addition, the assay also demonstrated high sensitivity (70%) for selected classes of compounds such as neuroleptic, cardiac, antifungal, anticancer drugs. In contrast, assay sensitivity was lower for anti-inflammatory compounds, antibiotics, and reverse transcriptase inhibitors. We conclude that the high-throughput and high-content automated screening assays using iPSC-derived hepatocytes is feasible and can facilitate safety assessment or drugs and chemicals.

ABSTRACT FINAL ID: 2530 Poster Board -117
TITLE: Clinical Analysis of Ocular Injuries from Vapor Exposure to Chloropicrin and Hydrogen Fluoride

AUTHORS (FIRST INITIAL, LAST NAME): R. D. Causey, J. Lakin, H. Cheng, J. G. Lehman, and A. L. Ruff.
INSTITUTIONS (ALL): US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD.
KEYWORDS: Toxic Industrial Chemicals, Mouse Model, Ocular Injury

ABSTRACT BODY: Many toxic industrial chemicals (TICs) are widely used and produced in large amounts. For these reasons, they present a significant hazard for an opportunistic terrorist attack. Although accounts of accidental ocular injuries from many TICs have been widely recorded, the TICs specific risk to ocular injury is unknown. Many TIC-induced ocular injuries can result in chronic ocular health issues, and the underlying causes of these injuries are unclear. It is important to understand the ocular threat that these toxicants present and to develop effective clinical strategies for treatment of these injuries. Chemicals such as Chloropicrin, hydrogen fluoride, and others found on the chemical terrorism risk assessment list are in widespread usage across many industries; therefore, we selected these TICs for study. We developed a mouse exposure model that utilizes a “vapor cup” for direct vapor exposure to the cornea. Dose response studies were conducted, and clinical injury was evaluated by slit-lamp microscopy across a 12-week time span. Within 24 hours postexposure, gross epithelial injury was observed. Other features of injury included lens swelling, iris ischemia/necrosis, corneal neovascularization, and corneal opacity. These features began to appear as early as two days postexposure. Injury assessment will be further examined on the molecular level with target microarray analysis and protein marker analyses using corneal buttons. Disclaimer: The views expressed in this abstract are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the US Government. The experimental protocol was approved by the Animal Care and use Committee at the US Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for Care and use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544) as amended. This research was supported by an interagency agreement between NIH/NIAID and the USAMRICD.

2013 Society of Toxicology Annual Meeting
Late-Breaking Abstracts

ABSTRACT FINAL ID: 2531 Poster Board -118
TITLE: Characterization of the Immune Response in a Murine Anaphylaxis Model

AUTHORS (FIRST INITIAL, LAST NAME): Y. Zhou[1], B. Lundell[1], J. Li[2], D. M. Colleluori[2], and R. White[1].
INSTITUTIONS (ALL): [1]WuXi AppTec, Inc., St. Paul, MN; [2]WuXi AppTec, Inc., Philadelphia, PA.
KEYWORDS: anaphylaxis, immunotoxicity, Type 1 Hypersensitivity

ABSTRACT BODY: The purpose of this study was to develop a small animal model for assessing anaphylaxis (Type I hypersensitivity) when evaluating materials used in the design or development of medical devices and biologics. Female C3H/HeJ mice were administered subcutaneously three induction doses of saline (negative control), horse serum (positive control 1), or ovalbumin (positive control 2) on Days 0, 2 and 4. On Day 21, each mouse received an intravenous injection of a respective challenge dose and was observed for signs of anaphylactic reaction. Body temperatures were monitored using microchips pre- and post- each induction and challenge dose. Approximately 24 hours after the challenge dose, all animals were rendered unconscious with CO_2, exsanguinated for blood collection, and necropsied. Serum levels of IgG_1, IgE, histamine, and platelet activating factor (PAF) were measured. No abnormal clinical signs or body temperature change were noted in any of the animals during the induction phase. All negative control animals appeared normal at challenge. All of the animals challenged with known anaphylactic antigens (horse serum or ovalbumin) exhibited severe clinical symptoms, indicating a valid model. The clinical observations included erythema (feet/nose), puffiness (eyes/nose/fur), decreased/difficult/hyper respiration, abnormal gait, weakness in rear legs, head tilt, agitation, hyperactivity, decreased activity, recumbency, and hunched posture. Significant decreases in body temperature were noted in positive control animals beginning 10 minutes and continuing for up to 90 minutes after the challenge dose. At 60 minutes after the challenge dose, horse serum group had a body temperature of 83.6 ± 5.4 °F (n=14, $p < 0.0001$); ovalbumin group had a body temperature of 85.0 ± 6.0 °F (n=14, $p < 0.0001$) while saline group had a body temperature of 100.8 ± 0.6 °F (n=14). The body temperatures in positive control groups returned to normal range within 24 hours. These preliminary findings suggest it is a reliable murine anaphylaxis model.

ABSTRACT FINAL ID: 2532 Poster Board -119
TITLE: Phosgene Inhalation Increases Gelatinase Activity in Mouse Lung Lavage Fluid

AUTHORS (FIRST INITIAL, LAST NAME): J. Seagrave[1], A. Senft[1], D. Yu[1], W. Weber[1], R. M. Baron[2], L. Fredenburgh[2], and J. D. McDonald[1].
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KEYWORDS: phosgene, gelatinase, acute lung injury

ABSTRACT BODY: Phosgene is a toxic industrial chemical that might be used in terrorist attacks or in warfare. Inhalation of this gas results in oxidative stress and inflammation. The key feature of the acute lung injury induced by phosgene is massive pulmonary edema and alveolitis associated with disruption of airway epithelial tight junctions. These effects share pathophysiologic features of ALI induced by other redox insults to the lung and may escalate to acute respiratory distress syndrome. Long-term consequences can include fibrosis. Matrix metalloproteinases (MMPs) released primarily by monocytes/macrophages have been shown to contribute to the loss of tight junction function as well as degradation of the extracellular matrix, and have also been implicated in the pathophysiology of pulmonary fibrosis. We therefore assessed the cellular, protein, and MMP burden of the bronchoalveolar lavage fluid in mice 24 hr after exposure to filtered air or to inhaled phosgene at concentrations of 1, 2, or 5 ppm. Exposure to 5 ppm resulted in the deaths of 2 of 8 mice, while no deaths occurred in the lower exposure levels. Of the surviving mice, lavage fluid inflammatory cells, predominantly macrophages, were approximately doubled in the group exposed to 2 ppm, and suppressed in the group exposed to 5 ppm. However, lavage protein levels increased in a dose-dependent manner, reaching a peak greater than 50x control for the surviving animals exposed to 5 ppm phosgene. Gelatinase activity was assessed by zymography. The activity increased in a dose-dependent manner as a function of phosgene exposure to levels more than 200x control in the animals exposed to 5

ppm phosgene. These results are consistent with a role for MMPs in the loss of epithelial barrier function resulting from phosgene exposure.

ABSTRACT FINAL ID: 2533 Poster Board -120
TITLE: A Pediatric Model of Organophosphate-Induced Status Epilepticus in Freely Moving Juvenile Rats

AUTHORS (FIRST INITIAL, LAST NAME): E. Scholl[1], M. Lehmkuhle[1], J. McDonough[2], and F. Dudek[1].
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KEYWORDS: organophosphate, nerve agent, pediatric

ABSTRACT BODY: Organophosphate (OP) poisoning can result in status epilepticus (SE), a medical emergency which can become pharmacoresistant if treatment is delayed. Little to no data exists on pediatric models of OP-induced SE, even though the immature brain is likely to respond differently to OPs, and the optimal therapies are also likely to differ from adults. Our aim is to identify novel drugs that block pharmacoresistant OP-induced SE in children. Here, we describe progress on development of a pediatric model of OP-induced SE and its age dependence. EEG monitoring involved a novel miniature telemetry device, which allowed freely-moving behavior while pups were cohoused with the dam. Sprague-Dawley rats at postnatal day 7, 14, 21 and 28 (P7-P28) were implanted with the miniature telemetry device 1-2 days prior to treatment. Video-EEG recordings were obtained for up to 24 h while pups were exposed to a chemoconvulsant to elicit SE. Feasibility experiments were performed with juveniles using Li-pilocarpine, a muscarinic acetylcholine-receptor antagonist. The behavioral and electrographic responses to Li-Pilocarpine were age-dependent; P7 and P14 were quite different from adults. Furthermore, neuronal damage in the juveniles, assessed by FluoroJade B labeling, was also age-dependent and different from adult animals. Initial studies with DFP at P7, P14, P21, and P28 revealed age-specific behaviors distinct from those seen in adults. DFP and Li-Pilocarpine caused similar, but not identical, behaviors. Future studies with DFP will examine the EEG profile during and after SE in relation to neuronal damage, as assessed with FluoroJade B. Supported by the CounterACT Program, National Institutes of Health Office of the Director (NIH OD), and the National Institute of Neurological Disorders and Stroke (NINDS), Grant W81XWH-12-2-0122 as a subcontract from the US Army Medical Research Institute of Chemical Defense (MRICD).

ABSTRACT FINAL ID: 2534 Poster Board -121
TITLE: Efficacy of Pharmaceutical Therapies with Laser Debridement in Superficial Dermal and Deep Dermal Sulfur Mustard-Induced Injuries

AUTHORS (FIRST INITIAL, LAST NAME): F. M. Reid[1], E. M. Abele[1], J. Rhone[1], J. L. Plahovinsak[1], and J. Dillman[2].
INSTITUTIONS (ALL): [1]BBRC, Battelle, Columbus, OH; [2]USAMRICD, Aberdeen Proving Ground, MD.
KEYWORDS: sulfur mustard dermal lesions, laser debridement, Anti-inflammatory therapeutics

ABSTRACT BODY: The weanling swine model for sulfur mustard (SM)-induced superficial dermal (SD) and deep dermal (DD) lesions was used to evaluate the efficacy of diclofenac sodium and clobetasol propionate with and without systemically administered etanercept, a TNF-α inhibitor, on lesions with and without laser debridement. SD or DD lesions were generated by exposure to 400 µL of SM for 8 or 30 min, respectively and lesions evaluated out to 14 and 60 days. Evaluations included lesion area, growth, contracture, modified Draize scoring, reflectance colorimetry, transepidermal water loss, torsional ballistometry, laser Doppler, infrared imagery and histopathology. Patterns of treatments involving debridement indicated a mixture of results over time when compared to nondebrided and nontreated lesions. On Day 7, average scores from treatment combinations with debridement were significantly higher (more severe) than those from the nontreated and nondebrided groups. By Study Day 14, however, average scores from treatments with debridement were significantly lower (less severe) than those from the same treatments without debridement. By Days 45 and 60, none of the selected pairwise comparisons were significant for any of the endpoints observed with few exceptions. Results were consistent with and without etanercept. In conclusion, treatment with etanercept combined with diclofenac and clobetasol

demonstrated significant efficacy when compared to the nontreated control group, particularly for DD lesions. Debridement provided increased efficacy with respect to the endpoints of epidermal necrosis and re-epithelialization on Day 14 when compared to the treatment combinations without debridement. [This work was supported by the US Army Medical Research and Materiel Command under Contract W81XWH-11-D-0002, Task Order 0003.]

ABSTRACT FINAL ID: 2535 Poster Board -122
TITLE: Efficacy Testing of Treatment Paradigms for Cutaneous Sulfur Mustard Lesions

AUTHORS (FIRST INITIAL, LAST NAME): J. Plahovinsak[1], E. Abele[1], J. Rhone[1], F. Reid[1], and J. Dillman[2].
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KEYWORDS: Sulfur Mustard, Skin

ABSTRACT BODY: Sulfur mustard (HD)-induced dermal lesions in a weanling swine model were used to evaluate the efficacy of topical therapies and systemically administered anti-cytokine treatments. Specifically, this study evaluated the efficacy of diclofenac sodium (DICLO) and clobetasol propionate (CLO) with and without a systemically-administered tumor necrosis factor (TNF)-α inhibitor (adalimumab) or with and without a topical anti-oxidant, Vitamin E. Superficial dermal (SD) or deep dermal (DD) lesions were generated by exposure to 400 µL of HD for 8 or 30 min, respectively. Postexposure assessments on Days 2, 7 and 14 included lesion area, modified Draize scoring, photographs, reflectance colorimetry, transepidermal water loss (TEWL), laser Doppler imagery, torsional ballistometry, and infrared imagery. Tissues were excised on Day 14 for histopathology. Generally, SD lesions were healed (75–100% re-epithelialization) and were similar to untreated control skin by Day 14. Epidermal and dermal necrosis, collagenolysis and inflammation were minimal in untreated SD lesions and had slightly increased scores following systemic and/or topical treatments. Fibroplasia was significantly decreased in SD lesions by adalimumab without topical treatment. Generally, DD lesions were less well healed at Day 14 with severe dermal necrosis and less than 50% re-epithelialization. Untreated DD lesions were characterized by almost complete retention of the necrotic epidermis and significant dermal coagulation. Topical treatment of DD lesions with or without adalimumab treatment significantly decreased mean Draize scores and TEWL on Day 7. For DD lesions, Vitamin E alone significantly increased re-epithelialization relative to untreated controls. In summary, the data indicate that systemic adalimumab with DICLO+CLO and Vitamin E alone trended toward improved healing relative to all other therapeutic regimens when the primary and secondary parameters are considered. This work was funded by US Army Medical Research and Materiel Command; Contract W81XWH-11-D-0002, Task Order 3.

ABSTRACT FINAL ID: 2536 Poster Board -123
TITLE: Mercury Affects the Amyloid-beta Protein Levels in Rat Brain by Downregulates Neprilysin and Soluble Molecule of Low-Density Lipoprotein is Early Biomarker for Mercury-Induced Amyloid-beta Protein Accumulation

AUTHORS (FIRST INITIAL, LAST NAME): D. Kim, J. Park, and B. Choi.
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KEYWORDS: Mercury, Neprilysin, Low-Density Lipoprotein

ABSTRACT BODY: Mercury (Hg) is one of the most neurotoxic elements related to Alzheimer's disease (AD). Accumulation of Aβ plays a major role in the etiology of AD. Aβ is generated by the cleavage of amyloid precursor protein (APP) by beta-site APP-cleaving enzyme 1 (BACE1), and is degraded by neprilysin (NEP). In addition, the level of a soluble molecule of LRP (sLRP) in plasma is related the removal of Aβ from brain. Thus, this study was estimated a roles that mercury in Aβ accumulation in rat brain and investigated the sLRP by indicator of mercury-induced Aβ accumulation. Wister rats were divided four methylmercury groups (0, 20, 200, 2000 Hg µg/kg/day) for 4 weeks. We measured the mercury in whole blood (WB), frontal cortex (FC), hippocampus (HC) and cerebellum (CB). The mercury levels in all brain sections and WB showed dose-dependently increased. Also we examined the Aβ levels in cerebrospinal fluid (CSF), FC, HC and CB using ELISA kit, Aβ levels in CSF of high dose group were decreased by 56.71% compared with controls, and Aβ levels in HC were dose-dependently increased. We also examined the effect of mercury on regulation of APP, BACE1 and NEP in FC, HC and CB

using real time RT-PCR and western blotting, only NEP protein levels were significantly decreased in HC of high group (55.18%). We investigated the sLRP levels in plasma, our data showed that sLRP levels in plasma were negatively associated with mercury levels in WB and HC, and Aβ levels in HC, and positively associated with Aβ levels in CSF, and NEP protein levels of HC. Moreover, mercury-induced decreased of sLRP levels were the earliest event. These results imply that the methlymercury is easily passed through blood brain barrier. And mercury affects accumulation of Aβ in the HC by decreasing Aβ degradation. Supplementarily, sLRP levels in plasma are early biomarker for mercury-induced Aβ accumulation. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (NO. 2012-0001666).

ABSTRACT FINAL ID: 2537 Poster Board -124
TITLE: Genomic Dose Response in Lung after Nickel(II) Sulfate and Nickel Subsulfide Inhalation in Male F344 Rats

AUTHORS (FIRST INITIAL, LAST NAME): A. Efremenko[1], L. Pluta[1], M. Black[1], D. E. Dodd[1], A. Oller[2], and H. J. Clewell[1].
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KEYWORDS: nickel, genomics

ABSTRACT BODY: Nickel subsulfide (Ni3S2) was carcinogenic by inhalation in a 2-yr bioassay, while nickel (II) sulfate (NiSO4) was not. In order to determine whether these results reflect different modes of action or differences in nickel dosimetry, we compared the genomic dose response of lung airway tissue to inhaled NiSO4 and Ni3S2. Fischer F344 rats were exposed to aerosols via inhalation for one and four weeks (6 hours per day, 5 days per week) and lung airway tissues were processed using Affymetrix gene arrays. Benchmark Dose (BMD) modeling was performed and gene enrichment analysis was conducted using GeneGo. The number of statistically significant up and down regulated genes decreased markedly from one week to four weeks of exposure for both forms of nickel, as did the number of significantly enriched pathways, indicating that the cellular response to nickel exposure is a function of both concentration and duration, with evidence of cellular adaptation on repeated exposure. Pathway enrichment was similar for NiSO4 and Ni3S2 exposures, primarily reflecting inflammatory responses, developmental pathways, cell cycle control, and cytoskeleton remodeling. However, there were striking differences in the specific pathways that were altered and in the direction of gene expression change, suggesting that there may be important differences in the modes of action for the two compounds. Comparison of the BMDs for the two compounds suggests that the cellular uptake of nickel is about a factor of two to three greater at the same inhaled nickel concentration (mg Ni/cu.m.) when the exposure is to Ni3S2. Thus the failure to observe tumors in the NiSO4 bioassay may in part be due to the inability to generate a sufficiently high internal cellular nickel exposure from inhalation of this compound. (These studies were funded by NiPERA)

ABSTRACT FINAL ID: 2538 Poster Board -125
TITLE: Time-Dependent Genomic Response in Primary Human Uroepithelial Cells Exposed to Arsenite and Its Trivalent Methylated Metabolites for Up to 60 Days

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KEYWORDS: *in vitro*, arsenic, genomics

ABSTRACT BODY: Primary human uroepithelial cells were exposed continuously to arsenite (Asi) or one of its trivalent methylated metabolites, monomethylarsonous acid (MMA) or dimethylarsinous acid (DMA), over periods of up to 60 days. Cells were kept in media with concentrations of 0.1 µM Asi, 1.0 µM Asi, 0.3 µM MMA, or 3.0 µM DMA, and genomic responses were compared with unexposed cells at several time points (10, 20, 30, 40 and 60 days). Treated cells continued to proliferate over the full 60-day exposure period, in contrast to the untreated controls, which ceased proliferating after 20 days of culturing. A peak in the number of gene changes in the treated cells compared to untreated controls was observed

between 30 and 40 days of exposure, with substantially fewer changes at 10 and 60 days, suggesting remodeling of the cells over time. Consistent with this possibility, a number of genes were found for which the direction of expression change reversed between 20 and 30 days. Very few gene changes were observed at the lower Asi concentration, but at the higher Asi concentration enriched pathways included cell adhesion, cytoskeleton remodeling, apoptosis, immune response, and development. These results are similar to those observed after 24 hr exposures to mixtures of Asi, MMA and DMA in a previous study. Responses to MMA treatment were similar to those at the higher Asi concentration, while DMA treatment resulted in enrichment of somewhat different pathways, consistent with a mode of action for the toxicity of DMA different from that of Asi and MMA. (This research was supported by EPRI, Palo Alto CA.)

ABSTRACT FINAL ID: 2539 Poster Board -126
TITLE: Removal of the Sclerostin Gene Protects Against Lead-Induced Reduction of Vertebral Bone Mass and Strength

AUTHORS (FIRST INITIAL, LAST NAME): E. E. Beier[1, 2], L. Sun[2], T. Sheu[2], M. J. Zuscik[2], and J. E. Puzas[1, 2].
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KEYWORDS: Lead, Sclerostin, Osteoporosis

ABSTRACT BODY: Exposure to lead (Pb) from environmental sources remains an overlooked, but serious public health risk. We believe that intoxication from Pb, starting during childhood, results in disruption in the attainment of peak bone mass and predisposes an individual to osteoporosis. This decrease, in part, comes from depression of Wnt/β-catenin signaling, a critical anabolic pathway for the formation of bone. Wnt signaling dictates cell fate of mesenchymal stem cells, directing these cells to become osteoblasts or adipocytes. Because of the observed elevation in Wnt inhibitors sclerostin and DKK-1 following Pb exposure, we hypothesize that this represents a functional mechanism in how Pb decreases bone mass. In an effort to mitigate the Pb-induced decrease in bone mass, we used a mouse model devoid of the sclerostin gene (SOST-/-) and found that the decreases in vertebral bone quality and strength were prevented. We also observed that the percentages of Sca-1+ and CD105+ bone marrow stem cells were decreased with Pb in WT mice, but not in SOST-/- mice. Osteoblastic numbers and activity was improved, however, there was an increase in osteoclastic activity in the Pb-treated KO animals. Additionally, pharmacological activation of β-catenin using BIO proved to be uninhibited by Pb. Together these results suggest that Pb inhibits Wnt signaling upstream of β-catenin, and removal of sclerostin palliates Pb-induced reduction of vertebral bone density.

ABSTRACT FINAL ID: 2540 Poster Board -127
TITLE: Temporal Changes in Urinary Levels of Cadmium, N-acetyl-D-glucosaminidase and β2-microglobulin in Individuals with High Environmental Cadmium Exposure

AUTHORS (FIRST INITIAL, LAST NAME): H. Kim[1], D. Yim[1], S. Eom[1], S. Moon[1], C. Park[2], G. Kim[2], S. Yu[2], B. Choi[3], Y. Kim[1], and J. Park[3].
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KEYWORDS: Cadmium, N-acetyl-β-D-glucosaminidase, beta 2-microglobulin

ABSTRACT BODY: Cadmium (Cd) is a widely distributed metal that is toxic to the kidney and particularly to the renal tubules. If renal tubules are damaged by Cd, urinary excretion of N-acetyl-β-D-glucosaminidase (NAG) and beta 2-microglobulin (β2-MG) increases. This aim of this study was to describe the changing patterns of their urinary Cd, NAG, and β2-MG levels during a 3-year period. This follow-up study included 191 residents living in the vicinity of a copper refinery. Urinary levels of Cd, NAG activity, and β2-MG were measured, and their determinants and changing patterns were statistically analyzed using hierarchical general linear model (GLM) models. The natural logarithm of urinary Cd levels

decreased significantly over time. Sex and intake of locally cultivated rice were significant determinants of urinary Cd concentration. Urinary NAG activity decreased as time passed. Age and urinary Cd concentration were significant determinants of urinary NAG activity in subjects with urinary Cd concentrations ≥ 5 μg/g creatinine. In persons whose urinary Cd concentrations were more than 2 μg/g creatinine, diabetes was found to be a significant risk factor for high urinary NAG activity. In the group with urinary Cd concentrations ≥ 5 μg/g creatinine, diabetes and intake of domestic rice were associated with increased urinary β2-MG levels. The slope for temporal changes in urinary β2-MG levels was negative for subjects whose urinary Cd levels were <2 μg/g creatinine, but positive in those whose urinary Cd levels were 2–5 μg/g creatinine or ≥5 μg/g creatinine. The urinary β2-MG levels found in individuals whose urinary Cd levels were more than 2 μg/g creatinine suggests previous Cd-induced renal tubular damage.

ABSTRACT FINAL ID: 2541 Poster Board -128
TITLE: Comparison of Regional Brain Mn Accumulation in Welders, Smelters and Controls

AUTHORS (FIRST INITIAL, LAST NAME): Z. Long[1, 2], Y. Jiang[3], X. Li[4], J. Xu[1, 2], L. Long[4], W. Zheng[1], and U. Dydak[1, 2].
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KEYWORDS: Manganese, Magnetic Resonance Imaging, Welders

ABSTRACT BODY: Signal intensity indices from T1-weighted magnetic resonance images can be used as a semiquantitative *in vivo* indicator of Manganese (Mn) accumulation in the brain of Mn-exposed workers. Increases in these indices have been associated with gliosis and neurodegeneration. This study was aimed to compare regional brain Mn accumulation among smelters, welders and controls. Nine Mn-exposed smelters, 14 Mn-exposed welders and 23 age- and gender-matched controls were recruited from two factories in China. 3D T1-weighted MR images were acquired on a 3T Philips Achieva MRI scanner. Signal intensity indices were calculated as the signal ratio in the volumes of interest (VOI) in frontal cortex, posterior cingulated cortex, thalamus and hippocampus to a neck muscle reference region. Blood and urine samples were collected to measure Mn concentrations in erythrocytes and urine using inductively coupled plasma-atomic emission spectrophotometer. We found that smelters showed significantly higher Mn and Fe in erythrocytes and urine than welders ($p<0.01$ and $p<0.01$, respectively) and controls ($p<0.01$ and $p<0.01$, respectively). However, only welders showed significantly higher intensity indices in frontal cortex, thalamus and hippocampus than controls ($p<0.05$, $p<0.01$ and $p<0.01$, respectively). The thalamus index correlated with exposure years in welders ($R=0.618$, $p<0.05$) Overall, our study demonstrates that despite lower Mn levels in the erythrocytes and urine than smelters, welders have more elevated regional Mn accumulation in their brains. Thus, welders may be more prone to Mn-induced neural damage (Supported by NIH/NIEHS R21 ES-017498, National Science Foundation of China Grant #81072320 and 30760210).

ABSTRACT FINAL ID: 2542 Poster Board -129
TITLE: Differences in the Susceptibility to Cadmium-Induced Renal Tubular Damage and Osteoporosis According to Sex

AUTHORS (FIRST INITIAL, LAST NAME): Y. Kim[1], D. Yim[1], S. Eom[1], S. Moon[1], C. Park[2], G. Kim[2], S. Yu[2], B. Choi[3], J. Park[3], and H. Kim[1].
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KEYWORDS: Cadmium, β2-Microglobulin, Osteoporosis

ABSTRACT BODY: Cadmium (Cd) has been reported to induce nephrotoxicity and bone damage. This study aimed to estimate the risks for renal tubular damage and osteoporosis in individuals with long-term environmental Cd exposure. This cross-sectional study comprised 1,086 residents living in the vicinity of a copper refinery plant. Urinary Cd levels, bone

mineral density (BMD), and urinary β2-microglobulin (β2-MG) levels were measured, and their interrelationships according to sex were statistically analyzed. The geometric mean of urinary Cd level was significantly higher in female subjects (2.9 μg/g creatinine) than that in male subjects (1.6 μg/g creatinine). As the urinary Cd levels increased, the proportion of female subjects with β2-MG ≥ 300 μg/g creatinine also increased significantly, but this was not observed in the male subjects. The prevalence of osteoporosis in male subjects with urinary Cd levels ≥ 5 μg/g creatinine was significantly different compared with male subjects with urinary Cd levels < 5 μg/g creatinine. This difference was not observed in the corresponding female groups. The association between increased urinary excretion of β2-MG and decreased BMD was statistically significant only in the female subjects (odds ratio: 1.89, 95% confidence interval: 1.02–3.50). Multivariate analyses showed results with the same statistical significance. We suggest that an increased Cd body burden directly decreases the BMD in male subjects; however, in female subjects, it first induces renal microtubular damage, which can lead to osteoporosis.

ABSTRACT FINAL ID: 2543 Poster Board -130
TITLE: TEMTOX: A Novel Chemical Toxicity Test Revealing an EMT

AUTHORS (FIRST INITIAL, LAST NAME): S. Sanquer, L. Herry, C. Tomkiewicz, and R. Barouki.
INSTITUTIONS (ALL): INSERM UMR-S 747, Université Paris Descartes, Paris, France.
KEYWORDS: Regulatory Toxicity Testing, Epithelial-to-Mesenchymal transition (EMT), Dioxin

ABSTRACT BODY: Introduction: Epithelial-to-mesenchymal transition (EMT) is involved in numerous physiological and pathological processes such as embryogenesis, wound healing, chronic inflammation, fibrosis and cancer. Alteration of EMT is one of the mechanisms elicited by chemicals such as dioxin (Bui et al, Oncogene 2010, 28:3642-51). Unfortunately, its detection is generally based on qualitative techniques that are tedious to implement for large-scale studies, which explains the lack of systematic search for such a mechanism in regulatory toxicity tests. Therefore, we have developed a method, the TEMTOX test, for quantitatively characterizing an EMT process induced by a chemical substance. Methods: To design the TEMTOX test, we have taken advantage of the new functions that epithelial cells acquire when they undergo an EMT process: they are able, like mesenchymal cells, to contract a collagen gel. HepG2 cells were mixed with culture medium and a collagen solution. The mixture was poured into 6- to 24-well cluster plates and incubated at 37°C. Then, collagen gels were exposed or not to 25 nM dioxin. The extent of collagen gel contraction was simply assessed by daily measuring the diameter of the collagen gels. Changes in cytoskeleton after dioxin treatment were assessed by performing actin- and paxillin-immunostaining. Results: The exposure to dioxin of collagen gels prepared with HepG2 epithelial-like cells resulted in an enhancement of their contraction leading to a significantly lower diameter of the collagen gels as compared to control gels. Immunostaining of actin and paxillin further confirmed that dioxin promotes a change in the cytoskeleton of epithelial cells with the development of actin-containing extensions. In addition, dioxin promotes the expression of adhesion molecules with the appearance of paxillin dots on the cell membranes. Conclusion: TEMTOX is the first toxicity test that allows to visualize and quantify, in a very simple manner, the effects of chemicals on cell plasticity and some EMT steps, opening therefore the ways of performing quantitative high-throughput screening tests.

ABSTRACT FINAL ID: 2544 Poster Board -131
TITLE: Meta-Hydroxylated PCB 95 Is a More Potent Activator of RyR1 than Its Respective Para-Hydroxylated Metabolite in Myotubes

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KEYWORDS: noncoplanar PCBs, skeletal muscle, ECC

ABSTRACT BODY: PCBs are persistent, widespread environmental contaminants. Noncoplanar chiral PCBs such as PCB 95 have persisted at levels of concern to human health. Such PCBs interact with ryanodine receptors (RyRs), Ca^{2+} channels

broadly expressed in central and peripheral nervous systems and muscle. RyRs regulate release of Ca^{2+} from endoplasmic/sarcoplasmic (ER/SR), generating essential Ca^{2+} signals. PCB 95 and its 4-OH and 5-OH metabolites were synthesized and their sensitizing activity toward the type 1 isoform (RyR1) was compared using [^{3}H]ryanodine ([^{3}H]Ry) receptor binding and microsomal Ca^{2+} flux analyses. [^{3}H]Ry binding indicated a monotonic concentration-effect relationship ranging from 100nM to low µM of parent and hydroxylated PCBs. Both assays indicated the rank order of activity was 5-OH>parent>>4-OH. Single RyR1 channel current measured under voltage clamp indicated 5-OH PCB 95 stabilized the RyR1 open state by increasing mean open time and decreasing the mean close time, whereas 4-OH PCB 95 was minimally active. This structure-activity extends to other chiral PCBs. In whole cell Ca^{2+} imaging studies acute exposure to 5µM 4-OH PCB 95 did not alter excitation contraction coupling (ECC), whereas 5-OH PCB 95 significantly impaired ECC in addition to depleting Ca^{2+} stores. In subchronic studies, 300nM 4-OH PCB 95 was less potent than 5-OH PCB 95 at impairing ECC. These data identify meta-hydroxylated metabolites of PCB 95 as especially potent sensitizers of RyRs.

ABSTRACT FINAL ID: 2545 Poster Board -133
TITLE: An Ecological Risk Assessment of Trace Metal Concentrations in Sediments of Lagos Lagoon at Ikorodu

AUTHORS (FIRST INITIAL, LAST NAME): A. O. Adeogun, R. O. Ibor, and A. V. Chukwuka.
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KEYWORDS: trace metals, sediment, Lagos lagoon

ABSTRACT BODY: Inadequately treated industrial wastewater discharged into aquatic systems has been implicated in the deterioration of surface waters and elevated concentrations of toxic substances such as trace metals. To assess the recent contamination levels of trace metals in Lagos lagoon, the sediment samples of the Ikorodu segment of the lagoon receiving textile industry effluent were analyzed for levels of selected trace metals, (i.e. Fe, Cu, Mn, Zn, Pb, Cd, Ni, Cr and Co) over a 24-month period and compared with Average Shale Concentrations (ASC). Iron (Fe), Cd and Pb showed elevated concentrations in reference to the ASC at most of the sampling points. Qualitative evaluation of sediment using Contamination factor (CF) and Index of geo-accumulation (Igeo) gave similar depictions showing the Ikorodu segment as highly contaminated with Pb and Cd. The mean Pollution Load Index (PLI) (1.41) value indicated that the sediments were polluted significantly with higher values observed in the rainy season than the dry season. The Potential Ecological Risk (PER) for each trace element showed a low potential ecological risk for all metals except Cd and Pb reflecting serious and moderate ecological risks respectively. Subjection of sediment variables (Clay, Silt, water holding capacity (WHC), porosity, bulk density, organic carbon, N, P, K, Na, Mg, Exchangeable acidity, Conductivity, Fe, Mn, Cd, Cr, Pb, Co, Cu, Ni and Zn) to principal component analysis (PCA) showed that the granulometric properties (% clay and silt) of the sediments accounted for the highest variability (37.02%) and may be a major factor influencing the metal retention capacity of the sediment. The moderate risk recoded for Pb and the high risk priority recorded for Cd toxicity indicated that the lagoon was highly polluted and may pose health risks for aquatic biota and humans given the potential of these metals for ecotoxicological effects.

ABSTRACT FINAL ID: 2546 Poster Board -134
TITLE: Evaluation of Differential Cytotoxic Effects of the Oil Spill Dispersant Corexit

AUTHORS (FIRST INITIAL, LAST NAME): M. Ahuja[1], M. Zheng[2], J. S. Hayworth[2], P. Clement[2], and M. Dhanasekaran[1].
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KEYWORDS: Corexit, Environmental toxicity, Gulf Oil spill

ABSTRACT BODY: Introduction: The British Petroleum (BP) oil spill event has raised several ecological and health concerns. BP used Corexit to disperse the crude oil in the Gulf of Mexico to reduce toxicity and to prevent shoreline contamination. Nevertheless, portions of this oil/Corexit still remain in various Gulf environments, including the near shore beach environment. However, the use of Corexit itself has become a significant concern since the impacts of Corexit on human

health and environment is unclear. Aim: This study is an attempt to quantify the *in vitro* toxicity of Corexit by using cell lines from different tissues. Thus this study aims to provide indirect evidence to establish the effects of Corexit on terrestrial ecosystem and organisms. Methods: To achieve this we tested different concentrations of Corexit in four different mammalian cell lines under two varying metabolic conditions. We also conducted biochemical tests to determine the ROS generation and DNA degradation. Key Findings: The LC50 of Corexit in BL16/BL6 cell was 16 ppm, in 1321N1 cell line was 33 ppm, in H19-7 cell was 70 ppm, and in HK-2 cell line was 95 ppm under serum starved conditions. Interestingly, our results showed that Corexit exhibited dose-dependent cytotoxic effects with different levels of cytotoxicity on all the four cell lines. Prominently, the result elucidates the vulnerability of actively dividing cells in mammalian system such as bone marrow and gastrointestinal cells. ROS levels were remarkably elevated in the Corexit exposed cells demonstrating a role of oxidative stress in resultant cellular toxicity. Significance: The experimental results presented in this study raises genuine concerns about using high amounts of Corexit, a potential environmental toxin, in sensitive ocean environments.

ABSTRACT FINAL ID: 2547 Poster Board -135
TITLE: Identification of Acot1 As a Putative Marker of Perfluorododecanoic Acid Exposure-Induced Hepatotoxicity Based on Proteomic Analysis

AUTHORS (FIRST INITIAL, LAST NAME): H. Zhang, R. Cui, and J. Dai.
INSTITUTIONS (ALL): Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Beijing, China.
KEYWORDS: Perfluorododecanoic acid, 2-D DIGE, hepatotoxicology

ABSTRACT BODY: Perfluorododecanoic acid (PFDoA) is a member of the perfluoroalkyl acid (PFAA) family with has broad applications and a wide distribution in the environment. As the liver is the primary bioaccumulation site of PFAAs and its target organ, many studies have focused on the hepatotoxicity of PFAAs, but few have focused on the hepatotoxic mechanism of long-chain PFCs, especially in the translational level. Male rats were exposed to 0, 0.05, 0.2 and 0.5 mg/kg/day of PFDoA for 110 days. After two-dimensional difference in gel electrophoresis (2-D DIGE) and MALDI TOF/TOF analysis, 73 differentially expressed proteins between the control and the PFDoA treated rats (0.2 and 0.5 mg-dosed groups) were successfully identified. These proteins were mainly involved in lipid metabolism, amino acid metabolism, TCA cycle and pyruvate metabolism, gluconeogenesis and glycolysis, stress response, cytoskeleton-related proteins and other functions. Qantitative PCR and western blots were used to verify the veracity of 2-D DIGE. Additionally, the expression levels of Acot1 in the 0.5 mg/kg/d PFDoA group showed over six fold change in protein level compared to the control, as well as thousand fold changes in mRNA levels. Moreover, *in vitro* research was further to confirm that the expressional levels of Acot1 increased depending on the induction of PPARα and showed obvious dose-dependent induction. Acot1 could be a potential biomarker for hepatotoxicity of PFDoA.

ABSTRACT FINAL ID: 2548 Poster Board -136
TITLE: Effect of Perfluorononanoic Acid Exposure on Hepatic microRNAs and Lipid Regulated Genes in Mice

AUTHORS (FIRST INITIAL, LAST NAME): J. Wang, S. Yan, and J. Dai.
INSTITUTIONS (ALL): Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China.
KEYWORDS: perfluorononanoic acid, microRNA, hepatic effect

ABSTRACT BODY: Perfluoroalkyl chemicals (PFASs) are a class of highly stable man-made compounds, and its toxicological impact is currently of worldwide concern. In this study, the hepatic effects of perfluorononanoic acid (PFNA), a nine carbon backbone of perfluorocarboxylic acids (PFCAs), on mice were detected. PFNA administration resulted in increase of liver weight dose-dependently (0, 0.2, 1 and 5 mg/kg body weight, once a day for 14 days), and increase of hepatic triglyceride and cholesterol in median dose group, as well as serum transaminases in high-dose group. The mRNA level results indicated that PFNA exposure not only possess fatty acid oxidation effect, but also activated genes involved in fatty acid and cholesterol synthesis in mice liver. We further investigated the potential involvement of microRNAs (miRNAs) in the hepatic

effect of PFNA exposure using miRNA microarrays. Four (3 down, 1 up) and thirty-one (15 down, 16 up) miRNAs exhibiting at least 2-fold alteration ($P < 0.05$) were identified in 1 and 5 mg/kg/day PFNA treatment groups, respectively. Molecular network analysis of putative targets showed that the altered miRNAs potentially regulated many cellular processes and pathways, such as inflammatory response, endocytosis, and tumorigenic signature. The results indicated that PFNA can exert its hepatic effect, at least partially, by altering the levels of miRNAs.

ABSTRACT FINAL ID: 2549 Poster Board -137
TITLE: Circulating MicroRNA Profiles Altered in Mice After 28 Days Exposed to Perfluorooctanoic Acid

AUTHORS (FIRST INITIAL, LAST NAME): J. Dai and S. Yan.
INSTITUTIONS (ALL): Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Beijing, China.
KEYWORDS: Perfluorononanoic acid, Circulating miRNAs, Biomarker

ABSTRACT BODY: Perfluorooctanoic acid (PFOA) is stable man-made compound with many industrial and commercial uses. Concern has been raised that it may induce hepatotoxicity, immunotoxicity, developmental effects and some other toxicological effects. Circulating miRNAs as potential novel biomarkers have been found to be altered during several clinical diseases, but whether PFOA can alter the expression levels of circulating miRNAs is still unknown. Here, we explore differentially expressed circulating miRNAs in mice after exposure to PFOA. Using Taqman miRNA array analysis, we found that there were 24 circulating miRNAs altered in the 1.25 mg/kg/d PFOA group and 73 altered in the 5 mg/kg/d group. Six miRNAs, miR-28-5p, miR-32-5p, miR-34a-5p, miR-122-5p, miR-192-5p and miR-200c-3p, were selected for further validation using TaqMan Real-Time PCR Assays, and all of their expression levels were significantly increased after PFOA exposure. Among these miRNAs, the expression levels of miR-122-5p and miR-192-5p, which may be connected with liver injury, were sharply increased. In addition, expression levels of two other circulating miRNAs, miR-26b-5p and miR-199a-3p, which have been found to be associated with PFOA concentration in human serum, were elevated also. Our results demonstrated that circulating miRNA profiles could be altered by exposed to PFOA and some of them may indicate liver injury and act as potential novel biomarkers for PFOA exposure.

ABSTRACT FINAL ID: 2550 Poster Board -138
TITLE: Does Mercury Play a Role in Seal Strandings in the Northeast US?

AUTHORS (FIRST INITIAL, LAST NAME): T. Saddler, W. Smiley, and D. Stevens.
INSTITUTIONS (ALL): Life Sciences, Salish Kootenai College, Pablo, MT.
KEYWORDS: mercury, methylmercury, Harbor seal

ABSTRACT BODY: Every year seals are found stranded on various coastlines in the US. Often, no apparent cause for this behavior can be found, even after a thorough necropsy. Methylmercury (MeHg) is a potent neurotoxin and has been shown to cross the placental barrier, exposing fetuses. Fish is the main source of MeHg and seals are primarily piscivores, presumably leaving them vulnerable to mercury's neurodevelopmental effects. We were interested in evaluating the role MeHg may play in these strandings. For this preliminary study, archived necropsy samples from 33 pup and weanling Harbor seals (*Phoca vitulina*), collected from the northeastern US in 2009 and 2010, were used. Hg levels in this age group would be indicative primarily of *in utero* exposures. Total Hg concentrations were determined in muscle, liver, kidney and brain (cerebrum). Additionally, total Hg was also determined in the fur to test whether fur could be used as a noninvasive biomarker of mercury levels in these tissues. Selenium, often considered protective against the effects of methylmercury, was determined in brain to calculate a Se:Hg ratio for this target organ. Finally, brain MeHg levels will also be determined (still pending) to examine the extent of demethylation, a putative detoxification pathway in the formation of nonbioavailable mercury selenide. Average Hg levels were as follows in ppb (with standard deviation): muscle: 262 (129); liver: 689 (476); kidney: 717 (335); brain: 71 (40); fur: 4482 (2744). Average brain Se value (ppb) was: 224 (84). The individual Se:Hg molar ratio varied from approximately 2 for the highest exposed seals to over 20 for the seals showing the lowest Hg accumulation. Thus, with low brain Hg levels and beneficial Hg:Se ratios for all but a few seals, Hg could probably

be ruled out as an important factor in most of the strandings in this first small study. Finally, fur Hg showed a positive correlation with Hg in each of the tissue compartments. However, the r-squared values were low, ranging from 0.14 (brain) to 2.6 (kidney), so fur Hg is not a good quantitative biomarker of mercury exposure.

