2011 Annual Meeting Abstract Supplement

Late-Breaking and Grace Period Abstract Submissions

These abstracts will be available on-line through the Itinerary Planner until April 30, 2011.

50th Anniversary Annual Meeting & ToxExpo™
March 6–10, 2011
Washington, D.C.

www.toxicology.org
# 2011 Annual Meeting Abstract Supplement

## Poster Session* Schedule

### Monday Morning, March 7

9:30 AM to 12:30 PM

<table>
<thead>
<tr>
<th>SESSION TITLE</th>
<th>ABSTRACT NUMBERS</th>
<th>POSTER BOARD #s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypersensitivity: Methods and Mechanisms</td>
<td>80-98</td>
<td>101-119</td>
</tr>
<tr>
<td>Epigenetic Mechanisms</td>
<td>99-110</td>
<td>125-136</td>
</tr>
<tr>
<td>DNA Replication and Repair</td>
<td>111-122</td>
<td>137-148</td>
</tr>
<tr>
<td>Bioinformatic Profiling and Computational Pathway Prediction</td>
<td>123-159</td>
<td>212-248</td>
</tr>
<tr>
<td>Neurodegenerative Diseases</td>
<td>160-182</td>
<td>301-323</td>
</tr>
<tr>
<td>Cardiovascular Toxicology</td>
<td>183-218</td>
<td>325-360</td>
</tr>
<tr>
<td>Carcinogenesis I</td>
<td>219-250</td>
<td>401-432</td>
</tr>
<tr>
<td>Inflammatory Mediators in Disease Pathogenesis</td>
<td>251-264</td>
<td>435-448</td>
</tr>
<tr>
<td>Metals I</td>
<td>265-292</td>
<td>501-528</td>
</tr>
<tr>
<td>Metals II</td>
<td>293-314</td>
<td>537-548 and 601-610</td>
</tr>
<tr>
<td>Genotoxicity</td>
<td>315-343</td>
<td>613-641</td>
</tr>
<tr>
<td>Receptor and Receptor-Mediated Toxicity</td>
<td>344-379</td>
<td>701-736</td>
</tr>
<tr>
<td>Cell Signaling and Gene Regulation</td>
<td>380-416</td>
<td>801-837</td>
</tr>
<tr>
<td><strong>Visiting Student Poster Session</strong></td>
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<td><strong>BY INVITATION ONLY</strong></td>
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### Monday Afternoon, March 7

1:00 PM to 4:30 PM

<table>
<thead>
<tr>
<th>SESSION TITLE</th>
<th>ABSTRACT NUMBERS</th>
<th>POSTER BOARD #s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug Induced Liver Injury</td>
<td>420-443</td>
<td>101-124</td>
</tr>
<tr>
<td>Acetaminophen Hepatotoxicity</td>
<td>444-463</td>
<td>129-148</td>
</tr>
<tr>
<td>Risk Assessment: Computational Approaches, Analyses, and Applications</td>
<td>464-505</td>
<td>207-248</td>
</tr>
<tr>
<td>Animal Models in Toxicological Research</td>
<td>506-534</td>
<td>301-329</td>
</tr>
<tr>
<td>Animal Models in Toxicology</td>
<td>535-562</td>
<td>333-360</td>
</tr>
<tr>
<td>Inhalation and Cardiopulmonary Toxicology</td>
<td>563-609</td>
<td>401-447</td>
</tr>
<tr>
<td>Children’s Health/Juvenile Toxicology</td>
<td>610-630</td>
<td>501-521</td>
</tr>
<tr>
<td>Skin</td>
<td>631-648</td>
<td>525-542</td>
</tr>
<tr>
<td>Immunotoxicity: Methods and Evaluation</td>
<td>649-667</td>
<td>547-548 and 601-617</td>
</tr>
<tr>
<td>Mechanisms of immunotoxicity</td>
<td>668-698</td>
<td>618-648</td>
</tr>
<tr>
<td>Biological Modeling: Computational Approaches, Mixtures, Multiroute and Lifestage Applications</td>
<td>669-737</td>
<td>701-736 and 846-848</td>
</tr>
<tr>
<td>Kidney</td>
<td>738-774</td>
<td>801-837</td>
</tr>
<tr>
<td>Pharmaceutical Safety Assessment: Therapeutic Agents</td>
<td>775-812</td>
<td>901-938</td>
</tr>
</tbody>
</table>

*Late-Breaking or Grace Period Abstract Poster Sessions.

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### 2011 Annual Meeting Abstract Supplement

**Poster Session* Schedule**

**Tuesday Morning, March 8**
9:00 AM to 12:30 PM

<table>
<thead>
<tr>
<th>SESSION TITLE</th>
<th>ABSTRACT NUMBERS</th>
<th>POSTER BOARD #s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late-Breaking Abstracts - Session I</td>
<td>2700-2724</td>
<td>101-116 and 201-211*</td>
</tr>
<tr>
<td>Alternative Approaches to Animal Testing for</td>
<td>959-998</td>
<td>118-148</td>
</tr>
<tr>
<td>Toxicological Research</td>
<td></td>
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<tr>
<td>Alternatives to Mammalian Models for Testing</td>
<td>990-1021</td>
<td>217-248</td>
</tr>
<tr>
<td>Reproductive Toxicology I</td>
<td>1022-1048</td>
<td>301-327</td>
</tr>
<tr>
<td>Reproductive Toxicology II</td>
<td>1049-1076</td>
<td>333-360</td>
</tr>
<tr>
<td>Hepatotoxicity</td>
<td>1077-1107</td>
<td>401-431</td>
</tr>
<tr>
<td>Cholestasis, Lipid Homeostasis, and Liver Toxicity</td>
<td>1108-1120</td>
<td>436-448</td>
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<tr>
<td>Ah Receptor in Immune Regulation and Toxicity</td>
<td>1121-1139</td>
<td>501-519</td>
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<tr>
<td>ImmunoSafety Methods in Non-Rodents</td>
<td>1140-1152</td>
<td>522-534</td>
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<tr>
<td>Late-Breaking Abstracts - Session II</td>
<td>2725-2735</td>
<td>537-547</td>
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<tr>
<td>Stem Cell Toxicology</td>
<td>1153-1173</td>
<td>601-621</td>
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<tr>
<td>Nanotoxicology: Carbon Nanotubes, Carbon Nanoparticles, and Quantum Dots</td>
<td>1174-1197</td>
<td>625-648</td>
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<tr>
<td>Epidemiology and Exposure Evaluations</td>
<td>1198-1229</td>
<td>701-732</td>
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<tr>
<td>Exposure Assessments and Biomonitoring Applications</td>
<td>1230-1268</td>
<td>801-839</td>
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<tr>
<td>Oxidative Stress and Redox Biology</td>
<td>1269-1308</td>
<td>901-940</td>
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**Tuesday Afternoon, March 8**
1:00 PM to 4:30 PM–Exhibit Hall

<table>
<thead>
<tr>
<th>SESSION TITLE</th>
<th>ABSTRACT NUMBERS</th>
<th>POSTER BOARD #s</th>
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</thead>
<tbody>
<tr>
<td>Medical Devices</td>
<td>1312-1323</td>
<td>101-112</td>
</tr>
<tr>
<td>Neurotoxicity of Pesticides</td>
<td>1324-1358</td>
<td>114-148</td>
</tr>
<tr>
<td>Genetic Polymorphisms</td>
<td>1359-1374</td>
<td>201-216</td>
</tr>
<tr>
<td>Metal Neurotoxicity: Methylmercury and General</td>
<td>1375-1402</td>
<td>221-248</td>
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<tr>
<td>Mutagenecity</td>
<td>1403-1422</td>
<td>301-320</td>
</tr>
<tr>
<td>Methods in Biomarker Discovery and Validation</td>
<td>1423-1452</td>
<td>333-360</td>
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<td>Nanotoxicology</td>
<td>1453-1496</td>
<td>401-444</td>
</tr>
<tr>
<td>Drug Allergy, Pseudoallergy, IDRH, and Autoimmunity</td>
<td>1497-1515</td>
<td>501-519</td>
</tr>
<tr>
<td>Risk Assessment and Regulatory Policy Applications</td>
<td>1516-1540</td>
<td>524-548</td>
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<tr>
<td>Safety and Risk Assessment: Critical Characterizations for Chemicals and New Concerns</td>
<td>1541-1582</td>
<td>601-642</td>
</tr>
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</table>

*Late-Breaking or Grace Period Abstract Poster Sessions.

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# Late Mechanistic Toxicology

**Phase II:** Cellular Developmental Toxicology

**Phase III:** Late Developmental Toxicology

**Phase IV:** Late Risk Assessment: Conceptual Constructs and Current Controversies

**Phase V:** Arsenic

**Phase VI:** Chemical and Biological Weapons

**Phase VII:** Nanotoxicology: Metal Oxides, Silver, Gold, and Silica Nanoparticle Toxicity

**Phase VIII:** Pesticides: General

## Xenobiotic Biotransformation

<table>
<thead>
<tr>
<th>SESSION TITLE</th>
<th>ABSTRACT NUMBERS</th>
<th>POSTER BOARD #s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xenobiotic Biotransformation</td>
<td>1817-1836</td>
<td>101-120</td>
</tr>
<tr>
<td>Phase I and II Biotransformation Enzymes</td>
<td>1837-1864</td>
<td>121-148</td>
</tr>
<tr>
<td>Toxicology Education: K-12 and Beyond</td>
<td>1865-1880</td>
<td>201-216</td>
</tr>
<tr>
<td>Late-Breaking Abstracts-Session III</td>
<td>2736-2745</td>
<td>217-248</td>
</tr>
<tr>
<td>Safety Testing of Pharmaceuticals</td>
<td>1881-1893</td>
<td>301-313</td>
</tr>
<tr>
<td>Pharmaceutical Safety Assessment: Novel Methods</td>
<td>1894-1932</td>
<td>322-360</td>
</tr>
<tr>
<td>Risk Assessment: Conceptual Constructs and Current Controversies</td>
<td>1933-1967</td>
<td>407-441</td>
</tr>
<tr>
<td>Arsenic</td>
<td>1968-2009</td>
<td>501-542</td>
</tr>
<tr>
<td>Chemical and Biological Weapons</td>
<td>2010-2050</td>
<td>601-641</td>
</tr>
<tr>
<td>Nanotoxicology: Metal Oxides, Silver, Gold, and Silica Nanoparticle Toxicity</td>
<td>2051-2085</td>
<td>701-735</td>
</tr>
<tr>
<td>Pesticides: General</td>
<td>2086-2120</td>
<td>801-835</td>
</tr>
<tr>
<td>Late-Breaking Abstracts-Session IV</td>
<td>2766-2776</td>
<td>837-848</td>
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<tr>
<td>Metal Neurotoxicity: Manganese and Lead</td>
<td>2121-2151</td>
<td>901-931</td>
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## Wednesday Morning, March 9

**9:00 AM to 12:30 PM**

## Wednesday Afternoon, March 9

**1:00 PM to 4:30 PM**

## Toxicology of the Gulf Oil Spill

<table>
<thead>
<tr>
<th>SESSION TITLE</th>
<th>ABSTRACT NUMBERS</th>
<th>POSTER BOARD #s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late-Breaking Abstracts-Session V</td>
<td>2777-2787</td>
<td>101-111</td>
</tr>
<tr>
<td>Nanotoxicology: In Vitro or Ex Vivo Studies</td>
<td>2154-2184</td>
<td>118-148</td>
</tr>
<tr>
<td>Late-Breaking Abstracts-Session VI</td>
<td>2788-2811</td>
<td>201-224</td>
</tr>
<tr>
<td>Developmental Basis of Adult Disease</td>
<td>2185-2203</td>
<td>230-248</td>
</tr>
<tr>
<td>Cellular Effects of Natural Product Extracts</td>
<td>2204-2236</td>
<td>301-333</td>
</tr>
<tr>
<td>Persistent Organic Compounds (POPs)</td>
<td>2237-2256</td>
<td>341-360</td>
</tr>
<tr>
<td>Pharmacokinetics and Disposition</td>
<td>2257-2285</td>
<td>401-429</td>
</tr>
<tr>
<td>Risk Assessment: Models and Approaches for Inhaled Agents</td>
<td>2286-2302</td>
<td>432-448</td>
</tr>
<tr>
<td>Mechanistic Assessment of Chemical Mixtures</td>
<td>2303-2316</td>
<td>501-514</td>
</tr>
<tr>
<td>Mechanisms of Aspiration Injury and Airway Disease</td>
<td>2317-2337</td>
<td>516-536</td>
</tr>
<tr>
<td>Late-Breaking Abstracts-Session VII</td>
<td>2812-2821</td>
<td>537-547</td>
</tr>
<tr>
<td>Toxicology of the Gulf Oil Spill</td>
<td>2338-2342</td>
<td>601-605</td>
</tr>
<tr>
<td>Endocrine Toxicology</td>
<td>2343-2377</td>
<td>614-648</td>
</tr>
<tr>
<td>Aquatic and Ecotoxicology</td>
<td>2378-2409</td>
<td>701-732</td>
</tr>
<tr>
<td>Food Safety and Nutrition</td>
<td>2410-2443</td>
<td>801-834</td>
</tr>
<tr>
<td>Late-Breaking Abstracts-Session VIII</td>
<td>2822-2861</td>
<td>901-940</td>
</tr>
</tbody>
</table>

*Late-Breaking or Grace Period Abstract Poster Sessions.*

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# 2011 Annual Meeting Abstract Supplement

## Poster Session* Schedule

**Thursday Morning, March 10**  
8:00 AM to 12:00 NOON—East Registration Area

<table>
<thead>
<tr>
<th>SESSION TITLE</th>
<th>ABSTRACT NUMBERS</th>
<th>POSTER BOARD #s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternatives to Mammalian Models</td>
<td>2509-2542</td>
<td>101-134</td>
</tr>
<tr>
<td>Alternatives to Animal Models in Toxicology</td>
<td>2543-2575</td>
<td>137-169</td>
</tr>
<tr>
<td>Disease Prevention</td>
<td>2576-2593</td>
<td>177-194</td>
</tr>
<tr>
<td>Developmental Neurotoxicity: General</td>
<td>2594-2629</td>
<td>201-236</td>
</tr>
<tr>
<td>Cancinogenesis II</td>
<td>2630-2661</td>
<td>241-272</td>
</tr>
</tbody>
</table>

**Thursday Morning, March 10**  
8:00 AM to 12:00 NOON—Room 202

<table>
<thead>
<tr>
<th>SESSION TITLE</th>
<th>ABSTRACT NUMBERS</th>
<th>POSTER BOARD #s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grace Period Abstracts-Session I</td>
<td>2862-2875</td>
<td>101-116</td>
</tr>
<tr>
<td>Grace Period Abstracts-Session II</td>
<td>2876-2887</td>
<td>117-130</td>
</tr>
<tr>
<td>Grace Period Abstracts-Session III</td>
<td>2888-2902</td>
<td>133-147</td>
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<td>Grace Period Abstracts-Session IV</td>
<td>2903-2924</td>
<td>149-178</td>
</tr>
<tr>
<td>Grace Period Abstracts-Session V</td>
<td>2925-2934</td>
<td>201-210</td>
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The abstracts provided in this supplement are accessible online from the Itinerary Planner which is available through April 2011.
ABSTRACT FINAL ID: 2700 Poster Board #101
TITLE: A REVIEW OF DEVELOPMENTAL TOXICITY SCREENING USING ZEBRAFISH LARVAE

AUTHORS (LAST NAME, FIRST NAME): Diekmann, Heike1; Jones, Matt1; Dodd, Andrew1; Hill, Adrian J.1
INSTITUTIONS (ALL): 1. ADMET/Zebrafish, Evotec (UK) Ltd, Abingdon, Oxon, United Kingdom.
KEYWORDS: Developmental toxicity, Zebrafish, Teratogenicity and embryotoxicity.

ABSTRACT BODY: One of the most prominent and expensive causes of candidate drug failure is toxicity identified late in drug discovery. The pharmaceutical industry is therefore highly motivated to reduce this attrition by integrating new predictive assays earlier in the screening cascade to help identify these liabilities. Zebrafish have gained increasing popularity for drug testing over the past decade due to numerous advantages over other vertebrates, including the use of an in vitro-like assay format requiring only single milligrams of test compound to obtain a swift, full dose response readout and the transparency of the zebrafish larva, which decreases the need for invasive procedures and dissection. To evaluate its zebrafish developmental toxicity assay, Evotec has tested 90 blinded compounds and compared the outcome against results from mammalian embryofoetal developmental toxicity studies. Fertilized eggs (n=14 per concentration) were exposed to a six point standard aqueous concentration range throughout embryogenesis, followed by the assessment for 28 different morphological endpoints. Since uptake by zebrafish larvae is compound dependent and can vary significantly even between similar compounds, the actual body burden (ng/larva) was determined at the respective NOECs and LOECs via LCMS. Overall, the majority of drugs testing positive in traditional mammalian assays produced similar results in zebrafish larvae, although the specific phenotypes observed were different for some compounds. Some false negative results were explained due to a lack of compound absorption by the larva. Nevertheless, taking into account the interpretation from bioanalysis results, the overall predictivity for all compounds tested was 89.4%. This zebrafish assay is therefore considered a suitable screening tool for evaluating developmental toxicity and has the potential to be used efficiently early in drug discovery due to its higher throughput and lower assay costs compared to regulated mammalian developmental toxicity assays.

ABSTRACT FINAL ID: 2701 Poster Board #102
TITLE: DEVELOPMENT OF A NOVEL ASSAY PLATFORM FOR VISUAL ASSESSMENT OF HESC DERIVED NEURAL PROGENITOR CELL MIGRATION IN RESPONSE TO NEUROTOXICANTS

AUTHORS (LAST NAME, FIRST NAME): Powe, Allan C.1; Hodges, Kathryn L.1; Chilton, Jamie M.1; Herber, Renee L.2; Hulkaner, Keren L.1; Stice, Steven L.1
2. Platypus Technologies, LLC, Madison, WI, United States.
KEYWORDS: human neural progenitor cells, high throughput toxicity testing, cell migration.