ABSTRACT FINAL ID: 2551 Poster Board -139
TITLE: Seafood Safety following the Deepwater Horizon Disaster

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KEYWORDS: Seafood Safety, Deepwater Horizon Oil Spill, Polycyclic Aromatic Hydrocarbons (PAHs)

ABSTRACT BODY: The 2010 Deepwater Horizon oil spill led to the release of ~4.5 million barrels of crude oil into the Gulf of Mexico, making it the largest oil spill in US history. Crude oil contains a number of petrogenic Polycyclic Aromatic Hydrocarbons (PAHs) suspected of causing adverse effects including immunosuppression, developmental abnormalities, and cancer through aberrant activation of the Aryl Hydrocarbon Receptor (AhR). The potential bioaccumulation of these PAHs in the Gulf marine biota represents a long-lasting risk for adverse human health through consumption of tainted seafood. Subsistence fishing communities throughout the Gulf region are especially susceptible to PAH toxicity because these communities consume greater than average amounts of seafood. In response to this concern, this study is measuring PAH contamination in Gulf shellfish species including brown as well as white shrimp, blue crab, oysters, and several fin-fish (red snapper, grouper, mackerel, speckled trout). In keeping with the principles of community based participatory research, the samples are collected by our community partners in coastal Alabama, Louisiana, and Mississippi. PAHs were extracted from samples in the presence of deuterated standards, then quantified and characterized by gas chromatography mass spectrometry. Seafood PAH contamination has been detected at levels exceeding 1.3 µg/gm tissue, with levels in oysters>blue crab>brown shrimp>white shrimp = finfish. The toxic potential of these extracts was analyzed using the EPA-approved Chemically Activated Luciferase gene eXpression(CALUX) bioassay to measure AhR activation. The results are compared to a TCDD dose response to calculate the total toxic equivalency (TEQ). Using a maximal daily intake (NOAEL) of 4 pg/kg/day TEQ, the CALUX assay results revealed that the current US Food and Drug Administration consumption guidelines are inadequate to protect individuals who, like those in Gulf fishing communities, consume above average amounts of Gulf Seafood. This work is supported by U19ES020676 and T32ES07254.

ABSTRACT FINAL ID: 2552 Poster Board -140
TITLE: Antiproliferative Activity of *Chromoloaena odorata L.* (Asteracea) Leaf Extract on Human Colon Cancer Cell Lines

AUTHORS (FIRST INITIAL, LAST NAME): O. E. Ola-Davies[1], J. E. Mata[2], and O. Odunola[3].
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KEYWORDS: C. odorata, CaCo2 cells, Apoptosis

ABSTRACT BODY: Ethanolic leaf extract from *C. odorata L.* (Asteraceae) used traditionally as anticancer agent was evaluated *in vitro* for the *in vitro* antiproliferative activity against Human colon cancer cell lines (CaCo2). The extract was fractionated by flash and vacuum liquid chromatographic techniques using methanol, n-hexane, ethyl acetate, acetone and distilled water. Thin Layer Chromatography (TLC) was used to identify active fractions. The apoptotic activity of active fractions was determined using Cell Death Detection Enzyme Linked Immunosorbent assay with cisplatin, a known anti cancer chemotherapeutic agent as positive control. All the fractions demonstrated significant antiproliferative activity against CaCo2 cell lines. Each of the chromatographic fractions (1-5) from *C. odorata* showed cytotoxic effect on CaCo2 cells with fraction 2 having the minimum 50% growth inhibition concentrations (IC50). Further purification and separation of this fraction produced six fractions tagged C1 - C6. All these fractions also showed cytotoxicity in CaCo2 cells with least IC50 of 0.05 µg/ml produced by fraction C3. TLC analysis showed the presence of alkaloids, tannins, saponins, phlobatannin, and

flavonoids in the extract. This finding suggests that the induction of apoptosis may be one of the mechanisms through which the fractions are exerting their antiproliferative activity in the cancer cell lines. Further studies are on going on detailed characterization and extensive biological evaluation of the most active component of the extract.

ABSTRACT FINAL ID: 2553 Poster Board -141
TITLE: *In Vitro* Cytotoxic Activity of *Rhus trilobata* Extracts in Caco-2 Cells

AUTHORS (FIRST INITIAL, LAST NAME): B. E. Sánchez Ramírez[1], L. Varela-Rodríguez[1], E. Salas Muñoz[1], A. García-Triana[1], M. González-Horta[1], and P. Talamás-Rohana[2].
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KEYWORDS: cytotoxicity, Polyphenols, Rhus trilobata

ABSTRACT BODY: Aqueous infusions of *Rhus trilobata* (Skunkbush sumac) have been used in traditional medicine for cancer treatment. The aim of this study is to evaluate the cytotoxic activity of *Rhus trilobata* extracts in Caco-2 cells. Skunbrush was recollected in May and November; extracts were prepared using only shrub stems, boiling for aqueous infusion, by maceration in 70% methanol or in hexane. Solvents were evaporated in rotavapor and dried extracts were reconstituted and tested for *in vitro* (i) cytotoxicity against Caco-2 cells, using the MTT cytotoxicity assay; (ii) antioxidant activity, analyzed by measuring the scavenging activity of DPPH radicals; (iii) polyphenols content, determined by Folin–Ciocalteu Assay; and (iv) qualitative composition, characterized by phytochemical analysis and HPLC. Results showed that all extracts tested had *in vitro* cytotoxic activity against Caco-2 cells in a similar concentration to Vincristine; methanol extract showed the highest cytotoxicity. Folin-Ciocalteu Assay revealed a high concentration of polyphenols by 165 mg/g of shrub stems that agree with antioxidant activity. Qualitative composition showed the presence of alkaloids, flavonoids, anthocyanins, coumarins, tannins, and triterpenes; HPLC assay confirmed the presence of different types of polyphenols in crude extracts. These results demonstrated that, additional to medical uses of *Rhus trilobata* as antiviral, anti-inflammatory and astringent agent, this bush produces a high number of compounds that could be used in the treatment of some kind of cancer. The high polyphenol content present in extracts showed promising potential antioxidant activity. Studies are in progress to a better characterization of the bioactive molecules. Grant FOMIX CHIH-2010-01-143572.

ABSTRACT FINAL ID: 2554 Poster Board -142
TITLE: Biomarkers of Zinc Deficiency

AUTHORS (FIRST INITIAL, LAST NAME): Y. Nkrumah-Elie[1], J. Kirkwood[1], J. Stevens[1], R. Tanguay[1], C. Chung[2], J. King[2], K. Brown[3], and E. Ho[1].
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KEYWORDS: Zinc Deficiency, Biomakers, Metabolomics

ABSTRACT BODY: Zinc deficiency can result in DNA damage, increased sensitivity to oxidative stress and can contribute to an increased susceptibility to environmental toxins and the development of chronic disorders such as cancer, insulin resistance, and immune dysfunction. Despite the established interaction between zinc deficiency and susceptibility to toxins, zinc status is seldom considered when monitoring the impact of environmental stresses in the population. A major barrier in the field is the lack of reliable biomarkers for human zinc deficiency. To examine novel biomarkers of zinc deficiency in humans, zinc levels and other functional indices were assessed in plasma samples from a controlled feeding trial in healthy men to induce zinc depletion. No significant differences in plasma zinc levels (current clinical biomarker) were detected at baseline, after 6 wk of zinc deficiency, and after 4 wk of zinc repletion. Zinc depletion did cause a significant increase in DNA damage that was reversed with zinc repletion. Untargeted, unbiased metabolic profiles were also evaluated in plasma samples obtained at each dietary phase using LC-MS/MS technology. Principle component analysis demonstrated metabolite clustering among dietary groups. A total of 12 different metabolites were significantly ($P<0.05$) up

or down regulated (log 2-fold change) during dietary zinc depletion relative to baseline and was reversed with zinc repletion. Methylhistidine was identified as the most significantly increased (8-fold change) metabolite resulting from zinc depletion. This research identified unique zinc-dependent processes and novel metabolomic signatures responsive to zinc deficiency. These functional parameters may be used as biomarkers for zinc status in humans and aid in identifying individuals with altered susceptibility to environmental stresses. Research examining the interaction between zinc status and toxicants such as arsenic and PAHs are an important area of future work.

ABSTRACT FINAL ID: 2555 Poster Board -143
TITLE: Effects of Cobalt Dietary Supplementation on Cobalt Body Burden, Steady-State Levels and Selected Biochemical Parameters

AUTHORS (FIRST INITIAL, LAST NAME): B. E. Tvermoes[1], B. Finley[2], J. Otani[2], K. Unice[3], D. Paustenbach[2], and D. Galbraith[2].
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KEYWORDS: Cobalt, Dietary Supplements, Biomonitoring

ABSTRACT BODY: Concerns have recently been raised regarding blood cobalt (Co) concentrations in individuals with Co-containing hip implants. However, there is little information available regarding the relationship between blood Co concentrations and health effects. Therefore, we measured blood Co concentrations in individuals consuming off-the-shelf Co dietary supplements. In our study, five healthy adult male and five healthy adult female volunteers ingested 1.0 mg Co/day of a commercially available Co supplement for an average of 31 days. Predosing whole blood Co concentrations were found to be ≤ 0.5 µg/L. Mean Co blood concentrations in males and females after an average of 31 days of dosing were 16 µg Co/L (9.6-32 µg Co/L) and 33 µg Co/L (7.3-91 µg Co/L), respectively. Most individuals reached a steady-state condition after 2 weeks of dosing. To assess the potential physiologic effects of Co supplementation, several clinical parameters were studied, including creatine kinase-myocardial band (CK-MB), red blood cell (RBC), hematocrit (Hct), hemoglobin (Hgb), thyroid stimulating hormone (TSH), and free thyroxine (T4). There were no clinically significant differences between values at baseline and Day 31 for these parameters, suggesting that there were no cardiac (CK-MB), endocrine (TSH and T4), or hematological (RBC, Hct, and Hgb) effects at these doses. Overall, our results indicate that Co blood concentrations ranging from 9.6 to 91.4 µg/L are attained when consuming Co supplements at the recommended dosage, and that clinical effects do not occur at these blood Co concentrations. Given that most hip implant patients have blood Co concentrations less than 10 µg/L, we believe these findings suggest that the vast majority of individuals with Co-containing hip implants are not likely to be at risk of developing Co-related systemic health effects.

ABSTRACT FINAL ID: 2556 Poster Board -144
TITLE: Cobalt Blood Concentrations and Health Effects in Adult Volunteers During a 90-Day Cobalt Supplement Ingestion Study

AUTHORS (FIRST INITIAL, LAST NAME): D. Paustenbach[1], B. E. Tvermoes[2], J. Otani[1], K. Unice[3], B. Finley[1], and D. Galbraith[1].
INSTITUTIONS (ALL): [1]ChemRisk, San Francisco, CA; [2]ChemRisk, Boulder, CO; [3]ChemRisk, Pittsburgh, PA.
KEYWORDS: Cobalt, Dietary Supplements, Biomonitoring

ABSTRACT BODY: Cobalt (Co) supplements are available for sale in the US but little is known regarding the efficacy of these supplements or the Co body-burden experienced by the users of these over the counter supplements. Further, concerns have been raised regarding Co exposures and potential health effects in patients with Co-containing hip implants. In this study, five healthy adult males and four healthy adult females ingested ~1.0 mg Co/day of a commercially available Co supplement for a three month period. Average predosing whole blood Co concentrations were ≤ 0.8 µg/L. The mean Co blood concentrations in males and females after approximately 90 days of dosing were 20 µg/L (12.4-32.9 µg/L) and 61 µg/L (6.2-117 µg Co/L), respectively. The average creatine kinase-myocardial band (CK-MB), red blood cell (RBC), hematocrit (Hct), hemoglobin (Hgb), thyroid stimulating hormone (TSH), and free thyroxine (T4) levels were within normal reference ranges for males and females at day 90. We also studied several clinical variables in the volunteers, including hearing,

vision, and cardiac function. These were assessed prior to study onset, at the study midpoint, and just after study completion. No significant changes in hearing, vision or heart function were detected at any time point studied. We believe that our findings offer a useful comparative benchmark for populations currently exposed to Co. Our findings indicate that ingestion of over the counter Co supplements leads to blood Co concentrations generally much higher than those measured in most metal-on-metal (MoM) hip implant patients (10 μg/L or less), which suggests that most implant patients are not at risk of developing Co-related systemic health effects.

ABSTRACT FINAL ID: 2557 Poster Board -145
TITLE: Aflatoxin Adsorption by Smectite Clays

AUTHORS (FIRST INITIAL, LAST NAME): A. G. Marroquin-Cardona[1], A. L. Barrientos-Velázquez[3], Y. Deng[3], N. J. Mitchell[2], and T. D. Phillips[2].
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KEYWORDS: Aflatoxin, Smectite, Adsorption

ABSTRACT BODY: Aflatoxins are toxic fungal products found in foods. Montmorillonite, a mineral in the smectite group, has been reported to sorb aflatoxins and to reduce their bioavailability after oral administration. There are six different categories of smectites and in the present work we investigated the sorption ability for aflatoxin B1 (AFB1) and the mineral characterization for various smectite samples. Fourier transformed infrared (FTIR), and X-ray diffraction analysis (XRD) were used to determine mineral identity, and X-ray fluorescence (XRF) to reveal elemental composition. Adsorption isotherms for AFB1 were done at pH 6.5 using 11 dilutions of toxin ranging from 0.4 ppm up to 8 ppm with 0.1 mg of clay. Sorption ability was assessed by Qmax (capacity) and Kd values. In total two montmorillonites (Na and Ca), two saponites (from Australia and Spain), a nontronite, a beidellite and one hectorite were analyzed. Results of XRD and FTIR verified the identity of the minerals. XRF data showed expected trends of Na content for Na-rich montmorillonite. Hectorite had the highest concentration of Ca and calcite peaks were observed in the XRD pattern. This clay also had the highest levels of Sr. Ca-rich montmorillonite occupied the second position in Ca and Sr content and the first one in Zn concentration. Nontronite had the highest amount of Fe while beidellite had the highest levels of Al. The saponites had a high content of Mg, as expected. The AFB1 adsorption analyses revealed differences in binding ability. Montmorillonites depicted the highest Qmax values (~0.4 mol AFB1/kg of clay) followed by saponite (Spain), beidellite, saponite (Australia), hectorite and nontronite. This research suggests that cations exchanged in smectites may play an important role in the AFB1 sorption ability of smectite clays. Funded by USAID LAG-G-00-96-90013-00 and NIH 1RO1MD005819-01.

ABSTRACT FINAL ID: 2558 Poster Board -146
TITLE: Toxicological Evaluation of *In Vitro* and *In Vivo* Effects of Jambu Oleoresin

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KEYWORDS: Jambu Oleoresin

ABSTRACT BODY: Jambu Oleoresin (extracted from *Spilanthes acmella* var. oleracea) use as a flavoring agent is expanding. To further evaluate its' toxicological properties three genotoxicity assays, *in vivo* effect on cholinesterase activity, three dermal toxicity assays, ocular irritation, acute and 28-day oral toxicity assays were conducted using multiple commercial batches of Jambu Oleoresin. Significant toxicological results were found in pilot dosing studies, micronucleus, cholinesterase, dermal and oral toxicity assays. Jambu Oleoresin elicited positive responses in the mouse lymphoma assay indicating chromosomal damage. Pilot dosing studies for the mouse micronucleus test resulted in convulsions and mortalities following intraperitoneal (1,000 and 2,000 mg/kg) and oral (100-1,000 mg/kg) dosing. Two female rats displayed convulsions followed by death in the cholinesterase study (500 mg/kg). Jambu Oleoresin produced transient irritant effects

but was classified as a nonirritant to intact and abraded skin. Depending on the evaluation criteria (Kay and Calandra, CPSC, or EEC), Jambu Oleoresin was classified as a non to mild eye irritant. Behavioral and anatomical abnormalities as well as mortalities were seen with acute oral doses of 200-5,000 mg/kg. The LD50 was calculated to be 213 and >2,000 but <5,000 mg/kg in females and males, respectively. The 28-day oral toxicity range finding study showed similar toxic effects to the acute study (200-1,000 mg/kg/day). Different batches of Jambu Oleoresin demonstrated inconsistent behavioral and anatomical toxicity as well as mortality, especially in females, in different assays. The significant toxicological effects, and differences in effects between commercial batches, suggest batch specific characterization is warranted in future evaluations of Jambu Oleoresin.

ABSTRACT FINAL ID: 2559 Poster Board -147
TITLE: Nephrotoxicity of Melamine, Cyanuric Acid, and Their Combination in Newborn F344 Rats

AUTHORS (FIRST INITIAL, LAST NAME): G. Gamboa da Costa[1], L. Loukotková[1], L. S. Von Tungeln[1], G. Olson[2], R. L. Sprando[3], D. G. Hattan[3], C. B. Stine[4], R. Reimschuessel[4], and F. A. Beland[1].
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KEYWORDS: Melamine, Cyanuric acid, Nephrotoxicity

ABSTRACT BODY: The intentional adulteration of pet food ingredients with melamine (MEL) and a number of its derivatives, including cyanuric acid (CYA), caused kidney failure and death of hundreds of cats and dogs in the USA. Co-exposure to low levels of these compounds elicits nephrotoxicity due to the formation of melamine cyanurate crystals in the nephrons. In previous experiments we evaluated the dose-response for a co-exposure to MEL and CYA in adult F344 rats for periods of up to 90 days. In order to evaluate the relative sensitivities of adult versus newborn rats to the nephrotoxic effects of these triazines, we have now assessed the dose-response of a co-exposure of F344 rats from postnatal day 1 (PND1) to weaning (PND21). Groups of male and female rats (24 rats/sex/dose) were gavaged daily with 0 (control), 0.63, 1.25, 2.5, 5, 10, 20, or 40 mg/kg bw of MEL and CYA, or with 100 mg/kg bw MEL or CYA. The individual exposures to MEL or CYA failed to produce any noteworthy observations, as was previously observed in adult rats. However, the newborn rats were substantially less sensitive than the adult rats to the combined exposures to MEL and CYA as assessed by general observations, histopathology, wet-mount analysis of melamine cyanurate crystals in the kidneys, and clinical chemistry. Although the reasons for the lower sensitivity of the newborn rats are still under investigation, we hypothesize that physiologic differences in the developing kidney (e.g. lower concentration of the urine filtrate) may reduce the ability of MEL and CYA to co-precipitate as melamine cyanurate in the nephrons. Funded by FDA IAG 224-12-0003 / NIH AES12013 between the FDA/NCTR and the NIEHS/NTP.

ABSTRACT FINAL ID: 2560 Poster Board -201
TITLE: Jacketed External Telemetry Acquisition: Environmental RF Spectrum Analysis to Optimize Wireless Data Transmission

AUTHORS (FIRST INITIAL, LAST NAME): S. Tichenor, D. Regalia, R. Kaiser, and H. Holzgrefe.
INSTITUTIONS (ALL): Charles River Laboratories, Reno, NV.
KEYWORDS: JET jacketed external telemetry, radio frequency interference, Bluetooth data acquisition

ABSTRACT BODY: Utilization of Jacketed External Telemetry (JET) in preclinical toxicology studies for continuous unrestrained data collections has become a preferred approach to less sensitive snap shot data collections such as ECG and blood pressure from restrained animals. JET relies on Bluetooth technology, a short wavelength radio transmission in the 2.4 GHz band, to transfer data from the JET device to the acquisition system. JET reduces hardwired connections and enables individually ascribable, continuous data signals. Variable background radio frequency (RF) interference is common in most animal rooms due to ambient contributions, principally from proximal electrical equipment and wireless networks.

Above a critical threshold level of ~-60 decibels per milliwatt (dBm), RF interference may corrupt JET data transmission, and in extreme cases, result in signal failure. As such, the prestudy characterization of ambient RF noise is necessary to ensure the integrity of JET data transmission during scheduled recording sessions. Accordingly, we characterized the background 2.4 GHz environment in 126 study rooms employing a portable RF spectrum analyzer (Wi-Spy, MetaGeek, Boise, ID). This survey indicated that approximately 90% of the study room environments were amenable to JET technology as demonstrated by RF amplitudes ≤ 60 dBm for at least 85% of the 2.4 GHz band range. In the remaining rooms, the RF noise exceeded empirical JET interference threshold amplitude of -60 dBm for at least 15% of the 2.4 GHz band range. Some rooms occasionally lacked any open frequencies, completely blocking Bluetooth JET transmissions. Therefore, prospective RF characterization of JET study suites with a suitable spectrum analysis device is essential to assure continuous high fidelity data collections using JET or other Bluetooth-based data streaming devices. Failure to do so may result in otherwise unrecoverable data loss.

ABSTRACT FINAL ID: 2561 Poster Board -202
TITLE: Derisking Cardiovascular Hypotension Using Highly Predictive Preclinical Low-Bulk Screening

AUTHORS (FIRST INITIAL, LAST NAME): T. Brabham[1], J. Heyen[2], M. Hemkens[2], J. Guzova[3], L. Warren[4], W. Hu[5], T. Schroeter[4], and J. W. Davis[3].
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KEYWORDS: Cardiovascular, Hypotension, Safety

ABSTRACT BODY: With increasing costs in developing effective safety derisking strategies, gaining cardiovascular safety data via predictive low bulk requiring assays, offers a clear drug-candidate API advantage. Our novel screening funnel compared specified chemical classes in development vs. known modulators of blood pressure; to establish high confidence in translation across assays including: rat aortic ring, anesthetized rat and conscious rat telemetry models. The established translation detected significant cardiovascular effects in the aortic ring (~30–100% relaxation) and anaesthetized rat (>15% decreased systolic and diastolic blood pressure) assays. A key relationship in developing this strategy included the establishment of an EC_{10}, as a concentration relevant to observed *in vivo* effects. Four-point concentration response curves were conducted in KCl precontracted rat aorta rings to identify the EC_{10}; coupled with comparisons to predicted target efficacious concentrations, to establish theoretical safety margins. Follow up testing of lead compounds with desirable safety margins in anaesthetized and conscious rat models confirmed hypotensive effects at plasma exposure concentrations near an EC_{10}. Our *in vitro* investigations demonstrated that specific compounds also decreased phosphorylation of myosin light chain (p-MLC) in a manner that correlated with potency in rat aortic rings. Consequently, a high throughput screen utilizing “In Cell Westerns” to detect p-MLC was used for added SAR and drug-candidate prioritization. Collectively, these data suggest the novel rat cardiovascular screening funnel is capable of predicting drug-mediated hypotensive risk with high confidence, and aids in advancement of compounds with acceptable cardiovascular profiles.

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Late-Breaking Abstracts

ABSTRACT FINAL ID: 2562 Poster Board -203
TITLE: Direct Evidence for PDCD10 (CCM3) Playing an Important Role in the Initiation, Promotion and Progression of Cavernous Angioma

AUTHORS (FIRST INITIAL, LAST NAME): Y. He[1, 2], H. Zhang[1], A. Vortmeyer[1], S. Peng[3], and W. Min[1].
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KEYWORDS: CCM3, vascular malformation, cavernous angioma

ABSTRACT BODY: Cerebral cavernous malformations (CCMs) account for 10–15% CNS vascular malformations, but the cellular and molecular events causing CCMs remain poorly characterized. CCM1, CCM2 and CCM3 genes have been identified by lineage analysis from human CCM. Recently we have reported that CCM3-mediated VEGFR2 signaling was critical for vascular development by ubiquitous or specific deletion in the vascular endothelium. Here we report mice bearing CCM3 specific deletion in SMC/pericyte and bearing CCM3 global inducible deletion all can generate human-like CCM lesions, even in as young as E11.5 embryos. Pathological CCM lesions in CCM3 SMCs/pericytes-specific knockout (smKO) old mice were same as human lesions comprising closely clustered, enlarged capillary channels (caverns) with a single layer of endothelium without mature vessel wall elements or normal intervening brain parenchyma. In the CCM initiation stage, CCM3 deletion caused abnormal vasculogenesis-de novo vascular malformation, while SMC/pericyte still covered the vessel. In promotion stage, the lesion showed abnormal vasculature, disturbed blood flow, thrombosis, hypoxia, inflammation, angiogenesis. Finally the lesion entered into progress stage and demonstrated multistage hemorrhage, infiltration and fibrosis. Our conclusion is that CCM3 deletion plays very important role in the whole process of cerebral cavernous malformations.

ABSTRACT FINAL ID: 2563 Poster Board -204
TITLE: Activation of Nrf2-Regulated Gene Expression in Cardiomyocytes in Response to Acute Doxorubicin-Induced Oxidative Stress

AUTHORS (FIRST INITIAL, LAST NAME): K. K. Nordgren and K. B. Wallace.
INSTITUTIONS (ALL): Biomedical Science, University of Minnesota Medical School, Duluth, MN.
KEYWORDS: Doxorubicin, NRF2, Cardiac

ABSTRACT BODY: Doxorubicin (DOX) is a widely prescribed treatment for a broad scope of cancers, but clinical utility is limited by the cardiomyopathy/congestive heart failure that occurs in 20% of patients. DOX-induced cardiotoxicity is due, in part, to reactive oxygen species (ROS) formation, which can result in lipid peroxidation and damage to DNA, RNA, and proteins. A major cellular defense mechanism against oxidative stress is activation of the Nrf2-antioxidant response element (ARE) signaling pathway, which transcriptionally regulates expression of genes associated with antioxidant defense. Normally, the cytosolic form of transcription factor Nrf2 is bound to reduced-Keap1 protein and targeted for proteosomal degradation. In cases of increased ROS, Keap1 is oxidized, releasing Nrf2, which then translocates to the nucleus, binds promoter ARE motifs, and controls expression of genes whose protein products are involved in the detoxication of ROS. We have shown previously that DOX redox cycles on the mitochondrial respiratory chain to release ROS and cause oxidative injury in cardiac tissues. We hypothesize that the oxidative stress induced by DOX alters the Keap1/Nrf2 paradigm leading to increased Nrf2 protein by inhibiting Keap1-mediated Nrf2 degradation, resulting in activation of ARE regulated genes. Exposure of H9c2 rat cardiomyocyte cells to DOX resulted in a time and dose-dependent decrease in nonprotein sulfhydryl groups. Associated with this was a near 2-fold increase in Nrf2 protein but no change in Keap1 protein. Although the expression of the Nrf2 gene (*Nfe2l2*) was not altered by DOX, several of the Nrf2-regulated down-stream transcripts were significantly increased, including *Gstp1* (2.3-fold, $p<0.01$), *Ugt1a1* (1.8-fold, $p<0.05$), and *Nqo1* (1.8-fold, $p<0.01$). These results are consistent with our hypothesis that acute DOX exposure induces an antioxidant gene transcription response that is facilitated, at least in part, by an inhibition of Keap1 protein-mediated degradation of Nrf2. (This work was supported in part by a grant from the 3M Co.)

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ABSTRACT FINAL ID: 2564 Poster Board -205
TITLE: Histopathologic Evaluation of Chronically Implanted Telemetry Devices for Blood Pressure Measurement in Rhesus Monkeys

AUTHORS (FIRST INITIAL, LAST NAME): R. Kaiser, J. A. Chilton, S. D. Tichenor, H. H. Holzgrefe, and D. J. Meyer.
INSTITUTIONS (ALL): Charles River, Reno, NV.
KEYWORDS: Cardiovascular, Telemetry, Pathology

ABSTRACT BODY: Telemetric blood pressure monitoring (TBP) of nonhuman primates in repeat-dose toxicology studies provides for the collection of high-fidelity physiologic data, implementing the 3Rs by combining endpoints and minimizing animal use. However, the histopathological impact of long term TBP implantation remains largely uncharacterized. Accurate histopathological evaluation of target organs is a critical determination in chronic enabling toxicology studies. Accordingly, we evaluated the gross pathology and histopathologic effects following chronic TBP implantation (7 months, n=6) by light microscopic examination of selected tissues. TBP devices (TA11PA-C10-TOX-LA, DSI, St. Paul, MN) were surgically implanted with attendant femoral artery catheterization. Tissues evaluated by gross and light microscopic examination included those associated with the implantation site (femoral artery, proximal muscle, connective tissue) and major target organs including brain, kidney, heart, liver, spleen, and lung. There were no gross pathology findings in any of the tissues. No TBP-related histopathological changes were noted in any of the target organ tissues. There was minimal to mild fibrosis at the subcutaneous implantation site with regeneration of muscle underlying the telemetry device. The femoral artery exhibited varying degrees of intimal thickening and nonocclusive thrombosis with compensatory neovascularization of the surrounding tunica adventitia. Two of 6 femoral arteries had focal minimal to mild chronic mural inflammation. These data demonstrate that chronic, subcutaneously implanted TBP devices employing femoral catheterization were well tolerated for up to 7 months in rhesus monkeys. The absence of any associated histopathological changes in the major target organs enabled the unambiguous interpretation of histopathologic endpoints, while TBP provided a high-fidelity physiologic background, facilitating robust toxicological assessments that are not possible in standalone study designs.

ABSTRACT FINAL ID: 2565 Poster Board -206
TITLE: Acclimation of Dogs to Jacketed External Telemetry and Assessment of Heart Rate and Blood Pressure over a 17 Week Period

AUTHORS (FIRST INITIAL, LAST NAME): K. Norton and R. Billing.
INSTITUTIONS (ALL): Safety Pharmacology, Charles River Laboratories, Senneville, QC, Canada.
KEYWORDS: Blood pressure, Telemetry, Heart Rate

ABSTRACT BODY: The ICH S7a, M3, S6 and S9 note that cardiovascular safety pharmacology assessments can be incorporated into toxicology studies. Historically this approach has been rare; but, it is now becoming a common request in order to evaluate potential effects from repeat dosing, whilst at the same time reducing animal usage. However, combining studies is not without complication, (e.g. surgical placement of blood pressure transmitter, ensuring animals are acclimated to the jacketed system), confirming implant viability for the duration of the study and interpretation of pathology related to transmitter placement. The purpose of this investigation was to optimize the acclimation time, verify that transmitters remained viable for at least 17 weeks and determine what, if any, macroscopic findings were noted at the surgical site. Beagle dogs were instrumented with blood pressure transmitters and a 10-day recovery period. Subsequently ECG leads and a jacket were placed on the animals and baseline heart rate and blood pressure assessed for 6 days. Animals were then dosed with vehicle or Thoirdazine at 5, 10 and 20 mg/kg and heart rate, blood pressure and ECG intervals assessed. At Week 17 the dosing regime was repeated to ensure catheter patency and verify the magnitude of change relative to the first dosing occasion. During the acclimation period baseline heart rates stabilized within 3 days. Administration of Thoridazine resulted in a dose dependent decrease in blood pressure, a compensatory reflex tachycardia and QTc interval prolongation, on both dosing occasions with a similar magnitude of response. Baseline assessments on Week 17 were comparable with Week 3 indicating no drift in the blood pressure signal. A review of macroscopic findings showed minimal

effects, consistent with findings noted in safety pharmacology studies, and were considered easy to differentiate from potential treatment related effects. Incorporation of CV assessments on toxicology studies is feasible and can potential increase overall knowledge of potential CV effects whilst reducing animal usage.

ABSTRACT FINAL ID: 2566 Poster Board -207
TITLE: Exposure of the Tracheally Intubated Rat to Aerosolized Lunar Dust Surrogate JSC1A-VF

AUTHORS (FIRST INITIAL, LAST NAME): P. Gerde[1, 2], B. Blomgren[3], G. Prisk[4], and E. Selg[1].
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KEYWORDS: inhalation, mineral dust, intratracheal intubation

ABSTRACT BODY: Exposure to inhaled mineral dusts is a long-known cause of acute as well as chronic disease in humans. The causative exposures can either be episodic high-level exposures or more chronic low-level exposures. One particular example of the former scenario is the risk of high level exposures to mineral dusts during manned missions to the moon or other extraterrestrial bodies. However, few methods are available for studying biological effects in laboratory animals following controlled inhalation exposures to higher concentrations of respirable particles. The purpose of this study was to perform inhalation exposures of tracheally intubated rats to high concentrations of the lunar dust surrogate JSC1A-vf. The test material was aerosolized using the Dustgun generator to produce aerosols with a mass median aerodynamic diameter of 2.6 µm at aerosol concentrations of ~3 mg/L. Using an active dosage measuring system based on the measured local aerosol concentration in the breathing zone combined with the animal ventilation pattern, two groups of animals were exposed one at a time to target lung burdens of 1 and 3 mg JSC1A-vf. Animals were euthanized immediately after exposures and lungs were harvested for analysis of the mineral dust content based on the dust silica component, using tissue digestion and a spectrophotometric method. Results show that the target levels were reached within one hr exposure time and less than 30 aerosol exposure cycles with resulting lung burdens of respectively 1.2±0.0 and 2.8±0.5 mg dust (n=4, SD). Light microscopy investigation of the exposed lungs showed an even distribution of the inhaled dust. By providing a high dose-rate inhalation exposure method, the aerosol technology can be a useful alternative to intratracheal instillation as a method to enable high-level episodic exposures in toxicological studies. Research supported by the Swedish National Space Board.

ABSTRACT FINAL ID: 2567 Poster Board -208
TITLE: *In Vitro* Systems Toxicology Approach to Investigate the Effects of Repeated Cigarette Smoke Exposure on Respiratory Tract Tissue Cultures

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KEYWORDS: organotypic tissue models, whole cigarette smoke, system biology

ABSTRACT BODY: Cigarette smoke (CS) is a complex mixture of chemicals eliciting oxidative stress and inflammation, which induce smoking-related disorders in respiratory tissues. Human tissue engineered models have been developed, that may mimic the clinical situation more closely than primary monolayer cultures, thus providing more meaningful risk assessment tools. The aim of our study is to investigate the impact of CS on different human organotypic respiratory tract tissue cultures (bronchial, nasal, buccal and gingival) which are in primary contact with CS upon inhalation *in vivo*. We modeled the smoking behavior of a light smoker during one day by repeatedly exposing the tissues to a total of 4 cigarettes with one hour interval between each cigarette. The air/liquid interface of each culture allows a direct exposure to whole smoke using Vitrocell systems smoking machine. These organotypic cultures derived from cells from nonsmoker donors and contain fibroblasts in addition to epithelial cells. First, we simultaneously exposed all tissues to various CS concentrations ranging

from 8% to 35% (diluted with humidified air ([vol/vol]) or to only humidified air (control). We found that gingival tissue was more sensitive to CS exposure than the other tissues based on cell viability assay. Secondly, all tissues were exposed in parallel to two doses of whole CS (10% and 16% inducing less than 20% cell death) or to humidified air. Various endpoints (e.g., gene and microRNA expression, CYP1A1 activity, proinflammatory markers release, differential cell counts, trans-epithelial electrical resistance and cytotoxicity measurements) were then captured at different postexposure times. Using computational approaches we identified the *in vivo* biological perturbations which can be represented by these *in vitro* respiratory tract tissues exposed to whole CS.

ABSTRACT FINAL ID: 2568 Poster Board -209
TITLE: 3D Organotypic Cultures of Human HepaRG Cells: A Tool for *In Vitro* Toxicity Studies

AUTHORS (FIRST INITIAL, LAST NAME): P. Gunness[1], D. Mueller[2], V. Shevchenko[3], E. Heinzle[2], M. Ingelman-Sundberg[1], and F. Noor[2].
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KEYWORDS: 3D cultures, HepaRG, hepatotoxicity

ABSTRACT BODY: Drug-induced hepatotoxicity is difficult to predict. The use of animals in research studies is highly controversial due to ethical reasons and interspecies differences in drug metabolism and toxicology. There is a dire need for the development of *in vitro* human hepatic models that can be employed to more accurately assess drug-induced hepatotoxic effects. In this study, long-term 3D organotypic cultures of the human hepatoma HepaRG cell line was obtained using a high-throughput hanging drop method. The organotypic cultures were maintained for 3 weeks and assessed for: (a) liver-specific functions, including cell metabolism, phase I and III enzyme activities, (b) expression of liver-specific proteins and (c) dose response to the hepatotoxicant, acetaminophen. Our results show that the organotypic cultures maintain high liver-specific function over 3 weeks of culture. The production rates of albumin and glucose as well as CYP2E1 activity were higher in the 3D versus the 2D cultures. The IHC analyses illustrate that the organotypic cultures express liver-specific markers such as albumin, CYP3A4 and CYP2E1 throughout the cultivation period. Acute toxicity studies reveal that the organotypic cultures are more sensitive to acetaminophen than the 2D cultures. Therefore, taken all together, the results from our study suggest that the 3D organotypic HepaRG cultures may be a promising *in vitro* tool for the more accurate assessment of drug-induced hepatotoxicity.

ABSTRACT FINAL ID: 2569 Poster Board -210
TITLE: Hepatoprotective Activity of *Enantia chlorantha* Stem Bark Extracts against Acetaminophen-Induced Liver Damage in Rats

AUTHORS (FIRST INITIAL, LAST NAME): O. E. Adebiyi and O. M. Abatan.
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KEYWORDS: hepatoprotection, acetaminophen, *Enantia chlorantha*

ABSTRACT BODY: The study evaluated the hepatoprotective activity of solvent extracts (hexane, chloroform, ethyl acetate and methanol) of *Enantia chlorantha* stem bark in liver injury induced by acetaminophen, using silymarin (100 mg/kg p.o.) a known hepatoprotective agent, as standard. The degree of hepatoprotection was determined by measuring levels of serum transaminases (AST and ALT), alkaline phosphatase, bilirubin, albumin, and total protein levels. The results showed that all the solvent extracts of *E. chlorantha* stem bark (500 mg/kg, p.o.) significantly ($P<0.05$) reduced the elevated serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and bilirubin in acetaminophen-induced hepatotoxicity. Similarly the total serum protein was significantly ($P<0.05$) increased by all the solvent extracts. Histological examination of the liver tissues showed no visible lesions in livers of animals treated with the solvent extracts. Our findings may suggest that E. chlorantha stem bark extracts possess hepatoprotective activity. The hexane extract of stem bark of the

plant possesses the highest hepatoprotective activity compared to other extracts. This may suggest that the purification and isolation of active compounds from *E. chlorantha* may serve as useful source for treatment in liver damage.

ABSTRACT FINAL ID: 2570 Poster Board -211
TITLE: Alcohol Pretreatment Enhances Acetaminophen-Induced Hepatotoxicity in Cyp2e1(-/-) Mice but not in Cyp1a2/2e1 (-/-) Double-Knockout Mice

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KEYWORDS: Acetaminophen Hepatotoxicity, Alcohol, Cytochrome P450

ABSTRACT BODY: Cytochrome P450 (CYP) 2E1 is believed to contribute to the alcohol-mediated increase in acetaminophen (APAP)-induced hepatotoxicity in humans. Alcohol has been shown to enhance APAP-induced hepatotoxicity in Cyp2e1(-/-) knockout mice, suggesting Cyp2e1 alone is not necessary for increased sensitivity. Alcohol pretreatment is known to increase other CYPs that convert APAP to the hepatotoxic metabolite, *N*-acetyl-*p*-benzoquinone imine, including CYP1A2 and CYP3A. Furthermore, Cyp1a2/2e1(-/-) double-knockout mice have been shown to be more resistant than wild-type mice to APAP-induced hepatotoxicity. In the present work, the contribution of mouse Cyp1a2 to the alcohol-mediated increase in APAP-induced hepatotoxicity was investigated in Cyp1a2/2e1(-/-) mice and compared with Cyp2e1(-/-) mice. Serum alanine aminotransferase (ALT) levels revealed that APAP was not toxic to Cyp1a2/2e1(-/-) mice at doses as high as 2.0 g/kg; in contrast, 2.0 g APAP/kg was hepatotoxic in Cyp2e1(-/-) mice. Alcohol pretreatment augmented APAP-induced hepatotoxicity in Cyp2e1(-/-) but not in Cyp1a2/2e1(-/-) mice. Hepatic levels of reduced glutathione were similar in both mouse lines. Alcohol pretreatment increased hepatic Cyp3a in both mouse lines, but was two-fold greater in Cyp1a2/2e1(-/-) than in Cyp2e1(-/-) mice. These results suggest that Cyp1a2 contributes to the alcohol-mediated increase in APAP-induced hepatotoxicity in mice. In the absence of both Cyp1a2 and Cyp2e1, Cyp3a exhibits no beneficial or detrimental contribution.

ABSTRACT FINAL ID: 2571 Poster Board -212
TITLE: Development of a Human 3D Biomimetic Liver Construct for Predicting Physiology and Toxicology

AUTHORS (FIRST INITIAL, LAST NAME): N. Senutovitch[1], R. DeBiasio[1], B. Gough[1], D. Taylor[1], B. Usta[2], M. Yarmush[2], and L. A. Vernetti[1].
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KEYWORDS: Biomimetic Liver, Biosensors, Oxidative Stress

ABSTRACT BODY: The early identification of drug-induced human hepatotoxicity is essential to improve the safety of new drugs and to decrease the time and cost of development. To address this need, we are constructing a 3D human liver model. It consists of a microfluidic chamber layered with human primary hepatocytes, stellate, endothelial and Kupffer cells to model the liver acinus. A subset of the cells are modified to create fluorescence-based biosensors of cell functions (sentinel cells). The goal is to elucidate the mechanisms of action (MOA) of known and unknown compounds and to create a predictive platform. Biosensors for oxidative stress, ROS production, mitochondrial function, apoptosis, as well as other hepatocyte molecular activities are being developed. Sentinel cells will also report the migration and activation of Kupffer cells and stellate cell division in regions of injury. Experiments in both HepG2 and primary human hepatocytes demonstrate that the sentinel cells differentiate the response to controls menadione and CCCP, from the response to the hepatotoxic drug nefazodone. There was no response to the nonhepatotoxic drugs trazodone and buspirone. Combining data from the sentinel cell biosensors with standard fluorescent indicators we demonstrate that CCCP and menadione induced a reduction in mitochondrial function, produced ROS, cytochrome C release accompanied by caspase-3 activation, the

signature MOA events leading to intrinsic apoptosis. The use of live readout biosensors to discriminate MOA over extended treatment periods will provide new tools to better understand the complexity of drug-induced hepatotoxicity. Future aims include demonstrating the viability of this model for toxicity studies of 4 to 6 weeks and exploring the use of human pluripotent stem cells to detect idiopathic hepatotoxicity from distinct genetic backgrounds.

ABSTRACT FINAL ID: 2572 Poster Board -213
TITLE: Use of B-CLEAR® Human Sandwich-Cultured Hepatocytes to Screen Compounds for Cholestatic Potential

AUTHORS (FIRST INITIAL, LAST NAME): T. Marion, W. Smith, R. Hart, C. Hubert, R. St. Claire III, and K. Brouwer.
INSTITUTIONS (ALL): Qualyst Transporter Solutions, Durham, NC.
KEYWORDS: hepatic transporters, hepatocytes, cholestasis

ABSTRACT BODY: Inhibition of the bile salt export pump (BSEP), which is localized to the canalicular membrane of hepatocytes and is the major efflux route for bile acids, has been implicated as a potential mechanism for drug-induced cholestasis and hepatotoxicity. Current screening methods using transfected models have not shown a strong correlation between BSEP inhibition potency and clinical cholestasis or hepatotoxicity. Bile acid homeostasis is tightly controlled through many mechanisms including multiple transport proteins that take up bile acids from the blood and efflux them into bile. The relative extent of inhibition of both uptake and efflux determines the intracellular accumulation of bile acids. A potential inhibitor's intracellular concentration is also important since it determines the extent of transport inhibition and drives toxicity. Thus, an *in vitro* model that has all transport proteins expressed and functional as *in vivo* is necessary to evaluate uptake and efflux simulataneously, as it occurs *in vivo*. The goal of this study was to measure changes in uptake, efflux, and intracellular concentration of taurocholic acid (TCA) in B-CLEAR® human sandwich-cultured hepatocytes following exposure to the BSEP inhibitors pioglitazone (PIO), bosentan (BOS), or clozapine (CLOZ), and to relate the extent of inhibition to the intracellular concentrations of the inhibitors. Cells were exposed to each inhibitor and [3H]TCA or d8-TCA for 10-20 minutes, and lysates of cells and cells+bile pockets were analyzed by scintillation counting or LC-MS/MS for accumulation of TCA and inhibitor. Results indicated that all three compounds inhibited canalicular efflux of TCA and caused its intracellular accumulation to increase. The extent of compound accumulation also corresponded to efflux inhibition and TCA accumulation, with PIO>CLOZ>BOS. These results indicate that both uptake and efflux processes, as well as intracellular concentration, are important parameters in evaluating compounds' cholestatic potential.