ABSTRACT BODY: Migration of neural progenitors is an important process for the proper development and maintenance of the nervous system. Exposure to neurotoxicants during development can interfere with neural progenitor migration and lead to nervous system defects (for review see Rice and Barone, 2000). In fact, certain toxicants are known to interfere with neural stem cell migration (e.g., Moors, et al., 2009). Recent publications advocate the development of in vitro cell culture systems to identify and prioritize potential human developmental neurotoxicants among >80,000 untested commercial chemicals (Coecke et al., 2007; Gibb, 2008; Lein and Fryer, 2005). To that end, we are developing a high throughput screening (HTS) amenable assay to measure the migration of human embryonic stem cell (hESC) derived neural progenitors (hNP1™; Aruna Biomedical) by using a novel 96-well based cell migration assay platform (Oris™ Cell Migration Assay; Platypus Technologies). Stopers create central exclusion zones within the wells; cells are plated outside the zones and migrate inward once the stoppers are removed. At the end of the assay period, cells that have migrated into the central zones are stained and detected using fluorescence plate readers and/or visualized by microscopy. Using the Oris™ assay, we demonstrated that cytochalasin D, a disruptor of actin microfilaments, inhibits hNP1™ migration with an IC50 of ~15 nM. We also show that neurotrophic factors, such as basic fibroblast growth factor (bFGF), can accelerate neural progenitor migration as high as two-fold. Thus, this assay
ABSTRACT FINAL ID: 2702 Poster Board #103
TITLE: ASSESSMENT OF IMMUNIZATION AGAINST RECOMBINANT HUMAN BONE MORPHOGENETIC PROTEIN-2 (DIBOTERMIN ALPHA) ON REPRODUCTION AND DEVELOPMENT IN RABBITS

AUTHORS (LAST NAME, FIRST NAME): Wright, David J.; Minck, Dan; Nowak, John; Warner, Garvin; Cukierski, Mark

INSTITUTIONS (ALL): 1. Drug Safety, Pfizer, Groton, CT, United States. 2. Division of Metabolism and Endocrinology Products, FDA, White Oak, MD, United States. 3. Alnylam Pharmaceuticals, Cambridge, MA, United States.

KEYWORDS: BMP-2, Development, Fetus.

ABSTRACT BODY: Recombinant human bone morphogenetic protein (rhBMP-2, diboterin alpha) has clinically established bone forming activity at the site of administration. The protein in combination with implantable carriers has been approved for use in spinal surgery, fracture repair and certain dental and maxillofacial indications. Previous studies demonstrated that direct intravenous administration of rhBMP-2 to rats and rabbits did not produce developmental toxicity. In the present study, does were immunized against BMP-2, and the antibody response and developmental effects were evaluated. Female New Zealand White rabbits received 4 intramuscular injections (on pre-mating days 1, 8, 22, and 43 [3 days before mating]) of saline and Titermax® (Group 1) or rhBMP-2 (2 mg/dose) and Titermax® (Group 2). On GD29, fetuses were examined, and maternal and fetal anti-BMP-2 titer levels and neutralizing activity were assessed. There were no effects of immunization, anti-BMP-2 titer levels, or neutralizing capability on fetal external, visceral, or skeletal development. On GD 29 in Group 2, anti-BMP-2 antibodies were detected in 17 out of 18 does and in 127 of 151 fetuses. Fetal anti-BMP-2 antibody levels were similar to those in the does, and pregnancy did not boost the immune response to BMP-2 (based on sequential measurements in does only). Only a small number of fetuses (n = 4) displayed detectable neutralizing anti-BMP-2 antibodies, but there were no associated effects on the fetuses. Slight delays in ossification were associated with reduced fetal weights in 4 separate fetuses, but these fetuses did not display anti-BMP-2 antibodies with neutralizing activity. Therefore, there was no correlation between anti-BMP-2 antibodies with neutralizing activity and fetal development, including ossification. These results indicate that immunization of rabbits against rhBMP-2 did not affect embryo/fetal viability or morphological development.

ABSTRACT FINAL ID: 2703 Poster Board #104
TITLE: COMPARISON OF MEHG-INDUCED TOXICOGENOMIC RESPONSES ACROSS IN VIVO AND IN VITRO MODELS USED FOR DEVELOPMENTAL TOXICOLOGY

AUTHORS (LAST NAME, FIRST NAME): Robinson, Joshua F.; Theunissen, Peter T.; van Dartel, Dorien A.; Pennings, Jeroen L.; Faustman, Elaine M.; Piersma, Aldert H.


KEYWORDS: Toxicogenomics, development, metals.

ABSTRACT BODY: Toxicogenomic evaluations may improve toxicity prediction of in vitro-based developmental models, such as whole embryo culture (WEC) and embryonic stem cells (ESC), by providing a robust mechanistic marker which can be linked with responses associated with developmental toxicity in vivo. While promising in theory, toxicogenomic comparisons between in vivo and in vitro models are complex due to inherent differences in model characteristics and experimental design. We compared available toxicogenomic data assessing the impact of the known teratogen, methylmercury (MeHg) across a diverse set of in vitro and in vivo models to investigate the impact of experimental variables (i.e. model, dose, time) on our comparative assessments. We evaluated common and unique aspects at both
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

the functional (Gene Ontology) and gene level of MeHg-induced response. At the functional level, we observed
stronger similarity in MeHg-response between mouse embryos exposed in utero (2 studies), ESC, and WEC as
compared to liver, brain and mouse embryonic fibroblast MeHg studies. These findings were strongly correlated to the
presence of a MeHg-induced developmentally-related gene signature. In addition, we identified specific MeHg-induced
gene expression alterations associated with developmental signaling and heart development across WEC, ESC and in
vivo systems. However, the significance of overlap between studies was highly dependent on traditional experimental
variables (i.e. dose, time). In summary, we identify promising examples of unique gene expression responses which
show in vitro–in vivo similarities supporting the relevance of in vitro developmental models for predicting in vivo
developmental toxicity.

ABSTRACT FINAL ID: 2704 Poster Board #105
TITLE: THE EMBRYONIC STEM CELL TEST FOR PREDICTION OF TERATOGENICITY: ROCHE HISTORICAL DATA AND
MILESTONES TOWARDS FULL AUTOMATION

AUTHORS (LAST NAME, FIRST NAME): McGinnis, Claudia; Schaefer, Nicole; Iaiza, Patrick; Hochstrasser, Remo;
Fattinger, Christof; Kolaja, Kyle; Weiser, Thomas; Singer, Thomas; Clemann, Nicole

INSTITUTIONS (ALL): 1. Non-Clinical Safety, Hoffmann-La Roche, Basel, Switzerland. 2. Discovery Technologies,
Hoffmann-La Roche, Basel, Switzerland.

KEYWORDS: EST, teratogenicity, automation.

ABSTRACT BODY: The Embryonic Stem Cell Test (EST) was validated and implemented at Roche several years ago and
has since shown proven value in predicting for in vivo adverse developmental effects. This poster will show historical
EST data obtained over the past 6 years, as well as results on new assay adaptations that have been developed. In
addition, a major project is underway to fully automate the EST assay, leading to higher throughput and faster
turnaround times. As part of this project a novel device has been developed that allows for automated handling and
consistent culture of embryonic bodies.

ABSTRACT FINAL ID: 2705 Poster Board #106
TITLE: PRIMARY HUMAN SEROTOLI CELLS IN THE DEVELOPMENT OF IN VITRO MODELS TO ASSESS TESTICULAR
TOXICITY

AUTHORS (LAST NAME, FIRST NAME): Brown, Caitlin W.; Pietruska, Jodie; Hixon, Mary

INSTITUTIONS (ALL): 1. Pathology and Lab Medicine, Brown University, Providence, RI, United States.

KEYWORDS: Primary Human Cells, Testis, MEHP.

ABSTRACT BODY: We have previously shown that the PI3K/Akt signaling pathway plays a role in protecting germ cells
in the postnatal and/or adult testis following toxicant exposure in vivo. We are using primary adult human Sertoli cells
to develop in vitro models to assess the effects of toxicants on the adult testis. These cells are GATA4 and Sox9
positive by Western blot. Western analyses identified Akt1 as a prominent isofrom in primary human Sertoli cell
cultures. Total P-Akt (Ser473) as well as the downstream target GSK3-beta are increased after a 60 minute exposure to
100μM MEHP. Treatment with the PI3K inhibitor Wortmannin showed complete inhibition of total P-Akt (Ser473) as
well as a decrease of phosphorylation of GSK3-beta. Exposure of the Sertoli cells to a range of doses of MEHP in vitro
(0.1μM, 1μM, 10μM, 100μM and 250μM) did not result in overt cytotoxicity. We are currently developing an hTERT
immortalized line of these cells, which will aid in future experiments to study the effects of toxicant exposure and to
elucidate the signaling networks involved.
ABSTRACT FINAL ID: 2706 Poster Board #107
TITLE: PRIORITIZED DIFFERENTIATION OF STRESSED BLASTOCYST-DERIVED STEM CELLS YIELDS TOXICOLOGICAL BIOMARKERS

AUTHORS (LAST NAME, FIRST NAME): Rappolee, Daniel A.; Xie, Yufen; Zhou, Sichang; Slater, Jill A.; Awonuga, Awoniyi O.

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KEYWORDS: Biomarkers, Stem cells, Reproductive toxicity.

ABSTRACT BODY: Our lab has demonstrated for the first time, that stem cells derived from the embryo undergo “compensatory” and “prioritized” differentiation in response to stress. Three stressors, benzopyrene, hypoxia, and hyperosmolar sorbitol activate stress enzymes over a long dose range. However, at low stress doses where there is no significant decrease in stem cell accumulation. At these low stress doses stress enzymes mediate metabolic effects but no effects on differentiation. Only at higher doses associated with significant decrease in stem cell accumulation rates, does stress-induced stress enzyme activity mediate differentiation. Since stress induces the next essential differentiated functional lineage when decreased stem cell proliferation occurs, we call this “compensatory” differentiation. We have found that stress induces pluripotent embryonic stem (ES) cells and trophoblast stem (TS) cells to differentiate to early essential lineages. But, stress suppresses differentiation to later essential lineages. Thus we call this second phenomenon “prioritized” differentiation. In a study of stressed TS cells, microarray analysis showed that nearly 300 mRNA transcript types were upregulated after 24hr. Compared with unstressed differentiation, there was an enhancement of the many markers of early lineages and suppression of later lineages. We propose that the ratio of early lineage markers/later lineage markers should yield a robust measure of toxicological stress. In addition, decrease in stem cell proliferation rates are coupled with an increase in the ratio of early differentiation markers/later differentiation markers. Thus both diminished stem cell proliferation and the ratio of prioritized differentiation markers produces biomarkers for toxicological reproductive stress.