ABSTRACT FINAL ID: 2573 Poster Board -214
TITLE: Calorie Restriction Feminizes the Hepatic Expression of Drug Metabolizing Enzymes and Transporters in Mice

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INSTITUTIONS (ALL): Kansas University Medical Center, Kansas City, KS.
KEYWORDS: xenobiotic metabolism, phase-II detoxification, liver

ABSTRACT BODY: A recently proposed mechanism of aging is decreased xenobiotic detoxification capabilities with age and thus accumulation of damaged macromolecules. Therefore, we hypothesized that xenobiotic metabolism and disposition in liver is likely altered by calorie restriction (CR), which is the best known dietary anti-aging intervention. Male C57BL/6 mice were fed an ad libitum or CR (15, 30, or 40%) diet for one month (n=5), followed by mRNA quantification of 98 xenobiotic processing genes (XPGs) in liver, including 7 uptake transporters, 39 phase-I enzymes, 37 phase-II enzymes, 10 efflux transporters, and 5 transcription factors. In general, 15% CR was not sufficient to alter the mRNA expression of the XPGs, whereas 30 and 40% CR increased the mRNAs of 33 XPGs (for instance organic anion transporting polypeptide Oatp1a4, cytochrome P450s Cyp1a1 and 4a14, most flavin-containing monooxygenases, most sulfotransferases, many UDP-glucuronosyltransferases, and most glutathione *S*-transferases) and decreased 28 XPGs (for instance Oatp1a1, many carboxylesterases, catechol-*O*-methyl transferase, and some efflux transporters). Many of the XPGs that were altered by CR at mRNA levels have gender-divergent expression, which is regulated by the stimulatory or inhibitory effects of male-pattern growth hormone secretion. Among the 61 XPGs that were altered by CR, the mRNA profiles of 55% were feminized

by CR in male mice, possibly due to decreased levels of growth hormone by CR. In conclusion, CR alters the mRNA expression of over half of the 98 XPGs quantified in livers of male mice, and over half of these alterations appear to be due to feminization. (This study is funded by NIH grants ES-009649 and DK-081461.)

ABSTRACT FINAL ID: 2574 Poster Board -215
TITLE: Leptin Deficiency Dysregulates Nrf2 Function on Lipid Accumulation in White Adipose Tissue and Liver Induces Dyslipidemia and Glucose Intolerance

AUTHORS (FIRST INITIAL, LAST NAME): J. Xu, A. Donepudi, V. More, S. Kulkarni, L. Li, B. Yan, and A. L. Slitt.
INSTITUTIONS (ALL): Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI.
KEYWORDS: Obesity, diabetes, NRF2

ABSTRACT BODY: Nuclear Factor E2-Related Factor-2 (Nrf2) signaling in response to chemical-induced injury has been well described, whereas its participation in lipids transport, clearance and homeostasis is relatively unknown. The study herein determined the impact of Nrf2 deficiency on lipid metabolism and the related obesity and diabetes in Lepob/ob mice. Lepob/ob mice (OB) with targeted Nrf2 deletion (OB-Nrf2KO) were generated. Nrf2KO mice exhibited increased white adipose tissue mass, larger adipocyte size, and increased adipogenic marker gene expression of Peroxisome proliferator-activated receptor γ and Fatty acid-binding protein 4. However, OB-Nrf2KO exhibited decreased white adipose tissue mass and impaired adipogenic gene expression compared to OB mice. Nrf2 deficiency prolonged hyperglycemia in response to glucose challenge, impaired insulin signaling and induced insulin resistance in Lepob/ob mice. Hepatic triglycerides and cholesterol content was increased in Nrf2KO mice, in conjunction with decreased Small heterodimer partner and increased Fibroblast growth factor 21 expression; but leptin-deficiency blocked Nrf2 function of inducing lipid accumulation and tended to decrease hepatic lipid accumulation and steatosis. Nrf2 deficiency enhanced VLDL secretion, decreased exogenous lipid clearance rate, which likely contributes to the increased plasma triglyceride content in OB-Nrf2KO mice. Additionally, OB-Nrf2KO mice exhibited decreased serum total cholesterol, LDL-, and HDL-cholesterol content. The current study herein demonstrates that Nrf2 deficiency inhibited adiposity, reduced hepatic steatosis, impaired glucose tolerance and insulin signaling, but induced insulin resistance and dyslipidemia in Lepob/ob mice.

ABSTRACT FINAL ID: 2575 Poster Board -216
TITLE: Chronic Exposure of Disinfectant Byproduct Bromodichloromethane Causes Nonalcoholic Steatoheaptitis, a Hepatic Manifestation of the Metabolic Syndrome

AUTHORS (FIRST INITIAL, LAST NAME): R. Seth[1], A. Kumar[2], S. Das[1], M. Kadiiska[2], E. J. Tokar[3], M. Waalkes[3], G. Michelotti[4], A. Diehl[4], and S. Chatterjee[1].
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KEYWORDS: bromodichloromethane, DBP, NASH

ABSTRACT BODY: Childhood and adolescent rates of obesity and overweight are assuming pandemic proportions in the last decade. Obesity is associated with strong risks of development of chronic inflammatory liver disease and metabolic syndrome following a second hit from the built environment. The present study tests the hypothesis that free radical metabolism of low chronic exposure of bromodichloromethane (BDCM), a disinfection byproduct of drinking water causes nonalcoholic steatohepatitis (NASH), mediated by cytochrome P450 isoform CYP2E1 and adipokine leptin. Using diet-induced obese mice (DIO), mice deficient in CYP2E1 and spontaneous knock out of the leptin gene, we show that BDCM caused increased lipid peroxidation and increased tyrosine nitration in DIO mice, events dependent on reductive metabolism of CYP2E1. DIO mice exhibited increased hepatic leptin levels, higher proinflammatory gene expression and Kupffer cell activation. Obese mice exposed to BDCM also showed profound hepatic necrosis, Mallory body formation, collagen deposition, higher alpha smooth muscle actin expression, events that are hallmarks of NASH. The absence of CYP2E1 gene in mice that were fed with a high-fat diet did not show NASH symptoms and were also protected from hepatic

metabolic alterations in Glut1, phosphofructokinase and phospho carboxykinase gene expressions (involved in carbohydrate metabolism) and UCP-1, PGC1alpha, SREBP1C and PPAR-gamma genes (involved in hepatic fat metabolism). Mice lacking the leptin gene were significantly protected from both NASH and metabolic alterations following BDCM exposure, suggesting that higher levels of leptin induction of BDCM and its corresponding reductive metabolism in the liver might contribute to the development of NASH and metabolic alterations in obesity. (NIH-R00ES19875 to S.C.)

ABSTRACT FINAL ID: 2576 Poster Board -217
TITLE: Knowledge-Based Model for Assessment of Drug-Induced Liver Injury

AUTHORS (FIRST INITIAL, LAST NAME): B. Rakic[1], G. Apic[1], V. Veljovic[1], and R. Russell[2].
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KEYWORDS: Hapatotoxicity, DILI, off-target pharmacology

ABSTRACT BODY: Drug-induced liver injury (DILI) has become a leading cause of severe liver disease and therefore poses a major clinical and regulatory challenge. Many new *in vitro* assays and preclinical animal models are being developed to help screen compounds for the potential to cause DILI. The aim of this study is to develop and validate an expert computational model for assessing the potential of drug-induced liver toxicity based on the chemical structure. We manually annotated around 9850 gene expression observations from all to date available studies—104 articles related to drug-induced hepatotoxicity from both *in vivo* and *in vitro* studies. In parallel we manually annotated 5000 compounds reported in the literature to be hapatotoxicant. Moreover, for each hepatotoxicant we manually annotated all metabolizing enzymes, nuclear hormone receptors, other affected proteins and dysregulated genes in the context of DILI, and all the literature references for each relation in the knowledge base. Based on this knowledge we have developed rules and models for predicting possible hepatotoxicity of a drug, based on its structural similarity to known hepatotoxicants, its toxicogenomic signature and biological network. In our study we tested our model on a model hepatotoxicant, acetaminophen and confirmed several types of hepatotoxicities. The model outlines proposed underlying molecular mechanism. This study confirms a proof of principle for a knolwdge based DILI assessment.

ABSTRACT FINAL ID: 2577 Poster Board -218
TITLE: Use of Novel Renal Biomarkers to Assess the Nephrotoxic Effects of Melamine and Cyanuric Acid in Pregnant and Nonpregnant Female Rats

AUTHORS (FIRST INITIAL, LAST NAME): O. Bandele[1], C. Stine[2], T. Crosby[2], E. Evans[2], T. Black[1], N. Olejnik[1], Z. Keltner[1], M. Scott[1], R. Reimschuessel[2], and R. Sprando[1].
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KEYWORDS: Kidney, Nephrotoxicity Biomarkers, Melamine

ABSTRACT BODY: Although routine clinical assessments of renal damage typically detect loss of kidney function, KIM-1, clusterin (CLU), and osteopontin (OPN) are novel biomarkers proposed to indicate earlier changes in renal integrity. The recent adulteration of infant formula and other milk-based foods with melamine (MEL) revealed a link between MEL ingestion and acute kidney injury in infants characterized by urinary tract stones that induced clinical and subclinical urolithiasis. Due to food safety issues regarding MEL ingestion, the effects of MEL and related analogues (e.g., cyanuric acid, CYA) in other sensitive groups (e.g., pregnant females) should be assessed. This study examined the ability of multiplexed renal biomarker immunoassays to detect and differentiate the effects of MEL and CYA in urine from pregnant and nonpregnant female Sprague Dawley rats gavaged for 10 days with 1000 mg/kg bw of either compound. Our results illustrate that KIM-1, CLU, and OPN can differentiate the severity of adverse effects induced by MEL and CYA in nonpregnant and pregnant rats. MEL-treated animals experienced adverse effects; however, pregnant rats were most sensitive as indicated by dramatic increases in SCr, BUN, and kidney weights which were associated with reduced body weight and extensive planar nonspherical renal crystals. These effects coincided with substantial increases in renal biomarkers levels that were detected within two days of exposure. Several rats in this group were removed due to severe

adverse effects. Overall, CYA caused no obvious toxic effects. However, one nonpregnant CYA-treated rat displayed effects similar to MEL-treated animals, including a concomitant increase in renal biomarker levels. Our work shows the ability of these novel biomarkers to detect early toxic effects of MEL and CYA and further substantiates multiplexed immunoassays of urinary proteins as a powerful, noninvasive method that may outperform traditional tests.

ABSTRACT FINAL ID: 2578 Poster Board -219
TITLE: A 3 Dimensional (3D) Human Kidney Model As a Predictive Preclinical Assay for Chronic Drug-Induced Kidney Injury (DIKI)

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KEYWORDS: 3D kidney model, kidney toxicity biomarkers, *in vitro* kidney toxicity

ABSTRACT BODY: DIKI accounts for 25% of acute renal failure cases and 5% of new drug failures. DIKI is difficult to predict in the preclinical setting and more predictive kidney models are needed to reduce the high-failure rate. We developed a 3D human kidney tissue system that has more human relevance than 2D systems and validated it against drugs known to cause DIKI. Immortalized human renal cortical epithelial cells (hRCEC) grown in a hydrogel mixture developed 3D interconnected tubular structures by 2 weeks, were maintained for up to 8 weeks, and maintained expression of the proximal tubule epithelial cells markers: E-cadherin, cytokeratin 8/18/19, gamma glutamyl transferase1, and organic anion transporters 1 and 4. Functional assays demonstrated appropriate albumin and glucose uptake and increased production of cAMP in response to parathyroid hormone. EC50 values as determined by a nonlytic LDH release assay of both gentamicin and doxorubicin were significantly lower in the 3D kidney tissues (9mM and 2uM, respectively) than in 2D (22mM and >20uM) and lower than published values of other *in vitro* models. Soluble biomarkers of DIKI (kidney injury molecule-1 [Kim-1] and neutrophil gelatinase-associated lipocalin[NGAL]) demonstrated differences between the 3D model and 2D with both Kim-1 and NGAL measurement showing differential biomarker response to cisplatin, gentamicin and doxorubicin at varying concentrations (high v. low) and time points. This is one of the first models to not only look at function of hRCEC in a hydrogel system, but to also apply it in long term testing of drugs known to cause DIKI. The combination of a 3D human kidney model and nonlytic biomarker assays which are associated with early and late renal toxicity will be extremely valuable for reducing drug failures due to DIKI, cost of drug development and animals in preclinical testing.

ABSTRACT FINAL ID: 2579 Poster Board -220
TITLE: Novel near Infrared Agent for Noninvasive Imaging and Quantification of Glomerular Filtration Rate in Mice

AUTHORS (FIRST INITIAL, LAST NAME): J. D. Peterson[1, 2], G. Ho[2], J. Zhang[2], J. Jarrell[1], K. O. Vasquez[1], J. Delaney[2], M. Rajopadhye[2], and B. Bao[2].
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KEYWORDS: glomerular filtration rate, near infrared fluorescence, fluorescence molecular tomography

ABSTRACT BODY: The measurement of glomerular filtration rate (GFR) is the gold standard in kidney function assessment and is used to determine progression of kidney disease and drug-induced kidney toxicity. GFR is typically determined in preclinical animal models through measurement of radiolabeled inulin clearance from the circulation, requiring serial bleeding of multiple cohorts of animals. We have developed a near infrared (NIR) fluorescent-labeled form of inulin (ex/em = 670/685 nm) in a spectral region providing low background and high tissue penetration for *in vivo* application. Fluorescence molecular tomographic (FMT) imaging of an intravenous bolus of NIR-Inulin in SKH-1E mice provides 3D, quantitative fluorescent images of heart fluorescence over time (1-60 minutes postinjection). GFR was calculated using a two-compartment model (PK Solver 2.0), determining average rates of 270 ± 6 µL/min in normal mice. These results were comparable to kinetic studies in which multiple cohorts of mice receiving labeled Inulin were assayed ex vivo. In comparison, nephrectomized mice imaged by FMT showed a significant 2-fold decrease in GFR ($p < 0.005$), and mice treated with Cyclosporine A (80 mg/kg/day) for 14 days showed an expected 40% decrease in GFR ($p < 0.05$). All imaging results

correlated well with ex vivo plasma microplate assays showing increased levels of creatinine and blood urea nitrogen (BUN). In conclusion, FMT imaging of circulating NIR-inulin in the heart provides a noninvasive fluorescent imaging approach that requires very few mice (3-10 mice per group) to generate consistent GFR measurements and to detect the GFR changes induced by nephrectomy or drug toxicity. As neither blood nor urine sampling is required, and no labor-intensive microplate assays, GFR can be determined quickly after the imaging procedure is completed. These results illustrate the potential of this imaging approach to facilitate the study of kidney disease and the monitoring of drug safety.

ABSTRACT FINAL ID: 2580 Poster Board -221
TITLE: Ionizing Radiation-Induced MiR-320a Activated by p38 MAPK and JNK Enhances Radiation Sensitivity of HeLa Cells

AUTHORS (FIRST INITIAL, LAST NAME): Y. Tie, Z. Hu, H. Fu, R. Xing, J. Zhu, Z. Sun, and X. Zheng.
INSTITUTIONS (ALL): Beijing Institute of Radiation Medicine, Beijing, China.
KEYWORDS: miR-320, Radiation-Induced, Transcription regulation

ABSTRACT BODY: MicroRNAs (miRNAs) play important roles in numerous cellular processes, including development, proliferation, tumorigenesis, and apoptosis. It has been reported that miRNA expression is induced by ionizing radiation (IR) in cancer cells. However, the molecular mechanisms are not fully understood. In this study, the promoter region of miR-320a gene was identified, and we found that miR-320a expression increased linearly with IR doses and time after IR. Using ChIP, it was verified that ATF2, ELK1, and YY1 bound to the promoter of miR-320a and these transcription factors activated by p38 MAPK and JNK after IR regulated miR-320a expression. It was identified that CDK6, XIAP, and HMGB1 were miR-320a target genes during IR response. By targeting these genes, miR-320a could trigger G0/G1 arrest, induce apoptosis, and inhibit proliferation of cancer cells. These results suggested that miR-320a could contribute to the cellular DNA damage response and become useful for cancer radiotherapy.

ABSTRACT FINAL ID: 2581 Poster Board -222
TITLE: p53-Mediated Apoptosis by Marijuana Smoke Condensate Is Caused by Oxidative Stress in BEAS-2B Cells

AUTHORS (FIRST INITIAL, LAST NAME): D. Shin[1], H. Kim[1], S. Lee[2], S. Oh[3], and K. Chung[1].
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KEYWORDS: Marijuana smoke condensate, Oxidative stress, Apoptosis

ABSTRACT BODY: p53 is known to be crucial in regulating the DNA damage responses in lung epithelial cell. The aim of present study is to investigate the relevance of p53 in oxidative stress-mediated apoptosis induced by marijuana smoking. First, we confirm the oxidative stress induction of marijuana smoke condensate (MSC) in human normal epithelial (BEAS-2B) cells in DCF-DA assay. In addition to that, MSC up-regulated superoxide dismutase, catalase activity and their mRNA expressions. Second, to determine if apoptosis due to MSC is mediated by p53 in BEAS-2B cells, we observed caspase-3 activation and DNA fragmentation as markers of apoptosis. Increase of these markers demonstrated that p53 is relevant to MSC-induced apoptosis. MSC also increased mRNA levels of apoptosis genes (p53 and bax), but no change was observed in antiapoptosis gene (bcl-2). When exposed to MSC, p53, phospho-p53, and bax proteins increased in a dose dependent in western blotting. Apoptosis and apoptosis-related genes expression were partially blocked by an inhibitor of p53-dependent transcriptional activation (pifithrin-a). These results show that p53 is involved to MSC-induced apoptosis. Lastly, radical scavengers like superoxide dismutase, catalase, mannitol, and sodium selenite prevented MSC-induced apoptosis. This indicate that p53-mediated apoptosis is relevant to ROS. In conclusion, these results demonstrate that MSC increases p53-mediated apoptosis through oxdative stress in BEAS-2B cells and this may have broader implications for our understanding of lung diseases.

2013 Society of Toxicology Annual Meeting
Late-Breaking Abstracts

ABSTRACT FINAL ID: 2582 Poster Board -223
TITLE: Protective Effect of Prolactin in the Methamphetamine-Induced Neurotoxicity in Bovine Microvessel Endothelial Cells: An *In Vitro* Study

AUTHORS (FIRST INITIAL, LAST NAME): H. Rosas-Hernandez[1, 2], E. Cuevas[2], S. M. Lantz[2], M. G. Paule[2], S. F. Ali[2], and C. Gonzalez[1, 2].
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KEYWORDS: Methamphetamine, Prolactin, Blood-brain barrier

ABSTRACT BODY: Methamphetamine (METH) is a highly addictive drug of abuse which exerts its toxic effects by affecting the dopaminergic system. Recent reports suggest that METH disrupts the blood-brain barrier (BBB) by increasing the permeability, increased apoptosis and decreases tight junction proteins. The hormone prolactin (PRL), in several experimental models exerts protective vascular effects associated with the stimulation of endothelial cell proliferation. The aim of this study is to evaluate the possible protective effect of PRL in the METH-induced neurotoxicity in bovine microvessel endothelial cells (bMVEC). A primary culture of bMVEC was isolated by enzymatic digestions and differential centrifugation for an *in vitro* model of BBB. Confluent bMVEC monolayers (cultured 10-14 days) were treated with different concentration of METH (100 µM–2.5 mM). METH decreases bMVEC proliferation and increases apoptosis in a dose-dependent manner. Co-treatment of METH (1 mM) with PRL only at the concentrations of 10 an 100 nM protect against METH-induced decrease in cellular proliferation and apoptosis. However, at higher concentration of METH (2.5 mM) PRL fail to protect against the proliferation and partial protect against METH-induced apoptosis. PRL only at 10 and 100 nM increases proliferation however, does not affect apoptosis. These data suggest that PRL protects against METH-induced neurotoxicity in bMVEC. Further investigations are warranted to evaluate the mechanism involved and if these hormonal protective effects are extensive to other toxic effects of METH on BBB.

ABSTRACT FINAL ID: 2583 Poster Board -224
TITLE: Factors Affecting the 27K DNA Methylation Pattern in Asthmatic and Healthy Children from Locations with Various Environments

AUTHORS (FIRST INITIAL, LAST NAME): A. Rossnerova[1], E. Tulupova[1, 2], N. Tabashidze[1], J. Schmuczerova[1], M. Dostal[1], P. Rossner[1], H. Gmuender[3], T. Wittenberger[3], and R. J. Sram[1].
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KEYWORDS: DNA methylation, bronchial asthma, air pollution

ABSTRACT BODY: Gene expression levels are significantly regulated by DNA methylation. In this study we compare the methylation profiles in 200 blood samples of children (aged 7–15 years) with and without bronchial asthma from two regions in the Czech Republic with different levels of air pollution (a highly polluted Ostrava region and a control Prachatice region). Samples were collected in March 2010 when the mean concentrations of benzo[a]pyrene (B[a]P) measured by stationary monitoring were 10.1±2.4 ng/m3 in Ostrava Bartovice (5.6 times higher than in the control region). Significantly higher concentrations of other pollutants (benzene, NO2, respirable air particles and metals) were also found in Ostrava. We applied the Infinium Methylation Assay, using the Human Methylation 27K BeadChip with 27,578 CpG loci for identification of the DNA methylation pattern in studied groups. Results demonstrate a significant impact of different environmental conditions on the DNA methylation patterns of children from the two regions. These results are in agreement with gene expression profile data obtained in our previous studies. We did not find a difference in DNA methylation patterns between children with and without bronchial asthma in individual locations, but patterns in asthmatics from Ostrava differed from asthmatics from Prachatice. Further, we show differences in DNA methylation pattern depending on gender and urinary cotinine levels. Other factors including length of gestation, birth weight, and

length of full breastfeeding are suggested as possible factors that can impact the DNA methylation pattern in future life. Supported by the Ministry of the Environment of the Czech Republic (SP1b3/8/08) and the Grant Agency CR (P503/11/0084).

ABSTRACT FINAL ID: 2584 Poster Board -225
TITLE: Global and Epigenome-Wide Methylation Profiles in Human Fetal Liver Samples Characterized for Bisphenol A Exposure

AUTHORS (FIRST INITIAL, LAST NAME): M. S. Nahar, J. H. Kim, L. S. Rozek, M. A. Sartor, and D. C. Dolinoy.
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KEYWORDS: Bisphenol A, DNA methylation

ABSTRACT BODY: Environmental exposures can affect gene expression through one or more epigenetic mechanisms, especially during development. DNA methylation of CpG dinucleotides is a stable epigenetic mark that is often associated with developmental chemical exposure or nutritional status, illustrating its role in the developmental origins of health and disease. Bisphenol A (BPA), a high volume industrial chemical used in polycarbonate plastic and epoxy resin, is associated with endocrine disruption and epigenetic effects. In this study, we evaluated global and epigenome-wide DNA methylation profiles in healthy 1st and 2nd trimester human fetal liver samples obtained from the Laboratory for the Study of Human Embryology (LSHE) at the University of Washington, Seattle and characterized for tissue concentrations of BPA. We quantified percent methylation at long interspersed retrotransposons (LINE-1) via pyrosequencing and at CCGG sites throughout the genome via luminometric methylation assay (LUMA) to assess global DNA methylation (N=50). Among the fetal liver specimens, neither LINE-1 (β=0.019; p-value: 0.10) nor LUMA (β=0.019; p-value: 0.81) methylation was significantly associated with increasing total BPA levels after adjusting for sex and gestational age. A subset of the cohort was assigned to three exposure categories (N=6 each): low (<1.0 ng/g), intermediate (3.5-5.8 ng/g), and high (35-97 ng/g) total BPA, and examined for epigenome-wide profiles using MethylPlex-Next Generation Sequencing. We identified exposure dependent regions of altered methylation using the edgeR package in R software. Upon examination of CpG Islands (CGI), CGI shores (0-2kb from CGI), and CGI shelves (2-4kb from CGI), we observed increased methylation in CGIs and decreased methylation in CGI shores and shelves with higher BPA concentrations. Validation of these regions of altered methylation in the full sample set will be important in identifying biomarkers of exposures to facilitate risk assessment.

ABSTRACT FINAL ID: 2585 Poster Board -226
TITLE: Epigenetic Mechanisms of Environmental Estrogens Corruption of Immune Functions

AUTHORS (FIRST INITIAL, LAST NAME): W. Tang[1], T. Dao[1], R. Cheng[2], X. Hong[3], and X. Wang[3].
INSTITUTIONS (ALL): [1]Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; [2]Branch of Radiation Oncology, National Cancer Institute, Bethesda, MD; [3]Population, Family and Reproductive Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.
KEYWORDS: Epigenetics, polychlorinated biphenyls, Immune response

ABSTRACT BODY: Exposure to polychlorinated biphenyls (PCBs) has been demonstrated to cause a variety of adverse health effects on the reproductive, nervous and immune system. PCBs have been banned in the United States since 1979 but dietary exposure still occurs through PCBs-contaminated land animals and fish. Recent studies have supported exposure to environmental estrogens during early development (such as fetal or postnatal periods) alters the epigenetic modifications in the genome. These changes may conflict with the programmed “adaptive changes” made during early development and impede adaptability to later-life challenges and elevate disease risk. In this study, we investigated the epigenetics effect of PCBs on the biological immune functions in human. We employed the methylome data in a subsample of the human Boston Birth Cohort (BBC) participants who had blood collected at maternal, birth and two years after birth. These children are being prospectively followed from birth onwards to determine their postnatal growth, development and disease outcomes in response to environmental exposure. Promoter methylation of cytokines genes and their association to dysfunctional

immune response was determined. Also, we showed PCB153, the highest prevalence among the other PCBs in the BBC, changed the expression level and activity of epigenetic enzymes in human peripheral blood mononuclear cells. Specifically, these epigenetic alterations contribute to cytokine Ifnγ gene transcription. Herein, we provide the evidence that PCBs disrupt immune cell functions via epigenetic modifications of gene regulation. Once the specific epigenetic marks are set, they may maintain throughout life and continuously impact the immune system, resulting in increased susceptibility to inflammation/infection-related diseases like asthma, COPD, cardiovascular disease or cancer in later life.

ABSTRACT FINAL ID: 2586 Poster Board -227
TITLE: Modulation of Foxo Transcription Factors by Glutathione Depletion

AUTHORS (FIRST INITIAL, LAST NAME): A. Turkistani, L. Klotz, and X. Hou.
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KEYWORDS: FoxO, Glutathione

ABSTRACT BODY: Rationale: FoxO transcription factors are known mediators of insulin effects. Their activity is modulated by oxidative stress and redox signaling. Glutathione, the most abundant intracellular antioxidant, plays a pivotal role in maintaining cellular redox homeostasis. The aim of our study was to examine the effect of glutathione depletion on insulin signaling and FoxO protein activity. Experimental procedures: HepG2 human hepatoma cells were exposed to different concentrations of diethyl maleate (DEM) to deplete glutathione or to buthionine sulfoximine (BSO) to inhibit glutathione synthesis. Cells were then treated with insulin, followed by cell lysis. Analysis of insulin signaling was performed by Western blotting and immunodetection of Akt and FoxO phosphorylation. FoxO subcellular localization was investigated using GFP-tagged FoxO1. Results: Exposure of HepG2 cells to DEM at concentrations significantly depleting cellular thiols interfered with insulin-induced FoxO1a/FoxO3a phosphorylation at Thr-24/32, sites phosphorylated by Akt. Interestingly, the effect of DEM on Akt phosphorylation was delayed and less intense, suggesting that the DEM effect on FoxOs is independent of Akt activity. Pretreatment with 10 mM DEM for 1 hour significantly blocked the phosphorylation of FoxO1a/3a induced by stimulation of cells with insulin. Similarly, FoxO1/3a phosphorylation by insulin was significantly blocked after a 2-hour pretreatment with 3 mM DEM. On the other hand, an up to 48-hour pretreatment with BSO showed no effect on FoxO1a/FoxO3a insulin-induced phosphorylation. In line with these data, DEM overrode insulin-induced nuclear exclusion of FoxO1 and triggered nuclear accumulation of FoxO1. Conclusion: Our results show that DEM impairs insulin downstream signaling by modulating FoxO protein phosphorylation. This finding suggests that glutathione may be involved in regulating FoxO activity. Future experiments will serve to elucidate the mechanism of interaction between glutathione and insulin signaling.

ABSTRACT FINAL ID: 2587 Poster Board -228
TITLE: Cell-Based Multiparametric Analysis of Nefazodone-Induced Hepatotoxicity in HepG2 Cells Using High-Content Imaging

AUTHORS (FIRST INITIAL, LAST NAME): B. S. Mandavilli, R. J. Aggeler, A. Rukavishnikov, C. Pickens, U. Singh, H. Kang, K. Gee, B. Agnew, and M. S. Janes.
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KEYWORDS: Oxidative stress, apoptosis, Hepatotoxicity

ABSTRACT BODY: Oxidative stress plays an important role in the progression of several diseases including inflammation, atherosclerosis, and age-related degenerative disorders. Reactive oxygen species damage membrane bound lipids including the unsaturated fatty acids linoleic acid and arachidonic acid. This form lipid electrophiles, which can rapidly react with proteins and DNA to form adducts and chronic oxidative stress leads to cell death. Multiparametric measurements of oxidative stress, mitochondrial health and cell death by high content imaging provide a powerful platform to quantitate hepatotoxicity in HepG2 cells. Nefazodone is an antidepressant drug and has been shown to lead to hepatotoxicity in some patients. In this multiparametric study, we analyzed nefazodone-induced oxidative stress, lipid peroxidation, mitochondrial

membrane potential, apoptosis, and cell death in HepG2 cells using high-content imaging. Nefazodone treatment leads to increase in oxidative stress and lipid peroxidation-derived protein modifications, as well as a decrease in mitochondrial membrane potential. These changes lead to the induction of apoptosis and plasma membrane permeability. Rosiglitazone, a drug that does not cause hepatotoixcity did not show any changes in oxidative stress, mitochondrial membrane potential and there was no cell death in rosiglitazone-treated cells. Nefazodone produced 2-3 fold increase in oxidative stress and lipid peroxidation-derived protein modifications. Nefazodone treatment also led to cell death as evidenced by an increase in caspase activation and plasma membrane permeability when compared to controls. The nefazodone-induced multiparametric hepatotoxicity is attenuated by antioxidants like n-acetyl cysteine. The strategy described here to measure mulitiple parameters involved in nefazodone-induced hepatotoxicity and its ability to distinguish it from nontoxic molecules like rosiglitazone may have a broader applicability in cell-based preclinical toxicity measurements.

ABSTRACT FINAL ID: 2588 Poster Board -229
TITLE: Mechanisms of Acute Hematotoxicity by Hydroxylamine Sulfate

AUTHORS (FIRST INITIAL, LAST NAME): G. M. Prodanchuk and M. G. Prodanchuk.
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KEYWORDS: oxidative stress, hydroxylamine sulfate, poisoning

ABSTRACT BODY: Hydroxylamine and hydroxylamine sulfate widely used as intermediates in synthesis of pharmaceuticals, pesticides, dyes, caprolactam and other organic compounds. A case of acute poisoning by hydroxylamine sulfate was investigated in subjects (adults and children) who accidentally ingested the toxicant mislabeled as citric acid and sold to the general public at the market. All subjects recovered after being treated with one of the two established clinical protocols: (i) methylene blue, vitamin C and correction of acidosis, or (ii) alpha-tocopheryl acetate, alpha-lipoic (thioctic) acid and correction of acidosis. Upon examination of the medical records, we determined that in subjects with both mild and moderate/sever intoxication, the period for normalization of hematological parameters was significantly shortened in a group treated with alpha-tocopheryl acetate and alpha-lipoic acid. To investigate the mechanisms of acute hematotoxicity by hydroxylamine sulfate, and possible reasons for effectiveness of antioxidants, we conducted an experimental study in Wistar rats. Hydroxylamine sulfate caused acute hematotoxicity and oxidative stress (appearance of free oxidized iron in blood, reduced glutathione content and increased lipid peroxidation in liver) *in vivo*. *In vitro* experiments using rat thymocytes, using electron spin resonance spectroscopy and spin trap Fe2+(DETC)2, showed that cytotoxicity of hydroxylamine sulfate may occur due to two mechanisms: release of free NO from hydroxylamine sulfate, and/or formation of stable covalent adducts with heme iron. Both of these mechanisms may be responsible for alterations in oxygen transport of red blood cells, anemia and metabolic hypoxia. We conclude that oxidative stress is a key mechanism of acute hematotoxicity by hydroxylamine sulfate and that alpha-tocopheryl acetate and alpha-lipoic (thioctic) acid are effective antioxidant therapies for cases of acute poisoning.

ABSTRACT FINAL ID: 2589 Poster Board -230
TITLE: AHR Agonists Inhibit IGF2-Stimulated Human Breast Cancer Cell Growth

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KEYWORDS: aryl hydrocabon receptor, Igf2, breast cancer cell line

ABSTRACT BODY: High rates of obesity have been shown to increase breast cancer risk, increase breast cancer recurrence and increase breast cancer mortality in humans. In breast cancer cells, adipocyte secreted adipokines have been reported to promote tumor progression through several mechanisms. The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor and potential drug target that has historically been linked to toxicity. The objectives of this study were to investigate potential interactions between AHR signaling and mitogenic adipokine signaling in human estrogen receptor

(ER) positive breast cancer cells given that patients with ER positive breast tumors have been shown to be particularly sensitive to obesity associated increased cancer risk. Herein, adipocyte conditioned medium (adipo-CM), adipokine protein arrays and an IGF2 blocking antibody showed that adipo-CM contained high levels of several adipokines, is a potent inducer of cancer cell growth and that the adipokine insulin like growth factor 2 (IGF2) plays a major role in adipo-CM stimulated cancer growth. We discovered that treating breast cancer cells with AHR agonists 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) and SU5416 significantly inhibited adipo-CM and IGF2 stimulated breast cancer cell through an AHR mechanism. These results are the first to provide mechanistic evidence that IGF2 is an adipokine that plays a major role in adipo-CM stimulated breast cancer cell growth and that activation of the AHR pathway inhibits proliferative effects of IGF2.

ABSTRACT FINAL ID: 2590 Poster Board -231
TITLE: Protein Expression and Epigenetic Changes in Human T47D Breast Cancer Cells with Varying Intracellular ERα/ERβ Ratio Upon Exposure to 4OH-Tamoxifen

AUTHORS (FIRST INITIAL, LAST NAME): N. Evers[1], S. Boeren[2], J. Groten[1], J. Vervoort[2], and I. Rietjens[1].
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KEYWORDS: Epigenetic changes, Proteomics, ERα/ERβ ratio

ABSTRACT BODY: Scope: The aim of the present study was to characterize the effect of a varying intracellular ERα/ERβ ratio on partial ER agonist 4OH-tamoxifen induced protein expression in human T47D-ERβ breast cancer cells. T47D-ERβ cells show tetracycline dependent ERβ expression and can mimic physiologically relevant ERα/ERβ ratios. Experimental procedures: The T47D-ERβ cells were exposed to 0, 100, or 1000 ng/ml tetracycline to express respectively high, intermediate and no ERβ levels. After 24h cells with various ERβ expression were exposed to 0 or 300 nM 4OH-tamoxifen for 24h. In-gel digestion was performed on the T47D-ERβ protein samples which were measured by nanoLC-Orbitrap-MS and analyzed by MaxQuant, Perseus, and Cytoscape software. Results: At no/intermediate ERβ expression, up-regulated proteins were mostly involved in mRNA transport and mRNA processing, while down regulated proteins were mostly involved in intracellular transport and protein localization. In cells with a high ERβ expression most up-regulated proteins were involved in the induction of apoptosis by intracellular signals, and down regulated proteins were generally related to mRNA metabolic processes. Methylated (a.o. histone H3 when ERβ expression is high), acetylated (histone H3 when ERβ expression is high) and phosphorylated up-regulated proteins were found upon treatment with 4OH-tamoxifen as well as methylated down regulated proteins (a.o. histone H3.2 when ERβ expression is high). Conclusion: The present study gives insight in protein expression and epigenetic changes occurring in human T47D breast cancer cells with varying intracellular ERα/ERβ ratio exposed to 300 nM 4OH-tamoxifen compared to unexposed cells; these first proteomic data point towards the specific antiproliferative action of ERβ upon stimulation with 4OH-tamoxifen.

ABSTRACT FINAL ID: 2591 Poster Board -232
TITLE: RNA Sequencing Reveals New Aryl Hydrocarbon Receptor (AHR) Regulatory Targets in Human Breast Cancer Cells

AUTHORS (FIRST INITIAL, LAST NAME): T. B. Salisbury[1], D. Primerano[2], G. Boskovic[2], J. Fan [2], and J. Denvir[2].
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KEYWORDS: Aryl Hydorcarbon Receptor, RNA sequencing, breast cancer cell line

ABSTRACT BODY: Obesity is a risk factor for breast cancer, increases the risk for breast cancer recurrence and breast cancer associated mortality in humans. Adipocytes secrete factors known as adipokines that act on breast cancer cells to stimulate breast cancer cell proliferation. The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that has historically been reported to mediate the toxic effects of environmental toxicants. We have discovered that is some human breast cancer cells the AHR itself is important for mediating adipokine stimulated breast cancer cell growth. Based on these

results, we performed genome-wide RNA sequencing experiments to detect differences in gene expression between control and AHR knockdown MCF-7 human breast cancer cells. All genes showing statistically significant changes in expression as determined by RNA-seq were loaded into Ingenuity Pathway Analysis (IPA) and a Core Analysis was performed using default settings. Of the 634 RNA products uploaded, 496 were mapped to known entities by IPA. The top 15 biological functions by statistical significance were determined and the most statistically significant disease was Cancer (180 associated molecules: p-value 3.45 x 10^(-11)) and the most statistically significant molecular/cellular function was Cell Cycle (84 associated molecules: p-value 2.76 x 10^(-5)). We also found that several novel AHR regulated genes where associated with retinoblastoma protein (Rb) pathway genes. This later finding is consistent with prior reports showing that the AHR and Rb through physical interactions regulate gene expression. We are currently investigating whether AHR and Rb coregulated genes are important for adipokine-stimulated breast cancer cell growth.

ABSTRACT FINAL ID: 2592 Poster Board -233
TITLE: Proprotein Convertase 4 Is Overexpressed in Nonsmall Cell Lung Cancer and Induced by NNK

AUTHORS (FIRST INITIAL, LAST NAME): K. Brant and G. Leikauf.
INSTITUTIONS (ALL): University of Pittsburgh, Pittsburgh, PA.
KEYWORDS: Proprotein convertase, NNK

ABSTRACT BODY: Proprotein convertases (PCSKs) are serine endoproteinases which process latent peptide precursors to their biologically active form as they traffic through the cell. There is growing evidence that overexpression of PCSK proteins contributes to the pathophysiology of various types of cancer, including lung. However, studies examining the regulation of PCSK in airway epithelia, and the potential for PCSK activation by chemical carcinogens to contribute to malignant change are limited. Our data indicate that PCSK4, which has previously been characterized as a germ-line specific proprotein convertase, is overexpressed in nonsmall cell lung cancer cell lines compared with normal human bronchial epithelial (NHBE) cells. In addition, immunohistochemical analysis of human lung cancer tissue arrays show elevated PCSK4 expression in adenocarcinoma and squamous cell carcinoma compared with adjacent uninvolved tissue and airway epithelia from normal subjects. In order to determine if PCSK4 expression can be induced in response to chemical carcinogens, NHBE cells were stimulated with the tobacco-derived carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Western blot analysis of cell lysates show an increase in PCSK4 expression following treatment with 100 nM NNK compared with control-treated cells. Moreover, NNK induced a significant increase in cell-associated PCSK activity. When NHBE cells were pretreated with AG-1478, the effect was blocked, implicating a role for epidermal growth factor receptor activation in NNK-induced PCSK activity. These findings suggest that during the process of malignant change, cells acquire the necessary environment to overexpress and self-activate PCSK4. Moreover, stimulation of convertases such as PCSK4 by environmental carcinogens may enhance activation of peptide substrates that facilitate tumor progression.

ABSTRACT FINAL ID: 2593 Poster Board -234
TITLE: Determination of the Steady State and Half Life of Exogenous Formaldehyde Derived N^2-hydroxymethyl-dG Adduct in Rat Nasal Epithelium in a 28-Day Study

AUTHORS (FIRST INITIAL, LAST NAME): R. Yu[1], B. C. Moeller[2], G. L. Andrews Kingon[2], W. M. Bodnar[1], and J. A. Swenberg[1, 2].
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KEYWORDS: Formaldehyde, DNA adducts, Steady state and half-life

ABSTRACT BODY: Rationale: The evidence for formaldehyde causing leukemia is limited, and the question is whether formaldehyde can reach remote tissues such as bone marrow. We employed stable [$^{13}CD_2$]-formaldehyde exposure in a 28-day rat study, the endogenous DNA adducts (N^2-hydroxymethyl-dG) in both nasal epithelium and bone marrow, and the time for the exogenous adducts to reach the steady state and its half-life were determined at a more realistic and relevant exposure concentration of 2 ppm. Methodology: DNA was isolated and reduced prior to injection onto an Agilent HPLC-UV

for quantitation of nucleosides and adducts collection. DNA adducts were quantitated using a Waters nanoAcquity UPLC and Thermo Quantum Ultra-Triple-Quad. Results: The average amounts of endogenous adducts in rat nasal epithelium and bone marrow are 2.87 ± 0.65 (n=44) and 3.71 ± 1.85 (n=46) endogenous adduct/ 10^7 dG. And exogenous adducts in nasal epithelium were accumulated through 28-day exposure, and the time to reach the steady state is about 25–32 days; and the half-life was determined to be 5 to 8 days. No exogenous adducts were detected in rat bone marrow. Conclusions: No exogenous adducts were detected in bone marrow, which is in accord with previous 10 ppm exposure studies; further confirming the implausibility for formaldehyde causing leukemia. The half-life was much longer than previous 10 ppm exposure studies, which may be the result of N^2-hydroxymethyl-dG arising as a direct adduct and as a degradation product of DNA-protein crosslinks. These types of data may be used to replace default assumptions of linear extrapolation from high to low exposures previously used for risk assessments with science-based data, rather than default assumptions.

ABSTRACT FINAL ID: 2594 Poster Board -235
TITLE: The Role of Peroxisome Proliferator-Activated Receptor-β/δ (PPAR β/δ) in Lung Cancer

AUTHORS (FIRST INITIAL, LAST NAME): K. C. Pramanik[1], C. Khozoie[1], B. Zhu[1], F. J. Gonzalez[2], and J. M. Peters[1].
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KEYWORDS: Lung Cancer, Cell cycle, PPARbeta

ABSTRACT BODY: Lung cancer is one of the most invasive malignancies and is the leading cause of cancer death in the world for both men and women. In many studies, peroxisome proliferator-activated receptor β/δ (PPARβ/δ) has been implicated in the regulation of cell growth and differentiation in various cancers. The present study examined the role of PPARβ/δ receptor in the cell growth and carcinogenesis in a variety of lung cancer models. The expression of PPARβ/δ and its target gene ANGPTL4 was lower in human lung adenocarcinomas and carcinoid tumors as compared with nontransformed lung tissue. Further, ligand activation of PPARβ/δ in human A549 lung cancer cells inhibited cell proliferation without producing changes in anchorage-dependent clonogenicity. Pretreatment of human lung cancer cells with the PPARβ/δ ligand GW0742 in PPARβ/δ over-expressing A549 cells modestly enhanced G2/M arrest, indicating that over-expression and ligand activation of PPARβ/δ promotes lung cancer cells toward cell cycle arrest. Furthermore, epidermal growth factor (EGF) mediated chemotactic invasion and migration were attenuated by ligand-activation of PPAR β/δ in the A549 cells. AKT activation is known to promote cell cycle progression. Interestingly, results revealed that AKT phosphorylation is significantly inhibited in A549 cells over-expressed with PPARβ/δ as compared with controls. Collectively, these results suggest that over-expression and ligand activation of PPARβ/δ in the human lung cancer cell line A549 could inhibit cell proliferation and enhance cell cycle arrest via inhibition of AKT phosphorylation, while diminishing the migration and invasion response to EGF. This suggests that activation of PPARβ/δ could be useful not only for chemoprevention, but also chemotherapy due to its anti-metastatic effects in human lung cancer cell lines.

ABSTRACT FINAL ID: 2595 Poster Board -236
TITLE: Replication Past *S*-[4-(N^6-deoxyadenosinyl)2,3-dihydroxybutyl]GSH by DNA Polymerases

AUTHORS (FIRST INITIAL, LAST NAME): S. Cho and F. Guengerich.
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KEYWORDS: DEB-GSH conjugate, misincorporation, S-[4-(N6-deoxyadenosinyl)2,3-dihydroxybutyl]GSH

ABSTRACT BODY: The carcinogen 1,2,3,4-diepoxybutane (DEB) has been shown to cause glutathione (GSH)-dependent enhanced base-substitution mutations, especially A:T to C:G in *Salmonella typhimurium* TA1535 (*Chem. Res. Toxicol*. *23*, 1544. 2010) and *Escherichia coli* TRG8 cells (*Chem. Res. Toxicol*. *25*, 1522. 2012). We previously identified *S*-[4-(N^6-deoxyadenosinyl)2,3-dihydroxybutyl]GSH adduct as a major adduct in the reaction of *S*-(2-hydroxy-3,4-epoxybutyl)glutathione (DEB-GSH conjugate) with nucleosides and calf thymus DNA and *in vivo* in livers of mice and rats

treated with DEB (*Chem. Res. Toxicol*. *25*, 706. 2012). For investigation of the miscoding potential of the DEB-GSH conjugate-derived major DNA adduct, *S*-[4-(*N*^{6}-deoxyadenosinyl)2,3-dihydroxybutyl]GSH adduct, and the effect of GSH conjugation on replication of DEB, extension studies were performed in duplex DNA substrates containing the site specifically-incorporated *S*-[4-(*N*^{6}-deoxyadenosinyl)2,3-dihydroxybutyl]GSH adduct, *N*^{6}-(2,3,4-trihydroxybutyl)deoxyadenosine adduct, or unmodified deoxyadenosine by human polymerases η, ι, k, and REV1, bacteriophage polymerase T7, and *Sulfolobus solfataricus* polymerase Dpo4. Although dTTP incorporation was the most preferred opposite *S*-[4-(*N*^{6}-deoxyadenosinyl)2,3-dihydroxybutyl]GSH adduct, *N*^{6}-(2,3,4-trihydroxybutyl)deoxyadenosine adduct, or deoxyadenosine by all polymerases examined except REV1, dCTP misincorporation frequency opposite *S*-[4-(*N*^{6}-deoxyadenosinyl)2,3-dihydroxybutyl]GSH was significantly higher than that opposite the *N*^{6}-(2,3,4-trihydroxybutyl)deoxyadenosine adduct or deoxyadenosine by human polymerase k. These results indicate the relevance of GSH-containing adducts in enhanced A:T to C:G mutation produced by DEB. (Supported in part by USPHS grants R01 ES010546 and P30 ES000267.)

ABSTRACT FINAL ID: 2596 Poster Board -237
TITLE: Correlation between Activation of PPARγ and Resistin Downregulation in Mouse Adipocyte Cell Line by a Series of Thiazolidinediones

AUTHORS (FIRST INITIAL, LAST NAME): A. Sotiriou[1, 5], R. H. Blaauw[2], C. Meijer[1], L. H. Gijsbers[3], B. van der Burg[4], J. Vervoort[5], and I. M. Rietjens[1].
INSTITUTIONS (ALL): [1]Toxicology, Wageningen University, Wageningen, Netherlands; [2]Chiralix BV, Nijmegen, Netherlands; [3]LeadPharma Medicine BV, Nijmegen, Netherlands; [4]BioDetection Systems, Amsterdam, Netherlands; [5]Biochemistry, Wageningen University, Wageningen, Netherlands.
KEYWORDS: TZDs, Resistin downregulation, PPARγ

ABSTRACT BODY: Thiazolidinediones (TZDs) are a chemical class providing specific drugs for treatment of insulin resistance. The insulin sensitizing TZDs have been reported to be specific ligands for peroxisome proliferator-activated receptor gamma (PPARγ) which is a receptor that regulates adipogenesis and glucose homeostasis. Upon PPARγ activation by the TZD rosiglitazone, resistin, a 12.5 kDa polypeptide that is expressed at high levels in obese and type 2 diabetes mice and human was reported to be downregulated. The aim of the present study was to investigate whether for a series of related TZDs the level of activation of PPARγ detected by a recently developed reporter gene assay would correlate with their potential for resistin downregulation in mouse adipocytes. To this end a method for detection of resistin expression in mouse adipocytes was developed. The results obtained reveal a significant correlation (r^2=0.6221, r=0.788, $p<0.05$) between the EC_{50} for PPARγ activation in the reporter gene assay and that for resistin downregulation in mouse adipocytes. It is concluded that both the newly developed PPARγ reporter gene assay and the downregulation of resistin in mouse adipocytes might provide adequate bioassays for the screening of antihyperglycemic compounds in the framework of developing new therapeutic strategies for the treatment or prevention of diabetes. Because of the higher throughput of the PPARγ assay the resistin downregulation assays seems most suitable to be used as a second tier in a tiered screening strategy.

ABSTRACT FINAL ID: 2597 Poster Board -238
TITLE: Exosome, a New Mediator of Irradiation Bystander Effect

AUTHORS (FIRST INITIAL, LAST NAME): B. Tian[1], Y. Cong[2], H. Fu[1], Y. Cong[2], Y. Song[1], Z. Sun[1], and X. Zheng[1].
INSTITUTIONS (ALL): [1]Biochemistry and Molecular Biology Department, Beijing Institute of Radiation Medicine, Beijing, China; [2]Radiation Disease Therapy Department, Beijing institute of Radiation Medcine, Beijing, China.
KEYWORDS: Bystander effect, irradiation, Exosome

ABSTRACT BODY: Bystander effect of radiation is a key issue of radiobiology. Presentation the mechanism underlying bystander effect will be helpful for radiation protection and improving clinical radiation therapy. It had been revealed that communications between target cells and by stander cells mediated these nontarget effects through gap junctions,

releasing cytokines, reactive oxygen species and nitric oxide. Recently, function of exosome in cell communication has been largely explored. Exosomes, containing the bulk of RNAs (mRNA, miRNA, etc) and proteins, are secreted by immune cells, epithelial cells, tumor cells, etc. Exosome can be transported to distal part and exert its modulation functions. So function of exosomes in mediating the bystander effect was explored. Treatment with exosomes purified from 4Gy γ-irradiation conditioned Raji cell medium resulted in enhanced micronuclei rate and DSBs both in Raji and MCF-7 cells to compare to exosome from unconditioned Raji cell medium. Further, we confirmed that exosomes purified from irradiated mouse serums also could mediate the bystander effect in mouse fibroblasts NIH3T3 cells, even in human tumor cells, Raji and MCF-7. OH-scavenger DMSO and NO scavenger Carboxy-PTIO couldn't block exosome functions in mediating the bystander effect. But RNase A1 treatment did inhibit exosome function in mediating the bystander effect significantly. Then exosome miRNAs and proteins were compared before and after irradiation. Several differentially expressed miRNAs and proteins were identified. These results showed that exosome was a new mediator of irradiation bystander effect, and RNAs played important roles in exosome mediated bystander effect.

ABSTRACT FINAL ID: 2598 Poster Board -239
TITLE: Effect of Acrolein on DNA Damage and Repair in Lung Adenocarcinoma Cells

AUTHORS (FIRST INITIAL, LAST NAME): P. Sarkar and B. E. Hayes.
INSTITUTIONS (ALL): College of Pharmacy and Health Sciences, Texas Southern University, Houston, TX.
KEYWORDS: Acrolein, lung adenocarcinoma cells, DNA damage

ABSTRACT BODY: Acrolein (Acr), an unsaturated aliphatic aldehyde and ubiquitous environmental pollutant is a respiratory irritant. Acr-derived 1,N(2)-propanodeoxyguanosines (Acr-dG) are exocyclic DNA adducts formed following exposure to cigarette smoke or via lipid peroxidation. Earlier studies in our laboratory have shown that ACr induces expression of inflammatory and apoptotic markers in rat lung epithelial cells. Since Acr is a highly reactive electrophile, it can disrupt many biochemical pathways which may result in alteration of the transcriptional regulation of the genome. Formation of Acr-DNA adducts increases the potential for gene mutations that may lead to carcinogenesis. These mutations may result either from loss of genes responsible for DNA repair or genes involved in DNA damage. In the present study, DNA damage and repair genes that are susceptible to Acr were screened in human lung adenocarcinoma cells (A549) using real time PCR array. A total of 11 out of 84 genes analyzed showed significant changes following exposure to Acr after a 24-hour treatment period. Three genes showed a significant decrease in response to Acr while eight genes showed 2 to 4-fold increase. Interestingly, Acr treatment caused a 3-fold decrease in GADD45G, while RAD50 was increased by 4-fold. GADD45G is a stress responsive gene and has been shown to be altered in response to environmental stress. Acr-mediated changes in GADD45G are indicative of Acr-induced stress in in A549 cells. RAD50 is a DNA repair gene that may counteract ACr-DNA damage. The results of this study, using real time transcripts analysis, provide preliminary data suggesting that Acr affects DNA damage responsive genes. Further studies on these genes and their biological functions are underway. GRANT SUPPORT: Research infrastructure support was provided by grants G12RR003045 and CO6RR012537 awarded by the National Center for Research Resources, (NIH). The G12 program is now a part of the NIMHD and the C06 program is in the Office of Research Infrastructure Programs in the Office of the Director, NIH.

ABSTRACT FINAL ID: 2599 Poster Board -240
TITLE: Pyrosequencing: Applicability for Studying the DNA Damage-Induced Mutagenesis

AUTHORS (FIRST INITIAL, LAST NAME): I. G. Minko, L. F. Earley, K. Larlee, and R. Lloyd.
INSTITUTIONS (ALL): Center for Research on Occupational & Environmental Toxicology, Oregon Health & Science University, Portland, OR.
KEYWORDS: mutation analysis, DNA lesions, Pyrosequencing

ABSTRACT BODY: Site-specifically modified DNAs are routinely used in the study of DNA damage-induced mutagenesis. These analyses generally involve the creation of DNA vectors containing a lesion at a predetermined position, intracellular

replication, and determination of mutations at the target sequences. This has previously required the isolation of individual DNA clones, hybridization with radioactively-labeled probes, and verification of mutations by Sanger sequencing. In search for an alternative, less labor-consuming, inexpensive, and environmentally safe procedure, we evaluated the applicability of pyrosequencing to site-specific mutagenesis assays. This technique allows quantification of sequence variants in a mixed population of DNA molecules and is used to determine heterozygosity, ploidy, and mixed genotypes in heterogeneous samples. To test the sensitivity of pyrosequencing, DNA vectors were generated containing a single nucleotide variant in otherwise identical sequences, mixed at various ratios, and analyzed. Depending on the nucleotide identity and the local sequence context, sensitivity ranged from 1 to 5%, and the data were highly reproducible. Pyrosequencing-based procedures were then used to assess the mutagenic properties of the methyl-FAPY-dG lesion following replication of site-specifically modified DNA vectors in mammalian cells. Analyses of individual clones using the previous methodologies indicated that the lesion caused ~7% G to T, ~3.5% G to A, ~3.5% G to C substitutions, and ~2% deletions. The frequencies of the G to T and G to A substitutions measured by pyrosequencing were comparable to the values obtained by the traditional procedure, while G to C transversions and deletions could not be reliably detected. Additionally, pyrosequencing-based protocol was used to show a low mutagenic potential of methyl-FAPY-dG in *E. coli.* Collectively, our data demonstrate the applicability and limitations of pyrosequencing for the analyses of DNA samples generated in site-specific mutagenesis assays.

ABSTRACT FINAL ID: 2600 Poster Board -241
TITLE: Evaluation of 4-Methylimidazole, Styrene and 3-Methylindole in the Bacterial Reverse Mutation Test Using Induced Rodent Liver and Lung S9

AUTHORS (FIRST INITIAL, LAST NAME): C. Beevers[1], and R. H. Adamson[2].
INSTITUTIONS (ALL): [1]Covance Laboratories Ltd, Harrogate, North Yorkshire, United Kingdom; [2]TPN Associates, LLC, Germantown, MD.
KEYWORDS: 4-Methylimidazole, 3-Methylindole, Genotoxicity

ABSTRACT BODY: 4-Methylimidazole (4-MeI) is formed by the interaction of ammonia with reducing sugars and low levels have been identified as a by-product in coffee, soy sauce, wine, dark beers, soft drinks and caramel colors. 4-MeI has been reported to induce alveolar/bronchiolar tumors in mice but not rats. Its mechanism of action is unlikely to be due to genotoxicity as 4-MeI does not induce mutation in Salmonella typhimurium and does not induce micronuclei in rodent peripheral erythrocytes or bone marrow cells. However, the question of whether genetically reactive intermediates could be formed via lung-specific metabolism has not previously been addressed. 3-Methylindole (3-MeI) is a preferential pneumotoxicant. A number of lung-expressed human cytochrome P450 enzymes have been reported to metabolize 3-MeI to DNA-reactive intermediates that induce DNA strand breaks (as measured by the Comet assay). In addition the metabolism of 3-MeI by human lung-expressed cytochrome P450 enzymes, but not hepatic P450s, has been reported to elicit mutagenicity in S. typhimurium, thus indicating that 3-MeI may be a human pulmonary carcinogen. We investigated whether 4-MeI, 3-MeI and a reference compound styrene could induce mutation in five standard Ames strains of S. typhimurium using induced rat (F344/N) and mouse (B6C3F1) liver and lung S9 as a source of exogenous metabolism. The chemicals were tested in a GLP and OECD 471-compliant bacterial reverse mutation assay, using both plate-incorporation and preincubation methodologies, together with 10% S-9 metabolic activation. No induction of mutation (as measured by an increase in revertant colonies) was observed for any of these chemicals. We conclude that 4-MeI was not mutagenic in Salmonella typhimurium using either rodent liver or lung S9 for exogenous metabolism. Similarly, no mutation was observed with 3-MeI or styrene. This work was funded by the American Beverage Association.

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ABSTRACT FINAL ID: 2601 Poster Board -242
TITLE: The Effect of Environmental Pollutants on Nucleotide Excision Repair and DNA Damage

AUTHORS (FIRST INITIAL, LAST NAME): P. Rossner, A. Mrhalkova, K. Uhlirova, M. Spatova, A. Rossnerova, H. Libalova, J. Schmuczerova, A. Milcova, J. Topinka, and R. J. Sram.
INSTITUTIONS (ALL): Institute of Experimental Medicine, Prague, Czech Republic.
KEYWORDS: Nucleotide excision repair, DNA adducts, air pollution

ABSTRACT BODY: We studied the nucleotide excision repair (NER) response [mRNA and protein expression of XPE, XPC and XPA genes, unscheduled DNA synthesis (UDS)] and levels of bulky DNA adducts in human embryonic lung fibroblasts (HEL 12469 cells) after their treatment with benzo[a]pyrene (B[a]P; concentrations 1, 10, 25 μM) and the extractable organic matter (EOM; concentrations 1, 10, 25 μg/ml) from ambient air particles < 2.5 μm (PM2.5). EOMs were collected in winter and summer of 2011 in two Czech cities (Prague and Ostrava) with different levels and sources of air pollution. The effects of the studied compounds were analyzed in the presence and absence of the microsomal S9 fraction. mRNA expression was analyzed using quantitative PCR, protein expression by Western Blotting, UDS by incorporation of 5-ethynyl-2'-deoxyuridine and bulky DNA adducts by 32P postlabeling. While mRNA expression of XPE and XPC measured after treatment of the cells with B[a]P was mostly elevated, the effect on XPA mRNA levels was less pronounced. EOMs collected in summer tended to induce mRNA levels of XPC and XPA more efficiently than winter EOMs; the expression of XPE mRNA was negatively associated with levels of B[a]P in EOMs. B[a]P treatment induced protein expression of XPE, XPC and XPA in the absence, but not in the presence of the S9 fraction. All tested EOMs induced protein expression of XPE, but only in the absence of the S9 fraction. None of the tested compounds significantly increased UDS. Levels of bulky DNA adducts were elevated in samples treated with B[a]P. We observed a weak effect of winter EOMs on bulky DNA adduct levels, while summer EOMs did not induce bulky DNA adducts. In summary, the HEL cells respond to the treatment with B[a]P and EOMs by induction of NER, but the response is mostly not sufficient to protect the cells against DNA damage. Supported by the Grant Agency of CR (P503/11/0084 and P503/11/0142).

ABSTRACT FINAL ID: 2602 Poster Board -243
TITLE: Inflammatory Cell Derived Oxidants Drive Silica Nanoparticle Genotoxicity

AUTHORS (FIRST INITIAL, LAST NAME): A. Sullivan[1], T. R. Downs[1], M. E. Crosby[2], Y. Shan[1], and S. Pfuhler[1].
INSTITUTIONS (ALL): [1]Procter & Gamble Company, Mason, OH; [2]AstraZeneca Pharmaceuticals, Waltham, MA.
KEYWORDS: Genotoxicity, Oxidative stress and inflammation, Silica nanoparticles

ABSTRACT BODY: The safety profile and potential mode(s) of action (MoA) by which nanomaterials (NMs) may induce genotoxicity are not fully understood. In a previous study (Downs et al 2012), we demonstrated that an increase in DNA damage, as measured by the alkaline Comet assay (CA) and micronucleus assay following three i.v. applications, occurred at doses that showed a tissue damage mediated inflammatory response. Using the same amorphous silica NM and dose, 15nm Levasil® 200 at 50mg/kg, we examined a range of biomarkers after a single i.v. injection to male Wistar rats at 4, 8, and 24h time points. When liver tissue was analyzed for the transcriptional expression of genes involved in oxidative stress response (Ho-1) and inflammation (Ccl2) by qRT-PCR, these genes were upregulated at 4 and 8h respectively. Additionally, pathway analysis indicates strong responses in the areas of inflammation, apoptosis and immune response. A variety of inflammatory and immune processes occurred at all time points, whereas the apoptotic response peaked by 8h. The initial immune and inflammatory responses involve physical responses such as neutrophil activation and the presence of kupffer cells as well as cell signaling cytokines such as IL2 and IL6. This ultimately results in a strong complement system response as well as phagocytosis to remove damaged tissue. We found no significant increases in direct DNA damage in the liver after a single iv injection using the CA at any of the time points. A second experiment was performed under similar conditions to address earlier time points, 2 and 4h. To assess oxidative DNA damage the OGG1 modification of the CA was performed. The CA again showed no significant increase in direct DNA damage in the liver at either time point while the OGG1 modification indicated a small increase in oxidative DNA damage in response to the NM. Our results indicate that the

inflammatory response precedes the antioxidant response suggesting that the inflammatory cell derived oxidants cause DNA damage rather than the particles themselves.

ABSTRACT FINAL ID: 2603 Poster Board -245
TITLE: Pulmonary Toxicity and Its Mechanism of PHMG-Phosphate *In Vitro*

AUTHORS (FIRST INITIAL, LAST NAME): C. Park, D. Shin, H. Kim, and K. Chung.
INSTITUTIONS (ALL): Pharmacy, Sungkyunkwan University, Suwon, Republic of Korea.
KEYWORDS: PHMG phosphate, Humidifier disinfectants, Pulmonary toxicity

ABSTRACT BODY: The acute death of 10 people including pregnant women and children was reported by the Korea Centers of Disease Control and Prevention (KCDC) on August 31, 2011. Still, quite a few people are hospitalized. The victims had been suffering unidentified rapidly progressive lung fibrosis. According to KCDC's epidemiology and toxicology study, the cause of mysterious lung fibrosis might be humidifier disinfectants, which case has never been reported before. For this reason, we identified pulmonary toxicity of humidifier disinfectants containing polyhexamethyleneguanidine phosphate (PHMG-phosphate, CAS # 89697-78-9), such as Oxy® and Wiselect®, by using human normal bronchial epithelial (BEAS-2B) cells. Furthermore, to confirm the cause of unidentified lung disease, the toxicity and toxic mechanism of the humidifier disinfectant's main component, SKYBIO 1125® (contains 25% PHMG-phosphate) was examined on mouse macrophage cell line (RAW 264.7). The consumer products were brought out significant dose-dependent cytotoxic effects and ROS generation. Likewise, cytotoxicity and ROS generation were elevated by exposing PHMG-phosphate in RAW 264.7 cells. In addition, the noteworthy activation of nuclear factor (NF)-κB signaling pathway was analyzed. Taken together, these results suggest that main component of humidifier disinfectant may cause pulmonary inflammation and toxic effects. Our results represent valuable foundation to clarify correlation between humidifier disinfectants and pulmonary toxicity. Further studies should be executed to confirm the inflammation and fibrosis mechanism of lung damage by humidifier disinfectants and to comfort the victims.

ABSTRACT FINAL ID: 2604 Poster Board -246
TITLE: Interleukin-6 (IL-6) Effects on P450 Isoforms, Uptake and Efflux Transporters in Primary Cultured Human Hepatocytes with Stabilized Gene Expression

AUTHORS (FIRST INITIAL, LAST NAME): Q. Yang and A. P. Li.
INSTITUTIONS (ALL): *In Vitro* ADMET Laboratories, Advanced Pharmaceutical Sciences, Columbia, MD.
KEYWORDS: human hepatocytes, drug metabolizing enzymes, transporters, inflammatory cytokines

ABSTRACT BODY: A complicating factor towards the investigation of the down-regulatory effects of cytokines on P450 and transporter gene expression in primary cultured hepatocytes is the spontaneous down-regulation of the same genes with time in culture. We recently reported that human hepatocytes cultured for 7 days in a novel medium, Li's Differentiation Maintenance Medium (LDMM), had stabilized expression of key liver-specific genes at levels similar to that of the first day of culture. We thereby evaluated of the effects of the prototypical proinflammatory cytokine IL-6 in the 'LDMM-stabilized (LS)' human hepatocytes. Human hepatocytes from 4 donors were treated with 1, 5 and 20 ng/mL of IL-6 for time periods of 6, 12, 24 and 48 hrs. The LS-human hepatocyte cultures were found to be highly responsive to IL-6 induction of the inflammatory gene markers CRP and SOCS3, suggesting the expression of IL-6 receptors and the subsequent signaling pathways. Extensive dose-and time-dependent down regulation of the gene expression of CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4, and 3A5, uptake transporters SLCs (10A1, 22A1, 22A7), SLCOs (1B1, 1B3, 2B1), and efflux transporters (ABCB1, ABCB11, ABCC2, ABCC3, ABCC4, and ABCG2) were observed. Over 80% suppression of gene expression was observed for all P450 isoforms and uptake transporters. Efflux transporters in general were less responsive to IL-6 treatment, with maximum suppression ranged from 40-80%. The L-6 effects observed were substantially higher than that reported by others using the routine culture conditions where spontaneous down regulation occurs. The high sensitivity of the hepatocytes to IL-6 is attributed to the restored and stabilized expression of the genes studied, thereby allowing a higher

dynamic range of response. The results suggest that the LS-human hepatocytes represent a physiologically relevant model for investigation of the down-regulatory effects of xenobiotics and may be applicable for the evaluation of biotherapeutics.

ABSTRACT FINAL ID: 2605 Poster Board -301
TITLE: Study on Metabolism of 2-amino-9H-pyrido[2,3-b] Indole (AαC) in Microsome with HPLC-DAD-TOFMS Technique

AUTHORS (FIRST INITIAL, LAST NAME): J. Yu, Y. Fu, G. Zhao, S. Wang, and F. Xie.
INSTITUTIONS (ALL): Zhengzhou Tobacco Research Institute, Zhengzhou, Henan, China.
KEYWORDS: AαC, *in vitro* metabolism, HPLC-UV-QTOF

ABSTRACT BODY: AαC (2-amino-9H-pyrido [2,3-b] indole) is a mutagenic and carcinogenic compound formed during food cooking and cigarette smoking. Humans are chronically exposed to low levels of AαC through foods (grilled or pan-fried meats) and smoke inhalation (cigarette/wood smoke, diesel exhaust). To understand the metabolism of AαC and search for the possible biomarkers for further exposure assessment, *in vitro* metabolism of AαC in hepatic microsomes from human pools, monkeys, rats, mice and dogs was investigated using a metabolomic analysis approach with HPLC-UV-QTOF technique. The factors that may influence the metabolism of AαC were investigated and optimized, such as incubated time, microsomes protein concentration and sample pretreatment. Data was analyzed with Principle component analysis (PCA) and Metabolite ID. Results showed that the metabolism of AαC in different microsomes produced similar metabolites with different amounts ratio. And in the human microsomes, ten metabolites could be found, besides the several metabolites previously reported, some new compounds were observed, which may be three minor hydroxylated metabolites ([M+H]+, m/z ([M+H]+, m/z=200.0811) and one hydroxylated and methylated metabolite ([M+H]+, m/z 214.0975).

ABSTRACT FINAL ID: 2606 Poster Board -302
TITLE: Human Lethality Predictions from Exposure to VX Using a Physiologically Based Pharmacokinetic-Pharmacodynamic Model

AUTHORS (FIRST INITIAL, LAST NAME): T. R. Covington[1], C. D. Ruark[1], K. O. Yu[2], and J. M. Gearhart[1].
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KEYWORDS: VX, PBPK, lethality

ABSTRACT BODY: There are limited data available from human exposures to chemical warfare nerve agents (CWNAs) on which to base human lethality estimates. This results in animal data as the primary biological evidence for dose response estimates. Physiologically-based pharmacokinetic-pharmacodynamic (PBPK-PD) models have proven useful for extrapolating between species and routes in order to determine human risk estimates when insufficient human data are available. The physiological nature of PBPK-PD models allows for extrapolation between dose routes as well as between species; they can also better account for differences in absorption and uptake that can vary widely between routes. Using an existing PBPK-PD model for VX and published data on mortality dose-response relationships for an animal species, internal doses were simulated to develop new mortality dose-response curves from which internal LD50 doses may be estimated. The PBPK-PD model, using physiological parameters for the human, was then used to estimate external doses which would result in the same internal doses (internal LD50s) estimated for the animal LD50s. The resulting predicted human LD50 based on internal AChE activity in brain is consistent with a previously predicted human LD50. This method of lethality prediction was further enhanced through the use of Monte Carlo analysis by allowing for the incorporation of population variability in physiological and chemical specific parameters (such as body weight, baseline AChE levels or polymorphisms in metabolism) in order to develop a distribution of lethality estimates rather than single point estimates. These distributions better account for differences in response between more resilient or more susceptible subpopulations.
(This project received support from the Defense Threat Reduction Agency - Joint Science and Technology Office, Basic and Supporting Sciences Division under grant number CBM.NEURO.03.10.AHB.)

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ABSTRACT FINAL ID: 2607 Poster Board -303
TITLE: Induction of Cytochrome P450 (CYP)1A1 and CYP1B1, but not CYP1A2, Protein Expression in Human Lung Cell Line, H358, by 3-methylcholanthrene (MC): Implications for Pulmonary Carcinogenesis by Polycyclic Aromatic Hydrocarbons

AUTHORS (FIRST INITIAL, LAST NAME): S. Kondraganti, C. Chu, W. Jiang, and B. Moorthy.
INSTITUTIONS (ALL): Pediatrics, Baylor College of Medicine, Houston, TX.
KEYWORDS: CYP1A1, 3-Methylcholanthrene, Lung cell lne, 358

ABSTRACT BODY: Humans are constantly exposed to environmental carcinogenic polycyclic aromatic hydrocarbons (PAHs) through cigarette smoke, diesel exhausts, charcoal-broiled meats, etc. Cytochrome P4501A (CYP1A) enzymes play important roles in the activation of PAHs such as 3-methylcholanthrene (MC) to carcinogenic DNA-binding metabolites, which in turn contribute to pulmonary carcinogenesis. We recently showed that MC causes persistent induction of hepatic and pulmonary CYP1A1 in mice by mechanisms independent of the persistence of the parent compound, and that this phenomenon contributes to carcinogenesis by MC. Molecular regulation of CYP1A1 in human lung is not well understood as many lung cell lines such as A-549 fail to express CYP1A1 even after treatment with MC. In this study, we tested the hypothesis that MC elicits induction of CYP1A1 and 1B1 in human lung cell line, H358, which is derived from bronchoalveolar cells. H358 cells were exposed to MC (2.5 μM), and CYP1A1 enzyme activity was determined by measuring the activity of ethoxyresorufin O-deethylase (EROD) 8 h after treatment. CYP1A1, 1A2, and 1B1 apoprotein expression and mRNA expression were determined by Western blotting and real time RT-PCR, respectively. Real-time RT-PCR showed that 8 h treatment with 2.5 μM MC-induced mRNA of CYP1A1, CYP1A2, and CYP1B1 by 849-, 121-, and 10-fold, respectively. In Western blot analysis with a monoclonal human CYP1A1-specific antibody, basal CYP1A1 apoprotein expression was observed in H358 cells. MC elicited significant induction of CYP1A1 and 1B1 apoprotein in a time- and dose dependent manner. MC also caused an 8-fold induction of EROD (CYP1A1) activities. However, CYP1A2 apoprotein expression was not detected in control or MC-exposed cells. The results suggest that H358 could be a valuable cell line model to investigate the molecular mechanisms of regulation of CYP1 enzymes by PAHs, in relation to human carcinogenesis.

ABSTRACT FINAL ID: 2608 Poster Board -304
TITLE: High-Throughput Gene Silencing and mRNA Expression Analysis in Hepatocyte Sandwich Cultures

AUTHORS (FIRST INITIAL, LAST NAME): B. D. Hollingshead[1], L. M. Gauthier[2], J. W. Davis[1], and A. D. Burdick[1].
INSTITUTIONS (ALL): [1]Drug Safety Research and Development, Pfizer, Cambridge, MA; [2]Drug Safety Research and Development, Pfizer, Andover, MA.
KEYWORDS: Hepatocytes, siRNA, cytochrome P450

ABSTRACT BODY: Primary hepatocyte sandwich cultures are useful for a variety of research applications where maintenance of metabolic competency is essential. Hepatocytes cultured in a collagen-Matrigel™ sandwich recapitulate many architectural features of liver (e.g. gap junctions and bile canalicular networks) and can be cultured for longer time periods compared to monolayer collagen cultures. The Matrigel™ overlay complicates gene silencing by traditional reagent-based transfection methods. Here, we describe a siRNA delivery method in primary mouse hepatocytes that allows cells to be cultured with Matrigel™ overlay. To accomplish this we transfected freshly isolated primary mouse hepatocytes in a suspension containing 500,000 cells per ml with 100 nM siRNA immediately prior to plating on collagen-coated 96-well dishes. Two hours later, a Matrigel™ overlay was added to the adherent cells. Fresh media was added every 24 hours and cells were lysed 48-72 hours post transfection. This transfection method delivered greater than 80% mRNA silencing of constitutive (Cyp3a11 = 89%, Cyp3a13 = 86%) and rafampicin inducible (Cyp3a11 = 83%) cytochrome P450 gene expression with little decrease in cell viability as determined by ATP depletion assays. Additionally, basal Cyp3a11 and Cyp3a13 mRNA levels were greater than 4 fold higher than hepatocytes grown in nonsandwich culture conditions. The 96-well format of this method allows for high-throughput RNA processing and downstream quantitative PCR applications that reduce time and resource usage. This format is particularly useful when experiments requiring many different sampling conditions (such as pharmacologic dose-response curves) are required.

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ABSTRACT FINAL ID: 2609 Poster Board -305
TITLE: Developing a Framework for Identifying Systemic Toxicity for Chemical Compounds Used in Cosmetics using ToxCast and ToxRefDB

AUTHORS (FIRST INITIAL, LAST NAME): L. Truong[1], K. McLaurin[1], N. Kleinstreuer[1], S. Loisel-Joubert[2], G. Ouedraogo[2], R. Note[2], L. Le Capitaine[2], H. Noçairi[2], D. Dix[1], and M. Martin[1].
INSTITUTIONS (ALL): [1]NCCT, US EPA, Durham, NC; [2]L'Oréal, Paris, France.
KEYWORDS: cosmetics, systemic, toxicology

ABSTRACT BODY: The US EPA is collaborating with L'Oréal to develop high throughput screening (HTS) and nonanimal testing methods to assess potential systemic toxicity of chemical compounds present either as ingredients or traces of contaminants in personal care products, fragrances and cosmetics. The EPA ToxCast project is analyzing data generated on thousands of chemicals across rapid, automated HTS assays with human gene and protein targets including those which may play a role in systemic toxicity. The Toxicity Reference Database (ToxRefDB) is a repository of over 5,000 legacy animal studies on ~ 1,000 chemicals, and captures the animal studies using a standardized, multilayered effect vocabulary across various study types and species. The structure of ToxRefDB provides the ability to focus on specific study types, species and effect endpoints. HTS ToxCast data was available for 518 ingredients/traces of cosmetics. 178 of these 518 ingredients had *in vivo* subchronic (SUB) and chronic (CHR) studies using either oral or feed administration routes in ToxRefDB. Of the 178 chemicals, 125 had CHR rat studies, 103 with SUB rat, and 80 chemicals with both study types. As a first step to developing HTS and nonanimal testing methods, preliminary evaluation of the *in vivo* SUB and CHR data of the ingredients/traces of cosmetics was performed and identified liver as a prevalent target organ. For these ingredients from the chemical industry, the uterus was a more sensitive target organ than testis with the lowest effect level (LEL) at the top 5% of the distribution being 1.69 compared to 195 mg/kg/day, respectively. The list of chemicals with complimentary *in vitro* and *in vivo* toxicity data will inform the development of future predictive models for systemic toxicity that will help identify potential systemic toxicity of ingredients used in cosmetics for further testing. This abstract does not necessarily represent EPA policy.

ABSTRACT FINAL ID: 2610 Poster Board -306
TITLE: Interactive Web Application (Dashboard) for ToxCast Data Exploration

AUTHORS (FIRST INITIAL, LAST NAME): C. L. Strope, S. Watford, D. Reif, A. Frame, R. Judson, N. Baker, I. Shah, and M. Martin.
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KEYWORDS: Chemical risk assessment

ABSTRACT BODY: The USEPA ToxCast research program has generated over 1 million assay-chemical concentration response curves across over 600 high throughput screening (HTS) assays. The US EPA has developed a web-based interface (Dashboard) to synthesize data from the ToxCast project in Aggregated Computational Toxicology Resource (ACToR) database into customized information displays. The ToxCast Dashboard is built upon a flexible infrastructure, allowing multiple views of ToxCast data organized to support user-specific needs. The users select sets of chemicals and HTS assay data to focus on for data exploration and analysis. Data are organized by data-class to represent relevant information for decision needs. Currently the ToxCast Dashboard provides data-class views by assay technology and assay target family, but future improvement will expand viewing options. Within the Data Explorer mode, the user can view summarized information for the various classes of assays and more detailed hit-calling information across all selected chemicals. In contrast, the Chemical Explorer mode permits viewing of detailed concentration response plots and curve-fitting parameters for the selected data and chemicals. Chemical-specific scores containing information from each data-class is presented in a dynamic summary table with a default score (e.g., average AC50 across assays within the data class). Users can adjust score criteria or apply expert knowledge to modify scores of particular chemicals for a specific dashboard session, with all such session-specific decisions saved for transparency of decision making. Scores are carried over into the Prioritization Mode where the chemicals are ranked in a weight-of-evidence scheme including an implementation of the

Toxicological Prioritization Index (ToxPi). The ToxCast Dashboard will serve as a portal for ToxCast data access for risk managers and the public. This abstract does not necessarily reflect US EPA policy.

ABSTRACT FINAL ID: 2611 Poster Board -307
TITLE: Interaction of Cardiolipin and Acrolein with Cytochrome C and Implications: A Molecular Dynamics Study

AUTHORS (FIRST INITIAL, LAST NAME): I. Shrivastava[1], I. Bahar[1], and V. E. Kagan[2].
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KEYWORDS: Cytochrome c, Cardiolipin, Acrolein

ABSTRACT BODY: Acrolein is a highly toxic three carbon alpha-beta-unsaturated aldehyde, an environmental pollutant and a key component of cigarette smoke (Sarkar & Hayes, 2008, *Life Sci.* 85,188-195). Acrolein is also an endogenous by-product of lipid peroxidation. Several studies have suggested Acrolein to be a mitochondrial toxin causing mitochondrial dysfunction leading to hepatotoxicity and smoke-related diseases (Sun et al, 2006 *Mitochon.* 6,136-142). Acrolein is known to cause mitochondrial membrane permeability transition—a process leading to the release of proapoptotic proteins such as cytochrome c (cyt c) from mitochondria into the cytosol during apoptosis. Cytochrome c interaction with a mitochondrial anionic phospholipid, cardiolipin (CL) causes cyt c to gain peroxidase activity, induce cardiolipin peroxidation—an event essential for the the release of cyt c and development of apoptotic response (Kagan et al. Nat Chem Biol. 2005 4, 223-232). However, the characterization of cyt c - CL complex has been difficult by traditional structural methods. Here we use molecular dynamics (MD) simulations to study the mechanism of interaction of cyt c with CL and with acrolein. Our studies reveal mechanism of interaction between cyt c and CL, and the conformation of a highly stable complex formed between the two molecules. Simulations suggest that interaction of cyt c with either CL or acrolein significantly perturbed the folded structure of cyt c leading to its partial unfolding. We further identify specific residues on cyt c which act as attractors for binding acrolein molecules. These so called 'sticky' residues are mostly responsible of persistent intermolecular interactions causing partial unfolding of cyt c which may facilitate its permeation through the mitochondrial membrane permeability pore.

ABSTRACT FINAL ID: 2612 Poster Board -308
TITLE: Strengthening the Case for *In Silico* GTI Assessments

AUTHORS (FIRST INITIAL, LAST NAME): S. Stalford, C. G. Barber, L. Coquin, T. Hanser, P. N. Judson, J. D. Vessey, and R. V. Williams.
INSTITUTIONS (ALL): Lhasa Limited, Leeds, United Kingdom.
KEYWORDS: genotoxic impurities, (Q)SAR, negative predictions

ABSTRACT BODY: It is probable that guidance on genotoxic impurities (GTIs) will continue to support the regulatory acceptance of *in silico* predictions for mutagenicity. The basis of this acceptance is that predictive systems can rule out the possibility of mutagenic potential in GTIs. In this work, we present methods to strengthen predictions by 1) augmenting an expert predictive system with negative prediction algorithms and 2) generating a novel system to provide complementary predictions. Methods to enhance expert *in silico* predictive systems were investigated and applied to a set of knowledge-based structural alerts. These included a) adding a second system to check compound similarity in a reference set, and b) using boundary space around prediction nodes to make negative predictions. It was found that using a system to measure compound similarity in addition to structural alerts was feasible, with the absence of similarity to an active compound correlating with experimental inactivity. An evaluation of boundary space showed that this could also be used to make accurate negative predictions. As well as enhancing existing systems, a novel (Q)SAR model has been developed. This self-organising hypotheses network (SOHN) model offers a complementary methodology for making toxicity predictions which is data driven, transparent, and has the advantage of organising global and local models simultaneously in a hierarchical way. In initial mutagenicity prediction performance measures, SOHN models returns results comparable to other (Q)SAR

system approaches (accuracy>75%). Additionally the model presents relevant structural hypotheses along with useful confidence values and applicability domain assessments. Coverage of the typical chemical space defined for this endpoint was also good (> 90%). We have developed novel methods to extend expert rule-based predictive systems to explicitly make negative predictions and established novel machine-learning methods that provide transparent and accurate predictions for mutagenicity.

ABSTRACT FINAL ID: 2613 Poster Board -309
TITLE: Evaluation of Automated Blood Sampler (ABS) with Dried Blood Spot (DBS) Technology for Routine Rat PK Studies in Discovery DMPK

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INSTITUTIONS (ALL): Celgene, San Diego, CA.
KEYWORDS: Dried Blood Spots, Automatic Blood Sampling, Pharmacokinetics

ABSTRACT BODY: Dried blood spot (DBS) technology has recently generated a great deal of interest in drug discovery and development as an alternative to conventional blood sampling methods. The introduction of the automated blood sampling (ABS) systems has considerably streamlined the process of collecting blood samples and thus has contributed substantially to increased efficiency and throughput. This study evaluated the feasibility of using ABS incorporating the DBS technology for routine PK screening. Four internal compounds with different chemical templates were administered intravenously to male CD-IGS rats and blood samples were collected using the Instech ABS units. These compounds had pKa values ranging from 4–12, cLogP ranging from 1–4 and blood to plasma (BP) ratios ranging from 0.7-3. Full PK profiles from different groups of animals (n=3) were obtained over a 24-hr period. The groups were as follows: 1) Blood samples spotted directly on the DBS disks by the ABS, 2) Blood samples collected by ABS but manually spotted on the DBS disks, and 3) Blood samples collected by ABS, spun down and plasma component collected and processed using the conventional technique. For all compounds, the results from groups 1 and 2 compared very well. When compared to plasma PK (group 3), the average PK parameters generated from the automated DBS and manually spotted DBS, were highly comparable for 3 out of the 4 compounds after correcting for their corresponding BP ratios, with the lone exception being a compound with high BP ratio that exhibited ~2X lower CL. However, it is important to note that using automated DBS in lieu of plasma samples did not change our placement category of low or high clearance values based on our internal guidelines for progressing compounds into subsequent PK studies. Based on these results, we can conclude that the ABS system coupled with DBS collection is promising to obtain reliable full PK profiles with increased efficiency and throughput while gaining significant synergies from the advantages of both techniques.

ABSTRACT FINAL ID: 2614 Poster Board -310
TITLE: The Xenobiotic Transporter Gene Polymorphisms: Possible Association with Colorectal Cancer Risk

AUTHORS (FIRST INITIAL, LAST NAME): G. Ozhan and B. Alpertunga.
INSTITUTIONS (ALL): Pharmaceutical Toxicology, Istanbul University, Istanbul, Turkey.
KEYWORDS: OATP1B1, ABC1B1, Genetic polymorphisms

ABSTRACT BODY: Colorectal cancer is an important cause of death throughout the world and its aetiology involves the interaction of genetic and environmental factors. Transporter proteins are important in protecting organs from xenobiotics or toxins. Organic Anion Transport Protein 1B1 (OATP1B1) and ATP binding cassette transporter B1 (ABCB1) translocate a variety of substrates across extra- and intracellular membranes, and act as efflux proteins. The transporters are expressed in the apical membranes of excretory tissues, such as liver, kidney and intestine, and contribute to the elimination of toxic exogenous substances or metabolites. The transporters are characterised by the presence of genetic polymorphisms mainly represented by single nucleotide polymorphisms (SNPs), some of which having an impact on their activity. The aim of our study was to determine if polymorphisms in OATP1B1 and ABC1B1 genes were associated with colorectal cancer. For that, three OATP1B1 (388A>G, 11187G>A, 521C>T) and three ABC1B1 (1236C>T, 2677G>T/A, 3435C>T) variants were determined

using polymerase chain reaction-restriction fragment length polymorphism analysis in patients with colorectal cancer and healthy controls. Odds ratios (ORs) and 95% confidence intervals (CI) were estimated. In conclusion; OATP1B1 521T>C and ABCB1 1236C>T were statistically significantly associated with colorectal cancer risk (OR=2.66; 95% CI=1.31-5.41; p=0.0057 and OR=1.91; 95% CI=1.09-3.35; p=0.034, respectively). In haplotype-based analysis, OATP1B1 haplotype G388-T11187-T521 and ABCB1 haplotype C1236-G2677-T3435 might be associated with the development of colorectal cancer (OR=4.26; 95% CI=1.62-11.16; p=0.002 and OR=11.96; 95% CI=2.59-55.32; p=0.0004, respectively). In conclusion; OATP1B1 and ABC1B1 seem to play a role in colorectal cancer. The results may be a basis for studies on the interindividual variability in drug disposition and perhaps certain disease processes even though inheritance is complex and genetic factors interact with environmental factors.