ABSTRACT FINAL ID: 2707 Poster Board #108
TITLE: PHOSPHORAMIDE MUSTARD-INDUCED OVOTOXICITY FOLLOWING A SINGLE EXPOSURE IN RAT OVARIES

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INSTITUTIONS (ALL): 1. Department of Animal Science, Iowa State University, Ames, IA, United States.
2. Department of Physiology, University of Arizona, Tucson, AZ, United States. 3. Institut national de la recherché scientifique, Université du Québec, Pointe Claire, QC, Canada.

KEYWORDS: Ovary, Phosphoramide Mustard.

ABSTRACT BODY: Phosphoramide mustard (PM), the ovotoxic metabolite of the chemotherapeutic agent cyclophosphamide causes primordial and small primary follicle loss (30 μM, on d4 of culture; Petrillo et al., 2008). This study investigated the effect of single PM exposures on F344 rat ovarian follicle numbers. Postnatal day 4 (PND4) rat ovaries were cultured in control medium for 4d to allow development of large primary and secondary follicles prior to PM exposure. Ovaries were treated with medium containing vehicle control (CT) or PM at concentrations where primordial and small primary follicle loss has (30 μM) and has not (10 μM) been observed. Ovaries were maintained in culture for 8d, followed by histological evaluation and follicle classification and enumeration. Loss of contact between the oocyte and granulosa cells, suggesting advanced atresia, of large primary and secondary follicles were observed at both concentrations of PM. There was no effect (P > 0.05) of PM exposure on primordial (CT = 176.7 ± 50.7; PM 10 μM = 163.3 ± 56.7; PM 30 μM = 183.7 ± 34.3), small primary (CT = 187.3 ± 21.5; PM 10 μM = 201.3 ± 74.1; PM 30 μM = 158.7 ± 35.63), or large primary follicles (CT = 42.67 ± 3.5; PM 10 μM = 28.7 ± 9.8; PM 30 μM = 24.3 ± 15.9). In contrast, relative to control, single PM exposures at both concentrations caused loss (P < 0.05) of secondary follicles (CT = 13.7 ± 1.5; PM 10 μM = 2.3 ± 0.3; PM 30 μM = 4.3 ± 1.7). In summary, exposing ovaries that contain increased numbers of large pre-antral follicles to PM demonstrated PM-induced loss of contact between the oocyte and granulosa cells of large pre-antral
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

follies, with a reduction in the number of secondary follicles. Thus, these results raise concern over single exposures of PM for female ovarian function and support the use of the rat ovary culture system for mechanistic studies on PM-induced ovotoxicity.

**ABSTRACT FINAL ID:** 2708 Poster Board #109
**TITLE:** REPRODUCTIVE AND DEVELOPMENTAL TOXICITY OF FORMALDEHYDE: A SYSTEMATIC REVIEW AND SUPPORTING EVIDENCE

**AUTHORS (LAST NAME, FIRST NAME):** Duong, Anh 1; Steinmaus, Craig 1; McHale, Cliona M.2; Vaughan, Charles P.3; Bishop, Jack 4; Zhang, Luoping 1

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2. California Environmental Protection Agency, Sacramento, CA, United States.
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**KEYWORDS:** Formaldehyde, Reproductive/Developmental toxicity, meta-analysis.

**ABSTRACT BODY:** Formaldehyde, found ubiquitously in the environment, has been recently classified as a known human carcinogen. Its reproductive and developmental effects, particularly at critical windows of exposure, have long been suspected. However, studies in this area are limited with conflicting results, and previous reviews are inconclusive. We sought to systematically evaluate all weights of evidence regarding formaldehyde exposure and adverse reproductive and developmental effects to date, making sure to include all routes of formaldehyde exposure, doses and study populations, both humans and animals. We conducted an updated meta-analysis of recent data from human studies, combining all adverse pregnancy outcomes. The meta-analysis results showed an increased risk of all combined adverse pregnancy outcomes (1.54, 95% CI 1.27-1.88, p<0.001) and spontaneous abortion (1.76, 95% CI 1.20-2.59, p<0.002), in formaldehyde-exposed women, although differential recall, selection bias, or confounding could not be ruled out. Animal studies further demonstrated a consistently positive association between formaldehyde exposure and adverse pregnancy outcomes for multiple routes of exposure at a wide range of doses on several animal species. Thus results from the meta-analysis and animal data provided some evidence that formaldehyde can induce reproductive and developmental toxicity in both humans and animals, although further human epidemiologic studies, with appropriate evaluation and control of potentially important sources of bias and confounding, are needed. Future mechanistic studies are also needed to improve the current understanding of formaldehyde's systemic effects.

**ABSTRACT FINAL ID:** 2709 Poster Board #110
**TITLE:** SUPPRESSION OF SPERM FERTILITY BY MONOPHALTATE VIA INHIBITING SPERMATOZOOAL PHOSPHOLIPASE A2 ACTIVITY

**AUTHORS (LAST NAME, FIRST NAME):** Hara, Shuntaro 1; Sato, Hiroyasu 1, 2; Taketomi, Yoshitaka 1, 2; Murakami, Makoto 2

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**KEYWORDS:** monophthalate, phospholipase a2, spermatozoa.

**ABSTRACT BODY:** Phospholipase A2 (PLA2) catalyzes the hydrolysis of phospholipids at the sn-2 position, liberating free fatty acids and lysophospholipids. Immunohistochemical analysis revealed that various PLA2 enzymes express in male reproductive organs and spermatozoa. By using knockout mice and specific inhibitors, we recently found that among these PLA2 enzymes, group X secreted PLA2 (sPLA2-X) is released during sperm acrosome reactions and involved in fertilization. It had been reported that mono(2-ethylhexyl)phthalate (MEHP), a major metabolite of di(2-ethylhexyl)phthalate (DEHP), inhibits human platelet PLA2 activity. Thus we here investigated the effects of MEHP and DEHP on enzymatic activity of recombinant sPLA2-X and in vitro fertilization (IVF) using murine spermatozoa and eggs. As the results, we found that MEHP, not DEHP, inhibited sPLA2-X activity and suppressed IVF efficiency in a dose dependent manner. Furthermore, the number of incomplete dividing oocytes was increased when MEHP-treated spermatozoa were used for IVF. Addition of lysophosphatidylcholine, a catalytic product of PLA2, overcame the effect
of MEHP on IVF efficiency. These results indicated that MEHP might disrupt fertilization via the inhibition of sPLA₂-X and that sPLA₂-X activity in spermatozoa might be a target for endocrine disrupters.

**ABSTRACT FINAL ID:** 2710 Poster Board #111  
**TITLE:** PHARMACOLOGICAL EFFECT OF DEOXYPODOPHYLLOTOXIN: A MEDICINAL AGENT OF PLANT ORIGIN, ON MAMMALIAN NEURONS

**AUTHORS (LAST NAME, FIRST NAME):** Gao, Rong; Xu, Peng; Sun, Qin; Zhang, Shougang; Xiao, Hang
**INSTITUTIONS (ALL):** 1. Nanjing Medical University, Nanjing, Jiangsu, China.
**KEYWORDS:** deoxypodophyllotoxin, Dorsal root ganglion neurons, Sodium channels.

**ABSTRACT BODY:** Deoxypodophyllotoxin (DOP) is a natural product that can be isolated from a variety of medicinal herb plants. It is well known for its antitumor, antiviral, and anti-inflammatory activities. However, there are few investigations that address neurotoxic effect of DOP in animal nervous system. In this study, whole cell patch clamp and calcium imaging techniques were employed to investigate effects of DOP on electrophysiological properties and calcium regulation of rat dorsal root ganglion (DRG) neurons. DOP inhibited both tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) sodium currents in voltage clamp recording and caused a decrease in the number of action potentials (APs) in current clamp experiment. Suppressive and unfavorable effects of DOP on the kinetics of sodium currents in terms of excitability of DRG neurons may greatly contribute to its antitumor and anti-inflammatory activities. Moreover, DOP evoked increase of intracellular Ca²⁺ concentrations ([Ca²⁺]) in DRG neurons, and this effect may lead to neuronal cytotoxicity. This work was financially supported by the National Natural Science Foundation of China (30671785, 30971939) to Rong Gao.

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**ABSTRACT FINAL ID:** 2711 Poster Board #112  
**TITLE:** RETINAL REMODELING FOLLOWING GESTATIONAL LEAD EXPOSURE (GLE) ALTERS GABA-MEDIATED STRUCTURE AND FUNCTION

**AUTHORS (LAST NAME, FIRST NAME):** Hamilton, Ryan; Xiao, Weimin; Wang, Minhua; Frishman, Laura J.; Fox, Donald A.
**INSTITUTIONS (ALL):** 1. University of Houston, Houston, TX, United States.
**KEYWORDS:** Lead, Retina, Development.