ABSTRACT FINAL ID: 2615 Poster Board -311
TITLE: PBDEs, Metals, Dioxin TEQs, PCBs and Persistent Pesticide Exposure in Vietnamese Electronic Waste Recyclers

AUTHORS (FIRST INITIAL, LAST NAME): M. Hommel[1], A. J. Schecter[1], H. Quynh[2], D. Cheng[1], T. Tuyet-Hanh [3], N. Imran[1], and L. S. Birnbaum[4].
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KEYWORDS: E-Waste recycling, Vietnam, Organics and metals

ABSTRACT BODY: Increased global demand for technology and shorter life cycles for electronics have led to high volumes of electronic waste (e-waste). Developed nations often outsource e-waste to developing nations for recycling. Recycling worker studies typically focus on metals or brominated flame retardants. This pilot biomonitoring study of 10 women working as home based e-waste recyclers in Vietnam and 10 comparisons with no known chemical exposures measured dioxin CALUX TEQs, PBDE congeners, persistent organic pesticides, nondioxin like PCBs, and metals. Of PBDE congeners measured, only BDEs 153, 183 and 209 had detectable levels in 30% or more of the women. BDE 47, from Penta BDE, was detected only in one worker; BDE 153 (found in Octa PBDE mixtures often used in electronics) was reported in 95% of all participants. Median BDE 153 was 3.65 ng/g lipid in workers and 1.1 ng/g in the comparisons. Median BDE 153, 183, 209 and total BDEs were higher in workers. This suggests worker Deca and/or Octa, but not Penta PBDE, exposure. Total nondioxin like PCB totals were similar in each group. DDT and DDE were higher in workers and comparisons compared to U.S. adults, but no statistically significant differences were noted in the workers. Dioxin TEQs were higher in workers, measured using CALUX. A metals screen was performed for workers and comparisons. Median urine metal levels were significantly higher in workers for arsenous acid, monomethylarsonic acid, cobalt and mercury 3.6 v 2.6, 7.0 v 5.3, 0.6 v 0.4, 1.0 v 0.4 ug/g creatinine. Thus, e-waste workers may have higher levels of both certain pollutants and metals than comparisons, and in some cases levels in both groups are higher than in the US. The opinions expressed in this article are the authors' and not the views of NIH/DHHS. This study is supported by the NCI Intramural Research Program.

ABSTRACT FINAL ID: 2616 Poster Board -312
TITLE: *In Vivo* Pharmacokinetic and Pharmacodynamic Comparability Study of Moxetumomab Pasudotox, an Immunotoxin Targeting CD22, in Cynomolgus Monkeys

AUTHORS (FIRST INITIAL, LAST NAME): X. Chen[1], L. Iciek[1], S. S. Chuang[2], L. Chang[1], B. Wang[1], M. Liang[1], I. Vainshtein[1], R. Lee[1], A. Schneider[1], L. Roskos[1], and R. Dixit[1].
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KEYWORDS: Pharmacokinetic, comparability study, moxetumomab pasudotox

ABSTRACT BODY: Substantial manufacturing process and facility changes occurred during the clinical development of moxetumomab pasudotox, an immunotoxin composed of a murine anti-CD22 variable domain (Fv) linked to a truncated form of Pseudomonas exotoxin. Due to the multifunctional mode of drug action, a GLP *in vivo* comparability study was conducted in cynomolgus monkeys to determine whether the manufacturing process changes altered the pharmacokinetic

(PK) exposure and pharmacodynamic (PD) activity of moxetumomab pasudotox. Animals that prescreened negative for pre-existing antibodies were randomized by body weight and baseline CD22 lymphocyte counts to two treatment groups (n=12/group), and received intravenous administrations of 1 mg/kg Process 1 or Process 2 (new process) moxetumomab pasudotox on Days 1, 3, and 5. Blood samples were collected for PK, flow cytometry (for PD), and immunogenicity assessments. The 90% confidence intervals of the geometric mean ratios of PK exposure were within the 80-125% range. Despite a short PK half-life (~1 hour), moxetumomab pasudotox induced substantial suppression of CD20+ (CD22+) B lymphocytes in cynomolgus monkeys. The CD20+ and CD22+ lymphocyte counts were depleted to a similar extent and the immunogenicity incidences were similar across the two groups. The results demonstrated that the Process 2 (new process) material was comparable to the Process 1 material and supported continued clinical investigation of moxetumomab pasudotox in patients with B lymphocyte malignancies using the Process 2 material.

ABSTRACT FINAL ID: 2617 Poster Board -313
TITLE: Possibility of High-Exposure Group Sampling Regarding PCB and POPs from Questionnaire in Birth Cohort Study

AUTHORS (FIRST INITIAL, LAST NAME): E. Todaka[1], M. Watanabe[1], Y. Matsuno[1], and C. Mori[1, 2].
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KEYWORDS: PCB, POPs, cohort study

ABSTRACT BODY: Purpose: In Japan Environment and Children's Study (JECS, a Japanese national birth cohort study), a detail questionnaire survey regarding food intake has been conducted. In other hand, our previous studies indicated that blood PCB (polychlorinated biphenyl) level of mothers could be a good indicator to know the blood level of other persistent organic pollutants (POPs) in both mothers and their fetuses. In this study, we examined the correlation between maternal blood PCB level and sea food intake information in JECS's questionnaire to see the possibility to sample the high-exposure group regarding PCB and POPs from the questionnaire. Subjects and method: We measured the concentration of total PCB in blood from pregnant women who participated at the JECS in Chiba Prefecture in 2012. The number of the subjects was 282 (age: 17–43). Total PCB was analyzed from whole blood by using a gas chromatograph equipped with a packed column and electron capture detector (Packed column GC/ECD). Results: PCB was detected from 97% of nulliparous (average was 0.31 ± 0.14 ng/g-wet) and 93% of multiparas (average was 0.28 ± 0.13 ng/g-wet). The blood PCB level increased with age. We found strong correlation ($P<0.01$) between blood PCB level and intake of bonito, yellowtail and tuna in questionnaire. Discussion: Our present study indicated that the information of sea food intake in questionnaire has the possibility to sample the high-exposure group to PCB and POPs. If the highly exposed group can be sampled from the questionnaire survey, potential high risk group of fetuses can be detected. Our results show the possibility to use the questionnaire to detect potential high risk group in a cost and time effective method in a large scale cohort study. The current data of JECS is not the final data but tentative and limited data of Chiba Prefecture.

ABSTRACT FINAL ID: 2618 Poster Board -314
TITLE: Conceptual Exposure Model for Worker Health Risks Associated with Hydraulic Fracturing

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INSTITUTIONS (ALL): [1]CRA, Dallas, TX; [2]CRA, Little Rock, AR.
KEYWORDS: Risk Assessment, Hydraulic Fracturing, Worker Exposure

ABSTRACT BODY: Exponential growth in the implementation of hydraulic fracturing (i.e., fracking) technology, where various constituents are injected into deep shale formations to extract natural gas, has increased calls from the regulatory community and the public for a closer scrutiny. The expansion in fracking exploration has raised concerns over potential health risks to workers and surrounding communities. A critical component in a human health risk assessment is the conceptual exposure model (CEM) where all potential pathways and routes are identified and classified as complete or incomplete based on information for a given case. A properly constructed and executed CEM helps to focus risk assessment

on issues with the greatest potential for adverse effects. This presentation demonstrates a comprehensive CEM for ascertaining human health risks for gas rig workers potentially exposed to various hydraulic fracturing operations. The model derivation methodology consisted of querying literature for data on actual/potential exposures of workers to various fracking constituents and under all scenarios. The most frequently-documented pathways/routes were amalgamated into a CEM, which for the purpose of the poster presentation, is depicted as a graphics-enhanced, diagramatic flow chart. The CEM analysis shows that the workers may come into contact with various fracking constituents via inhalation, ingestion, and dermal contact. The potential pathways include exposure to dusts (silica sand) during proppant handling, particulates from combustion engines (compressors, pumps, etc), hydrocarbons from the well head (methane, hydrogen sulfide, etc), fracking fluid chemicals (glutardehyde, ammonium persulfate, etc) via direct handling, produced water constituents (radionuclides, etc) originating in play formations via flow back/storage, and drilling muds during bore advancement. These potentially-complete exposure pathways and routes merit further assessment on case-by-case basis when evaluating the occupational safety of hydraulic fracturing operations.

ABSTRACT FINAL ID: 2619 Poster Board -315
TITLE: Integrated Risk Assessment and Management for a Chemical Spill Emergency Response Associated with a Train Derailment

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KEYWORDS: Risk Assessment, Train Derailment, Emergency Response

ABSTRACT BODY: Chemical spill emergency responses (ER) such as those associated with train derailments are conducted in fast-paced, dynamic, and highly variable environments that require immediate protection of human health, ecological health, and infrastructure. Effective response requires a safe and timely restoration of service while minimizing environmental impacts. Given this compressed time scale and high pressure environment, critical risk assessment and management (RAM) steps must be planned, negotiated, and implemented in the right sequence and in concert with the myriad of other, rapidly-developing response activities. The current study developed a risk assessment framework that captures the critical components of an effective ER RAM for a train derailment. The ER RAM framework is intended to assist responders in ensuring that early actions lead not only to the continued protection of human health and the environment, but also to significant cost savings since data gathering and commensurate RAM is highly tailored to each incident. The framework presented was constructed using observations, lessons learned, and data gathered from multiple derailments in the US and Canada. The information and data gathering stage consisted of querying existing company-wide project files and interviewing project managers, risk assessors, clients and regulators on key components and timing of a derailment emergency response that led to successful risk-based site closures. The findings were compiled and transformed into a decision analysis tool that identifies, categorizes, prioritizes, and interlinks the key ER components, which include the emergency response, regulatory compliance, risk assessment, remediation, and restoration phases. A hierarchical flow chart was developed that identifies the critical ER events, risk-based decision management points, potential outcomes, and alternatives. The tool illustrates the importance of ecological and risk assessment-based decisions early in the ER life-cycle.

ABSTRACT FINAL ID: 2620 Poster Board -316
TITLE: Managing Public Health Risks Using Air Monitoring at a Former MGP Site

AUTHORS (FIRST INITIAL, LAST NAME): R. DeHate[1], B. Skelly[1], U. Desai[2], G. Johnson[2], and R. D. Harbison[2].
INSTITUTIONS (ALL): [1]GEI Consultants, Inc., Valrico, FL; [2]College of Public Health University of South Florida, Tampa, FL.
KEYWORDS: Risk Management, Risk Assessment, Manufactured Gas Plants

ABSTRACT BODY: Monitoring emissions from a former Manufactured Gas Plant (MGP) site during remediation was used to manage risks associated with inhalation of VOCs such as benzene, toluene, ethylbenzene, and xylenes; and contaminated particulates acting as an exposure conduit for PAHs and heavy metals. This risk management case study presents a USEPA-

approved air monitoring program implemented to manage human public health risks during remediation at a former MGP site located in the southeast U.S. Risk-based Acceptable Air Concentrations (AACs) were developed and a sampling regime established to monitor potential emissions to maintain contaminant concentrations below the AACs. The AAC for benzene was based on carcinogenic effects using the current IUR from the USEPA's IRIS database. The AACs for toluene, ethyl benzene, and xylenes were based on noncarcinogenic effects using the current RfC from the IRIS database. The AACs for the carcinogenic PAHs were based on carcinogenic effects using the current IUR from California EPA. The AAC for respirable particulate matter (PM10) was the National Ambient Air Quality Standard (NAAQS) for PM10 and was used as a surrogate for both the PAHs and heavy metals. Site-specific AACs were calculated using a target cancer risk (TR) value of 1x10-4 for carcinogens and a target hazard quotient (THQ) of 1 for noncarcinogens. The exposure duration used was based on a twelve-month project duration and an exposure time of 24-hours per day; equations, toxicity values and sources were based on USEPA's Regional Screening Levels website. A total 535 twenty-four hour time weighted samples (269 VOC samples and 266 PAH samples) were collected over the project duration. Only minor levels of VOCs and PAHs were detected and no results were above the AACs. These time-weighted averages demonstrate that the real-time air monitoring and control measures implemented at the Site effectively maintained concentrations below the AACs and were protective of public health.

ABSTRACT FINAL ID: 2621 Poster Board -317
TITLE: Exposure and Health Risk Assessment of Metals in Apple Juice

AUTHORS (FIRST INITIAL, LAST NAME): I. Bebenek[1], B. Tvermoes[2], A. Banducci[2], A. Monnot[3], K. Devlin[2], and A. K. Madl[1].
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KEYWORDS: Metals, Risk Assessment, Dietary exposure

ABSTRACT BODY: The American Academy of Pediatrics suggests that 6 oz of juice can count toward a serving of fruit. However, concerns have recently been raised about heavy metal contamination in apple juices. The metal contents of fruit juices depend on a number of factors, including the soil composition, external conditions during fruit growing and fruit harvesting, as well as the fruit juice manufacturing processes employed. Heavy metals such as aluminum, arsenic, chromium, copper, iron, manganese, mercury and zinc are and have been used in a number of herbicides, pesticides and fungicides in the U.S and worldwide. In an effort to understand possible presence of heavy metals in fruit juices and the potential implications for human health, we measured the concentration of several metals in apple juices in three commercially available brands of juices, three brands imported from China and three local organic brands. The apple juices were analyzed for arsenic, cadmium, manganese, lead, copper, zinc, aluminum, chromium, and mercury. The levels ranged from 0.0018- 0.0085 mg/L for total arsenic, 0.0002- 0.0007 mg/L for cadmium, 0.1727- 1.197 mg/L for manganese, 0.00084-0.0091 mg/L for lead, 0.0051- 0.1823 mg/L for copper, 0.0756- 3.2118 mg/L for zinc, 0.0383- 4.9130 mg/L for aluminum, 0.0052- 0.0179 mg/L for chromium, and non detect to 0.0001 mg/L for mercury. The levels of copper, chromium, zinc, mercury, cadmium and total arsenic in all samples were below the FDA maximum contaminant level for drinking water. However, in some juices the levels of aluminum, lead, and manganese exceeded the FDA maximum contaminant level for drinking water. Noncarcinogenic risk levels were estimated for aluminum and manganese because a chronic oral reference dose has been established for these two metals. In general, those consuming apple juices are the young and it is this population that may be more vulnerable to over exposure of heavy metals, especially lead. Thus, it is important to understand the implications of these findings in regards to children's health.

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Late-Breaking Abstracts

ABSTRACT FINAL ID: 2622 Poster Board -318
TITLE: New IRIS Trichloroethene Toxicity Criteria: Impact of Noncancer Hazard at Cleanup Sites in California

AUTHORS (FIRST INITIAL, LAST NAME): K. C. Day, T. Behrsing, E. Sciullo, B. Bosan, and M. Wade.
INSTITUTIONS (ALL): Department of Toxic Substances Control (DTSC), Cal/EPA, Sacramento, CA.
KEYWORDS: TCE, Risk Assessment, Noncancer Hazard

ABSTRACT BODY: Trichloroethene (TCE) is an industrial solvent mainly used as a degreaser for metal-cleaning operations. TCE has been detected in groundwater, indoor air, soil, and soil gas; with inhalation of vapors and ingestion of contaminated groundwater the primary routes of exposure. DTSC is investigating over 550 sites with reported TCE contamination. Recently, USEPA/IRIS released new toxicity criteria for TCE. Previously, CalEPA's Office of Environmental Health Hazard Assessment (OEHHA) toxicity criteria were used to evaluate cancer risk and noncancer hazard at cleanup sites. The USEPA cancer criteria are 2-to 8-fold more protective than OEHHA. The new IRIS noncancer inhalation reference concentration (RfC) and oral reference dose (RfD) are 300-fold and 1000-fold more protective and are based on multiple critical effects (immune, neurotoxicity, increased fetal cardiac malformations), from multiple studies. The impacts of the new TCE toxicity criteria are now becoming apparent. At sites, the noncancer threshold (hazard index-HI) may exceed 1 using the IRIS criteria, while the cancer risk is at the low end of the risk management (RM) range. We present three case studies demonstrating the impact of the new noncancer RfC and RfD: Site A where the proposed cleanup value for beneficial use of groundwater is 2.6 µg/L vs. 5 µg/L; Site B where 2 of the 4 homes had detected indoor air TCE concentrations greater than the noncancer HI of 2 µg/m3 and now require vapor intrusion mitigation; and Site C with potential vapor intrusion issues from modeled groundwater due to the TCE HI which was less than 1 using OEHHA criteria for residential and industrial but is now 10 and 3.9, respectively. These examples illustrate that noncancer threshold may now play more of a role in RM decisions. Consideration of the new noncancer TCE criteria during the five year review process may also indicate previously proposed cleanup/remediation is not protective of human health. Thus, the TCE criteria updates (particularly for noncancer) have important implications on site risks and cleanup.

ABSTRACT FINAL ID: 2623 Poster Board -319
TITLE: Biliary Hyperplasia Induced by a Selective GSK3β Inhibitor: Involvement of Bile Acid Transporter Interaction and Proinflammatory Cytokines

AUTHORS (FIRST INITIAL, LAST NAME): U. Andersson[1], A. Berg[3], A. Lindström[3], G. Arnerup[3], K. Stockling[2], B. Möller[2], H. Powell[2], C. Summers[2], M. Marcusson-Ståhl[2], C. Zettervall[1], and G. Kenna[2].
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KEYWORDS: Biliary toxicity, hyperplasia, GSK3beta

ABSTRACT BODY: The development of inhibitors of the serine/threonine protein kinase GSK3β has been hindered by preclinical toxicity. In particular, biliary hyperplasia has been a recurring issue in both rat and dog. We have performed investigative *in vivo* studies in rat and *in vitro* studies in both rat and dog biliary epithelial cells with AZD8926, a highly selective GSK3β inhibitor, to determine its mechanism of toxicity. Biliary epithelial cells were shown to be the primary targets of toxicity: prominent biliary epithelial cells with lamellar inclusions were observed after one week of exposure, prior to any other noticeable changes in the tissue. In support of this, an adaptive increase in the expression of bile acid transporters was observed in the periportal hepatocytes, demonstrated by qPCR and IHC. Release of proinflammatory cytokines from biliary epithelial cells was demonstrated both *in vivo* and *in vitro*, and following two weeks of treatment resulted in a mild-periportal inflammation. The release of cytokines from biliary epithelial cells *in vitro* reflected the pharmacological potency of the GSK3β inhibition, while nonactive metabolites failed to induce cytokine release. Proliferation of biliary epithelium was a secondary response to biliary injury and, most likely, a response to paracrine mitogens, but could not be observed *in vitro*. However, the proliferation did not appear to be a direct response to GSK3β inhibition, as neither beta-catenin stabilization nor increase in cyclin D1 expression was observed in biliary epithelial cells as

determined by immunohistochemistry. In summary, our data support a series of key steps that underpin the development of biliary toxicity by GSK3β inhibitors supported by both *in vitro* and *in vivo* studies which suggest a complex interplay of on- and off-target mechanisms.

ABSTRACT FINAL ID: 2624 Poster Board -320
TITLE: Hydroxypropyl-b-Cyclodextrin Mitigates Cytotoxicity of Drugs *In Vitro*

AUTHORS (FIRST INITIAL, LAST NAME): Y. Wu[1], D. Nickisher[2], C. Cheng[2], A. Walker[2], S. Madari[2], U. M. Hanumegowda[1], and S. P. Adams[1].
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KEYWORDS: Cyclodextrin, Inclusion complex, Mitigation

ABSTRACT BODY: Hydroxypropyl-b-cyclodextrin (HPBCD) is a chemically modified cyclodextrin used as an enabling excipient to improve *in vivo* drug delivery. It is widely used in preclinical and clinical stages of drug development. HPBCD has a cup-like structure with a hydrophilic exterior and a hydrophobic interior, giving it the ability to form inclusion complexes with hydrophobic drugs. By way of forming inclusion complexes, HPBCD not only improves solubility but also can potentially shield intrinsic toxicity attributed to physicochemical properties of drugs. Herein, we demonstrate the protective effect of HPBCD from cytotoxicity of selected drugs in Caco-2 cells. HPBCD by itself was cytotoxic to Caco-2 cells at higher concentrations and also interfered with cytotoxicity assay. After optimization of HPBCD concentration and method of cytotoxicity assay in presence of HPBCD, Caco-2 cells were treated with flurbiprofen, indomethacin, rofecoxib or flutamide in the presence or absence of HPBCD. Presence of HPBCD not only improved solubility but also mitigated or completely abolished cytotoxicity of these drugs. HPBCD has been previously reported to mitigate gastrointestinal toxicity of some of these compounds in rats. These results indicate that HPBCD can interfere with oral toxicity evaluation of novel compounds with intrinsic toxicity potential.

ABSTRACT FINAL ID: 2625 Poster Board -321
TITLE: Acute and Repeated Dose Toxicity Studies of Synthetic Derivatives of Triazole Incorporated Pyridazinone As New Class of Hypertensive Agent

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KEYWORDS: Pyridazinone, Repeated dose toxicity, Histopathological findings

ABSTRACT BODY: The pharmacological activity of 4,5-dihydro-6-phenyl-3(2H)-pyridazinones has been extensively studied and it is known for its cardiovascular effects. To support the safety of synthetic compound 6-(4-ethylphenyl)-2-(4-(4-chrorophenyl)-5-thioxo-4, 5-dihydro-1H-1,2,4-triazol-3-yl)-4,5–dihydropyridazin-3(2H)-one, it has been examined in an acute and in a 4-week repeated dose toxicity study in rats. Animals were divided into groups of 5 animals each. The compound (20 mg/kg body weight and 40 mg/kg body weight) was injected intraperitonealy after suspending in 1% carboxymethylcellulose solution in single dose resulted in no adverse events or mortality. Also, the compound administered as a daily dose of 40 mg/kg for 4 weeks by gavage resulted in no adverse events or mortality. No evidence or treatment-related toxicity was detected during both studies. Data analysis of body weight gain, food consumption, clinical observations, blood biochemical, haematology, organ weight ratios and histopathological findings did not show significant differences between control and treated groups. It is concluded that the synthetic compound orally administered to rats was safe and that no treatment-related toxicity was detected in both acute ip route of exposure and repeated dose (4 weeks) oral route of exposure (40 mg/kg of body weight) toxicity studies.

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ABSTRACT FINAL ID: 2626 Poster Board -322
TITLE: Optimization of Platelet-Rich Plasma Preparation for Platelet Aggregometry in Cynomolgus Macaques

AUTHORS (FIRST INITIAL, LAST NAME): A. Aulbach and L. Cregar.
INSTITUTIONS (ALL): Clinical Pathology, MPI Research, Mattawan, MI.
KEYWORDS: Platelet aggregometry, platelets, cynomolgus macaque

ABSTRACT BODY: Platelet function testing via platelet aggregometry is increasingly used in the preclinical safety assessment of novel compounds. There is a paucity of information regarding the preparation of platelet-rich plasma (PRP) for platelet aggregometry in species commonly used in preclinical safety studies, including Cynomolgus macaques (C. macaques). Extrapolation of PRP processing techniques from human literature are commonly applied to nonhuman primates (NHP); however, these methods are time consuming, and often require dilution of PRP to obtain a standardized platelet concentration. The primary goal of this investigation was to optimize the preparation of PRP in NHP in order to minimize time and PRP manipulation, which can lead to *in vitro* platelet activation. In addition, the effects of PRP platelet concentration (PRP-PC) on platelet aggregation were evaluated. Sodium citrate (3.2%) anticoagulated whole blood was collected from nine C. macaques and PRP produced via centrifugation. All use of animals for this investigation were done in accordance with USDA and IACUC requirements. Processing variables were sequentially adjusted and included: centrifugal force (150g to 900g), centrifugation time (1 to 15 minutes) and "resting times" (0 to 5 minutes) in order to optimize platelet counts and minimize sample handling. PRP obtained via the optimized procedure was used to assess platelet aggregation induced by ADP (20 µg/mL) via the Bio/DATA PAP8E. Aggregation endpoints were compared to PRP-PC to determine the acceptability of this method for platelet aggregometry. PRP produced via centrifugation at 400 g for 2 minutes followed by a 5 minute "resting" period consistently produced PRP-PC within an acceptable range for platelet aggregation. No significant effects on platelet aggregation were noted over the range PRP-PC tested. This optimized method efficiently produces PRP-PC in an acceptable range for platelet aggregometry, while minimizing sample manipulation and potential preanalytical *in vitro* platelet activation.

ABSTRACT FINAL ID: 2627 Poster Board -323
TITLE: IND-Enabling Safety Assessment of the First Stapled Peptide Clinical Candidate, ALRN-5281

AUTHORS (FIRST INITIAL, LAST NAME): C. J. Viau[1, 2], M. R. Bleavins[3], K. A. Olson[1], H. Cai[1], S. J. DeMarco[1], W. E. Jones[1, 4], A. M. Manning[1], and H. C. Chen[1].
INSTITUTIONS (ALL): [1]Aileron Therapeutics, Inc., Cambridge, MA; [2]CJV Toxicology, LLC, Shrewsbury, MA; [3]White Crow Innovation, LLC, Ann Arbor, MI; [4]Independent Biopharmaceutical Consultant, Burlington, MA.
KEYWORDS: GHRH, Growth Hormone, Peptide Drug

ABSTRACT BODY: ALRN-5281, a 29 amino acid analog of human growth hormone releasing hormone (GHRH), is being developed as a once-weekly subcutaneous (SC) injectable drug to treat adult growth hormone (GH) deficiencies amenable to GHRH replacement therapy. The chemically synthesized molecule is stabilized with hydrocarbon cross-links ("staples") to slow protease degradation, extend plasma half-life and reduce dosing frequency. A cell-based hERG assay (up to 0.6 µM) was negative for QT prolongation; *in vivo* rat studies (at up to 10 mg/kg SC) revealed no neurobehavioral or respiratory dysfunction; and a dog cardiovascular safety study (up to 10 mg/kg SC) identified no treatment-related dysfunction. Six-week studies in rats and dogs, dosed twice-weekly at 1, 3, or 10 mg/kg SC with a 4-week recovery period, demonstrated GH-mediated effects, including increases in plasma GH and insulin-like growth factor 1, higher body weight and food consumption, and slightly altered serum protein and lipid profiles. Other than resolvable injection site irritation and the induction of anti-product antibodies (APA; a small percentage of which were neutralizing ex vivo but showed no effect on GHRH-related activity *in vivo*), no off-target effects were observed. Acute plasma exposures increased sublinearly with dose, but repeated dosing resulted in significant accumulation due to low SC bioavailability, prolonged absorption and/or drug-sustaining APA. Genetic toxicology studies *in vitro* (Ames bacterial assay and chromosomal aberration assay in human peripheral lymphocytes) and *in vivo* (intravenous mouse bone marrow micronucleus assay at up to 15 mg/kg) were each

negative. Overall, results indicate that (1) ALRN-5281 was safe and well-tolerated, with no toxic or pharmacologic effects contributed by the novel hydrocarbon staples, and (2) the NOAEL and exposure assessments provide a robust safety margin for the planned phase 1 trials.

ABSTRACT FINAL ID: 2628 Poster Board -324
TITLE: Prevalence of Plasmodium Infection in Charles River Asian Nonhuman Primates

AUTHORS (FIRST INITIAL, LAST NAME): J. Simmons and D. Meyer.
INSTITUTIONS (ALL): Charles River, Reno, NV.
KEYWORDS: nonhuman primate, exogenous infectious agents, drug safety assessment

ABSTRACT BODY: Infection with plasmodium is common in nonhuman primates (NHP) where raised prior to shipment for use in biomedical research. Plasmodium infection in an immunologically competent animal usually does not cause disease. However, overt malarial disease can manifest if the animal is immunosuppressed by toxicology study-related stress or administration of an immunomodulatory biopharmaceutical. Some of the clinical/clinicopathologic findings such as weight loss, thrombocytopenia, anemia, and light microscopic changes in major target organs can confound the toxicologic assessment of a drug candidate. The reported prevalence of plasmodium infection in laboratory NHP ranges 0-43%1,2. Knowledge of the prevalence is essential when selecting animals for a toxicology study especially when evaluating an immunomodulatory biopharmaceutical and determining the cost-benefit of testing animals for plasmodium infection during selection for study placement. OBJECTIVE: To determine the contemporary prevalence of plasmodium infection in newly imported Asian origin NHP at Charles River from 2009–2012. PROCEDURES: Whole blood samples from 4,267 NHP were submitted to the Charles River Research Animal Diagnostic Services for real time PCR analysis for plasmodium. Briefly, DNA was isolated from whole blood samples using a QIAamp DNA Blood Mini kit, and DNA was then amplified for 45 cycles using a Plasmodium spp. genus reactive primer set. A control for PCR inhibition was included for every sample; negative and 10 copy and 100 copy positive controls were included for each batch of assays that were run. RESULTS and CONCLUSIONS: The overall prevalence was 2.2% and ranged from 0.2% to 2.8%. An explanation for the cohort with a prevalence of 0.2% is likely related to age and source of the animals; most were 2-3 years of age and came from one supplier. Cohorts with a prevalence of 2.8% generally consisted of sexually mature/older animals which likely had longer exposure to infection prior to shipment.

ABSTRACT FINAL ID: 2629 Poster Board -325
TITLE: Microscopic Observations following Repeated Prophalyatic Diphenhydramine Administration in Response to Monoclonal Antibody-Dependent Hypersensitivity Reactions

AUTHORS (FIRST INITIAL, LAST NAME): W. E. Maier[1], D. Patrick[1], J. Roden[1], S. Kelley[1], and R. Caldwell[2].
INSTITUTIONS (ALL): [1]General Toxicology, MPI Research, Mattawan, MI; [2]Preclinical Safety, AbbVie Inc., North Chicago, IL.
KEYWORDS: Diphenhydramine, Rats, Monoclonal Antibody

ABSTRACT BODY: Some therapeutic monoclonal antibodies (mAb) demonstrate cross-reactive binding to target epitopes in multiple species, requiring toxicologic testing in more than one species. Hypersensitivity reactions to mAbs can confound interpretation of direct (target) or indirect (immunogenicity) test item effects. In a 13-week safety study in rats, a human monoclonal antibody (mAb) was administered once per week at doses of 20, 60, and 200 mg/kg IV and 200 mg/kg SC. Beginning week 3, animals administered the mAb displayed postdose reactions with severe clinical signs of moribundity. Therefore, beginning Week 4, all animals (including controls) were given a hindlimb intramuscular (IM) injection of diphenhydramine (DPH, a histamine antagonist) to attempt to ameliorate histamine-induced hypersensitivity reactions. DPH administration correlated to decreased incidence and severity of clinical signs, thus allowing continuation of the study objectives. DPH administration to all animals continued for the remainder of the study. Due to impaired hindlimb function following repeated DPH administration, the DPH route of administration was changed to a sacral subcutaneous (SC) injection. DPH dependent microscopic changes were comparable among control and mAb-treated animals. The microscopic

changes observed in control animals will be described in this presentation. The DPH-dependent IM microscopic effects included myofiber degeneration/necrosis, fibrosis, and subacute/chronic inflammation in the muscle and axon/myelin degeneration in the nerve. DPH-dependent SC microscopic observations consisted of ulceration, hemorrhage, inflammation, and myofiber degeneration/necrosis. In conclusion, DPH administration successfully alleviated mAb-dependent hypersensitivity reactions in rats, but was associated with injection site macroscopic/microscopic findings via multiple routes of injection. SC administration of DPH was less debilitating as compared to IM administration.

ABSTRACT FINAL ID: 2630 Poster Board -326
TITLE: Drug-Induced Lysosomal Enlargement Indicative of Phospholipidosis (PLD) in Sandwich-Cultured Rat Hepatocytes (SCRH) Alters Transport of the Anionic Probe Substrates Taurocholate (TC) and Rosuvastatin (RSV)

AUTHORS (FIRST INITIAL, LAST NAME): B. C. Ferslew and K. L. Brouwer.
INSTITUTIONS (ALL): Pharmaceutical Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC.
KEYWORDS: Drug-Induced Phospholipidosis, Sandwich-Cultured Hepatocytes, Anionic Transport

ABSTRACT BODY: Drug-induced PLD is a lysosomal storage disorder characterized by lysosomal enlargement and hyperaccumulation of phospholipids in affected tissues (e.g., liver, kidney, muscle, CNS). Associations between drug therapy, induction of PLD and organ toxicity exist; whether PLD represents an adaptive response to drug therapy or a distinct toxicity is unknown. Organ specific and metabolically active *in vitro* models are needed in drug development to prospectively identify PLD-inducing compounds. In the present study, SCRH were examined as a potential model of hepatic PLD. Freshly isolated rat hepatocytes were seeded on 6-well BioCoat™ plates, overlaid with Matrigel™ and incubated for 48h with increasing concentrations of 2 prototypical hepatic PLD-inducing drugs (amiodarone [AMD: 1, 10 or 50µM] or chloroquine [CHQ: 0.1, 1 or 10µM]), the renal PLD-inducing drug gentamicin (GTM; 1mM), or vehicle control. Florescence microscopy of pretreated cells after 30-min incubation with 100nM Lysotracker Red was used to qualitatively assess lysosomal morphology. Lysosomal number and size were increased by 10 and 50µM AMD, and 1 and 10µM CHQ. GTM did not change lysosomal morphology. Cellular toxicity assessed by MTT (mean±SEM, n=4-5) was 9±8% and 43±9% for 10 and 50µM AMD, respectively; 17±14% and 28±16% for 1 and 10µM CHQ, respectively; and 26±13% for 1mM GTM. Hepatic vectorial transport of TC and RSV was assessed using B-CLEAR® technology (*in vitro* biliary clearance; CL_{Bile}=[$Accumulation_{Cells+Bile}$-$Accumulation_{Cells}$]/$AUC_{media\ 0\text{-}T}$). AMD and CHQ decreased CL_{Bile} of TC, and both $Accumulation_{Cells}$ and CL_{Bile} of RSV in a concentration-dependent manner; no changes in TC or RSV were noted with GTM. This is the first report of altered hepatic transport of anionic substrates (impaired TC biliary excretion and RSV uptake) secondary to drug-induced hepatic lysosomal dysfunction. SCRH are a promising model to study hepatic PLD *in vitro*. Supported by NIH GM41935.

ABSTRACT FINAL ID: 2631 Poster Board -327
TITLE: Biomarkers of Exposure to [$^{13}C_2$]-Acetaldehyde

AUTHORS (FIRST INITIAL, LAST NAME): C. D. Pastoor[1], B. C. Moeller[2], V. Sharma[3], L. B. Collins[3], W. M. Bodnar[3], and J. A. Swenberg[2, 3].
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KEYWORDS: Acetaldehyde, DNA adduct, Biomarkers of exposure

ABSTRACT BODY: Acetaldehyde (AA) is a known animal and human carcinogen found in a number of consumer and industrial products. AA is also a metabolite of ethanol through the enzyme, alcohol dehydrogenase (ADH). It is also endogenously produced as a by-product of normal cellular metabolism and respiration within the body. AA reacts with a large number of cellular macromolecules such as proteins and DNA. In DNA, acetaldehyde reacts at the N^2 position of deoxyguanosine (dG), forming the N^2-ethylidene-dG adduct. Being able to quantitatively measure DNA adduct levels at low

and high exposures of [$^{13}C_2$]-AA allows for determination of the internal dose of the [$^{13}C_2$]-AA versus the endogenous biomarker in cells. HepG2 human liver cells were exposed to [$^{13}C_2$]-AA for 6 hours at concentrations of 10, 50,100, 250, 500, 1000 μM, along with a control (0 μM). DNA was isolated, reduced, and digested prior to HPLC fraction collection and analysis using LC-MS/MS. Endogenous adduct formation remained relatively constant across increasing concentrations of [$^{13}C_2$]-AA,. The average endogenous N^2-ethyl-dG adduct level was 1.49 ± 0.26 (Avg. ± SD) adducts/10^7dG across all doses. Exogenous adduct formation showed a clear dose-dependent response with increasing [$^{13}C_2$]-AA exposure concentrations. There was a quantifiable increase in the formation of [$^{13}C_2$]-N^2-ethyl-dG adducts (~19% of endogenous) at the 100 μM exposure. Larger amounts of exogenous adducts were observed at concentrations ≥250 μM with exogenous and endogenous adducts being ~ equal at 250 μM. Additional studies are being conducted to understand the dose-response relationship of exogenous adduct formation following [$^{13}C_2$]-ethanol exposures and the effects of modulating ADH and ALDH2 expression. Understanding AA's effects in various cell lines is important for determining its potential hazard to humans.

ABSTRACT FINAL ID: 2632 Poster Board -328
TITLE: A Validated Method for the Quantitation of Exposure Biomarkers of Tobacco Specific N-Nitrosamines in Human Urine

AUTHORS (FIRST INITIAL, LAST NAME): S. Wang, G. Zhao, J. Yu, B. Wang, and F. Xie.
INSTITUTIONS (ALL): Zhengzhou Tobacco Research Institute, Zhengzhou, Henan, China.
KEYWORDS: exposure biomarker, Tobacco Specific N-Nitrosamines, human urine

ABSTRACT BODY: Tobacco Specific N-Nitrosamines (TSNAs) are listed as Group 1 and 3 carcinogens by the International Agency for Research on Cancer (IARC). Human are exposed to TSNAs through mainstream cigarette smoke and Environmental Tobacco Smoke. TSNAs consist of four chemical compounds: N-nitrosonornicotine (NNN), 4-methyl-N-nitrosamino-1-(3-pyridyl)-1-butanone (NNK), N-nitrosoanatabine (NAT), N-nitrosaoanabasine (NAB). NNK is metabolized to 4-methyl-N-nitrosamino-1-(3-pyridyl)-1-butanol (NNAL) in human body. NNAL, NNN, NAT and NAB are biomarkers of exposure of TSNAs in urine. An analytical method was developed to measure 4 biomarkers of TSNAs in urine using High Performance Liquid Phase Chromatography Tandem Mass Spectrometry (HPLC-MS/MS). Urine samples are deglucuronized with β-glucuronidaze/aryl sulfoctase and molecular imprinted solid phase extraction(MIPSE) with molecular imprinted polymers. The samples were analyzed in electron spray ionization mode with HPLC-MS/MS. The stable isotope labeled compounds of each TSNAs were used as internal standards during the analytical procedure. Recovery experiments were conducted using SupelMIP TSNAs cartridge and elution with a 9:1 mixture of Dichloromethane:methanol. NNAL, NNN, NAT, NAB recoveries from urine ranged from 94% to 107%. The LOD of biomarkers of TSNAs in human urine are from 2.5 to 5.0pg/mL. Intraday and interday precisions of method were ranged from 5.6% to 9.6%, The stability of the sample was also investigated. Results showed that the NNAL, NNN, NAT, NAB were stable in the sample for three days period. The lower matrix effect and higher sensitivity of goal compounds were achieved due to molecular imprinted polymers were used. Urine samples of smokers and nonsmokers were analyzed, there are obviously relativity between exposure level and levels of NNAL and NNN in human urine, concentration of NNAL and NNN in urine of smokers are several times higher than they in urine of nonsmoker.

ABSTRACT FINAL ID: 2633 Poster Board -329
TITLE: Determination of Styrene Oxide by Reversed-Phase, High-Performance Liquid Chromatography following Derivatization with *N*, *N*-Diethyldithiocarbamate

AUTHORS (FIRST INITIAL, LAST NAME): D. Falodun and R. M. Uppu.
INSTITUTIONS (ALL): Environmental Toxicology, Southern University and A&M College, Baton Rouge, LA.
KEYWORDS: Epoxide, RP-HPLC determination, Polystryrene plastics and coatings

ABSTRACT BODY: Styrene oxide (1,2-epoxyethylbenzene; SO) is the primary oxidative metabolite generated by vinyl epoxidation of styrene in biological systems. It is also an industrial intermediate en-route the synthesis of styrene glycol and

its derivatives, many of which are used in cosmetics, surface coatings, and agricultural and biological products. SO has been classified by IARC as a probable human carcinogen falling under Group 2A. The extensive use of SO and the lack of simple methodology for its determination are thus a major concern. Herein, we describe a reversed-phase, high performance liquid chromatography (RP-HPLC) method that allows quantitation of low levels of SO. The method involves derivatization of SO by excess *N*, *N*-diethyldithiocarbamate (DTC) in 0.05 M phosphate buffer, pH 7.0 for 15 min at 70 °C. Following acidification with H_3PO_4 (final pH *ca*. 2.0), which facilitates a near total decomposition of the unreacted DTC to diethyl amine and CS_2, aliquots (20 µL each) of the derivatized sample are analyzed by RP-HPLC using a Supelcosil LC-18-S (15 cm x 4.6 mm) column and a mobile phase consisting of 50% acetonitrile in water that also contained 0.1% trifluoroacetic acid (flow rate: 1 mL/min; detection: 278 nm). Under these conditions, the peaks for 2-hydroxy-2-phenyl-ethyl-DTC (major adduct) and 1-hydroxymethyl-1-phenyl-methyl-DTC (minor adduct) resolve with retention times of 4.28 and 5.26 min, respectively. The calibration curves for the major and minor adducts of SO-DTC are linear in the concentration range of 1 to 50 µM ($r^2 \geq 0.9998$; injection volume: 20 uL). The method is robust, and as low as 20 pmol of SO could be successfully detected. We are currently studying the usefulness of the methodology developed for analysis of free SO in styrofoam dinner ware and other packaging materials which could serve as potential sources of human exposure to SO. [Support from NSF (HRD-1043316) and the US DoED (PO31B040030) is acknowledged. Corresponding author's email: rao_uppu@subr.edu].

ABSTRACT FINAL ID: 2634 Poster Board -330
TITLE: 3D MRI-Based Histology Using Compact, High-Resolution MRI

AUTHORS (FIRST INITIAL, LAST NAME): Y. Schiffenbauer[1], C. Brami[1], R. Maronpot[4], R. Abramovitch[2], and A. Nyska[3].
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KEYWORDS: imaging, mri, MR-based histology

ABSTRACT BODY: Magnetic Resonance Imaging (MRI) is widely used in preclinical research and is a powerful method for *in vivo* assessment of phenotypes in murine models of disease. MR Histology (MRH) (Johnson et al) of fixed tissue specimens is gaining recognition as a technique to provide complimentary information to conventional histology, as numerous digital slices from any plane can be acquired in the intact sample followed by conventional histology. The purpose of this study was to investigate the capabilities of a new compact, high-performance MRI platform (M2, Aspect Imaging) in the field of MRH toxicology. MRH allows for rapid acquisition of 3D data of the entire target organ and allows for a more comprehensive assessment of the toxicological effects, which can then inform follow-on conventional histopathology. Here we present the results of *in vivo* MRI and *ex vivo* MRH of acute kidney injury (AKI) induced in mice by intramuscular injection of glycerol, producing abrupt rhabdomyolysis, associated with rapidly progressive renal dysfunction. Time course of the changes in renal morphology and cortico-medullary differentiation were evaluated *in vivo* on anesthetized mice using a compact MRI scanner. High-resolution MRH of the extracted kidneys was performed using a 10mm RF coil on the same compact MRI platform followed by H&E histology and further immunostained for apoptosis (TUNEL), proliferation (BrdU) and hypoxia (Pimonidazole). Changes in MR contrast were readily observed in affected kidneys *in vivo* as well as *ex vivo* indicating higher water content in the cortex as a result of edema and cell death. Administration of Gd-DTPA revealed delayed circulation, filtration and washout, demonstrating renal dysfunction. We have demonstrated the utility of compact, high-performance MRI and MRH as valuable tools to complement conventional toxicological studies by nondestructively providing 3D digital data sets, detailed morphological, functional, and quantitative data.

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ABSTRACT FINAL ID: 2635 Poster Board -331
TITLE: Formaldehyde Is a Major Source of the N^6-Formyllysine Protein Modification

AUTHORS (FIRST INITIAL, LAST NAME): B. Edrissi[1], K. Taghizadeh[2], B. C. Moeller[3], J. A. Swenberg[3], and P. C. Dedon[1, 2].
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KEYWORDS: N6-Formyllysine, Formaldehyde, Histone Modifications

ABSTRACT BODY: There is increasing recognition that aberrant protein modifications play an important role in the pathophysiology of many human diseases. N^6-Formyllysine, a chemical homolog of the biologically important N^6-acetyllysine, has recently emerged as a widespread modification of histone and chromatin proteins. Using novel ultrasensitive and specific liquid chromatography-coupled tandem mass spectrometry methods (LC-MS/MS) to quantify N^6-formyllysine, we aimed to investigate the sources as well as the fate of this protein modification. In addition to the DNA oxidation pathway we previously reported, we present evidence that endogenous formaldehyde is a source of lysine N^6-formylation and that this adduct is widespread among proteins in all cellular compartments. We observed both *in vitro* and *in vivo* that formaldehyde exposure leads to a dose-dependent increase in N^6-formyllysine protein adducts, with the use of $[^{13}C^2H_2]$-formaldehyde to dissect endogenous from exogenous sources. Importantly, exogenous N^6-formyllysine adducts were detectable in nasal epithelium of rats exposed to $[^{13}C^2H_2]$-formaldehyde by inhalation, but not in lung and liver. Furthermore, our investigation of histone deacetylases revealed that despite chemical similarity of N^6-formyllysine to N^6-acetyllysine, the former is refractory to removal by histone deacetylases. If not removed, N^6-formyllysine could accumulate to significant levels in histone proteins and interfere with their epigenetic regulatory roles. We will examine possible adduct accumulation in several additional tissues from rats exposed to $[^{13}C^2H_2]$-formaldehyde for 28 days and determine loss of N^6-formyllysine over a 7-day postexposure period. These results will provide information on the distribution and potential mechanisms for formaldehyde toxicity, through disruption of histone protein function by modification of conserved sites of lysine acetylation and methylation.

ABSTRACT FINAL ID: 2636 Poster Board -332
TITLE: Environmental Exposure Affects Metabolic Phenotypes in Bronchoalveolar Lavage Fluid from Otherwise Healthy HIV-1-Infected Subjects

AUTHORS (FIRST INITIAL, LAST NAME): Y. H. Park, S. Cribbs, J. Roede, B. Liang, and D. P. Jones.
INSTITUTIONS (ALL): Medicine, Emory University, Atlanta, GA.
KEYWORDS: Environmental Exposure, HIV-1, High-Resolution Metabolomics

ABSTRACT BODY: CD4 counts and viral loads are used as prognostic markers in human immunodeficiency virus type 1 (HIV-1) infected subjects. Keeping CD4 counts high can reduce complications of HIV-1 infection and extend one's life; however, respiratory infections continue to occur in subjects with high CD4 counts and low viral loads in otherwise healthy HIV-1 infected subjects. Therefore, it is necessary to determine the metabolic phenotype of otherwise healthy HIV-1 infected subjects compared to healthy control. High-resolution metabolomics was employed to identify metabolic phenotypes in bronchoalveolar lavage fluid (BALF) of otherwise healthy HIV-1 infected subjects compared to healthy controls. False discovery rate (FDR q=0.05) determined 115 significant metabolites from between healthy HIV-1 subjects and controls. Among these 115 features, environmental chemicals were identified extremely high in otherwise healthy HIV-1 infected subjects, including 4-methylimidazole (105.04 m/z), 1,2,3,7,8-Pentachlorodibenzofuran (360.85 m/z), S-Seven (368.96 m/z), and fipronil (436.94 m/z). GeneGo pathway analysis showed that N-acylethanolamine pathway was significantly different among two populations. Phosphatidylethanolamine, 690.49 m/z, in this pathway increased more than 5000 times in BALF of otherwise healthy HIV-1 infected subjects. In conclusion, HIV-1 patients with elevated CD4 counts may have impaired lung immunity due to environmental exposures and these environmental exposures may increase the risk for further infectious insults.

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ABSTRACT FINAL ID: 2637 Poster Board -333
TITLE: Urinary Flow Rate Data from NHANES 2009-2010

AUTHORS (FIRST INITIAL, LAST NAME): S. M. Hays[1], B. deCastro[2], L. Aylward[1], and B. Blount[2].
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KEYWORDS: NHANES, biomonitoring, urine

ABSTRACT BODY: The 2009 –2010 US National Health and Nutrition Examination Survey (NHANES) quantified the volume of urine samples collected from participants and asked the time of last urine void. This enabled calculation of urinary flow rate [ml/min], which can substitute for other methods of adjusting for hydration in estimating urinary analyte concentration. We computed sample-weighted statistics for the urinary flow rate data across several demographic and study characteristics for nearly 7,800 NHANES participants 6–84 years old. Urinary flow rates were significantly higher for adults 20–84 years old than for children 6–11 years old, but when adjusted for body weight, flow rates [ml/min-kg] were significantly higher in children 6–11 years-old (GM [GSE]: 0.0172 [0.0008]) than adults 20–84 years old (0.0108 [0.0002]). Body weight-adjusted flow rates were significantly lower among non-Hispanic blacks (0. 0087 [0.0002]) compared to non-Hispanic whites (0.0114 [0.0004]); significantly lower in participants with overweight/obese BMIs (0.0095 [0.0002]) and higher with underweight BMIs (0.0171 [0.0007]) compared to healthy BMIs (0.0131 [0.0006]); and significantly higher in urine samples collected during the afternoon (0.0114 [0.0003]) and evening (0.0127 [0.0003]) compared to morning (0.0102 [0.0003]). Urine flow rate data combined with urine analyte concentration allow calculation of excretion rates for analytes, such as creatinine and environmental pollutants. Body weight-adjusted urinary creatinine excretion rates extrapolated to 24 hours [mg Cr/day-kg] were consistently lower than predicted by formulas developed by Mage (Huber, et al., 2011) across age, sex, race/ethnicity, and BMI categories, and correlation between the natural log of NHANES and Mage excretion rates was low at 0.35. The patterns of flow rate in spot urine samples reported in the NHANES dataset can help inform the design of other biomonitoring studies and can be used in conjunction with environmental chemical concentration data to evaluate patterns in environmental chemical exposures.

ABSTRACT FINAL ID: 2638 Poster Board -334
TITLE: ASS and SULT2A1 Are Novel and Sensitive Biomarkers of Acute Liver Injury and Hepatotoxicity

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KEYWORDS: Argininosuccinate synthase, hepatotoxicity, biomarker

ABSTRACT BODY: Liver and kidney injury induced by bacterial endotoxins released following polytrauma and sepsis, or by chemical toxins and drugs, are among the leading causes of the multiple organ failure and death. Currently used ALT and AST biomarkers are not sensitive or specific for detecting the early stages of injury, which is vital for the effective management of these life-threatening conditions. Previously, we identified several hepatic proteins, including argininosuccinate synthase (ASS) and sulfotransferases, as novel biomarker candidates. In this study, we assessed serum levels of ASS and sulfotransferase SULT2A1 in acute hepatic injury induced by chlorinated compounds, bacterial endotoxin, MDMA, and acetaminophen using newly developed sandwich ELISA assays. The use of ASS and SULT2A1 as a duplex biomarker panel for liver injury confers advantages over existing 'surrogate' markers: (i) ASS and SULT2A1 accumulated in blood earlier than ALT/AST, (ii) the diagnostic window was much larger, with the increase ranging from 2 to 1000-fold depending on the magnitude of injury, (iii) ASS/SULT2A1 declined faster than ALT/AST upon resolution of damage, and (iv) ASS/SULT2A1 play roles in pathogenesis of hepatic injury, linking oxidative stress, liver function, and responses to toxic insults. Thus, our data suggest that ASS and SULT2A1 have superior characteristics for assessment of chemical and drug-induced liver injury or endotoxemia, and may be of high value for clinical applications.

2013 Society of Toxicology Annual Meeting
Late-Breaking Abstracts

ABSTRACT FINAL ID: 2639 Poster Board -335
TITLE: Can the Microbiome Influence Host Response to Toxicants?

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KEYWORDS: Microbiome, Hepatoxicity, Acetominophen

ABSTRACT BODY: The diverse bacterial population living in all of us is called the microbiome. Microbiome metabolism of endogenous compounds or xenobiotics may affect the host. Microbiome influence on host response to toxicants has not been fully explored. Multiple factors affect microbiome composition, including genetic background and diet. Utilizing a mouse model of hepatotoxicity, we investigated the influence of microbiome composition on the severity of acetaminophen toxicity. Strains dosed included C57L/J, A/J, SM/J, CBA/J, B6C3F1/J, BALB/cBYJ and C3H/HeJ. Fasted mice received a single oral dose of 300 mg/kg acetaminophen (n=4) or vehicle (0.5%CMC;n=3). Pre-and postdose fecal pellets were collected. Serum chemistry and liver histopatholgical analysis were performed 24 hrs postdose. Serum ALT and AST were similarly increased in all strains, but there were distinct differences in histopathologic injury scores (mild =1, moderate=2, marked=3, and severe=4). The BALBc and B63F1/J strains exhibited severe centrilobular necrosis scores(4.0) while the A/J and C3H/HeJ strains exhibited moderate scores (2 and 2.25). To evaluate the potential correlation between microbial gut community composition to liver histopathology scores we used 16S rRNA gene based pyrosequencing on the pre- and postdose fecal samples and compared microbial constituents of low responder and high responder mouse strains. Initial analyses of microbiome constituency indicated mouse strain, fasting and drug administration affected taxonomic composition. Interestingly, there were strain dependent differences detected in the microbiome, specifically the relative abundances of Lachnospiraceae, Ruminococcaceae, and Lactobacillaceae. These strain differences may affect host response. A greater N and omission of fasting will be used in future studies. We hypothesize that microbiome composition may play a role in the host's toxicologic response to some toxicants and potentially lead to adverse drug reactions. If true, the microbiome provides an easily accessible, predictive biomarker of host response.

ABSTRACT FINAL ID: 2640 Poster Board -336
TITLE: Direct Proteomics Analysis of Protein Oxidation in Blood Plasma of Alzheimer's Disease Patients

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KEYWORDS: Protein oxidation, Alzheimer's disease, Protein carbonylation

ABSTRACT BODY: Oxidative stress is a major source of protein damage, and has been closely related with ageing and ageing-related neurodegenerative diseases like Alzheimer's disease (AD). Researchers have found increased levels of oxidized and carbonylated proteins (that correlate with AD) by using immunological or chemical enrichment methods. In the present study, we aimed at identifying and quantifying protein carbonylation/oxidation in blood plasma samples from AD patients with conventional proteomics settings, and tried to elucidating the oxidative impact on proteins with AD progress. 218 human blood plasma samples (approved by Kuopio University Ethical Board) were pooled into eight age-matched groups based on gender and disease progression. Each of the pooled samples was independently digested three times by trypsin, and analyzed in duplicates by liquid chromatography online with Orbitrap Velos mass spectrometry (MS) using both higher energy collisional dissociation (HCD) and electron transfer dissociation (ETD) MS/MS. Lysine carbonylation/oxidation was selected for analysis as it is one of the most frequent carbonylated residues in-vivo. Besides accurate mass (10 ppm), we also evaluated peptide sequence coverage, presence of adjacent fragments on both N and C terminal of the modified lysine, and chromatography separation of modified and unmodified peptides. Based on mentioned criteria, one novel peptide belonging to serum albumin was implicitly identified and quantified as containing oxidized lysine. Oxidation occupancy of this peptide in AD and prone-AD patients was found significantly higher as compared to healthy

control. Our data has shown for the first time that oxidized proteins can be detected by standard proteomic settings without enrichment, and it also suggests that protein oxidation in blood plasma correlates with AD.

ABSTRACT FINAL ID: 2641 Poster Board -338
TITLE: Compounds Targeting Anaphase Promoting Complex Disrupt Mitosis and Induce Apoptosis in Cancer Cells

AUTHORS (FIRST INITIAL, LAST NAME): D. J. Saforo[1], B. C. Sils[1], C. A. France[1], B. F. Taylor[2], J. O. Trent[1], and J. C. States[1].
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KEYWORDS: mitotic arrest, apoptosis, anaphase promoting complex

ABSTRACT BODY: Mitotic spindle disrupting drugs such as the cancer chemotherapeutic paclitaxel induce mitotic arrest and apoptosis. Drug resistance is a major impediment to successful therapy. The anaphase promoting complex/cyclosome (APC/C) is an E3 ubiquitin ligase and is the master regulator of cell cycle promoting mitotic progression and licensing of the replication initiation complex. Activation of the spindle assembly checkpoint (SAC) by spindle disrupting drugs inhibits the APC/C and induces mitotic arrest. Thus, spindle poisons induce mitotic arrest in SAC-proficient (taxane-sensitive) cells, and checkpoint bypass in SAC-deficient (taxane-resistant) cells. Homology structure models for key interacting APC/C components were derived and used in drug design. ANAPC11 is the zinc RING finger protein and catalytic subunit that binds to the cullin subunit ANAPC2. These models were used for *in silico* screening of two sites on ANAPC2 to identify compounds that will interfere with ANAPC11 binding. Inhibition of APC/C is expected to induce mitotic arrest in SAC-proficient cells, and pseudo-G1 arrest in SAC-deficient cells. Testing of 19 compounds in 8 human cancer cell lines, and SV40-transformed and telomerase immortalized diploid fibroblasts indicates that 2 triazospiro derivatives (#'s 3, 3A1) & 3 substituted amino-2-propanols (#'s 8, 10, and 11) induce mitotic arrest and apoptosis in cancer and transformed cells but spare diploid cells. Compounds 3, 3A1, 10, and 11 were more potent than compound 8 in inducing cytotoxicity measured by short-term AlamarBlue viability assays and by colony forming ability for all cell lines tested. Compounds (8, 10, 11) induced apoptosis in A2780/CP70 and SKOV3 ovarian carcinoma cells, as indicated by increased caspase 3 activity, but not in telomerase immortalized human fibroblasts. These results suggest that compounds targeting the APC/C can induce mitotic arrest and kill cancer cells while sparing normal cells and may be an effective approach to developing new anticancer drugs.

ABSTRACT FINAL ID: 2642 Poster Board -339
TITLE: Perfluorooctane Sulfonate (PFOS)-Impaired Adipogenesis in Human Preadipocytes

AUTHORS (FIRST INITIAL, LAST NAME): J. Xu and A. Slitt.
INSTITUTIONS (ALL): Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI.
KEYWORDS: Perfluorooctanesulfonic acid, adipogenesis, perfluorooctane sulfonate

ABSTRACT BODY: Perfluorooctanesulfonic acid (PFOA) and perfluorooctane sulfonate (PFOS) are man-made fluorosurfactants and global pollutant. Since these fluoropolymers are resistant to degradation, they are widely used in the commercial and industrial applications, including coating for package and cooking pots, weather and stain-resistant clothing, and manufactory treatment for fabrics and carpets. Recent reports demonstrated that PFOA and PFOS exist in drinking water, dust, food package, breast milk, umbilical cord blood, as well as in the environment and wildlife. A previous study performed on mice reported that prenatal exposure of PFOA decreased neonatal body weight and survival in a dose-dependent manner. In addition, a significant reduction in mammary differentiation was detected in dams exposed to PFOA during gestation. Other compounds of concern regarding environmental exposure (e.g., bisphenol A, tributyltin, nonylphenol, and PFOA) have been identified as having obesogenic and/or prosteatotic properties. However, the effect of PFOA or PFOS on adipocyte differentiation or adipogenesis is not well described. In the current study, human preadipocytes was treated with PFOA or PFOS without or with antidiabetic drug of metformin or pioglitazone, and Nile red staining was performed, tended to identify the effects of PFOA or PFOS on the process of adipogenesis, and whether this effect will change or enhance the antidiabetic function of metformin or pioglitazone. The current results demonstrated that PFOS significantly decreased Nile red staining of preadipocytes without or with metformin or pioglitazone (by 23%, 22% and 23%,

respectively), suggesting PFOS treatment impaired adipogenesis. PFOA did not significantly affect adipogenesis in this pilot study. Metformin decreased the differentiation of adipocytes and impaired adipogenesis, whereas pioglitazone increased differentiation of adipocytes. Overall, PFOS impaired the process of adipogenesis, no significant effect of PFOA on adipogenesis was observed from the current study.

ABSTRACT FINAL ID: 2643 Poster Board -340
TITLE: Novel Molecular Targeted Therapeutics Prevents Pancreatic β Cell Death and Disease Progression in Preclinical Models of Type 1 Diabetes

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KEYWORDS: Diabetes, Drug discovery, kinases

ABSTRACT BODY: Type I diabetes (T1D) is an autoimmune disease which involves the death of insulin producing β-cells in the pancreas. In T1D, inflammatory/oxidative stresses cause dysfunctional signaling leading to β-cell damage and insulin deficiency. Presently, there are no drugs to stop slowdown or reverse T1D. Protein kinases (PK) are key components of these signaling mechanisms but these valuable mechanistic insights have yet to be translated into effective therapies to prevent T1D as traditional kinase drug development process is hampered by lack of specificity, high toxicity, cost and time. Our novel kinase drug platform technology bypasses these inherent difficulties to rapidly design a very small population of highly specific, less toxic, efficient drug candidates for kinase activation/inhibition. Drug candidates were designed against key regulatory kinases involved in β-cell dysfunction. The functional screening was performed in rodent pancreatic β cell lines RIN-M5F/INS1E using Streptozocin(STZ) toxin, as this model is widely used to study pancreatic β cell death. Drug candidates T149, T148, T138, T137, T119, and T117 modulating the activity of 3 key survival kinases protected against STZ-induced β cell death. The candidates were further studied in widely used STZ toxin based mice models of T1D. STZ was given once i.p to induce progressive pancreatic β cell death/ diabetes in C57bl/6 and sacrificed after 7 days for further analysis. Mice were co-treated with 5 or 10 mg/kg of drugs (i.e., injections) everyday starting at day 1 until day 5. Pancreatic β-cells survived when co-treated with drug candidates compared to diabetic mice, which were found to have lost most of the insulin producing cells. Further, drug candidates restored serum insulin levels as measured using ELISA. The STZ diabetic mice develop hyperglycemia starting at day 3 (>250 mg/dl) and the lead candidates was found to have prevented hyperglycemia. Our data confirms a novel approach to help pancreatic beta cell survival and prevent the progression of new onset T1D.

ABSTRACT FINAL ID: 2644 Poster Board -341
TITLE: Using a Protein-Protein Interaction Network to Investigate the Association between Autism Spectrum Disorders and Biological Systems Enriched by Psychoactive Pharmaceuticals in *Pimephales promelas* Fish Brains

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KEYWORDS: Idiopathic Autism, Psychoactive Pharmaceuticals, Protein Interaction Network

ABSTRACT BODY: Autism Spectrum Disorders (ASD) are complex neurological disorders and the prevalence in US is currently estimated to be 1 in 88 children. A majority of cases with idiopathic autism likely result from an unknown environmental trigger in genetically susceptible individuals. Environmental maternal exposure of a fetus to minute concentrations of pharmaceuticals and personal care products (PPCPs) and other compounds, via air, food, and water, is an interesting possibility that has not been sufficiently examined. Un-metabolized psychoactive pharmaceuticals reach drinking water through a variety of routes, including ineffectively processed sewage. Previous studies in our laboratory examined the extent to which gene sets associated with neuronal development were up- and down-regulated (enriched) in brains of fathead minnows treated with PPCPs at environmental concentrations. Here, we tested the hypothesis that these same gene sets were associated with ASD by analyzing the extent to which their protein products interacted with other proteins

in a protein-protein interaction network, composed of known ASD-associated gene products and their interaction partners or degrees. A network of 7212 proteins and 33,416 edges (connections) was generated and visualized by using the bioinformatics software package Cytoscape. We then analyzed previously enriched gene sets in the network based on average degrees. Using nonparametic Wilcox exact method, we found 8 significant gene sets (p-values <0.05) among 15 gene sets in total. Within the significant gene sets, we listed 20 key proteins that had higher degrees than other proteins. Most of these key proteins are associated with the growth, development and regulation of synaptic proteins. This study signifies the interconnection of key proteins with other proteins, and any perturbation in their expression may potentially disturb the network, subsequently contributing to or, potentially, causing neurological disorders like ASD.

ABSTRACT FINAL ID: 2645 Poster Board -342
TITLE: Neuregulin (NRG1) Induces a Dose-Response Increase in Epidermal Growth Factor Receptor 2 (ErbB2) in hMSCs: Potential Cell-Based Model of ErbB2 Variability on Mechanisms of Toxicity

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KEYWORDS: ErbB2, hMSCs, variability

ABSTRACT BODY: Doxorubicin and Trastuzumab (chemotherapeutic agents for breast cancer treatment) can cause cardiotoxicity alone and synergistically when combined. Predicting which patients will be harmed is not currently possible. Clinical, animal and cell culture studies have demonstrated the role of the ErbB2-NRG1 system in cellular protection and toxicity. However, model systems are needed to address the effects of different ErbB2 levels on mechanisms of Doxorubicin and Trastuzumab toxicity. The aim of this study was to determine whether NRG1 (ErbB3/4 ligand) could increase protein levels of ErbB2 and to examine the downstream pathway effects of increased ErbB2 levels in bone marrow-derived human mesenchymal stem cells (hMSCs). Cells were cultured in with 1, 10 or 100 ng/mL NRG1 for seven days. Cells were collected, lysates made and western blotting was performed. Cell viability was assessed by MTT and LHD assays in cells treated with 10 or 100 ng/mL NRG1 for five weeks in 96-well plates. No changes in cell viability occurred in either treatment group. ErbB2, ErbB4 and CyclinD1 protein levels were increased in a dose-dependent manner, and p27 protein levels were decreased after NRG1 treatment. These results indicate that NRG1-induced ErbB2 overexpression could affect cell cycle and proliferation potential of hMSCs. Furthermore, the dose-response increase in ErbB2 indicates that our model can be used to study the effects of ErbB2 variability on toxicity mechanisms. We are currently investigating the effects of NRG1-induced overexpression of ErbB2 on individual and combined toxicities of Doxorubicin and Trastuzumab. In the future, hMSCs from patients could be used to study interindividual variability and individual susceptibility to Doxorubicin and Trastuzumab toxicities.

ABSTRACT FINAL ID: 2646 Poster Board -343
TITLE: Carbaryl Cytotoxicity to Cultured Primary Human Melanocytes

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KEYWORDS: carbaryl, melanocytes, melanomagenesis

ABSTRACT BODY: Carbaryl (1-naphthyl methylcarbamate), a broad spectrum insecticide widely used in agriculture and domestic environment, has recently been associated with the development of cutaneous melanoma in an epidemiological cohort study with US farm workers also exposed to ultraviolet radiation, which is known to be the main etiologic factor for skin carcinogenesis. Although comprehensive and well designed, the epidemiological study does not allow to characterize the direct contribution of the insecticide and radiation in melanomagenesis. Several studies have explored the synergistic effect of certain chemicals to UV radiation, increasing its deleterious effects on the skin, and possibly contributing to tumor

development. In order to understand the possible mode of action of the association of UV and carbaryl exposure on melanomagenesis, *in vitro* techniques are widely employed. The first step of this project was to evaluate the carbaryl cytotoxicity to primary human melanocytes in culture. Carbaryl is highly lipossoluble and in order to perform the *in vitro* experiments an inclusion complex between carbaryl and hydroxypropyl beta cylcodextrin (HPβCD) was prepared to increase the insecticide water solubility. The mixture was then submitted to liophilization and further solubilized in melanocyte culture medium in different concentrations in order to investigate carbaryl cytotoxicity to melanocytes. Trypan Blue assay and Propidium Iodide labeling showed that carbaryl significantly reduced cell viability starting from 400µM, after 24 hours of treatment, in a dose- and time-dependent manner. Treatment with carbaryl 200µM resulted in severe change in cell morphology, with loss of the neural phenotype. The vehicle control was successful, once HPβCD did not induce cytotoxicity or cell morphology alteration to melanocytes in any of the concentrations tested. Noncytototoxic concentration of carbaryl to melanocytes (50µM) was selected to evaluate the concomitant effect of UV radiation and carbaryl to melanocytes.

ABSTRACT FINAL ID: 2647 Poster Board -344
TITLE: HPLC-UV-Vis and MALDI-TOF Analysis of Rat Serum, Urine, and Feces to Determine Lindane and Metabolites

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KEYWORDS: Pesticides, Analysis, Metabolism and disposition

ABSTRACT BODY: Metabolism of Lindane produces several metabolites but detection of lindane and metabolites are still imperfectly identified in the body. Therefore, an easy, fast and sensitive detection method is highly desirable. We have utilized HPLC-UV-Vis and MALDI-TOF to determine lindane and metabolites in urine, serum and feces. Samples were collected following treatment of rats with lindane (17.6 mg/Kg; 1/5 of LD50, orally for 4wks). Lindane and metabolites were extracted with hexane. The extracts were subjected to HPLC-UV-Vis analysis and confirmed with MALDI-TOF, HPLC spectrum of standard lindane were compared with that from the samples. HPLC spectra confirmed the presence of lindane and metabolites at different concentrations in urine (386-1652ppm), serum (207-371ppm) and feces (5.76-73.77ppm). Control samples did not show any peak corresponding to lindane. MALDI-TOF analysis of standard lindane showed a peak corresponding to 293 m/z whereas the urine and serum samples showed a major peak and feces samples showed a minor peak at 292-293 m/z corresponding to lindane and its metabolites. The concentration of lindane and metabolites in the feces were found to be very low compared to that detected from urine and serum samples. These results show that HPLC is sensitive for detecting lindane and metabolites and MALDI-TOF can identify specific metabolites of Lindane in samples by matching spectra peaks generated to the molecular weights of the metabolites. It further shows that minor quantity of lindane and metabolites are excreted through feces confirming that the major route for excretion of lindane and metabolites is through urine. Thus, HPLC-UV-Vis-MALDI-TOF analysis can be a reliable, noninvasive method of detecting lindane burden in serum, excretion in urine and feces. The HPLC-MALDI-TOF is highly sensitive to the point of detecting very low level (5 ppm) of lindane and metabolites in biological samples.

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ABSTRACT FINAL ID: 2648 Poster Board -345
TITLE: Pesticides Exposure Impairs Vascular Reactivity in Rat Aorta

AUTHORS (FIRST INITIAL, LAST NAME): N. Brinkley[1], C. Smith-Baker[2], J. B. Sapp[3], B. F. Bessac[4], A. O. Oyekan[5], and M. A. Yakubu[1, 5].
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KEYWORDS: Pesticides, lindane, vascular reactivity

ABSTRACT BODY: Exposure to pesticides continues to be of great public health concern as studies of moderate pesticide exposure found increased prevalence of neurotoxicity. But effects of exposure to acute low dose pesticides on vascular reactivity is lacking. We have investigated the effects of 2 or 4 weeks exposure to single pesticide (Lindane, 1/5LD50; SP) or multiple pesticides (aldrin, endosulfan, lindane, 4,4-DDT, and endrin: 1/100, 1/50, and 1/25 of the LD50, MP) on vascular reactivity. Changes in vascular reactivity was determined by ACh (10-8 -10-3 M) relaxation of phenylephrine (PE, 10-6 M) precontracted aorta and monitored by DI-720 system -DATAQ. In control, ACh dose-dependently relaxed PE-induced contraction which was attenuated by 2 wks lindane (reducing relaxation from 57 ± 5% to 36 ± 0.8%; while MP attenuated relaxation to 19.8 ± 5.8% and 19.0 ± 4.0% for 1/100 and 1/25 LD50; ACh 10-3 M, $p<0.05$, n=6). Paradoxically, 4 wks SP significantly enhanced relaxation to ACh from 60.5 ± 4.6% to 83.5 ± 8.8% (ACh 10-3 M, $p<0.05$, n=6) and MP had no significant effects. In aorta from dermal group (SP), relaxation to ACh was significantly attenuated from 59.4 ± 5.0 to 23.7 ± 6.0% (ACh 10-3 M, $p<0.05$, n=6). Vasoconstrictions to PE (10-8 -10-3 M) were significantly attenuated by 4 wks' oral SP and dermal MP without significant changes observed in 2 wks oral MP. These results demonstrate the adverse vascular effects of pesticides' exposure which is characterized by differential lost of vasorelaxation to ACh and vasoconstriction to PE. This is an indication that acute low dose exposure to pesticides as in real life scenario can contribute to development of cardiovascular dysfunctions, central vascular dementia and neurological diseases. Thus, to fully understand the vascular consequences of pesticides exposure and contribution to vascular dysfunctions the mechanism(s) behind this vasomotor impairment needs to be further investigated.

ABSTRACT FINAL ID: 2649 Poster Board -346
TITLE: Methomyl Poisoning: The Intentional Baiting of Wildlife with Fly Bait

AUTHORS (FIRST INITIAL, LAST NAME): J. P. Buchweitz[1], M. Bokhart[2], M. Johnson[1], A. Couch[1], and A. Lehner[1].
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KEYWORDS: Methomyl, Carbamate, Poisoning

ABSTRACT BODY: The poisoning of wildlife with fly bait containing the active ingredient methomyl is an intentional and illegal act in many jurisdictions. We describe a case of two animals poisoned by methomyl through consumption of tainted bait at multiple traps. Although thermally- and UV-labile, methomyl can be analyzed by GC/MS (gas chromatography/mass spectrometry) and is detected in abundance in bait samples; however, it is not readily observed in tissues, owing to its rapid metabolism and elimination. Accordingly, analysis of stomach contents discolored by the blue-coloring dye of the fly-bait formulation was reasoned to represent the best sample to yield direct evidence of the parent compound or its primary metabolite in the described case, rather than liver or kidney. Perplexingly, both methomyl and its oxime metabolite were singularly absent. A key find supporting its likely presence was GC/MS evidence for the coactive ingredient found in all fly bait formulations, Z-(9)-tricosene, in both bait and stomach contents. Derivatization of the stomach content extract with the trimethylsilyl (TMS) group stabilized residual methomyl-oxime and provided clear detection with retention time= 6.91 min by ion chromatography of the molecular ion (M+) at m/z=177. The intentional misuse of the pesticide product was further implied by an additional finding of caffeine in the bait and stomach contents. The source of the caffeine was most likely a carbonated beverage used to solubilize and sweeten the fly bait. While the use of derivatives for stabilizing

methomyl oxime in blood and other bodily tissues has been described in human poisoning incidents, their additional use in the analysis of ingesta warrants further consideration by veterinary diagnostic laboratories. Moreover, detection of coactive ingredients, such as Z-(9)-tricosene, aid in confirming the type of pesticide product involved in the poisoning incident.

ABSTRACT FINAL ID: 2650 Poster Board -347
TITLE: Preliminary Evaluation of the Mode of Action and Human Relevance of Liver Tumors in Rodents following Chronic Exposure to Imazalil

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KEYWORDS: Imazalil

ABSTRACT BODY: Chronic dietary administration of Imazalil has been shown to cause treatment-related liver tumors in CD1 mice and, at a dose exceeding the maximum tolerated dose, in Wistar rats. Dose-related hepatocellular findings seen above clear thresholds in subchronic mode of action (MOA) and chronic studies consisted of microsomal enzyme induction (CYP2B and CYP3A), increased liver weights, hypertrophy (reversible upon cessation of treatment) and/or vacuolation in both species and liver foci induction in the rat. Tumors were only observed at doses inducing hepatocellular effects. Imazalil is not genotoxic and further studies to evaluate the MOA of liver tumorigenicity were conducted. In studies with rats and mice, CAR activation was demonstrated by CYP induction and gene expression analysis. Humanized CAR/PXR mice had similar findings to wild type mice but with lower sensitivity. The potential of phenobarbital (PB) to induce hepatocellular effects in rats and mice was higher than imazalil's. Increased cell proliferation was observed following *in vitro* imazalil exposure of mouse hepatocytes with no responses seen in exposed human hepatocytes. Collectively, these data along with the negative genotoxicity demonstrate that imazalil, like other xenobiotics, induces mouse liver tumors through receptor-mediated sustained liver activation and ensuing cell proliferation. This MOA is not predicted to be relevant in humans at realistic exposure levels.

ABSTRACT FINAL ID: 2651 Poster Board -401
TITLE: Effects of Periconception Heavy Metal Administration to Mice on Indices of Chronic Disease in Offspring at Maturity

AUTHORS (FIRST INITIAL, LAST NAME): C. Camsari, A. D. Stanton, J. K. Folger, and G. W. Smith.
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KEYWORDS: Heavy metals, chronic diseases, mice

ABSTRACT BODY: A growing body of evidence indicates that the *in utero* environment can influence susceptibility of offspring to adult chronic diseases (e.g., diabetes, cardiovascular disease, and anxiety). Exposure to heavy metals such as cadmium and mercury has pronounced effects on human health. However, developmental programming effects caused by maternal administration of low concentrations of cadmium and mercury early in development have not been determined. We have previously shown that periconception administration of cadmium and mercury to CD1 mice increased anxiety-like behavior in offspring at adulthood. In the present studies, we hypothesized that maternal administration of a combination of cadmium and mercury during the periconception period would impact glucose homeostasis and reproductive traits of offspring at maturity. Trace amounts of cadmium chloride ($CdCl_2$) and methylmercury(II) chloride (CH_3HgCl) (0, 0.125, 0.5 or 2 mg/kg body weight) were subcutaneously administered to female CD1 mice for 4 days before conception and 4 days after conception. Body weights, abdominal adipose accumulation, glucose tolerance and indices of male fertility were measured in offspring at adulthood. For all doses of cadmium and mercury tested, body and adipose weights of male offspring were increased and glucose tolerance in male and female offspring at maturity was reduced relative to controls. Reduced testis weights, sperm motility and percentage of sperm with normal morphology were also observed for male offspring of dams administered the highest dose of cadmium and mercury during the periconception period. Results demonstrate combined

maternal cadmium and mercury administration immediately prior to and after conception significantly impacts glucose homeostasis and fertility traits of offspring at adulthood and support potential developmental programming effects of heavy metal exposure during the preimplantation period.

ABSTRACT FINAL ID: 2652 Poster Board -402
TITLE: The Role of Environmental Pollutants on Zebrafish Yolk Utilization—A Toxicity Assay

AUTHORS (FIRST INITIAL, LAST NAME): S. Kalasekar[1], M. Bondesson[1], E. Zacharia[2], N. Kessler[2], I. A. Kakadiaris[2], and J. Gustafsson[1].
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KEYWORDS: Zebrafish embryo toxicity

ABSTRACT BODY: Abnormal nutrient uptake can serve as a good indicator of abnormal cellular and organismal function. In larval zebrafish, the maternally derived yolk remains the sole source of nutrition until the larvae develop mouths to ingest exogenous food. It has been shown that larvae exposed to chemical pollutants (like pharmaceutical lipid regulator Clofibrate) in the water display diminished yolk uptake rates. The purpose of this study was to establish a screening model to identify other chemicals that may cause abnormal nutrient uptake. Transgenic zebrafish with GFP expression in the yolk (HGn50D) were used. The larvae were treated with chemicals and scored for retained yolk size. A user-friendly software package for the automatic analysis of images depicting the larvae was developed for high throughput quantification of fluorescence. Yolk sizes after treatment with clofibrate were used as reference points to establish treatment and quantification protocols. We then tested chemicals, including the pesticides prochloraz, butralin and imazalil, Liver X Receptor agonists TO 901317 and GW 3965, monoethylhexylphthalate, tetrabromo- and tetrachlorobisphenol A and tributyltin, and identified those that caused abnormal yolk uptake. Using this assay, we will be able to screen a large number of chemicals, and mixtures thereof, for their ability to affect yolk utilization. Abnormal yolk uptake can be either a cause or a consequence of a particular chemical's toxicity. Using this assay, we can determine concentrations of chemicals that, while not lethal, can still be toxic to the larvae. It is also possible that chemicals identified as causing abnormal yolk utilization can directly affect yolk assimilation or lipid transport in the zebrafish larvae.

ABSTRACT FINAL ID: 2653 Poster Board -403
TITLE: Comparison of Effects on Embryo-Fetal Development in Sprague-Dawley Rats of Two Salt Forms for Esomeprazole, Strontium Tetrahydrate and Magnesium Trihydrate

AUTHORS (FIRST INITIAL, LAST NAME): T. L. Edwards[1], D. G. Stump[1], Y. H. Kim[2], and K. H. Suh[2].
INSTITUTIONS (ALL): [1]WIL Research, Ashland, OH; [2]Hanmi Pharmaceutical Co. Ltd., Seoul, Republic of Korea.
KEYWORDS: Developmental Toxicity, Strontium, Sprague-Dawley Rat

ABSTRACT BODY: Esomeprazole strontium tetrahydrate (EST) contains esomeprazole, the S-enantiomer of omeprazole, a proton pump inhibitor. EST is being developed as a salt-exchanged version of esomeprazole magnesium trihydrate (EMT). In order to compare the effects of EST on embryo-fetal development with emphasis on bone development with those of EMT, comparator EMT groups were included at equimolar dose levels of the esomeprazole. The study was conducted at esomeprazole base dose levels of 0 (vehicle control), 14, 69, and 280 mg/kg/day administered by oral gavage from gestation day (GD) 6 through GD 17. Maternal body weights and food consumption were assessed. Laparohysterectomies were performed on GD 20 and the fetuses were weighed, sexed and examined for external, visceral and skeletal malformations and developmental variations. In addition, a satellite toxicokinetic phase was administered the same dose levels from GD 6 through 20 and strontium levels were determined in maternal and fetal plasma and fetal liver. Strontium and calcium levels were determined in maternal and fetal femurs and fetal femur length was measured on GD 20. All rats were systemically exposed to esomeprazole and dose proportionality, accumulation and Tmax were similar between equivalent EST and EMT dose levels. Maternal toxicity was evidenced by reductions in mean body weight, body weight gain,

and food consumption during the treatment period in the 280 mg/kg/day equimolar esomeprazole dose levels of the EST and EMT groups; however, these maternal toxicities extended into the 69 mg/kg/day equimolar esomeprazole dose level for EMT administration. Developmental toxicity was not observed at any dosage level of EST or EMT. There were no adverse effects directly related to the administration of the strontium salt; the maternal toxicity noted in the EST and EMT groups was attributed to esomeprazole and not the metal salts.

ABSTRACT FINAL ID: 2654 Poster Board -404
TITLE: Comparison of Effects on Pre- and Postnatal Development in Sprague-Dawley Rats of Two Salt Forms for Esomeprazole, Strontium Tetrahydrate and Magnesium Trihydrate

AUTHORS (FIRST INITIAL, LAST NAME): D. G. Stump[1], T. L. Edwards[1], D. L. Brown[2], Y. H. Kim[3], and K. H. Suh[3].
INSTITUTIONS (ALL): [1]WIL Research, Ashland, OH; [2]WIL Research, Hillsborough, NC; [3]Hanmi Pharmaceutical Co. Ltd., Seoul, Republic of Korea.
KEYWORDS: Prenatal and Postnatal Development, Strontium

ABSTRACT BODY: Esomeprazole strontium tetrahydrate (EST) contains esomeprazole, the S-enantiomer of omeprazole, a proton pump inhibitor. EST is being developed as a salt-exchanged version of esomeprazole magnesium trihydrate (EMT). In order to compare the effects of EST on pre and postnatal development with emphasis on bone development with those of EMT, comparator EMT groups were included at equimolar dose levels of the esomeprazole. Two studies were conducted; the first utilized standard rodent diet and the second utilized a reduced calcium and vitamin D diet to determine if the reduced mineral and vitamin levels potentiated EST toxicity. The studies were conducted at esomeprazole dose levels of 0 (vehicle control), 14, 69, 138 and 280 mg/kg/day by oral gavage from gestation day 6 through lactation day 21. The studies were conducted in general accordance with the ICH Guideline S5 (R2), Section 4.1.2. Esomeprazole and strontium levels in maternal and pup plasma, strontium levels in maternal milk and pup liver, and strontium and calcium levels in maternal and pup femurs were determined. Pup femurs were measured for length and examined histopathologically. Bone morphometry was conducted on maternal and pup tibias to assess epiphyseal plate thickness. Esomeprazole levels in plasma, milk, tissue and bone increased with increasing equimolar doses in the EST and EMT groups. Strontium levels increased with increasing dose in the EST groups only and calcium levels remained similar across the EST and EMT dose groups, regardless of diet. Maternal toxicity, F_1 neonatal toxicity and F_1 developmental toxicity, including neurobehavioral development, were noted at similar equimolar dose levels regardless of EST or EMT administration or the type of diet fed. There were no adverse effects directly related to the administration of the strontium salt; the toxicity noted from EST and EMT was attributed to esomeprazole and not the metal salts.

ABSTRACT FINAL ID: 2655 Poster Board -405
TITLE: Comparison of Effects on Juvenile Toxicity in Sprague-Dawley Rats of Two Salt Forms for Esomeprazole, Strontium Tetrahydrate and Magnesium Trihydrate

AUTHORS (FIRST INITIAL, LAST NAME): D. L. Brown[1], T. L. Edwards[2], D. G. Stump[2], Y. H. Kim[3], and K. H. Suh[3].
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KEYWORDS: Juvenile Toxicology, Strontium

ABSTRACT BODY: Esomeprazole strontium tetrahydrate (EST) contains esomeprazole, the S-enantiomer of omeprazole, a proton pump inhibitor. EST is being developed as a salt-exchanged version of esomeprazole magnesium trihydrate (EMT). In order to compare the effects of EST on juvenile toxicity with an emphasis on bone development with those of EMT, comparator EMT groups were included at equimolar dose levels of esomeprazole. The study was conducted at esomeprazole base dose levels of 0 (vehicle control), 70, 140 and 280 mg/kg/day administered by oral gavage to juvenile rats from postnatal day (PND) 7 through PND 35, followed by a 14-day recovery period. Body weights, food consumption, balanopreputial separation and vaginal opening were assessed. Ophthalmic examinations, clinical pathology, and anatomic

pathology were performed at both the primary and recovery necropsies. Blood samples for strontium analysis and bone samples for strontium and calcium analyses were collected from all animals. A toxicokinetic phase was conducted with the same dosing regimen to assess plasma esomeprazole levels. Exposure to esomeprazole generally increased as EST or EMT dosage increased on PND 7 and PND 35. Strontium levels in plasma and bone increased with increasing dose in the EST groups only and calcium levels in bone remained similar across the EST and EMT dose groups. During the dosing phase, reduced survival, alterations in erythrocyte parameters, reduced mean body weights and food consumption, intestinal metaplasia of the glandular stomach, and perivascular infiltrates in the lungs were noted in the 70, 140 and 280 mg/kg/day equimolar esomeprazole dose levels of the EST and/or EMT groups. Partial reversibility of toxicity was noted at the recovery phase. There were no adverse effects directly related to the administration of the strontium salt; the juvenile toxicity noted in the EST and EMT groups was attributed to esomeprazole and not the metal salts.

ABSTRACT FINAL ID: 2656 Poster Board -406
TITLE: Identifying Vascular Disrupting Chemicals Using Zebrafish

AUTHORS (FIRST INITIAL, LAST NAME): C. W. McCollum[1], C. Hans[2], S. Shah[2], F. Merchant[3], M. Bondesson-Bolin[1], and J. Gustafsson[1].
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KEYWORDS: Vascular toxicity, Zebrafish

ABSTRACT BODY: Human health has been shown to be affected by the increasing presence of environmental pollutants and teratogens in the forms of pesticides and industrial products, some of which are bioaccumulative. Using zebrafish as a model system, US EPA has spearheaded the investigation of the toxicity of a large number of various pesticides and industrial chemicals (Padilla et al., 2012). US EPA has also ranked chemicals by their biological activity signature for being potential vascular disrupting compounds (pVDCs) (Kleinstreuer et al., 2011). Here, we present results of an extensive ongoing screen for VDCs using a transgenic zebrafish strain that expresses GFP in endothelial cells, Tg(kdrl:GFP). The screened chemicals were chosen from the US EPA ToxCast phase I library, and in particular selected for predicted vascular disrupting capacity. Embryos were exposed to the chemicals over 3 days and assessed for vascular abnormalities in the intersegmental vessels (ISVs) and caudal vein plexus. Under normal conditions, ISVs sprout from the dorsal aorta at approximately 22 hpf and extend dorsally, then link to form the dorsal longitudinal anastomotic vessel. Perturbations in angiogenesis, represented by ISV sprouting, were visualized in real-time by confocal timelapse microscopy. The degree of vascular defects was quantified by using quantitative image analysis. Out of 65 screened compounds, 11 were shown to disrupt normal angiogenesis in zebrafish embryos.