**ABSTRACT BODY:** GLE produces supernormal scotopic electroretinograms (ERGs) in children, adult primates and rats with a correspondingly increased number of rod photoreceptors and bipolar cells in rats and mice. Our current goals were to determine in adult control and GLE mice: scotopic ERG function, the number of GABAergic amacrine cells (ACs), and the localization and expression levels of retinal GABAergic transport and metabolic proteins and their mRNA levels. C57BL/6 females were exposed to 55 ppm lead during gestation until postnatal day (PN) 10. Peak [BPb] was ~22 µg/dL at PN10 and by PN30 GLE [BPb] were not different from controls. ERGs were assessed before and one hour after an intravitreal GABA injection. Confocal studies performed on fixed-frozen vertical sections assessed morphometry, protein localization and expression levels. Westerns and RT-qPCR analyzed protein and gene expression levels, respectively. Surprisingly, in GLE mice ERG amplitudes were not different from controls. GABA produced supernormal b-waves in controls, but not GLE mice, indicating GABAergic alterations. Confocal and Westerns showed that GLE did not change the number of GABAergic ACs or expression of the GABA synthesizing enzymes GAD65 or GAD67. However, GLE significantly increased expression of plasmalemmal GABA transporters GAT1 (neuronal) and GAT4 (neuronal/glial) and the vesicular GABA transporter (VGAT) as well as the number of VGAT-immunoreactive (IR) horizontal cell processes in the outer plexiform layer and of GABA transaminase (GABA-T)-IR mitochondria in Müller glial cell outer nuclear layer processes. RT-qPCR results were consistent with these findings. Thus, GLE induced novel GABAergic remodeling (increased transporters, mitochondria and sprouting) that likely lowered the GABA “set-point” in GLE retinas and prevented supernormal scotopic ERGs in the absence or presence of exogenous GABA. These findings are consistent with the Fetal Basis of Adult Disease and implicate GLE as a risk factor for adult-onset neurodegenerative disorders. Supported by NIH Grants ES012482, EY06671, EY07551 and EY07024.
**ABSTRACT FINAL ID:** 2712 Poster Board #113

**TITLE:** GENDER-BASED DIFFERENCES IN GENE EXPRESSION PROFILES IN HIPPOCAMPUS FOLLOWING POSTNATAL LEAD EXPOSURE

**AUTHORS (LAST NAME, FIRST NAME):** Anderson, David; Sonnenahalli, Harikrishnadhar; Vadigepalli, Rajanikanth; Schneider, Jay

**INSTITUTIONS (ALL):** 1. Thomas Jefferson University, Philadelphia, PA, United States.

**KEYWORDS:** microarray, RNA, lead.

**ABSTRACT BODY:** The study of sex-related differences in outcome from lead (Pb) exposure has received little systematic attention. Yet, it appears that sex may be a modifier of the association between lead and brain development/function and neuropsychological development. Thus, the present study was performed to examine the influence of sex on gene expression profiles in the hippocampus in rats exposed to Pb. At weaning, male and female Long-Evans rats, housed 4 per cage, were fed diets containing lead acetate (1,500 ppm) or control diet without lead for 30 days. Animals were then euthanized and hippocampi removed, RNA extracted, QA/QC analyzed, amplified, and hybridized to Affymetrix Rat Gene 1.0 ST RNA Arrays. Males and females had comparable blood and brain Pb levels. Analyses have identified a small number of transcripts (N = 53) differentially expressed in control male and female animals as well as numerous transcripts (N = 249) significantly altered in expression by Pb exposure regardless of sex. Additionally, 120 transcripts were found to be differentially expressed in males compared to females. While there were a number of transcripts significantly altered by Pb in both sexes, over twice as many transcripts had significantly increased expression in Pb exposed males compared to Pb exposed females and over twice as many transcripts had significantly decreased expression in Pb exposed females compared to Pb exposed males. Data analyses indicate effects of Pb on a variety of pathways and functions including effects on genes related to ion fluxes and signaling, transcriptional mechanisms, cytoskeleton, myelin formation, and blood brain barrier function as well as genes related to stress responses and response to oxidative stress. These results suggest that even a brief postnatal exposure to Pb can have effects on the expression of a wide variety of genes in the brain and that the response of the male and female brain to Pb exposure is somewhat different. Supported by ES015295.

**ABSTRACT FINAL ID:** 2713 Poster Board #114

**TITLE:** NEUROPROTECTION BY NIMODIPINE AGAINST ADULT-ONSET MEHG NEUROTOXICITY IS ROUTE-DEPENDENT: NEUROMOTOR AND COGNITIVE EFFECTS

**AUTHORS (LAST NAME, FIRST NAME):** Newland, Marshall C.; Bailey, Jordan M.; Johnson, Josh E.; Cummings, Craig W.; Hoffman, Dan J.; Hutse, Blake A.; Ravis, William R.; Lin, Yuh J.

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**KEYWORDS:** Methylmercury, Calcium Channel Blocker, Neuroprotection.

**ABSTRACT BODY:** BACKGROUND. One mechanism by which chronic methylmercury (MeHg) may exert its neurotoxicity is by disrupting intracellular calcium homeostasis, with a consequent increase in intracellular Ca++ in vulnerable neurons. METHODS. To evaluate this hypothesis functionally, adult BALB/c mice were exposed chronically to 0 or 15 ppm of Hg (as MeHg) via drinking water and to nimodipine, an L-type Ca++ channel blocker with action in the CNS. Nimodipine was administered orally in diets (0, 20, or 200 ppm of nimodipine) or subcutaneously (s.c.) with sustained release pellets. These exposures produced about 0, 2, or 20 mg/kg/day of nimodipine. Endpoints included rotorod performance and spontaneous running. Mice meeting a set of pre-defined criteria were euthanized and a survival analysis was conducted. For the dietary study, incremental repeated acquisition (IRA) of behavioral chains was used to test cognitive function. Here, a different chain was presented to test the animals' ability to re-acquire a new response sequence. A “performance” condition in which the same chain was produced every session served as a control. RESULTS. Running decreased, rotorod performance deteriorated, and death rates increased in MeHg-exposed mice receiving no nimodipine or nimodipine in s.c. pellets. Dietary exposure to 20 or 200 ppm partially or completely
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

prevented this MeHg-neurotoxicity. MeHg impaired performance on the IRA task, and this was partially or completely blocked by dietary nimodipine, depending on dose. Nimodipine was detected in the blood of mice receiving the drug in s.c. pellets but not in the blood of mice consuming it in the diet, probably because of extensive first-pass metabolism. CONCLUSION. Dietary exposure to nimodipine was neuroprotective against neuromotor and cognitive effects of chronic, adult-onset MeHg exposure but this protection was not associated with detectable blood nimodipine. [Supported by NIH ES003299.]

ABSTRACT FINAL ID: 2714 Poster Board #115
TITLE: THE NEUROTOXIC EFFECTS OF TRIBUTYL Tin ON MALE F1 RATS BY CONTINUOUS EXPOSURE FROM THE FETUS STAGE THROUGH THEIR DEVELOPING STAGES

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KEYWORDS: tributyltin, neurotoxicity, development.

ABSTRACT BODY: Neurotoxicity is one of the major toxic effects of tributyltin (TBT). In our previous study, the mean values of F1 rats exposed to TBT via the placenta and their dams’ milk for total locomotion distance and instance of wall rearing in open field tests were significantly lower than those in the control. The problem remained to determine the neurotoxic effects of exposure to TBT on the developing stages after weaning. We therefore evaluated the effects of TBT on behavior by continuing exposure from fetus to 9 weeks of age for the comparison with the results among the rats exposed to TBT via the placenta and their dams’ milk and the rats exposed by their food only. F1 rats were exposed to TBT in utero and postnatally via their dams’ milk by the dams’ chow containing TBT at 0 and 125 ppm. After weaning, they were either fed chow containing TBT at 0 or 125 ppm until 9 weeks of age. The pups at 9 weeks were composed of the control (control-control: CC), the group exposed by their food only (control-TBT: CT), that exposed by the placenta and their dams’ milk (TBT-control: TC), and the continuing exposure group (TBT-TBT: TT). Open field tests and prepulse inhibition (PPI) tests were performed. In open field tests, the mean values of total locomotion distance in the CT and TT groups were significantly lower than that in the CC group. For the TC group, the mean value of locomotion distance between 15-20 min was significantly lower than that of the control. There were no significant differences in the PPI tests among the groups. These results suggest that TBT exposure over the developing stages inhibits behavior of F1 rats.

ABSTRACT FINAL ID: 2715 Poster Board #116
TITLE: DYSREGULATION OF BDNF-TRKB SIGNALING IN PRIMARY HIPPOCAMPAL NEURONS BY LEAD (PB2+)

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KEYWORDS: Lead, neurotoxicity, BDNF.