ABSTRACT FINAL ID: 2657 Poster Board -407
TITLE: Fetal TCDD Exposure Downregulates Sox9 Expression in the Developing Mouse Prostate

AUTHORS (FIRST INITIAL, LAST NAME): A. J. Schneider, R. W. Moore, and R. E. Peterson.
INSTITUTIONS (ALL): School of Pharmacy, University of Wisconsin-Madison, Madison, WI.
KEYWORDS: Sox9, Prostate, TCDD

ABSTRACT BODY: 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is causatively linked to developmental defects in a variety of vertebrate species, including humans. We have previously shown that fetal exposure to TCDD (5 µg/kg on E15.5) severely impairs prostate development in C57BL/6J mice. Initial stages of normal prostate development involve urogenital sinus epithelium (UGE) budding into the surrounding urogenital sinus mesenchyme (UGM). After exposure to TCDD, budding is patterned differently in some regions of the urogenital sinus and completely absent from other regions. Here we show by both *in situ* hybridization and immunohistochemistry that TCDD downregulates SRY-box containing gene 9 (Sox9) expression in the UGE. Sox9 is a transcription factor vital for proper development, as haploinsufficiency is generally perinatally lethal in both humans and mice. Importantly, Sox9 has recently been shown to be essential for prostate

development in the mouse. We have confirmed these findings by knocking out Sox9 in the UGE. However, unlike previous Sox9 knockout mouse models, we are able to present *in vivo* data showing that no prostatic buds develop in any region of the urogenital sinus when Sox9 is knocked out in the UGE. Together these results suggest that it is the TCDD-induced downregulation of Sox9 that causes abnormal prostate development in exposed mouse fetuses. It is known that sox9b (the zebrafish Sox9 homolog) expression is downregulated after TCDD exposure and that this downregulation is the direct cause of jaw malformations. To our knowledge, this is the first evidence that TCDD can negatively affect Sox9 expression in mammals. (Supported by NIEHS grant ES01332.)

ABSTRACT FINAL ID: 2658 Poster Board -408
TITLE: Assessment of Reproductive Toxicity of Isopropyl-3-Hydroxybutyrate (IPHB) in Rats

AUTHORS (FIRST INITIAL, LAST NAME): D. Kossor[1], and J. H. Charlap[2].
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KEYWORDS: teratogenesis, reproduction

ABSTRACT BODY: Isopropyl-3-hydroxybutyrate (IPHB) is a potential sustainable alternative solvent for cleaning products. IPHB is expected to hydrolyze *in vivo* to yield isopropanol and beta-hydroxybutyrate (BHB). Although several previous *in vitro* studies had concluded that BHB was teratogenic, the potential toxicity of BHB has not been determined *in vivo*, and therefore, the present study was done to assess potential reproductive effects of IPHB following oral administration (gavage) in rats that may be due to the production of BHB. In an OECD 422 guideline study, groups of 12 male or female Sprague Dawley rats were given single daily doses of IPHB (0, 250, 500 or 1000 mg/kg in deionized water). Males received 14 doses prior to mating, throughout mating until the day prior to euthanasia for a total of 28–29 doses. Toxicity phase females were dosed from study day 0 until the day prior to euthanasia for a total of 28–29 doses. Reproductive phase females received 14 doses prior to pairing and dosed until lactation day 3 for a total of 40–52 doses. Clinical observations, food consumption and body weights were recorded, and all rats were subjected to a functional observational battery. There were no adverse effects on food consumption, body weights or behavior related to IPHB treatment. Indices of reproductive performance were unaffected by IPHB treatment at all dose levels. There were no adverse effects on mean pup body weights or body weight gains and no remarkable clinical findings or macroscopic findings for F1 pups at all dose levels. All rats survived to scheduled necropsy and tissues were collected for histopathological evaluations. There were no IPHB-related clinical pathology (hematology, coagulation, serum chemistry) alterations or histologic changes in the males and repeat-dose toxicity phase females. A dose level of 1000 mg/kg/day was considered to be the no-observed-adverse-effect level (NOAEL) for repeat dose toxicity or reproductive toxicity of IPHB in rats. The present study provides conclusive evidence that oral IPHB exposure is not associated with developmental or reproductive toxicity in rats.

ABSTRACT FINAL ID: 2659 Poster Board -409
TITLE: Effects of Bisphenol A on Ovulation after Perinatal or Adult Exposure

AUTHORS (FIRST INITIAL, LAST NAME): D. Acuña-Hernández, T. Moore-Ambríz, and I. Hernández-Ochoa.
INSTITUTIONS (ALL): Department of Toxicology, Cinvestav-IPN, Mexico City, Mexico.
KEYWORDS: Bisphenol A, Ovulation

ABSTRACT BODY: Oocytes are released from the ovary during ovulation to be fertilized by sperm cells. Since some studies have suggested that high levels of bisphenol A (BPA), a plasticizer that leaches from plastics into food and water, alters ovulation, this study evaluated whether exposure to the current tolerable daily intake (TDI, 50 µg/kg/d) of BPA alters ovulation, and whether this effect depends on the exposure during a perinatal or an adult age. As for the perinatal exposure, pregnant C57BL/6J female mice (n = 6-8) were exposed orally to 50 µg/kg/d BPA, diethylstilbestrol (DES, 10 µg/kg/d, positive control) or corn oil daily from gestational day 10.5 to postnatal day (PD) 25, and female offspring were evaluated on PD 26. As for the adult exposure, 39 days old female mice (n = 5–7) were exposed orally to BPA (50 µg/kg/d), DES or corn oil daily for 12-15 d. Effects of BPA on ovulation were assessed as the changes in the number of oocytes

released into the oviduct in response to exogenous gonadotropins, as diameters of preovulatory follicles, as well as the changes in the numbers of preovulatory follicles and corpora lutea in ovarian histological sections. Our data showed that BPA treated mice had similar numbers and diameters of preovulatory follicles, as well as similar numbers of oocytes and corpora lutea to control mice regardless of the window of exposure. These data suggest that exposure to the TDI of BPA during a perinatal or an adult age does not affect ovulation. We cannot exclude the possibility, however, that effects at molecular levels are involved. Conacyt-Mexico CB-167678.

ABSTRACT FINAL ID: 2660 Poster Board -410
TITLE: Effects of Various Cryoprotectants on Bull Sperm Quality, DNA Integrity, and Oxidative Stress Parameters

AUTHORS (FIRST INITIAL, LAST NAME): E. Coskun[1], U. Tasdemir[2], S. Buyukleblebici[3], B. P. Tuncer[2], T. Ozgurtas[4], F. N. Aydin[4], O. Buyukleblebici[3], and I. S. Gurcan[5].
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KEYWORDS: cryoprotectants, bull sperm, DNA integrity

ABSTRACT BODY: The objectives of this study was to compare the effects of type and concentration of cryoprotectants glycerol (G), ethylene glycol (EG) and dimethyl sulfoxide (DMSO) on the plasma membrane and DNA integrity as well as antioxidant activity of cryopreserved Eastern Anatolian red bull sperm from 3 bulls. The pooled ejaculates were split into seven equal experimental groups and diluted with the modified base extender to a final spermatozoa concentration of 15 million/ml. Before the straws were plunged into liquid nitrogen at -196C, they were cooled slowly with a step by step cooling process. Frozen straws were thawed individually at 37C for 30s in a water bath to analyse progressive motility and sperm motion characteristics as well as membrane integrity using hypo-osmotic swelling test. Biochemical assays were performed in a spectrophotometer using commercial kits. DNA damage was evaluated by Comet Assay using Image Analysis System. 6%G exhibited the greatest percentages of CASA (43.7±2.92%) and progressive (26.4±2.64%) motilities when compared to the other groups (P<0.001). 6%G and 6%EG showed the greatest values of preserved membrane integrity (P<0.001). 6%DMSO and 3%EG+3%DMSO resulted in greater chromatin damage than the other groups (P<0.001). The antioxidant activities of GPx, GSH, and CAT as well as the total antioxidant activity were affected by the type of cryoprotectant; notably, 2%G+2%EG+2%DMSO yielded the lowest activities when compared to the other groups (P<0.001). In conclusion, no advantages were found in using EG or DMSO to replace G in bull sperm cryopreservation. Freezing with cryoprotectant 6% G yielded the best postthaw sperm characteristics for Eastern Anatolian Red bull spermatozoa.

ABSTRACT FINAL ID: 2661 Poster Board -411
TITLE: Effects of Low-Dose Developmental Perfluorooctanoic Acid (PFOA) Exposure on Mammary Gland Development and Pubertal Timing in CD-1 Mice

AUTHORS (FIRST INITIAL, LAST NAME): D. K. Robinson[1, 2], M. B. Macon[1, 2], A. Filgo[1, 2], C. Reed[2], J. Stanko[2], M. Strynar[2], and S. Fenton[2].
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KEYWORDS: Perfluorooctanoic Acid (PFOA), mammary gland, puberty

ABSTRACT BODY: Perfluorooctanoic acid (PFOA) is a persistent and ubiquitous environmental toxicant that has been shown to alter normal growth and development in mice. Low dose exposures to PFOA during the perinatal period prevent normal mouse mammary development and may also alter the timing of other pubertal events. Other chemicals are known to affect the mammary gland at lower doses than other crucial pubertal events, such as vaginal opening (VO), first estrus, and first normal cycle, all of which are governed by various related, but not identical, endocrine signaling pathways. Human studies

have identified delayed menarche in females living in close proximity to PFOA contaminated areas in the US. No single study has examined the effects of PFOA on all pubertal events in prenatally exposed mice. Our goal was to investigate the effects of a low dose PFOA exposure on the timing of critical pubertal events in the CD-1 mouse, such as mammary gland whole mounts, vaginal cytology, VO assessment, and steroid hormone levels. Timed pregnant CD-1 mice received oral gestational exposure (d 1 to 17) to vehicle, 0.01, 0.1, 0.3 or 1.0 mg/kg of PFOA. Female offspring were assessed for markers of puberty using mammary gland whole mounts and vaginal smearing, with collection of serum at estrus. PFOA did not alter the occurrence of VO or time until the first normal cycle. Time to first estrus was unchanged, with the exception of the 0.3 mg/kg dose group where it was delayed by approximately 3 days. Delayed mammary gland development was observed at all time-points for 0.3 mg/kg and 1.0 mg, and as low as 0.01 mg/kg for some timepoints. Alterations in mammary development but not other pubertal timing indicators demonstrate that PFOA may alter some but not all of the endocrine signaling pathways involved in pubertal timing and mammary gland development seems to be most sensitive to PFOA. This abstract does not represent NIEHS policy.

ABSTRACT FINAL ID: 2662 Poster Board -412
TITLE: A Comparison of Chinese and Mauritian Cynomolgus Macaque Immunotoxicology Endpoints

AUTHORS (FIRST INITIAL, LAST NAME): D. E. Wilkins, R. Young, and C. M. Satterwhite.
INSTITUTIONS (ALL): Laboratory Sciences, Charles River Laboratories, Preclinical Services, Reno, NV.
KEYWORDS: T-cell dependent response, immunophenotyping, immunotoxicology

ABSTRACT BODY: Cynomolgus macaques (*Macaca fascicularis*) are commonly used for preclinical toxicology assessments, and are largely imported from China, Indonesia, and the island of Mauritius. Chinese cynomolgus macaques (CCM) are commonly utilized at our site; however, the use of Mauritian cynomolgus macaques (MCM) is increasing due to the isolated nature of their origin and restricted genetic diversity. Interorigin differences have been reported between Mauritian and Chinese CM; however, a comparison of immune endpoints between the two origins has yet to be reported. We compared immunophenotyping, and T-cell dependent antibody response (TDAR) data collected from CCM and MCM at our site over two years. For Immunophenotyping analysis, whole blood samples were analyzed for T-lymphocyte subsets, B lymphocytes, and natural killer (NK) cells. Interestingly, CCM presented with higher frequencies of B lymphocytes than MCM (CMC B-lymphocyte mean and median percentages were 27.1% and 30.21% and MCM mean and median values were 12.9% and 12.6%), whereas MCM presented with greater frequencies of NK cells (mean and median values were 17.3% and 16.2% for MCM, and 10.5% and 10.19% for CCM, respectively). To determine if the variations in counts between CCM and MCM had a functional impact on B-lymphocytes, we also evaluated T-cell dependent antibody responses. CCM and MCM received either 750 µg keyhole limpet hemocyanin (KLH) mixed 1:1 with Incomplete Freund's Adjuvant (IFA), or 10 mg of KLH reconstituted in sterile water. Preliminary center point titer data indicate that CCM and MCM generated comparable IgM and IgG titers on Days 10, 14, and 21, post KLH immunization. Collectively, these data demonstrate that interorigin differences in lymphocyte cellularity exist between CMC and MMC; however, these differences to not appear to impact B-lymphocyte function, or preclude the use of Mauritian cynomolgus monkeys for the assessment of immunotoxicology endpoints.

ABSTRACT FINAL ID: 2663 Poster Board -413
TITLE: Characterization of the Role of Cannabinoid Receptors on Dendritic Cell Maturation

AUTHORS (FIRST INITIAL, LAST NAME): J. Suarez-Martinez[1, 2], N. Kaminski[2], and R. Crawford[2].
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KEYWORDS: Dendritic cells, cannabinoid receptors, maturation

ABSTRACT BODY: Dendritic cells (DC) play a crucial role as antigen presenting cells in coordinating CD4+ and CD8+ T cell responses. Previously we have shown that the antiviral response (HIV and influenza) in CB1-/-CB2-/- mice was markedly

increased compared to WT due to a deregulation of IL-17 producing T cells primarily attributable to DC. The role of the cannabinoid receptors CB1 and CB2 on DC function and susceptibility to exogenous and endogenous-derived cannabinoid compounds such Δ9-tetrahydrocannabinol (Δ9-THC) and anandamide (AEA) has not been characterized. The objective of the present study was to investigate differences between C57Bl/6 (WT) and CB1-/-CB2-/- mice in precursor and mature bone marrow -derived DC (bmDC). Freshly isolated bone marrow cells from WT mice demonstrated a modestly higher percent of lineage negative precursors and a significantly lower percent of lineage committed cells in comparison to CB1-/-CB2-/- mice. We also found that the main populations of precursors in fresh bone marrow were the macrophage and DC precursors (MDP) with no difference in percentage of these between the two strains. BmDC precursors were expanded with GMCSF for 9 days. During this expansion we followed DC precursors of classical and monocyte lineage and characterized differences between the two mouse strains as assessed by percentages of these populations. After the expansion, bmDC precursors were then stimulated with lipopolysaccharide (LPS), treated with Δ9-THC or AEA for 24 hours and evaluated by flow cytometry. With LPS stimulation CD11c, MHCII and CD86 expression was higher in CB1-/-CB2-/- vs. WT. Treatment with Δ9-THC (10 μM) and AEA (10μM) after LPS stimulation attenuated CD86 expression in comparison to the vehicle control. These studies suggest a role by CB1 and/or CB2 on DC maturation as evidenced by the differences in surface marker expression in both progenitor and mature DC populations. (Work supported by DA007908.)

ABSTRACT FINAL ID: 2664 Poster Board -414
TITLE: Sensitization to Acid-Hydrolyzed Wheat Protein by Transdermal Administration to BALB/c Mice

AUTHORS (FIRST INITIAL, LAST NAME): R. Adachi[1], R. Nakamura[1], S. Sakai[1], Y. Fukutomi[2], and R. Teshima[1].
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KEYWORDS: acid-hydrolyzed wheat protein, transdermal sensitization, gluten

ABSTRACT BODY: An increasing number of studies have shown that hydrolyzed wheat protein (HWP) can induce IgE-mediated hypersensitivity by skin contact. However, there has been no study of the sensitizing potential of HWP. In 2011, we reported five patients in Japan, who were primarily sensitized to HWP in facial soap and experienced wheat-dependent exercise-induced anaphylaxis after ingesting normal wheat products. As of December 20, 2012, more than 1,600 patients with such an allergy have been reported in Japan, and most patients used the same facial soap containing acid-HWP (HWP1). In this study, the possibility of transdermal pathway for sensitization to HWP1 was investigated using BALB/c mice, and compared with that of gluten. HWP1 or gluten (500 μg/mouse) was transdermally administered using patches. After four cycles of sensitization for 3 days/week, active systemic anaphylaxis (ASA) was induced by intraperitoneal injection of the antigen, and rectal temperatures, scores of anaphylactic responses, and plasma histamine levels were determined. Because HWP1 was included in facial soap, the effect of detergent (SDS) on the sensitizing potential was also investigated. Transdermal administration of HWP1-induced dose-dependent production of IgE and IgG1. After sensitization for 4 weeks, intraperitoneal injection of HWP1 caused ASA, leading to decreased rectal temperatures, increased anaphylaxis scores, and increased plasma histamine levels. In addition, splenocytes harvested after ASA produced IL-4, IL-5, and IL-10 by re-stimulation with HWP1. Transdermal exposure to gluten also induced IgE and IgG1 production, and intraperitoneal injection of gluten also induced ASA, only in mice sensitized in the presence of SDS. This study shows that HWP1 has a transdermally sensitizing potential to activate key immune pathways, whereas its allergenicity may be different from that of gluten.

2013 Society of Toxicology Annual Meeting
Late-Breaking Abstracts

ABSTRACT FINAL ID: 2665 Poster Board -415
TITLE: Hydrocarbon Oil Induces Autoimmune Diffuse Alveolar Hemorrhage: Analysis of Lung Infiltrating Cells

AUTHORS (FIRST INITIAL, LAST NAME): P. Prasad[2, 3, 1], I. Valera[1, 3], M. C. Fishbein[1, 4], and R. Singh[2, 3, 4].
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KEYWORDS: Hydrocarbon Oil, Diffuse Alveolar Hemorrhage, Dendritic Cells

ABSTRACT BODY: A community comparison study in people living in houses with increased hydrocarbon oil [2,6,10,14-tetramethylpentadecane (TMPD)] in house dust near an oil field waste site in New Mexico found a higher prevalence of systemic lupus erythematosus (SLE) and related rheumatic diseases in exposed as compared to unexposed population. This suggests a possible role of exposure to environmental agents in inducing autoimmune diseases. Indeed, a single injection of TMPD induces a SLE-like disease in mice. Intriguingly, different strains of mice develop different manifestations of SLE. For example, C57BL/6 and C57BL/10 mice, but not other strains tested, develop diffuse alveolar hemorrhage (DAH), a serious pulmonary complication of SLE. DAH is fatal in over 50% of cases, and without any effective treatment. To investigate the mechanisms underlying DAH development, we administered 0.5 ml TMPD or saline intraperitoneally once to C57BL/6 mice. Histopathology of lungs, thus far show that 11 of 14 (78%) TMPD exposed, compared to none of controls (n=11), developed mild to severe pulmonary hemorrhage/inflammation/vasculitis, within 2 weeks of injection. Histopathology of liver, kidney and spleen did not show any hemorrhage or major inflammation in TMPD exposed and control mice. Utilizing immunostaining and flow cytometry, we found a profound cellular infiltration with CD11c+ CD11b+ dendritic cells (DC), TCRβ+ T cells, monocytes and granulocytes in lungs of TMPD-exposed mice. In addition, there were increased plasmacytoid DC (pDC) in lungs, lung draining lymph nodes and bone marrow of TMPD exposed mice as compared to controls. Ongoing studies will determine whether a local expansion or preferential recruitment and migration from the bone marrow to lungs accounts for increased lung accumulation of DCs that then elicit local inflammatory response.

ABSTRACT FINAL ID: 2666 Poster Board -416
TITLE: TNFR2: A Sex-Related Factor in Multiple Sclerosis Susceptibility

AUTHORS (FIRST INITIAL, LAST NAME): S. C. McKarns and R. M. Howard.
INSTITUTIONS (ALL): Department of Surgery, University of Missouri, Columbia, MO.
KEYWORDS: TNF, Autoimmune, Multiple Sclerosis

ABSTRACT BODY: TNF plays a dual role in neurodegenerative diseases. While TNF receptor type 1 (TNFR1) pathways are neurodegenerative, TNFR2 signaling is predominantly neuroprotective. These findings support the hypothesis that blockade of membrane-bound TNF/TNFR2 signaling by nonselective anti-TNF therapy is the molecular basis exacerbation of multiple sclerosis (MS). TNFR2-mediated protection in preclinical models has been associated with neuronal and oligodendrocyte survival and function during episodes of remyelination. In this study, we now provide an additional, alternative immune-based pathway for TNFR2 mediated neuroprotection in EAE, an animal model of MS. Greater than 90% of female, but few male, C57BL/6 TNFR2-/- myelin oligodendrocyte glycoprotein (MOG)-specific (2D2) T cell receptor (TCR) transgenic mice spontaneously develop experimental autoimmune encephalomyelitis (EAE). B cell and CD4 T cell infiltration into the CNS correlates demyelination, increased production of IFN-γ and IL-17, and elevated sera titers of MOG35-55-specific IgG2b. The number of FoxP3+ Tregs was not deficient in the TNFR2-/- 2D2 mice; however, Treg autonomous functional defects as well as resistance of CD4 FoxP3-/- responder T cells correlated with EAE. Our results identify a sex-related TNFR2-dependent regulation of autoimmune susceptibility and suggest a role for TNFR2 in maintaining functional tolerance of autoreactive CD4 T and B cells. In addition to providing a new resource to study T and B cell interactions, TNFR2-/- 2D2 mice also provide a unique opportunity to address the question of why women are more susceptible to MS than men.

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Late-Breaking Abstracts

ABSTRACT FINAL ID: 2667 Poster Board -417
TITLE: System Biology Approach for Immunological Mediated Toxicities: A New Paradigm for Predictive Testing and Risk Assessment of Skin and Respiratory Sensitization Potential

AUTHORS (FIRST INITIAL, LAST NAME): C. A. Ryan[1], M. Baccam[1], L. Foertsch[1], X. Wang[1], G. Carr[1], R. J. Dearman[2], I. Kimber[2], and F. Gerberick[1].
INSTITUTIONS (ALL): [1]Procter & Gamble, Cincinnati, OH; [2]University of Manchester, Manchester, United Kingdom.
KEYWORDS: Chemical sensitizers, System biology, Genome-wide expression

ABSTRACT BODY: The objective of our system biology approach is to generate a global view of transcriptional changes, protein changes and/or metabolism changes induced in target cells exposed to a wide array of sensitizing chemicals with the goal of identifying common functional and regulatory pathways and/or molecules that play central roles in immunological toxicities. A set of 15 nonsensitizers (NON), 15 skin sensitizers (SS) and 14 low molecular weight respiratory sensitizers (RS) were tested in THP-1 and BEAS-2B cells. An initial experiment testing 3 skin and 3 respiratory sensitizers, along with 3 nonsensitizing chemicals (from the set) was conducted with 9 replicates of test chemicals and 12 replicates of vehicle control. The remaining chemicals in the set were tested using 6 replicates of test chemicals and 9 replicates of vehicle control. Cells were treated with the chemicals for 24 hours then harvested for global gene expression analysis using the Affymetrix GeneTitan® platform. Transcript profiling based on statistical analysis was performed to identify significant changes in gene expression. Most chemicals, though not all, elicited a robust change versus vehicle control. As expected, no single gene is sufficient to discriminate between the three classes of chemicals. In addition, some similarities in response between the two cell lines were noted. Further analyses of the differentially expressed genes using an enrichment approach will be conducted to define patterns of gene expression which identify biological pathways that are shared by or are unique to each of the chemical groups.

ABSTRACT FINAL ID: 2668 Poster Board -418
TITLE: A Robotized BG1 Luc Assay to Detect Estrogen Receptor (ER) Agonists and Antagonists

AUTHORS (FIRST INITIAL, LAST NAME): M. Stoner[1], C. Z. Yang[1], G. J. Kollessery[1], A. Wong[1], and G. D. Bittner[1, 2].
INSTITUTIONS (ALL): [1]CertiChem, Inc., Austin, TX; [2]The University of Texas, Austin, TX.
KEYWORDS: estrogen receptor, gene reporter assay, robotized

ABSTRACT BODY: Robotized MCF-7 cell proliferation assays and manual versions of BG1-Luc gene reporter assays are under validation by ICCVAM/NICEATM to detect ER agonists for estrogenic activity (EA) and antagonists for antiestrogenic activity (AEA). A subset of test substances used in MCF-7 and BG1-Luc validation studies was chosen to characterize a robotized BG1-Luc assay recently developed by using the technology platform pioneered in MCF-7 validations studies. The robotized BG1-Luc assay for EA, like the robotized MCF-7 cell proliferation assay, demonstrates high accuracy and sensitivity for both ER agonists and antagonists. For example, 9 out of 11 test substances have lower EC50 values (chi-squared test, $p<.05$) in the robotized BG1-Luc assay compared to the manual BG1-Luc assay. Additionally, the detection and quantification of antagonist activity is robust and reproducible for known ER antagonists such as tamoxifen, 4-OH-tamoxifen and raloxifene. These robotized BG-1Luc EA and AEA protocols include Confirmation Assays in which test substances are co-treated with the antiestrogen ICI 182,780 or with 17beta-estradiol (nonsaturating vs. saturating concentrations), respectively, to ensure that ER agonist or antagonist activity is correctly identified. In brief, robotized BG1-Luc assays enhance the utility of the ICCVAM/NICEATM-approved BG1-Luc manual assays by increasing sensitivity, sample throughput and reproducibility.

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ABSTRACT FINAL ID: 2669 Poster Board -419
TITLE: Assessing Endocrine Disrupting Activities of Four Bisphenols Using Zebrafish Larvae

AUTHORS (FIRST INITIAL, LAST NAME): R. Hao[1], M. Bondesson[1], P. Balaguer[2], and J. Gustafsson[1, 3].
INSTITUTIONS (ALL): [1]Department of Biology and Biochemistry, University of Houston, Houston, TX; [2]Institut de Recherche en Cancérologie de Montpellier, Montpellier, France; [3]Department of Biosciences and Nutrition, Karolinska Institute, Huddinge, Sweden.
KEYWORDS: Bisphenols, Zebrafish, Endocrine Disrupting Chemicals

ABSTRACT BODY: Endocrine disrupting chemicals (EDCs) are of concern since they may cause health problems, such as abnormal sexual differentiation, infertility, metabolic disorders, obesity and certain types of cancers. Bisphenol A (BPA), which is widely used for epoxy resin and polycarbonates plastics, has been extensively reported as an estrogenic EDC in the past few years. Therefore, the plastics industry is gradually introducing new BPA substitutes generating a need for an assessment of their estrogenic disrupting activities. As the estrogen signaling pathways are conserved from fish to mammals, zebrafish have become an emerging model for investigating estrogenic EDCs. Here, we investigated the estrogenic disrupting activities of BPA, Bisphenol C (BPC), Bisphenol AF (BPAF) and Bisphenol AP (BPAP) using zebrafish larvae as a model. Larvae were exposed to bisphenols to examine estrogenic effects, or co-exposed together with ethinylestradiol (EE2) to examine anti-estrogenic effects. Expression of estrogenic biomarkers vitellogenins (vtgs) and cyp19a1b was strongly up-regulated by EE2 and the four bisphenols, indicative of estrogenic activity. However, co-exposure of the larvae to EE2 and three of the bisphenols (BPA, BPAF and BPC) repressed the EE2-induced transcriptional activation of the biomarkers, suggesting anti-estrogenic effects. We also used Tg(5xERE:GFP) transgenic zebrafish, which contains five copies of an estrogen response element and the green fluorescent protein (GFP) reporter, to monitor the estrogenic effects of the bisphenols. GFP expression in the reporter fish was increased after exposure to the bisphenols, which is in agreement with the induced expression of the biomarkers by bisphenol treatment. We conclude that the four bisphenols exhibited estrogenic activities to different extents while BPA, BPC and BPAF also exhibited anti-estrogenic activities in zebrafish larvae.

ABSTRACT FINAL ID: 2670 Poster Board -420
TITLE: Acute Exposure to Ozone and Particulate Matter Activates the Hypothalamic-Pituitary-Adrenal Axis

AUTHORS (FIRST INITIAL, LAST NAME): E. Thomson, D. Vladisavljevic, S. Mohottalage, P. Kumarathasan, and R. Vincent.
INSTITUTIONS (ALL): Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON, Canada.
KEYWORDS: air pollution, hypothalamic-pituitary-adrenal axis, gene expression profile

ABSTRACT BODY: In addition to the considerable literature establishing a link between air pollution and cardiovascular disease, a number of recent epidemiological studies show associations between air pollution and central nervous system and behavioural outcomes, including cognitive impairment, depression, and suicide. While animal studies have demonstrated neurotoxicity following exposure to air pollutants, the biological mechanisms underlying neurobehavioural impacts are not well understood. In the present study we sought to gain insight into possible mechanisms underlying systemic effects of pollutant exposure through multiorgan profiling of gene responses. Following acute (4 h) inhalation exposure of male Fischer-344 rats to particulate matter (0, 5, 50 mg/m3) and ozone (0, 0.4, 0.8 ppm), expression of a panel of genes was evaluated in the lungs, heart, liver, kidney, spleen, cerebral hemisphere, and pituitary by real-time polymerase chain reaction immediately or 24h postexposure. Pollutant-induced changes included altered expression of genes involved in antioxidant response, xenobiotic metabolism, inflammatory signalling, and endothelial dysfunction. The pattern of gene expression, though exhibiting some interorgan differences, was remarkably similar across organs for a set of genes, including increased expression of redox and glucocorticoid-sensitive genes and decreased expression of inflammatory genes, suggesting a possible hormonal effect. Activation of the hypothalamic-pituitary-adrenal axis was confirmed through measurement of increased plasma levels of the glucocorticoid-signalling adrenocorticotropic hormone, and there was a corresponding increase in plasma corticosterone. The experimental data are consistent with epidemiological associations of

air pollutants with extracardiopulmonary health outcomes, and suggest a possible mechanism through which air pollutants may exert neurobehavioural effects.

ABSTRACT FINAL ID: 2671 Poster Board -421
TITLE: Effect of High-Fat Diet on Metabolism and Distribution of Fullerene C60 in Mice

AUTHORS (FIRST INITIAL, LAST NAME): A. Novokhatny[1], T. Fennell[1], R. Snyder[1], S. McRitchie[3], W. Pathmasiri[1], J. Brown[2], A. Lewin[1], and S. Sumner[1].
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KEYWORDS: metabolomics, C60 Fullerenes, disposition

ABSTRACT BODY: Exposure to carbon nanoparticles could occur in environmental, occupational, or medicinal settings due to their use in engineering, skin care, and therapeutic products. Determining the metabolism and distribution of carbon nanoparticles in different physiological states is needed to understand the potential for therapeutic response or adverse health outcomes. In this investigation, mice fed high fat diets, and mice fed diets normal in fat levels were administered, via tail vein injection, vehicle (saline with 5% polyvinylpyrrolidone) or 0.75 mg/kg [14C]C60 fullerene in vehicle. Urine, blood and tissues were collected for up to 72 hr after dosing. The concentration of radioactivity did not test significantly different between mice fed diets high in fat and mice fed diets normal in fat. The distribution of radioactivity was similar between these groups, with the highest distribution (dpm/g tissue) in lung > spleen > liver > kidney > heart. Metabolomics analysis revealed that mice fed high fat diets had higher levels, in urine, of hippurate, creatinine, and 2-hydroxy-3-methylvalerate compared with mice fed diets normal in fat. Mice exposed to C60, independent of diet, had increased levels of 3-methyl-2-oxovalerate. Compared to the vehicle exposed groups, C60 exposed mice fed high fat diets, but not normal fat diets, had increased levels of aspartate and 2-hydroxy-3-methylvalerate, while tartrate and nitrosodimethylamine were decreased for mice fed the normal fat diets. Cytokines GM-CSF, IL-13, IL-17, IL-5, and TNFα were increased in the serum of mice high fat diets, compared with mice fed normal fat diets, and C60 exposure resulted in an increase in cytokines TNFα, MIP-1α, IL-17, IL-10, GM-CSF, and Eotaxin in both study groups. This study reveals that C60 distributes similarly within the two study groups, but impacts the endogenous metabolism differently. Funded by RTI & NIEHS U19ES019525.

ABSTRACT FINAL ID: 2672 Poster Board -422
TITLE: Cellular Response of Small Airway Epithelial Cells and Human Microvascular Endothelial Cells in a Coculture System following Exposure to MWCNT

AUTHORS (FIRST INITIAL, LAST NAME): B. Talkington[1], C. Dong[2], J. Dymacek[3], D. Schwegler-Berry[1], V. Castranova[1], N. L. Guo[2], and Y. Qian[1].
INSTITUTIONS (ALL): [1]Pathology and Physiology Research Branch, NIOSH, Morgantown, WV; [2]Mary Babb Randolph Cancer Center, West Virginia University, Morgantown, WV; [3]Lane Department of Computer Science and Electrical Engineering, West Virginia University, Morgantown, WV.
KEYWORDS: multiwalled carbon nanotubes

ABSTRACT BODY: Nanotechnology, particularly the use of multiwalled carbon nanotubes (MWCNT), is a rapidly advancing field with implications for advancement in a variety of disciplines such as biomedical, electrical, and thermal research. A major route of exposure to MWCNT in both occupational and environmental contact is inhalation. While many studies showed adverse effects to either the lung epithelium or vascular endothelium upon MWCNT exposure, *in vitro* results did not often correlate with *in vivo* effects. Therefore, a more relevant cellular model to mimic *in vivo* exposure was needed. This study sought to create a coculture system in which both human small airway epithelial cells (SAEC) and human microvascular endothelial cells (HMVEC) were grown in coculture so as to resemble an alveolar-capillary interaction. Exposure of the epithelial layer to MWCNT-induced (at a low dose relevant to *in vivo* lung burden) multiple changes in the

endothelial barrier including an increase in ROS, actin rearrangement, loss of VE-cadherin, and potentially increased permeability. An increase in endothelial angiogenic ability, as well as overall increases in secreted VEGFA, ICAM1, and VCAM1 protein expression, was noted after epithelial exposure. Additionally, alterations to both SAEC and HMVEC mRNA and miRNA levels were noted after MWCNT exposure. This coculture system identified that epithelial exposure to MWCNT-induced multiple changes to the endothelium, potentially through cell signaling mediators, and suggested that the coculture system is an improved *in vitro* method to study the pulmonary toxicity and potential signaling pathways of MWCNT exposure.

ABSTRACT FINAL ID: 2673 Poster Board -423
TITLE: Uptake of Nanosilica in an Oral Epithelia Model As Assessed with FM1-43 Dye

AUTHORS (FIRST INITIAL, LAST NAME): M. Best[1], G. Phillips[1], C. Fowler[2], J. Rowland[2], and J. Elsom[1].
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KEYWORDS: nanomaterials, nanosilica, cytotoxicity

ABSTRACT BODY: Silica is an abundant natural material with wide utility. It is increasingly being utilised in nano form within a range of consumer products to include dentifrices. Given the variety of nanosilica specifications available, existing toxicological data on silica cannot always be easily extrapolated and applied to nanomaterials. The purpose of this research was to assess the potential cytotoxicity and uptake of nanosilica *in vitro* using an oral epithelial model, and to compare the data with other nanomaterials used in oral healthcare products, and with the respective bulk material. The use of FMI-43 dye presents a model of assessing nanoparticle uptake, without modifying the characteristics of the nanomaterials. The materials assessed were nanozinc oxide, nanotitanium dioxide, nanosilica and nanohydroxyapatite, alongside their respective bulk constituents. All were characterised for size and morphology using scanning electron microscopy, and administered in concentrations ranging from 0.031% to 0.250%w/v in DMEM/Ham's F-12 (without L-glutamine) for 5 minutes at 37°C to monolayers of nonkeratinised oral epithelia cells (H376). Cytotoxicity was assessed using the lactate-dehydrogenase and MTT cell viability assays. To investigate uptake, test materials were diluted in 50µM FM1-43/media and read at 540nm in a fluorometer. Confocal microscopy was used to verify the assay. Cytotoxicity results showed most materials were well tolerated within the *in vitro* oral epithelia model. Zinc oxide was the exception, showing cytotoxicity in both bulk and nano form. Silica was the only material in nano form to show uptake using both the FM1-43 assay and confocal microscopy. Results clearly demonstrate uptake of nanosilica *in vitro* and the potential of FM1-43 dye for assessing uptake of unmodified and nonfluorescent nanomaterials in oral epithelia.

ABSTRACT FINAL ID: 2674 Poster Board -424
TITLE: Unique Nanoparticle Properties Confound Fluorescent-Based Assays Widely Employed in Their *In Vitro* Toxicity Testing and Ranking

AUTHORS (FIRST INITIAL, LAST NAME): W. W. Polk[1], and K. Dreher[2].
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KEYWORDS: Nanoparticle, vascular toxicity, High-Throughput Screening

ABSTRACT BODY: Nanomaterials are a diverse collection of novel materials that exhibit at least one dimension less than 100 nm and display unique chemical and physical properties due to their nanoscale size. An emphasis has been put on developing high-throughput screening (HTS) assays to characterize and rank the toxicities of these materials in a manner consistent with the vision of the Toxicology in the 21st Century Initiative. Research reported here demonstrates that many of the available HTS fluorescent-based assays can be confounded by nanoparticles and that their use without careful controls can result in both false positive and false negative errors. Effects are demonstrated for assays measuring

cytotoxicity, oxidative stress, and nuclear content using commercially available metal oxide nanoparticles ranging in size from 7 to 250 nm and varying in crystalline structure. The confounding effects are demonstrated in a variety of primary human endothelial cell types and are shown to be more severe within cells than would be anticipated from analytic analysis. Moreover, the effects are demonstrated to be particle dependent and not predictable by elemental composition, purity, or primary particle size. The establishment of an improved technique is shown to completely alter the mechanistic understanding of titanium oxide nanoparticle toxicity within endothelial cells. It is concluded that a majority of available HTS assays have the potential to be impacted by particle interference and quality analysis is necessary for each particle within a model system prior to the initiation of screening programs. (This abstract dose not represent EPA policy. This work was partially funded by the USEPA—UNC Cooperative Training Agreement CR-83515201)

ABSTRACT FINAL ID: 2675 Poster Board -425
TITLE: Exposure Characteristics for the Nanomaterials Released from Two Multiwall Carbon Nanotube Workplaces: A Spray Coating Workplace in a Cleanroom and a Chemical Vapor Deposition Synthesis Workplace

AUTHORS (FIRST INITIAL, LAST NAME): J. Ji, D. Woo, S. Lee, and G. Bae.
INSTITUTIONS (ALL): Center for Environment, Health and Welfare Research, Korea Institute of Science and Technology, Seoul, Republic of Korea.
KEYWORDS: Multiwall Carbon NanoTube, Nanomaterial Exposure, Occupational Exposure

ABSTRACT BODY: Exposure to nanomaterials was assessed in two MWCNT workplaces. First, during the spray process in a cleanroom, the nanotubes were sprayed in a chamber fitted with an exhaust duct system. The front door of the spraying chamber was completely closed, but rear of the chamber was partially open. Throughout a series of spray processes, three detectors—a dust monitor, nanoparticle aerosol monitor and an aethalometer—counted and characterized particles escaping the chamber. Concentrations of particle and black carbon emitted by the spraying were assessed assuming zero in the cleanroom. Very low concentrations of black carbon, 0.4ug/m3, were observed during the processes. In a cleanroom, low concentrations of nanomaterials were detected to be emitted from a spraying chamber into the workplace. The level of particles reaching the workplace was sufficiently low to have made their detection difficult in a normal environment. Target nanomaterial and nonintended incidental nanomaterials were released during spraying. Despite the exhaust duct system in the process chamber, workers would be exposed to some particles if the chamber were partially open. The exhaust duct system was not enough to remove all the particles released in the chamber. Second, we investigated for the characteristics of nanomaterials exposed in a MWCNT CVD synthesis workplace. Particles' number and surface area concentrations were monitored using a SMPS, an OPC and a nanoparticle aerosol monitor (NAM). An aethalometer assessed the mass concentration of black carbon particles. We suggest an analysis method using the four real time aerosol detectors for a MWCNT workplace in general indoor background environment. Although incidental or background nanoaerosols are not of primary interest in exposure assessment, they contributed to the most measured data.

ABSTRACT FINAL ID: 2676 Poster Board -426
TITLE: Effect of Titanium Dioxide Nanoparticle Size on Cellular Immune Activation in U937 Cell Line

AUTHORS (FIRST INITIAL, LAST NAME): A. Engin[1], E. Engin[2], and B. Karahalil[1].
INSTITUTIONS (ALL): [1]Department of Toxicology, Gazi University, Faculty of Pharmacy, Ankara, Turkey; [2]Ankara University, Biotechnology Institute, Ankara, Turkey.
KEYWORDS: Titanium dioxide nanoparticle, Neopterin, U 937 cell line

ABSTRACT BODY: Titanium dioxide (TiO2) is widely used in consumer products and medical devices because of its advantageous combination of physicochemical and biological properties. Due to human exposure characterization, their potential entry through various routes of the body suggests that nano-sized TiO2 could pose a widespread exposure risk to humans. Nanoparticle (NP)-induced oxidative stress and proinflammatory responses are well correlated with their surface area. Pteridines of all oxidation states have been shown to act anti- or pro-oxidatively, depending on the special conditions

of the experiment and take part in the inflammatory conditions. Therefore, the aim of our study was to investigate whether the TiO2 NPs may alter the synthesis of cellular immune activation marker, neopterin. U937 cells were exposed to five different concentrations (1 μg/ml – 100 μg/ml NP) of 25 and 10 nm diameter TiO2 NPs for 48 hours. To determine toxicity levels of NPs, cell viability was estimated by MTT test. Neopterin concentrations were measured by commercially available enzyme immunoassay kit, ELISA. Concentration-dependent toxicity of 10 nm and 25 nm TiO2 particles was weakly increased in U937 cells ($p>0.05$). However at higher concentrations, neopterin synthesis was significantly increased after 48-hour exposure to 25 nm TiO2 NPs ($p<0.05$). Our study demonstrated that particle size and amount of effect the toxicity of NPs. On the other hand, 25 nm TiO2 NPs may induce neopterin synthesis and immune activation more effectively than the 10 nm ultrafine particles. This study indicated that the surface characteristics of NPs might be considered as an important factor in the alteration of immune markers. Acknowledgement We are grateful to Dr. Nathalie Herlin-Bomie and Dr. Marie Carriere who provided TiO2 NPs as a gift to be used in the project entitled “NanoLINEN” under the program New Indigo supported by FP7. This study was supported by Gazi University, Scientific Research Projects Division, 02/2012-23.

ABSTRACT FINAL ID: 2677 Poster Board -427
TITLE: Lysosomal and Mitochondrial Destabilization Induced by Exposure to SiO_2 Nanoparticles in A549 Cells

AUTHORS (FIRST INITIAL, LAST NAME): A. Solorio-Rodriguez, V. Escamilla-Rivera, M. Uribe-Ramirez, and A. De Vizcaya-Ruiz[1].
INSTITUTIONS (ALL): Toxicology, CINVESTAV, Mexico City, Mexico.
KEYWORDS: Nanotoxicology, Lysosomes and mitochondria, SiO2

ABSTRACT BODY: Amorphous SiO_2 nanoparticles (SiO_2NPs) are intensively being used in pigments, pharmaceuticals, electronics, and food additives. Generation of reactive oxygen species has been described as a main mechanism of toxicity; however interaction with relevant cell structures, such as lysosomes and mitochondria, could represent a key factor of their potential biological and toxic effects. The aim of this study was to evaluate if exposure to SiO_2NP in human alveolar epithelial A549 cells could alter lysosome and mitochondria, and compromise cellular homeostasis. A stable dispersion of SiO_2NPs was obtained with bovine serum albumin (BSA), and characterized (average size and zeta potential) in water and culture medium, using dynamic light scattering, laser doppler electrophoresis and scanning electron microscopy (SEM). Cell viability was determined with the MTT assay and the alteration of lysosome and mitochondria by flow cytometry using acridine orange and JC-1, respectively. The primary size of SiO_2-NPs was 10-20 nm, average size in water and culture medium was 290-370 nm and 170-600 nm, respectively. Zeta potential in culture medium was -11 to -12 mV, yet dispersion improved with BSA, confirmed by SEM. Exposure of A549 cells to 6.25, 12.5, 25, 50 and 100 μg/ml showed no significant cytotoxicity at 6, 12, 24, 48 and 72 h after exposure. However, 12 h after the exposure to 25 and 50 μg/mL of SiO_2NPs a moderate alteration of lysosomal integrity and a decrease in mitochondrial membrane potential were observed. Our results suggest that amorphous SiO_2NPs were readily incorporated into the cell, induced lysosomal and mitochondrial destabilization, at noncytotoxic concentrations and at an early time-point, indicating cellular stress. Thus, cellular components integrity is a relevant target for SiO_2NPs exposure, and the consequence of their alteration, in terms of cellular homeostasis should be further studied to understand their potential toxicity and assure safe use of new nanomaterials. Acknowledgement: ICyTDF 396/10 for partial financial support.