ABSTRACT BODY: The developing brain is vulnerable to the toxic effects of Pb2+ and is manifested as impaired cognitive function and intellectual capacity. Pb2+ is a non-competitive NMDA receptor (NMDAR) antagonist and NMDAR downstream signaling regulates transcription of neurotrophins such as brain derived neurotrophic factor (BDNF). BDNF is a neurotrophin that plays a key role in central nervous system development, modulation of synaptic transmission and synaptic plasticity and learning and memory. Recent studies from our lab have shown that Pb2+ exposure during the period of synaptogenesis of hippocampal (Hipp) neurons in culture alters Pro-BDNF protein levels and impairs BDNF basal release (Neal et al., Tox. Sci. 116: 249, 2010). Using the same Hipp neuron culture system, we now show that Pb2+ exposure decreases transcript specific BDNF mRNA (p<0.05) and this may be mediated by altered
levels of methyl CpG binding protein 2 (MECP2), a protein responsible for transcriptional silencing. The effect of Pb2+ on BDNF mRNA transcripts was specific to exons IV and IX with no change in exons I and II. The exon IV promoter has multiple sites for the transcriptional repressor protein MeCP2 and NMDAR activation reduces MeCP2 occupancy of the promoter for exon IV. Since Pb2+ exposure inhibits NMDAR function we anticipated an effect on MeCP2. Our data shows decreased nuclear expression of MeCP2 in both the total and phosphorylated state after Pb2+ exposure (p<0.05). The tropomyosin-related kinase receptor B (TrkB) is the cognate receptor for BDNF. Consistent with altered expression of BDNF, we observed a decrease in total TrkB levels and decreased phosphorylation at tyrosine 816. The later indicates reduced activation of the receptor. In summary, these findings suggest that Pb2+ exposure during the period of synaptogenesis of Hipp neurons in culture alters BDNF-TrkB signaling. These Pb2+-induced changes may be responsible for the early life effects of Pb2+ on neuronal development, synaptic plasticity and learning and memory [Supported by ES06189 to TRG]

**ABSTRACT FINAL ID:** 2716 Poster Board #201

**TITLE:** POWER ANALYSIS OF LEARNING AND MEMORY DATA FROM A NHP EPPND STUDY

**AUTHORS (LAST NAME, FIRST NAME):** Cappon, Gregg D.; Grantham, Lonnie; Bowman, Christopher J.; Chmielewski, Gary; Hurtt, Mark E.

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**KEYWORDS:** Statistical Power, Non-human Primate, Learning and Memory.

**ABSTRACT BODY:** The increase in biologics research has renewed emphasis on methods used to assess developmental toxicity in NHPs. Often the initial reaction has been to adapt traditional approaches used in small laboratory animals to NHPs. One example is a recent regulatory request to assess cognitive function in offspring in an NHP enhanced pre-and postnatal development (ePPND) study. One favored NHP learning and memory test is the object discrimination and reversal (ODR) task using a modified Wisconsin General Testing Apparatus. ODR endpoints are days to achieve learning criterion, days to first reversal, and the number of reversals completed. To evaluate the potential to detect a treatment-related change with this test, we performed a power analysis using data from control NHPs (n=10) from an ePPND conducted as per FDA request. With an n=10 per group, α=0.05 and a one-sided t-test, the learning phase of the ODR provided 88, 41, and 14% power in predicting 100, 50 or 20% increase from control. A sample size of 30 NHPs is needed to have an 80% power in predicting a 50% decrement in performance. For comparison, a similar analysis was performed using learning and memory data (Cincinnati Water Maze; CWM) from control rats from 3 PPNPD studies. The rat data used was the time to solve the maze averaged over all of the days tested. With n=10 per group, α=0.05 and a one-sided t-test, the CWM provided ~99, 83, and 28% power in predicting a 100, 50, or 20% increase from control. Increasing sample size to the routinely used 20 rats increased the power to detect a 50% change to ~98%, although the test is still not sensitive to a 20% change (45% power). Based on this simplified approach to power analysis, the rat CWM test affords about twice the detection power as the NHP ODR, and increasing the rat n to 20 enhances this power advantage. Due to the practical limitations on the number of juvenile NHPs available for evaluation, the data obtained from the ODR learning and memory test is not reliable for use in making safety assessments.
ABSTRACT FINAL ID: 2717 Poster Board #202
TITLE: THE DEVELOPMENTAL NEUROTOXICITY OF LEAD CHLORIDE IN RAT PRIMARY AGGREGATING BRAIN CELL CULTURES

AUTHORS (LAST NAME, FIRST NAME): van Vliet, Erwin; Hogberg, Helena T.; Hartung, Thomas
INSTITUTIONS (ALL): 1. EHS, Johns Hopkins University, Baltimore, MD, United States.
KEYWORDS: Developmental neurotoxicity, Lead, Primary culture.

ABSTRACT BODY: There is concern that exposures to environmental chemicals contribute to the increasing incidence of neurodevelopmental disorders in children. A chemical well known to adversely affect CNS development is lead. The current acceptable blood lead concentration is 10μg/dL or 0.48μM (WHO), however scientific evidence advises no safe level of lead exposure. This study aimed to investigate the adverse effects of lead chloride on processes of neurodevelopment in rat primary aggregating brain cell cultures. Firstly, processes of neurodevelopment were studied by the quantitative measurement of genes expressed in different cell types (nestin in neuroprecursor cells, neurofilament-200 in neurons, S100B in astrocytes and Myelin Basic Protein (MBP) in oligodendrocytes). RT-PCR analyses were performed over 35 days in vitro. Results showed that nestin expression significantly decreased over time, suggesting a reduction in neuroprecursor cells. Neurofilament-200 and S100B expression significantly increased over time, indicating neuronal differentiation and astrocytic proliferation/differentiation. MBP expression remained stable. Next, aggregating cultures were treated with lead chloride (0.1, 10μM) from day 7 to 14 or day 7 to 21. Treatments significantly increased the expression of Nestin and S100B at 10μM. In contrast, neurofilament-200 and MBP expression were significantly decreased in a concentration dependent manner, indicating an effect on neurons and oligodendrocytes. For further study, treated cultures (day 7-21) were analyzed by mass spectrometry based metabolomics. The data showed differences in metabolite levels between control and treated cells in a concentration dependent manner. Further analysis of the altered metabolites should give mechanistic insight into the developmental neurotoxicity of lead. This study demonstrates that gene expression and metabolomic analysis can be sensitive endpoints for developmental neurotoxicity. Moreover, lead affected neurodevelopment at <0.48μM, which suggests the lowering of current acceptable blood lead levels to protect public health.

ABSTRACT FINAL ID: 2718 Poster Board #203
TITLE: APPLICATION OF MICRO ELECTRODE ARRAYS AS AN EMERGING TECHNOLOGY FOR DEVELOPMENTAL NEUROTOXICITY: EVALUATION OF DOMOIC ACID-INDUCED EFFECTS IN PRIMARY CULTURES OF RAT CORTICAL NEURONS

AUTHORS (LAST NAME, FIRST NAME): Hogberg, Helena T.; Novellino, Antonio; van Vliet, Erwin; Hartung, Thomas; Bal-Price, Anna K.
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2. In-Vitro Methods Unit, European Commission Joint Research Centre, Ispra, Italy.
KEYWORDS: developmental neurotoxicity, multi electrode recording, domoic acid.

ABSTRACT BODY: Due to lack of knowledge only a few industrial chemicals have been identified as developmental neurotoxicants. Current developmental neurotoxicity (DNT) guidelines (OECD and EPA) are based entirely on in vivo studies that are time consuming and costly. Consequently, there is a high demand to develop alternative in vitro methods for initial screening to priorities chemicals for further DNT testing. One of the most promising tools for neurotoxicity assessment is the measurement of neuronal electrical activity using micro electrode arrays (MEAs) that provides a functional and neuronal specific endpoint. Here, for the first time the endpoint was evaluated to be suitable for the detection of potential developmental neurotoxicants. Primary rat cortical neurons grown on MEA chips were characterized for different cell markers over time, using immunocytochemistry. To detect DNT effects the electrical activity was measured after 4 weeks of exposure to the potential developmental neurotoxicant domoic acid (DomA). Primary cortical neurons could be a promising in vitro model for DNT testing since some of the most critical neurodevelopment processes e.g. progenitor cell commitment, proliferation and differentiation of astrocytes and
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

maturation of neurons are present. Long-term exposure to a low concentration (50 nM) of DomA increased the basal spontaneous electrical activity as measured by spike and burst rates. Moreover, the effect induced by the GABAA receptor antagonist bicuculline was significantly lower in the DomA treated cultures than in the untreated ones. The MEA measurements indicate that chronic exposure to DomA changed the spontaneous electrical activity leading to the possible neuronal mal functioning. The obtained results suggest that MEAs could be a useful tool to identify compounds with DNT potential. (Support in part by The Swedish Research Council)

ABSTRACT FINAL ID: 2719 Poster Board #205
TITLE: DEVELOPMENT OF A TRANSCRIPT PROFILE FOR FASTING AS A CONFOUNDING VARIABLE IN TOXICOGENOMIC STUDIES

AUTHORS (LAST NAME, FIRST NAME): Xu, Ke1,2; Kirchberg, Christopher3; Gomez, Shawn1,2; Morgan, Kevin1,3,4
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KEYWORDS: Fasting, Toxicogenomic, Circadian rhythm.

ABSTRACT BODY: Fasting has a major impact on hepatic gene expression and is thus a potentially important confounding variable during the interpretation of toxicogenomics studies. Animals may be fasted as part of the experimental protocol or in response to compound exposure, being off of their food due to illness. Our goal was to select a group of fasting-response transcripts that could serve as a flag for fasting effects. Previously published material (Morgan et al, 2005, Toxicol. Pathol, 133:136-145), derived from Affymetrix U34A gene arrays used to explore rat liver gene expression in response to fasting, was explored for fasting flag candidates. In this study, data were collected 0 and 16 hours after food was withdrawn. Approximately 1100 transcripts were statistically-significantly (p < 0.001) dysregulated at t = 16 hr versus 0 hr of fasting. A list of 10 flag genes, 3 up (Ech1, Dci, Acot1/2) and 7 down (Scd1, Pklr, Pygl, Csad, Fasn, Acly, Dak), was selected based on p-value, fold change, and known biological function in relation to fasting, whilst excluding those associated with circadian rhythm (GSE 8988, Almon et al. (2008), JPET 326:700-716). A study from the GEO Database (GSE5509, Spicker et al., 2008, Toxicological Sciences 102(2): 444-454) on chemical effects in the rat liver was tested using the transcript flag set, and the flag set indicated significant evidence of potential fasting effects (sick and off of food?) with two compounds (n-methylformamide, ANIT). The development of such flags for confounding variables could provide a valuable tool within software packages designed to assist in the interpretation of large-scale omics data sets.

ABSTRACT FINAL ID: 2720 Poster Board #206
TITLE: BIOLOGICAL EFFECTS OF PSYCHOACTIVE PHARMACEUTICALS PRESENT IN THE ENVIRONMENT: TESTING ALTERED BEHAVIOR AND GENE EXPRESSION IN FATHEAD MINNOWS

AUTHORS (LAST NAME, FIRST NAME): Joshi, Parag P.1; Thomas, Dr. Michael A.1
INSTITUTIONS (ALL): 1. Biological Science, Idaho State University, Pocatello, ID, United States.
KEYWORDS: Aquatic ecotoxicology, Behavior and gene expression analysis, Psychoactive pharmaceuticals.

ABSTRACT BODY: Metabolically active pharmaceuticals have been detected in waste water, streams and drinking water. These pharmaceuticals end up in the environment by two major routes: (i) 30-90% of the administered doses are excreted after intake and (ii) improper disposal of drugs by industries. Psychoactive pharmaceuticals are a class of frequently prescribed drugs which are observed environmentally. Direct adverse effects of these drugs at environmental concentrations are unknown. In our study we dosed juvenile fathead minnows with fluoxetine 10 µgL-1, venlafaxine50 µgL-1 and carbamazepine 100 µgL-1, individually and in a mixture. Fish were tested for altered behavior after 14 days of exposure, recording responses to an approaching predator model. After 18 days of exposure we extracted mRNA from brain tissue to test effects on gene expression. Using GSEA (Gene Set Enrichment Analysis) we compared fathead gene expression profiles to human phenotypes. We predicted that these drugs would have an effect on behavior and gene expression. Also, these drugs are observed in mixtures environmentally, we predicted
ABSTRACT

For chemical warfare nerve agents (CWNA) with low volatility, such as VX, the most likely route of exposure would be from dermal absorption. Also, very small amounts of CWNAs are typically required to cause systemic toxicological effects (including death), making the early absorption phase critical. Unfortunately, interpretation of the available dermal exposure data can be complicated by uncertainty in the various factors that can affect absorption. Some of these issues include: the actual exposed surface area which is influenced by the vehicle used for dosing; varying regional skin differences such as skin thickness and perfusion; lateral diffusion in the skin; and differences between what is actually absorbed into the skin versus what is absorbed into the bloodstream from the skin. This last issue can affect whether the animal being exposed continues to absorb the chemical even after the skin surface has been cleaned. In order to further explore the influence of some of these issues, an existing physiologically-based pharmacokinetic-pharmacodynamic (PBPK-PD) model for VX was used in conjunction with available dermal exposure data from the literature. This PBPK-PD model can simulate exposure to various species by adjusting species-specific parameters such that variations in absorption could be explored not only within a species but across species. Monte Carlo analysis was used to simulate variations in the dermal exposure parameters to explore the resulting variations in absorption as compared to the published data. These results demonstrate the usefulness of mathematical models in terms of explaining variability in results as well as the importance of accurate and detailed documentation of the actual exposure parameters. (This work was funded in part by the Defense Threat Reduction Agency Joint Science and Technology Office (CBM.NEURO.03.10.AHB.005))

ABSTRACT FINAL ID: 2722 Poster Board #209

TITLE: PREDICTED TOXICITY OF 2,5-DIMETHYLFURAN USING COMPUTATIONAL DATA GAP ANALYSES

AUTHORS (LAST NAME, FIRST NAME): Phuong, J.¹; Kim, S.¹; Johnson, D. E.²; Zhang, Luoping¹


KEYWORDS: 2,5-Dimethylfuran, Computational Toxicology, Computational Assessment.

ABSTRACT BODY: 2,5-Dimethylfuran (DMF), a potential biofuel candidate, is classified as hazardous in the workplace due to high flammability and potential irritant effects to eyes, skin, and mucous membranes. Limited toxicology data on DMF are available but it includes evidence of induced chromosome changes in cultured mammalian cells and DNA damage in bacteria. There is no evidence of mutagenicity in Ames bacterial tests. Biomonitoring studies of cigarette smokers report DMF to be present in sampled exhaled air, blood, and urine, suggesting an examination of the correlation to lung disease. DMF represents a proposed alternative chemical for biofuel substitution where significant data gaps exist. Using open source computational software to predict potential toxicity on human health by carcinogenicity, non-cancer effects, and environmental toxicity, we examined DMF and 50 other evaluable
ABSTRACT FINAL ID: 2723 Poster Board #210

TITLE: CHEMICAL DISRUPTION OF LIMB MORPHOGENESIS IN A PREDICTIVE VIRTUAL EMBRYO MODEL

AUTHORS (LAST NAME, FIRST NAME): Knudsen, Thomas B. 1; Rountree, Michael R. 1; DeWoskin, Robert S. 2; Kleinstreuer, Nicole C. 3; Sipes, Nisha S. 1; Chandler, Kelly J. 3, 1; Singh, Amar V. 4; Spencer, Richard M. 4


KEYWORDS: computational toxicology, developmental and reproductive toxicity, systems biology.

ABSTRACT BODY: ToxCast profiles the bioactivity of hundreds to thousands of chemicals via high-throughput screening (HTS) and computational methods (www.epa.gov/ncct/toxcast/). Many ToxCast assays are relevant to signaling pathways and cellular processes in development, posing the need to integrate these data to predict effects on the embryo. One approach is cell-based computer models (www.compucell3d.org/). Early limb-bud development was selected as a prototype, elaborating a chick model (Poplawski et al. 2007) to simulate mammalian limb-bud outgrowth through formation of the apical epidermal ridge (AER), zone of polarizing activity, and expansion of mesenchyme to a prechondrogenic stage as driven by genes/signals including FGF4, FGF8, FGF10, SHH, BMP, GLI3, DHAND. As proof of principle, we challenged the model with an episode of differential cell death. In the first example, cell death was randomly targeted to AER or mesenchyme. Cells were given the risk to die in each region at probabilities ranging from 3% to 75%. An incremental distortion of limb-bud outgrowth resulted when the probability of mesenchymal cell death >30%; however, the AER model uniquely predicted a disproportionate effect on mesenchymal lineages. A second example modeled 5-fluorouracil (5FU) exposure [Price et al. 2003]. 5FU was applied to the model for a 3h exposure window where proliferating cells at mitosis became apoptotic with a probability proportional to the dosage. The model recapitulated dysmorphogenesis foreshadowing dose-dependent micromelia or oligodactyly. These findings suggest that a ‘virtual embryo’, which systematically implements key genetic signals/responses with coordinated actions of fundamental cellular behaviors, can provide a novel research platform for translating in vitro data into predictive models of developmental toxicity that are quantitative, biologically-plausible and empirically-based. [This abstract does not necessarily reflect EPA policy.]
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

ABSTRACT FINAL ID: 2724 Poster Board #211
TITLE: MEASUREMENT AND MODELING OF METABOLISM OF INDUSTRIAL CHEMICALS

AUTHORS (LAST NAME, FIRST NAME): Terfloth, Lothar¹; Tarkhov, Aleksey²; Gasteiger, Johann¹; Melanie, Bausen²; Fabian, Eric³; Finking, Robert³; Wiench, Karin³; Bernshausen, Thorsten³
KEYWORDS: Metabolism, Chemical reactivity, Risk Assessment.

ABSTRACT BODY: This poster presents the results of the recently terminated OLIMPIC project which was funded by CEFIC-LRI. Within the OLIMPIC project the metabolism of 30 industrial chemicals in rats was investigated by experimental measurements. These 30 chemicals with high relevance to the chemical industry were selected from the following compound classes: diketones (linear, unsaturated, and/or cyclic), quinolines (di-chloro substituted, N-methylated), isoquinolines, triazolopyrimidines, sulfonamides, and organophosphates. The metabolites of the chemicals were determined by LC/UV and LC/MS/MS after standard incubations in rat liver S9-fraction in a cofactor (NADPH) containing buffer system at 37 °C. The detailed metabolism will be exemplarily shown in this poster, e.g. for the food dye Curcumin (E100). In addition, the in vitro metabolism of the industrial chemicals was compared with in silico predictions in order to identify discrepancies between the different methods and to elucidate how the predictive power of in silico models for metabolism can be further enhanced. For example, the in silico methods correctly predict the main metabolite of Curcumin resulting from an O-demethylation reaction. The assessment of the quality and improvement of in silico methods are prerequisites for replacing or minimizing the use of animal models in risk assessment. Furthermore, a novel approach for distinguishing observable from non-observable, but potential metabolic sites in a chemical which is based on physicochemical descriptors of the atoms involved in the degradation reaction will be presented. For aromatic hydroxylation reactions the models have a predictability of up to 97.8% in a 10-fold cross-validation. These models are very valuable in assisting the experimental determination of metabolites.

ABSTRACT FINAL ID: 2725 Poster Board #537
TITLE: MICRORNA CHANGES IN RAT MESENTERY AND PLASMA ASSOCIATED WITH DRUG-INDUCED VASCULAR INJURY

AUTHORS (LAST NAME, FIRST NAME): Thomas, Roberta A.¹; Frazier, Kendall S.¹; Thomas, Heath C.¹; Scicchitano, Marshall S.¹
KEYWORDS: miRNA, drug-induced vascular injury.