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ABSTRACT FINAL ID: 2678 Poster Board -428
TITLE: Systemic Consequences of Intranasally Administered Silver Nanoparticles in Mice

AUTHORS (FIRST INITIAL, LAST NAME): L. L. Eckert, B. Eppert, V. Carreira, M. Krishan, and M. Genter.
INSTITUTIONS (ALL): Department of Environmental Health and Center for Environmental Genetics, University of Cincinnati, Cincinnati, OH.
KEYWORDS: Silver Nanoparticles, Intranasal delivery

ABSTRACT BODY: Silver nanoparticles (AgNPs) are seeing increased use in manufactured products because of their bactericidal properties. Previous work has shown these particles to be harmful via multiple routes of administration *in vivo*, including IP and intranasal. The present study sought to extend prior published work from this lab by the use of lower doses of silver nanoparticles and additional endpoints. Male C57BL/6J mice were treated by intranasal instillation with uncoated 25 nm AgNPs (0–100 mg/kg) and sacrificed one week later. Multiple organs were collected for histopathology and silver distribution analysis by autometallography. Blood was collected at sacrifice for complete blood count analysis. Spleens underwent additional analysis to evaluate differential cell counts, lymphocyte populations, and hemosiderin content. Various brain subregions were evaluated for expression levels of genes involved in oxidative stress response and inflammation by qPCR. Histopathological analysis revealed few organ abnormalities, with minor alterations in spleen morphology being the most pronounced. Autometallography revealed silver deposition in the brain as well as in disal organs such as the liver at doses as low as 10 mg/kg. Blood parameters in all treatment groups were within normal limits. Increases were seen in the percentage of monocytes and macrophages, as well as decreases in the percentage of lymphocytes, in the spleens silver-treated mice. *Hmox1* expression was elevated dose-dependently in the hippocampus of treated groups. Changes in the distribution of cell populations in the spleen and elevated expression of oxidative stress-related genes in the hippocampus warrant further investigation into the potential toxic effects of intranasal administration of silver nanoparticles. This work was supported by NIH/NIEHS T32ES016646, NIH/NIEHS P30-ES006096, and the University of Cincinnati University Research Council.

ABSTRACT FINAL ID: 2679 Poster Board -429
TITLE: Effects of Exposure to Bisphenol A during Early Development on Behavior in the Radial Arm Maze and Neuron Number in the Prefrontal Cortex of Adult Rats

AUTHORS (FIRST INITIAL, LAST NAME): R. N. Sadowski[1], L. M. Wise[2], P. Y. Park[2], S. L. Neese[3], S. L. Schantz[1, 3], and J. M. Juraska[1, 2].
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KEYWORDS: bisphenol A, prefrontal cortex, endocrine disruptor

ABSTRACT BODY: Previous work has shown that exposure to bisphenol A (BPA) during early development can affect behavior and sexual differentiation of the brain in rodents, although few studies have examined effects on cognitive behavior and associated areas of the brain. The current study examined if developmental BPA exposure alters behavior in the 17-arm radial maze and the total number of neurons and glia in the medial prefrontal cortex (mPFC) in adulthood. Long-Evans hooded rats were bred in the laboratory, and pregnant rats were orally exposed to corn oil (vehicle), 4 µg/kg, 40 µg/kg, or 400 µg/kg throughout pregnancy. After parturition, pups were given daily oral doses of oil or BPA, corresponding to those given during gestation, from days 1–9. At weaning (day 23), blood serum was collected from offspring in each litter to assess levels of thyroxine (T4). Beginning in adulthood, 1 male and 1 female from each litter were trained on the radial arm maze for 6 days a week for 4 weeks. Several weeks following completion of training, brains were removed, coronally sectioned, and stained with methylene blue/azure II. The number of neurons and glia in the mPFC were quantified stereologically with the optical fractionator. Levels of T4 at weaning age were significantly increased in males and females that received 4 µg/kg and decreased in those receiving 40 µg/kg compared to rats receiving vehicle ($p<.05$). Completed behavioral results indicate that early exposure to BPA did not alter radial arm maze performance in males or females.

Additionally, preliminary results suggest that BPA does not alter the number of neurons or glia in the mPFC, although 1-3 animals/sex/group remain to be assessed. In conclusion, although alterations in T4 were evident at weaning, low doses of BPA during early development do not affect radial maze behavior or anatomy of the mPFC in adult males or females.

ABSTRACT FINAL ID: 2680 Poster Board -430
TITLE: Effects of Developmental Toxicants on microRNA Expression during Differentiation of LUHMES Neuronal Progenitor Cells

AUTHORS (FIRST INITIAL, LAST NAME): L. Smirnova, H. Hogberg, S. Martos, T. Dao, and T. Hartung.
INSTITUTIONS (ALL): Center for Alternatives to Animal Testing (CAAT), Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.
KEYWORDS: microRNA, developmental neurotoxicity

ABSTRACT BODY: miRNAs represent a class of small noncoding RNA molecules, which bind to target mRNAs thereby repressing their translation. miRNAs were shown to regulate neural development as well as cellular responses to toxicants. Rapid (8 days) and homogeneous differentiation of human neuronal precursor cell line (LUHMES) into dopaminergic neurons suggests this cell line as appropriate *in vitro* model for developmental neurotoxicity testing. We have adapted the LUHMES differentiation protocol for toxicant exposure. In addition, we have explored the possibilities to develop a 3D human neuronal model using LUHMES cells and the constant gyratory shaking as used for the 3D rat primary aggregating brain cell model. The differentiation under modified conditions was characterized by RT-PCR with neuronal marker genes and miRNAs. We observed strong induction of the neuronal markers (β-III-Tubulin, synapsin I, DAT and TH) as well as neural-specific/enriched miRNAs (mir-124, mir-9, mir-132, mir-133b) during neuronal differentiation. Then expression of neural specific miRNAs during LUHMES differentiation under exposure to developmental neurotoxicants (Lead Chloride and Valproate) was analyzed by qRT-PCR. mir-153 was the most downregulated miRNA under Lead exposure and was only slightly reduced in VPA-treated samples. Exposure to VPA but not Lead reduced mir-133b and induced mir-7. On the protein coding gene level exposure to Lead significantly downregulated TH and DAT expression as well as slightly downregulated β-III-Tubulin, while exposure to VPA reduced expression of TH only. In conclusion, differentiation of the LUHMES cell line allows investigating the role of miRNAs in chemical-mediated developmental toxicity. Our results show substance specific miRNA regulation. Further experiments and analysis of miRNA and their targets expression contribute to understanding developmental neurotoxicity mediated by selected toxicants.

ABSTRACT FINAL ID: 2681 Poster Board -431
TITLE: Using Zebrafish Embryos to Screen for Neurotoxicants: The Case of Valproic Acid and Its Derivatives

AUTHORS (FIRST INITIAL, LAST NAME): A. Riu[1], R. Cabrera[2], R. H. Finnell[2], J. Gustafsson[1], and M. Bondesson[1].
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KEYWORDS: Zebrafish, Neurotoxicants, embryonic development

ABSTRACT BODY: In the United States, 3% of babies are born with major birth defects, such as neural tube defects, and 3 to 8% of children are diagnosed with neurobehavioral disorders, including attention deficit hyperactivity disorder, autism and learning disabilities. A vast range of studies provide evidence that exposure to environmental pollutants or drugs during pregnancy can impact normal brain development and function in embryos. One such drug is the antiepileptic medication valproic acid (VPA; Depakene), which is extensively prescribed to treat seizures, bipolar disorders and migraine headaches. However, it is also known to have hepatotoxic and teratogenic side effects. Consequently, there is a need to develop VPA-derivatives that could better control seizures but without any side effects. We used an *in vivo* model with transgenic zebrafish (Danio rerio) to screen for neurotoxic potentials of VPA and several VPA-derivatives. Embryos of three different transgenic zebrafish (zf) lines were used to assess the neurotoxicity: Tg(Nestin:GFP), Tg(Ngn-1:eGFP) and Tg(Mnx1:GFP), in which GFP expression is driven by transcription factors expressed in neuroepithelial stem cells, dopaminergic progenitor

cells and secondary motor neurons, respectively. After VPA exposure, zf embryos displayed nervous system defects, with a decrease of neuroepithelial stem cells, a migrational defect of the neuronal expressing cells, and a lack of neurite sprouting. The VPA analogs tested so far induced severe motor neuron defects as well. We used a locomotor assay to investigate the behavior of the exposed embryos, and found increased locomotor activity for VPA-exposed embryos and decreased activity for one of the VPA-derivates. To summarize, we have developed a coherent *in vivo* model to assess the effects of chemicals on different neurotoxicological endpoints during embryonic development.

ABSTRACT FINAL ID: 2682 Poster Board -432
TITLE: Sex-Dependent and U-Shaped Behavioral Toxicity of Methylmercury and Prenatal Stress

AUTHORS (FIRST INITIAL, LAST NAME): D. Weston[1], H. Ishitobi[1], B. Rausch[2], S. Pelkowski[1], and D. Cory-Slechta[1].
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KEYWORDS: methylmercury, stress, neurodevelopment

ABSTRACT BODY: Both developmental methylmercury (MeHg) exposure and prenatal stress (PS) cause hypothalamic-pituitary-adrenal axis hyperactivation and mesocorticolimbic and hippocampal dopamine and serotonin-based behavioral and neurochemical dysfunctions. This study examined whether PS can enhance behavioral toxicity of MeHg in a fixed interval (FI) behavioral paradigm in rats born to dams given 0, 0.5 or 2.5 ppm MeHg in drinking water from 2.5 weeks prior to breeding through lactation, with/without PS (immobilization) on gestational days 16–17. On the FI 1 min schedule, reinforcement followed the first lever press after each 1 min interval. Sex-dependent behavioral toxicity was observed. In females, MeHg reduced FI response rates in a nonlinear fashion, with greater reductions at 0.5 as compared to 2.5 ppm. Correspondingly, 2.5 MeHg + PS further reduced FI rates to levels associated with 0.5 MeHg alone, while 0.5 MeHg + PS attenuated the MeHg-induced decreases. The FI overall rate reductions partially reflected decreased run rates, while enhanced increases in postreinforcement pause time (time to the first response after reinforcement delivery that reflects fixed interval length) occurred with 2.5 MeHg + PS. Males showed no effects. However, PS in males increased postreinforcement pause times and interresponse times during a subsequent extinction (withholding reward) challenge. In females, MeHg ± PS differences seen with FI 1 min re-occurred following a shift to FI 3 min, and in males increased postreinforcement pause times were seen in both the 0.5 + PS and 2.5 + PS groups. Mean blood mercury levels were 8 and 39 µg/ml and brain 338 and 1140 ng/g, respectively, for the 0.5 and 2.5 exposures in 6-7 day old pups. Greater behavioral toxicity of lower compared to higher levels of MeHg has significant implications for human developmental MeHg studies. Additionally, MeHg effects may be enhanced by stress indicative of cumulative risk. EPA RD 83457801 and P30 ES001247.

ABSTRACT FINAL ID: 2683 Poster Board -433
TITLE: Elucidating the Risk of Prenatal Nicotine Exposure in Offspring Cognitive Deficits

AUTHORS (FIRST INITIAL, LAST NAME): M. Buabeid, A. Alhowail, S. Bhattacharya, M. Ahuja, M. Dhanasekaran, K. Parameshwaran, and V. Suppiramaniam.
INSTITUTIONS (ALL): Department of Pharmacal Sciences, Harrison School of Pharmacy, Auburn University, Auburn, AL.
KEYWORDS: AMPA, Nicotine, Cognitive

ABSTRACT BODY: Maternal smoking during pregnancy has been reported as a strong risk factor for neurobehavioral alteration in offspring. This project elucidates the effect of prenatal nicotine exposure (PREN), which causes offspring cognitive deficits. Rodent model of PREN, where nicotine is infused (6mg/kg/day) via minipumps to pregnant dams, resulted in cognitive deficits in offspring. Here we demonstrate that basal synaptic transmission and long-term potentiation (LTP)are significantly decreased in the hippocampal Schaffer collateral-CA1 synapses of PREN offspring. Moreover, amplitude and frequency of AMPAR mediated spontaneous excitatory postsynaptic currents (sEPSCs) recorded from PREN hippocampal slices were significantly reduced ($p< 0.01$). The deficits in LTP and basal synaptic transmission were accompanied by alterations in the function and expression of synaptic α7/β2 nicotinic acetylcholine receptors (nAChRs). In

addition, co-immuno-precipitation studies in rat hippocampi revealed that α7/β2 nAChRs complex is associated with vessicularglutamate transporter (VGlut), postsynaptic density protein-95 (PSD-95) and synapse-associated protein (SAP102). This interaction was disrupted in PREN rats. Interestingly, α7 but not β2-nAChRs co-immunoprecipitated with ERK1/2. The blockade of α7 nAChR with 50nM methyllycaconitine in control animals impaired LTP. Western blots studies in rat PREN hippocampi revealed that synaptic proteins expressions of PSD-95 and SAP102, which are involved in regulating the organization of pre and postsynaptic components of nicotinic and glutamatergic synapses, were significantly decreased in PREN rodents. Taken together, our study suggests that exposure of nicotine during pregnancy will alter nAChRs expression and function in offspring, which lead to modification of glutamatergic transmission in the hippocampus resulting in memory deficits in PREN rodents.

ABSTRACT FINAL ID: 2684 Poster Board -434
TITLE: Developmental Nicotine Exposure Leads to Deregulation of NMDA Receptors Resulting in Deficits in Hippocampal Synaptic Plasticity and Memory

AUTHORS (FIRST INITIAL, LAST NAME): S. Bhattacharya, M. Buabeid, A. Alhoail, M. Ahuja, D. Bhattacharya, M. Dhanasekaran, K. Parameshwaran, and V. Suppiramaniam.
INSTITUTIONS (ALL): Department of Pharmacal Sciences, Auburn University, Auburn, AL.
KEYWORDS: NMDA, Nicotine, memory

ABSTRACT BODY: Smoking during pregnancy has been reported as a strong risk factor for learning and memory deficits in offspring.Consistent with these observations, animal studies from our and other laboratories have shown that developmental nicotine exposure (dNIC) results in learning and memory deficits.We have recently demonstrated that nicotine (6mg/kg /day), given as infusion via minipumps to pregnant rats resulted in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA)-glutamate receptor dysfunctions leading to memory deficits in offspring. In the current study, we illustrate how α7/β2 nicotinic acetylcholine receptors (nAChRs) modulation leads to alteredsynaptic NMDAR expression and function resulting in impaired plasticityand memory in dNIC offspring.The deficits in long term potentiation (LTP) in dNIC pupswere accompanied by decreasedexpression and function of α7/β2receptors leading to impaired synaptic NMDAR function. The NMDAR-mediated miniature synapticcurrents (mEPSCs) were altered indNICs withsignificant reduction in current amplitude and frequency ($p< 0.01$) that correlated well with decreased single channel open probability and conductance of synaptic NMDARs.In addition, there is decreased expression of NMDAR subunit NR1, Ephrine B2 (EphB2),Post Synaptic Density-95 (PSD-95)and Calmodulin Kinase-II (CaMK-II) in dNIC hippocampi.Modulation of α7-nAChRs by specific agonists, N-(3R)-1-azabicyclo[2.2.2]oct-3-yl-4-chlorobenzamide (PNU-282987)dose dependently enhanced synaptic NMDA receptor mediated currents, which was paralleled by significant improvement in LTP and spatial memory in 4-6 weeks old dNIC offspring. These findings indicate that dNIC regulates nAChRs and thereby modulates synaptic NMDAR expression and function leading todecreased synaptic plasticity and memory.

ABSTRACT FINAL ID: 2685 Poster Board -435
TITLE: Behavioral Impairment Caused by Short-Term Exposure of Firemaster® 550 in Adult Zebrafish

AUTHORS (FIRST INITIAL, LAST NAME): J. M. Bailey[1], A. N. Oliveri[1], H. Stapleton[2], and E. D. Levin[1].
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KEYWORDS: Toxicology, Behavior, Zebrafish

ABSTRACT BODY: Firemaster® 550 (FM 550) is a flame retardant mixture containing both organophosphate and brominated aromatic compounds. Today FM 550 is heavily used in polyurethane foam found in residential furniture as a replacement for the phased out flame retardant mixture known as PentaBDE, which contained polybrominated diphenyl ethers. However, few studies have investigated the potential health effects of FM 550. With adult zebrafish of both sexes we assessed the short-term neurobehavioral effects of exposure to FM 550 with exposure for 24 hours at concentrations of

0.3, 1 or 3 mg/l using .0012% DMSO to facilitate solution. DMSO alone was the vehicle control (N=16 fish per exposure condition). Two hours following exposure the fish were assessed for 1) sensorimotor response and habituation in a tap/startle test and 2) novel environment diving response. For the tap/startle test, fish were placed in test arenas 6 cm in diameter with 2 cm water. After 5 min in the arena, 10 taps, one per min, were delivered by solenoids beneath each arena. The swimming distance for the 5 seconds after each tap was assessed by Noldus Ethovision® software. The data were log transformed and subtracted from the distance swum for the five seconds prior to the tap to provide a normalized measure of the increase in swimming caused by the taps. To measure novel tank diving response (a predatory avoidance response), fish were placed in novel tanks (1.5 l) for 5 min and distance from the bottom of the tank and swimming distance was measured using Ethovision. FM 550 exposure caused a significant ($p<0.005$) increase in sensorimotor response to repeated taps. Dunnett's tests showed that the 1 and 3 mg/l doses caused significant ($p<0.05$) hyper-reactivity relative to control. No significant FM 550 effect was apparent on the novel tank diving response or overall swim speed. These data suggest short-term exposure to FM 550 is disruptive to sensorimotor response but not escape and exploratory behavior. (Support ES010356)

ABSTRACT FINAL ID: 2686 Poster Board -436
TITLE: Fluoride Toxicity in Cerebellum: Role of Bergmann Glia Cells

AUTHORS (FIRST INITIAL, LAST NAME): D. I. Ramirez[1, 2], M. A. Flores-Méndez[2], L. R. Hernandez-Kelly[2], A. Ortega[2], and L. M. Del Razo[1].
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KEYWORDS: fluoride, glial cells, protein synthesis

ABSTRACT BODY: Fluoride (F) is a drinking water contaminant, intake of water with fluoride levels ≥ 1.5 mg/L can result in a neurodegenerative disease. Fluoride has an important and significative impact in brain development and differentiation that often leads to learning and memory deficits. However, the molecular mechanisms associated to F exposure are far for being established. Bergmann glia cells (BGC) are a type of radial glia, which does not undergo the typical astrocytic conversion that occurs after birth. This cell type is found in the molecular layer of the cerebellum surrounding glutamatergic synapses between parallel fibers and Purkinje cells. BGC participate actively in the removal and recycling of glutamate from the synaptic space. Also, these cells provide neurons with lactate in what now is known as the astrocyte/neuronal lactate shuttle.Therefore, a continuous dialogue between neurons and their surrounding glial cells is important for proper brain function. Taking into consideration that Purkinje cells are target of F-induced degeneration, in this contribution, we hypothesized that the effect of this ion might be the result of impairment of glutamate neurotransmission and in particular of glia/neuronal interactions. To this end, we used the well-established culture system of chick cerebellar BCG. Exposure of the cultured cells to F concentrations as those found in tap water of contaminated regions, results in a time and dose dependent decrease in [^{35}S]-methionine incorporation into newly synthetized polypeptides. Phosphorylation experiments demonstrated a F-induced increase in the phosphorylation pattern of the eukaryotic elongation factor 2 (eEF2), suggesting that the elongation step of protein synthesis is one of the targets of F toxicity. The kinase responsible for eEF2 phosphorylation is a Ca^{2+}/calmodulin dependent kinase (eEF2K). Accordingly, we were able to demonstrate a F-triggered $^{45}Ca^{2+}$ influx. Our results favor the notion that F neurotoxicity might as main targets the surrounding glial cells.

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Late-Breaking Abstracts

ABSTRACT FINAL ID: 2687 Poster Board -437
TITLE: Serum Glial Fibrillary Acidic Protein As a Biomarker of Neurotoxicity in Rat Kainic Acid Model

AUTHORS (FIRST INITIAL, LAST NAME): R. L. Hayes[1], S. Mondello[1], D. Johnson[1], N. Denslow[2, 1], J. Streeter[1], and O. Glushakova[1].
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KEYWORDS: GFAP, biomarker, neurotoxicity

ABSTRACT BODY: Background: Current evaluations of neurotoxicity in clinical and preclinical studies rely on imaging, neurophysiological, neurobehavioral and postmortem histopathological evaluations. This study describes a novel approach of assessment of toxicity to the central nervous system (CNS) using serum level of glial fibrillary acidic protein (GFAP) as a biomarker following exposure to a neurotoxicant, kainic acid (KA), in rats. Methods: Neurotoxicity in adult rats was induced by subcutaneous injections of KA (9 mg/kg). A status epileptic for at least 2h after KA injection was confirmed in all animals. Serum and CSF levels of several biomarkers were examined using ELISA at different time points. Neuronal degeneration and the level of the biomarkers in brain tissue were analyzed using immunohistochemistry. Results: The results demonstrate a significant increase in the CSF level of GFAP at 24h following KA treatment. In the brain, the increased GFAP levels were observed at 24 and 48h in selected regions associated with neurodegeneration assessed using Fluoro-Jade and H&E. A similar temporal profile of GFAP levels was observed in serum. Serum levels GFAP increased significantly at 48h, whereas at 24 and 72h its level was higher but not significantly different as compared to control. The increase of GFAP was correlated with the increased levels of neuronal biomarkers of CNS injury including ubiquitin C-terminal hydrolase-L1 (UCH-L1) and αII-spectrin break down products (SBDP145, SBDP150 and SBDP120) assessed in CSF. Conclusion: This study demonstrated the validity of the use serum GFAP levels for assessment of neurotoxic damage. The accumulation of GFAP in serum is correlated with spatiotemporal profiles of biomarkers of CNS injury assessed in CSF and is associated with progression of gliosis and neurodegeneration. This study is an important step in development of novel strategies for safety assessments of new drugs and diagnostics using serum biomarker assays.

ABSTRACT FINAL ID: 2688 Poster Board -438
TITLE: *In Vitro* Cytotoxic Effects of Deepwater Horizon Oil Spill Tarmats

AUTHORS (FIRST INITIAL, LAST NAME): D. Bhattacharya[1], M. Zheng[2, 1], M. Ahuja[1], P. Clement[2], and M. Dhanasekaran[1].
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KEYWORDS: Gulf Oil spill, Tarmat, Environmental toxicity

ABSTRACT BODY: Introduction: The Deepwater Horizon oil spill (also known as the BP oil spill) is one of the major oil spills in the history that impacted the Gulf of Mexico. BP used Corexit 9500A (dispersant) to disperse the spilled oil. A portion of undispersed crude oil which did not have sufficient amount of Corexit dispersant formed an emulsion which is referred as “mousse” (semisolid deposits), which eventually interacted with near shore sediment to form “tarmat” (buried oil deposits). The tarmat has been found in large amounts along the Gulf region and the beach users in Florida, Alabama, Mississippi and Louisiana are often exposed to tarmat fragments. However, the effects of tarmat deposits on the health of human and the ecosystems (animals, fish, and birds) have not been studied. Aim: Evaluate the *in vitro* cytotoxic effects and the mechanism of toxicity of tarmat in hippocampal (neuron), kidney (nephron) and epithelial cells. Methods: Water accommodated fraction of tarmat was used in the present study. Cytotoxicity was elucidated by the MTT assay and cellular morphology assessment. Markers of oxidative stress and apoptosis were studied for the mechanism of toxicity. Statistical analysis was performed using Sigma-stat. Key Findings: Tarmat induced dose-dependent cellular toxicity. Tarmat significantly inhibited the cell viability in the hippocampal (H19), kidney (HEK 293) and epithelial (MCF-10A) cells. Distinctive cellular morphological changes associated to cytotoxicity were observed. Tarmat generated significant amount of reactive oxygen species. Significance: The data show that further studies that can elucidate the effects of tarmat on neurophysiological and neurochemical changes in an animal model.

2013 Society of Toxicology Annual Meeting
Late-Breaking Abstracts

ABSTRACT FINAL ID: 2689 Poster Board -439
TITLE: Effects of Cell Phone Radiation on Rat Brain

AUTHORS (FIRST INITIAL, LAST NAME): E. Cuevas[1], M. E. Wyde[2], S. M. Lantz[1], B. R. Robinson[1], H. Rosas-Hernandez[1], R. W. Hamilton[1], P. C. Howard[3], N. J. Walker[2], M. G. Paule[1], and S. F. Ali[1].
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KEYWORDS: Neurotoxicity, Oxidative stress, Cell phone radiation

ABSTRACT BODY: Cell phone radiofrequency (RF) energy has been associated with increased risk for brain damage, including blood brain barrier disruption. This study assessed the effects of specific absorption rates (SAR) of cell phone RF radiation on rat brains as measured by different biomarkers. Adult male and female Harlan Sprague-Dawley rats were exposed to Global System for Mobile Communication (GSM) or Code Division Multiple Access Interim Standard 95 (CDMA IS-95) modulation at 3, 6, or 9 W/Kg. Animals were exposed 18.7 hours /day (10 min on, 10 min off cycles) for 8 weeks. Control rats were housed in similar chambers without RF exposure. One hour after the last exposure, rats were sacrificed by decapitation and their forebrains were dissected and immediately frozen for subsequent western blot analysis probed with GPX, SOD, Claudin-5, and heat-shock proteins family (HSP90, HSP70, HSP27) antibodies. Brains were also sectioned and analyzed for astrogliosis (GFAP) and stress markers (RAGE and HSP90 antibodies). GSM exposure did not produce any significant changes in GPX, SOD-1 and Claudin-5 in males or females. CDMA IS-95 exposure significantly decreased in GPX and SOD-1, while increasing Claudin-5, in males and females. GSM exposures did not produce any significant changes in HSP90, HSP70, and HSP27 levels in either males or females. CDMA IS-95 exposure significantly increased HSP90, HSP70 and HSP27 at 3, 6, and 9 W/Kg in females but only HSP27 and HSP90 at 9 W/Kg in males. At 9 W/Kg in both genders, exposure to either GSM or CDMA modulation increased astrocytosis and number of RAGE- and HSP90-positive cells. These results indicate that the effects of different types of cell phone RF are dose- and gender-dependent. Studies are currently underway to elucidate the observed gender differences and to evaluate whether RF exposure causes neuronal damage in specific brain regions. (FDA/NCTR IAG # 224-070-0007) (NIH IAG # Y1ES1027).

ABSTRACT FINAL ID: 2690 Poster Board -440
TITLE: Neuroprotective Effects of *Scutellaria lateriflora* Against Oxidative Stress-Induced Cell Death

AUTHORS (FIRST INITIAL, LAST NAME): M. Lohani[1], M. Ahuja[2], R. Amin[2], D. Schwartz[1], D. Shannon[3], A. Romando[4], B. Kemppainen[1], and M. Dhanasekaran[2].
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KEYWORDS: Scutellaria lateriflora, Neuroprotection, antioxidant

ABSTRACT BODY: Introduction: Oxidative stress plays an important role in the pathogenesis of neuronal diseases, resulting in cellular damage and increasing the risk for cell death. Bioactive compounds (phytochemicals) present in the medicinal plants neutralize or scavenge unstable/toxic reactive free radicals and thus preventing them from attacking vital components of cells. *Scutellaria lateriflora*, a native plant of North America has been used by Americans as a nerve tonic for more than 200 years. However, the neuroprotective effects of *Scutellaria lateriflora* are not fully understood. Therefore, the objective of this study is to investigate the antioxidant effects of *Scutellaria lateriflora*. Experimental Procedures: Neuroprotective effects were evaluated against using hydrogen peroxide induced cytotoxicity using cell viability assay. The antioxidant potential of alcoholic extracts of *Scutellaria lateriflora* was determined in rodent brain tissue, differentiated hippocampal (H19-7) and pheochromocytoma (PC-12) cells. One way ANOVA was used for finding statistically significant differences between each mean value at $P \leq 0.05$ Results and Conclusion: The ethanolic extract scavenged the hydroxyl radicals in the brain homogenate. The alcoholic extracts significantly scavenged reactive oxygen species generated in both H19-7 and PC-12 cells. Decreased ROS generations correlated strongly with increased cell viability. Furthermore, alcoholic extract of *Scutellaria lateriflora* dose-dependently suppressed caspase 3, caspase 9 and cytochrome C expression.

Therefore, the findings of the research indicate that *Scutellaria lateriflora* is a potent neuroprotectant against oxidative stress-induced cell death.

ABSTRACT FINAL ID: 2691 Poster Board -441
TITLE: Guidance to Develop and Maintain Competency for Neurotoxicity Assessments

AUTHORS (FIRST INITIAL, LAST NAME): S. S. Anand[1], P. Mukerji[1], L. A. Malley[1], and L. P. Sheets[2].
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KEYWORDS: neurotoxicity assessment, testing procedures, proficiency

ABSTRACT BODY: This presentation proposes best practices for assessing neurotoxicity of chemicals and pesticides, based on a functional observational battery (FOB), a computer-automated test of motor activity (MA), and neuropathology. The OECD (TG424) and USEPA (OPPTS 870.6200) test guidelines require laboratories to demonstrate the sensitivity and reliability of the devices, suitability of testing procedures, and minimize observer variability. This is accomplished by conducting experiments periodically using positive control chemicals known to affect FOB (behavioral, autonomic, neuromuscular, sensorimotor, physiologic, and central nervous system functions), MA (increases and decreases), and neuropathology (peripheral and central nervous system). When initially developing the competency, positive control experiments should include both sexes and multiple doses, and should employ training and performance metrics to evaluate personnel proficiency. Further positive control studies are required periodically and after changes to the facility, but animal use is minimized by using one sex and one dose if personnel remain the same. Experimental variables are controlled by testing in replicates, blind observation, sound attenuation, counterbalancing, acclimation in the neurobehavioral laboratory, proceeding from the least interactive to the most interactive assessments, daily instrument verifications, and use of the same observer for a given study. Interpretation of data should give consideration to the dose response, the number of parameters affected, primary vs. secondary effects (neurotoxicity vs. systemic toxicity), inherent variability of the endpoint, baseline data, and historical control data. Data and recommendations involving the approach, reference chemicals (e.g., carbaryl, triadimefon, acrylamide, trimethyltin, amphetamine) and doses provided in this presentation are intended to improve the efficiency and consistency of procedures and to improve the quality of neurotoxicity data across laboratories.

ABSTRACT FINAL ID: 2692 Poster Board -442
TITLE: Proteasome Inhibitors Do Not Inhibit HtrA2/Omi to Induce Peripheral Neuropathy

AUTHORS (FIRST INITIAL, LAST NAME): J. J. Senn[1], V. Csizmadia[1], P. Hales[2], L. Dick[3], and V. Kadambi[1].
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KEYWORDS: Neuropathy, Neurotoxicty, Proteasome Inhibitors

ABSTRACT BODY: Based on unprecedented efficacy, the proteasome inhibitor (PI) bortezomib is now a cornerstone of multiple myeloma treatment. Nevertheless, in a subset of patients bortezomib causes peripheral neuropathy and this can limit its potential benefit to patients. Although the mechanism of bortezomib associated neuropathy is unknown, we have previously suggested that it is related to the mechanism of action. Recently Arastu-Kapur et al. have reported that the serine protease HtrA2/Omi was inhibited by bortezomib and not by carfilzomib. Further, since HtrA2/Omi is involved in neuronal survival they suggested that this off target inhibitionby bortezomib could be the mechanism underlying bortezomib associated peripheral neuropathy. To confirm and extend these published results, we investigated the effects of these two PIs on HtrA2/Omi activity in recombinant enzyme assays, in SH-SY5Y neuroblastoma-, and wild type and HtrA2/Omi double negative mouse embryonic fibroblast cells (MEF). In contrast to the results of Arastu-Kapur et al., our results clearly demonstrated that neither bortezomib nor carfilzomib inhibits HtrA2/Omi in recombinant enzyme assays at concentrations up to 100µM. As a positive control we used Ucf-101 an HtrA2/Omi specific inhibitor which in our assay behaved in a manner consistent with the published literature . Similarly, in MEF cells, only Ucf-101 prevented the

degradation of validated HtrA2/Omi substrates eIF4G1 and UCH-L1, while neither bortezomib nor carfilzomib prevented the degradation of these two substrates. In conclusion, we have assessed the protease activity of HtrA2/Omi both *in vitro* with purified enzyme and in cultured cells and we find that neither PI inhibits this protease. Therefore we think it is unlikely that PI associated peripheral neuropathy is caused by off target inhibition of HtrA2/Omi. Further research is needed to understand the side effects of PIs.

ABSTRACT FINAL ID: 2693 Poster Board -443
TITLE: Iron Overload Hemochromatosis Is Associated with Elevated Anxiety in Mice

AUTHORS (FIRST INITIAL, LAST NAME): J. Chang, A. Sukumaran, and J. Kim.
INSTITUTIONS (ALL): Department of Pharmaceutical Sciences, Northeastern University, Boston, MA.
KEYWORDS: Anxiety, Hemochromatosis, Iron overload

ABSTRACT BODY: Mutations in hemochromatosis (HFE) gene disrupt iron metabolism and lead to iron overload condition called hereditary hemochromatosis. Such mutations are commonly found in Caucasian population of European descent and are responsible for increased iron deposition in tissues, resulting in liver cirrhosis, cardiomyopathy and premature death. While several studies have suggested the relationship between iron status and mental illness, such as schizophrenia and autism, the effect of hemochromatosis on behavioral patterns is not clearly understood. Hence, we examined behaviors of Hfe-deficient mice, a mouse model that recapitulates iron overload hemochromatosis in humans. Using 8-week-old Hfe-knockout (Hfe-Ko) and their wild-type control (WT; 129S6/SvEvTac) mice, marble-burying test was conducted to determine response to natural tendencies of rodents to bury marbles. During 10-min test, Hfe-Ko mice buried less marbles (20 ± 1%; n=13; mean ± SEM) compared with WT mice (56 ± 1%; n=10; $p<0.001$), possibly due to increased anxiety in a novel environment. To directly test anxiety, elevated plus maze paradigm was employed. Hfe-Ko mice, compared with WT mice, traveled less distance during 5-min test (5.0 ± 0.3 vs. 6.8 ± 0.4 meters, respectively; p=0.003) and displayed reduced number of entries to the open arm (3.5 ± 0.8 vs. 8.4 ± 2.2 entries; p=0.022) with increased latency (130 ± 37 vs. 21 ± 10 seconds; p=0.021). These data indicate increased levels of anxiety due to iron overload upon Hfe deficiency. In addition, there was no significant difference in motor coordination, tested by rotarod device, between the two genotypes, eliminating a possibility that our findings above resulted from impaired motor functions. These results assert that mice with genetic iron overload display elevated anxiety in response to new environment. Our model provides genetic basis for a relationship between iron overload and mental/emotional disturbances, such as schizophrenia and autism. Supported by NIH R00 ES017781.

ABSTRACT FINAL ID: 2694 Poster Board -444
TITLE: Role of Nicotinic Acetylcholine Receptors(nAChRs) in Methylmercury (MeHg)-Induced Calcium Dysregulation in Rat PC12 Cells and Mouse Spinal Cord Neurons (SCNs)

AUTHORS (FIRST INITIAL, LAST NAME): M. Rios[1, 2], G. Ramanathan[2], and W. Atchison[2, 1].
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KEYWORDS: Methylmercury Neurotoxicity, Nicotinic Acetylcholine Receptors, Intracellular Calcium

ABSTRACT BODY: MeHg alters Ca^{2+}_i homeostasis in neurons *in vitro* and *ex vivo*. Sustained elevations in $[Ca^{2+}]_i$ can activate various signal transductions cascades leading to cell degeneration and severe pathological effects. We investigated the role that nAChRs play in MeHg-induced Ca^{2+}_i dysregulation. nAChRs increase $[Ca^{2+}]_i$ by intra- and extracellular mechanisms. Two model systems were used: primary cultures of mouse SCNs and differentiated PC12 cells; both express nAChRs of several phenotypes. SCNs and PC12 cells were exposed to [MeHg] of 0.1, 0.2, 0.5, 1 µM and 1, 2, 5 µM, respectively. To measure $[Ca^{2+}]_i$ changes induced by MeHg, we used microfluorimetric imaging with fura-2AM. MeHg caused a biphasic increase in fura-2AM fluorescence ratio in both cell types. MeHg-induced $[Ca^{2+}]_i$ elevation occurs in two kinetically-distinct phases; in other cells these have been demonstrated to result from 1) Ca^{2+} release from intracellular stores (Phase 1) and 2)

extracellular Ca^{2+} influx (Phase 2). In both cell types, the time to onset of the two phases was [MeHg]-dependent. To test whether nAChRs contribute to MeHg-induced cytotoxicity pathways in the two cell types, two nonspecific and one specific antagonist, hexamethonium (HEX), mecamylamine (MEC) and methyllycaconitine (MLA), respectively, were used. We tested whether these compounds delayed the time to onset of MeHg-induced effects. HEX and MLA significantly delayed the onset of MeHg-induced increase in the 1st but not 2nd phase for SCNs, indicating an effect on intracellular stores. In PC12 cells, MEC delayed both phase 1 and phase 2 at 1 and 2 μM, but not 5 μM [MeHg]. Thus, both MLA-sensitive homomeric, and HEX/MEC-sensitive heteromeric nAChRs contribute to MeHg-induced changes in $[Ca^{2+}]_i$. However, the different nAChRs subtypes act in a cell-type specific mechanism, and affect the two phases of $Ca^{2+}{}_i$ differently. Supported by NIEHS grant R01ES03299 and NINDS grant R25NS065777.

ABSTRACT FINAL ID: 2695 Poster Board -445
TITLE: The Effects of Low Dose Lead Exposure on the GABAergic System in Developing Zebrafish

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KEYWORDS: Lead, GABA, Zebrafish

ABSTRACT BODY: Lead (Pb) is a very stable element that is found in the environment and targets multiple organ systems including the heart, bone, kidney, intestine, reproductive system, and the central nervous system (CNS). The CNS is the most sensitive system during early development due to rapid cell proliferation, migration, differentiation, and complex cell interactions. The Centers for Disease Control recently revised the blood level of concern of 10 μg/dL to a current reference value of 5μg/dL due to research providing evidence that low dose lead exposure causes neurodevelopmental alterations in children including a reduced IQ and a decreased learning and reading ability and an increased odds ratio for attention deficit hyperactivity disorder. There is an abundance of research on lead neurotoxicity, but the mechanisms of neurotoxicity are not yet completely understood. One of the key components of CNS development is the GABAergic system. Gamma-aminobutyric acid (GABA) is one of the primary inhibitory neurotransmitters in the adult brain. However, during development GABA acts as an excitatory neurotransmitter which contributes to cell proliferation, neuron migration and growth, and synapse formation. There are multiple studies that show the location of GABA in the zebrafish brain. However, little is known on the adverse effects of chemical exposure on GABA during development. To characterize impacts of lead exposure on the GABAergic system, zebrafish embryos were exposed to 10, 50, or 100 ppb (μg/L) lead or a control treatment shortly after fertilization through 24, 48, 60, and 72 hours post fertilization (hpf). Expression of seven genes with roles in the GABAergic pathway including GABA synthesizers, transporters, and receptors were analyzed using quantitative PCR (qPCR). Analysis identifying gene expression alterations are dose and developmental time point specific. Current and future work is utilizing immunohistochemistry (IHC) to further our understanding of the interaction between lead and altered GABA expression in the developing brain.

ABSTRACT FINAL ID: 2696 Poster Board -446
TITLE: Neurotoxicity of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)

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KEYWORDS: 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, PhIP, Neurotoxicity

ABSTRACT BODY: The heterocyclic amine and suspect carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is an abundant amino-imidazoazaarene formed during high-temperature cooking, particularly in meat. It may be consumed

in single high doses and/or chronically at lower doses. Structurally related compounds have been found to affect activity of enzymes relevant to dopamine metabolism. Both PhIP and a major metabolite have also been shown to cross the blood-brain-barrier. Further, exposure to heterocyclic amines has also been linked to tremor in humans, although the mechanism is not well understood. The neurotoxic effects of PhIP have not been well examined. The goal of these experiments was to perform an initial characterization of the neurotoxic effects of PhIP exposure *in vitro* and *in vivo*. Preliminary experiments indicate that after a 24-hour exposure in primary neuronal cultures, PhIP is selectively toxic to dopaminergic neurons. Dose-response studies are being completed. Competitive uptake studies in SY5Y cells, using PhIP and tritiated dopamine suggest that PhIP may not enter cells through the dopamine transporter. Given the lipophillic structure of PhIP, it is possible that it may pass directly through cell membranes, which will need to be evaluated using radiolabeled PhIP. Stereotaxic infusion of PhIP into the medial forebrain bundle was also conducted in rats at a dose and regimen similar to that of other classic Parkinson's disease (PD) models. Preliminary pathological examination indicates that in the substantia nigra of PhIP-treated rats, degenerating, silver-positive cells with a morphology characteristic of dopaminergic neurons are observed. Taken together, these data suggest that PhIP neurotoxicity and its potential relevance to PD should be evaluated further.

ABSTRACT FINAL ID: 2697 Poster Board -447
TITLE: Notch Signaling As a Therapeutic Target for Parkinson's Disease

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KEYWORDS: Neurodegeneration, Parkinson's Disease, Notch Signaling

ABSTRACT BODY: Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder characterized by the progressive degeneration of dopaminergic (DA) neurons of the substantia nigra and associated intracytoplasmic inclusions (Lewy bodies). Because PD results from a complex interaction between genetic and environmental factors, a thorough understanding of these factors is essential for the development of effective therapeutic interventions. The role of the Notch pathway in neurological disorders such as stroke, Alzheimer's disease and PD has begun to be unraveled in recent years. Among the various accepted experimental models of PD, neurotoxicants still remain the most popular tools for producing DA cell death both *in vitro* and *in vivo*. Among the effective neurotoxicants, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is the most studied and widely used for modeling environmental causes of PD in both nonhuman primates and rodents in efforts to evaluate neuroprotective agents and signaling pathways in PD. In the present studies, we targeted the Notch pathway both in *in vitro* and *in vivo* studies using gamma-secretase inhibitor (GSI) and Notch1 antisense (NAS) transgenic mice (which exhibit greatly diminished Notch1 expression) and MPTP. Here, using biochemical and molecular biology techniques, we show that SNc DA neurons and striatal terminals in GSI and NAS mice are protected from MPTP-induced degeneration. Tyrosine hydroxylase and dopamine transporter immunoreactivity in the ventral midbrain SNc and striatum are more protected from MPTP in NAS mice over its saline-treated controls. Microglial activation, depletion of striatal fibers and dopamine and the development of motor deficits are significantly less in notch1 inhibited experiments. Together, these proposed studies suggest new mechanisms that may be involved in the pathogenesis of PD and that might be exploited in the development of novel therapeutics aimed at slowing or halting neurodegeneration in patients with PD.