ABSTRACT BODY: Identification of a pathogenic mechanism of drug-induced vascular injury (DIVI) and acquisition of validated clinical and nonclinical noninvasive methods for monitoring vascular integrity are significant hurdles in drug development. The ideal biomarker for vascular injury is noninvasive, stable, and precedes (yet persists during) injury. Circulating microRNA (miRNA) represent a pool of prospective noninvasive and stable biomarkers. There is growing evidence that miRNAs are involved in regulating cardiac and vascular inflammation, therefore, vascular biology may represent an exciting and underexploited realm for miRNA biomarker development. There is currently no literature describing miRNA expression as it relates specifically to DIVI. To this end, fenoldopam, dopamine, and yohimbine were selected for study of DIVI as they each exert vasodilatory effects due to antagonism of DA1, a1/a2/DA1/DA2, and a2 receptors, respectfully; fenoldopam and dopamine reproducibly induce mesenteric arterial lesions in rats while yohimbine does not. Changes in mesenteric miRNAs correlating with DIVI were observed. There were 44 and 34 miRNAs observed as increasing in a statistically significant manner with a fold change of 2 or greater in mesenteries of fenoldopam- and dopamine-treated rats, respectively, with 13 of these miRNAs shared. None of these changes were observed in mesenteries of rats treated with yohimbine. 3 miRNAs (mirR-134, -409-3p and U87) were also upregulated in serum with either fenoldopam or dopamine, suggesting their possible roles as peripheral biomarkers. Two miRNAs were statistically increased with dopamine treatment (miR-401 and -7a) but were not changed in the mesentery.
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

Vascular injury-specific miRNA changes in rat mesentery and serum, when correlated temporally with vascular pathology, have the potential to provide a stable, noninvasive circulating biomarker for DIVI. This is the first work, to our knowledge, describing miRNA expression changes correlating with DIVI in the rat.

ABSTRACT FINAL ID: 2726 Poster Board #538
TITLE: DISORDERED PORPHYRIN METABOLISM: A POTENTIAL BIOLOGICAL MARKER FOR AUTISM RISK ASSESSMENT

AUTHORS (LAST NAME, FIRST NAME): Echeverria, Diana1; Heyer, Nicholas J.2; Woods, James S.1
KEYWORDS: porphyrins, autism, children.

ABSTRACT BODY: Heme (ferro-protoporphyrin) plays a critical role in neurologic development, both in mitochondrial energy (ATP) production and in neurotransmitter receptor synthesis and processing. Impaired heme synthesis is associated with mitochondrial dysfunction, selective loss of synaptic connections, and a variety of behavioral and neurologic disorders. Heme synthesis is highly accelerated during perinatal development to meet these needs. Consequently, porphyrins formed during heme synthesis are excreted in the urine in concentrations that exceed those of adults by 2-5-fold. In recent studies, we found that urinary porphyrin levels among neurotypical (NT) children decline to adult levels between 2 and 12 years of age. In contrast, porphyrin levels remain elevated throughout this period among children with autism (AU), but not with other neurologic disorders, suggesting that impaired heme synthesis may be etiologic in AU. Logistic regression models of age-adjusted associations between porphyrin levels and AU vs NT indicated significant associations of 6- and 5- and 4-carboxyl porphyrins with AU, with odds ratios = 1.65, 2.36 and 2.03, respectively. Additionally, predictive models based on these urinary porphyrins identified as many as 27% of AU cases from among all AU and NT subjects with a specificity of 96%. These findings suggest that at least 27% of AU cases are characterized by disordered heme synthesis and suggest that urinary porphyrin measures may have clinical utility as a biomarker for autism risk. Supported by P30ES07033 from the NIEHS, the Autism Research Institute, and the Wallace Research Foundation.

ABSTRACT FINAL ID: 2727 Poster Board #539
TITLE: METABOLOMICS APPROACH TO DISCOVER BIOMARKERS FOR CISPLATIN-INDUCED NEPHROTOXICITY USING URINARY 1H NMR SPECTRAL DATA IN RATS

AUTHORS (LAST NAME, FIRST NAME): Kim, Kyu-Bong1,2; Um, So Young2; Chung, Myeon Woo2; Jung, Seung Chul2; Oh, Ji Seon3; Kim, Seon Hwa4; Na, Han Sung2; Lee, Byung Mu2; Choi, Ki Hwan4
KEYWORDS: metabolomics, nephrotoxicity, biomarkers.

ABSTRACT BODY: The primary objective of current study was to discover and characterize surrogate biomarkers which are able to predict nephrotoxicity induced by cisplatin using urinary proton nuclear magnetic resonance (1H NMR) spectral data. A procedure of 1H NMR urinalysis using pattern recognition was proposed for evaluation of nephrotoxicity of cisplatin in rats. The nephrotoxic compound was expected to induce necrosis to nephrons and this was confirmed through blood and urinary clinical chemistry and histopathology. Cisplatin (5 or 20 mg/kg) was intraperitoneally (i.p.) administered to Sprague-Dawley (S-D) rats and urine was collected every 24 h for 6 days. The lower dose (5 mg/kg) of cisplatin was treated for two days consecutively and the higher dose (20 mg/kg) of it was single administered. Animals were sacrificed 2 days or 8 days post-dosing to determine blood clinical chemistry and histopathology. 1H NMR spectroscopy revealed evidently different clustering between control and cisplatin treatment in global metabolic profiling through principal component analysis (PCA) and partial least square (PLS)-discrimination analysis (DA). In targeted profiling, endogenous metabolites of N-acetylglycine, cinnamate, dimethylamine, hippurate,
niacinamide, 2-oxoglutarate, lactate, cis-aconitine, 3-indoxylate, propionate, nicotinate, creatine, acetate, ethanol, phenylacetate, benzoate, fumarate, allantoin, phenylacetate, and salicyluracil were selected as putative biomarkers for nephrotoxicity by cisplatin. Comparison of our rat 1H NMR PLS-DA data with renal histopathological changes suggests that 1H NMR urinalysis can be used to predict or screen nephrotoxicity caused by cisplatin.

ABSTRACT FINAL ID: 2728 Poster Board #540
TITLE: GENES SIGNATURES FROM PHTHALATE TREATED MOUSE LIVER COMPARED WITH KNOWN TOXICITY
SIGNATURES INDICATE CANDIDATE BIOMARKERS FOR OTHER ENVIRONMENTAL POLLUTANTS

AUTHORS (LAST NAME, FIRST NAME): Mitic Potkrajač, Dragana1; Apic, Gordana1; Bonner, Frank1; Russell, Robert B.2
KEYWORDS: biomarker, toxicogenomic signature, hepatotoxicity.

ABSTRACT BODY: The aim of this study was to predict candidate markers for environmental pollutants affecting the liver by analysis of gene expression data from mouse liver and toxicogenomic signatures of the chemical used. Applying an in silico systems biology approach to toxicogenomic signatures we found several candidate biomarkers. Phthalates are environmental pollutants, known for their hepato- and reproductive toxicity. We analyzed a set of 35 significantly dysregulated genes from mouse liver treated with 1150mg/kg/day diethylhexylphthalate for 3 days (1). We predicted hepato- and developmental toxicity for this signature via comparison to gene expression toxicity signatures from 290 compounds of known toxicity (involving nearly 30 000 chemical to gene associations). The predictions were done using a network-based approach that considers prior knowledge of genes, proteins and chemicals and their associations with each other and to thousands of pathologies. From the 35 dysregulated genes, 27 were used for prediction and about 10 were present through known signatures of about 40 chemicals, including several environmental pollutants (2). The study shows that the incorporation of indirect evidence and comparison with known toxicity signatures for chemicals could serve to facilitate biomarker discovery and validation. (1). Currie RA et al, 2005. Gene ontology mapping as an unbiased method for identifying molecular pathways and processes affected by toxicant exposure: application to acute effects caused by the rodent non-genotoxic carcinogen diethylhexylphthalate. Toxicol Sci. 2005 Aug;86(2):453-69 2. Guruge KS et al, 2006. Gene expression profiles in rat liver treated with perfluorooctanoic acid (PFOA). Toxicol Sci. 2006 Jan;89(1):93-107

ABSTRACT FINAL ID: 2729 Poster Board #541
TITLE: MICRONRNAS IN BIOFLUIDS AS MARKERS OF HUMAN DRUG-INDUCED LIVER INJURY

AUTHORS (LAST NAME, FIRST NAME): Starkey Lewis, Philip J.1; Dear, James W.2; Platt, Vivien1; Simpson, Kenneth J.3; Craig, Darren G.3; Antoine, Daniel J.1; French, Neil1; Moggs, Jonathan4; Goldring, Christopher E.1; Park, Kevin1
INSTITUTIONS (ALL): 1. Molecular & Clinical Pharmacology, University of Liverpool, Liverpool, United Kingdom. 2. University/BHF Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, United Kingdom. 3. Scottish Liver Transplantation Unit, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom. 4. Investigative Toxicology, Translational Sciences, Novartis Institutes for Biomedical Research, Basel, Switzerland.
KEYWORDS: Acetaminophen, Drug-Induced Liver Injury, micro RNA.

ABSTRACT BODY: New biomarkers of liver injury are urgently required in the clinic and in preclinical pharmaceutical evaluation. MicroRNAs (miRNAs) potentially represent a new class of biomarkers which are stable and sensitive, and some miRNA afford tissue-selective expression. Here, we have examined these molecules in humans with acetaminophen poisoning. We hypothesised that circulating miRNAs will be raised in patients with liver injury, will correlate with established disease markers and miRNA concentration will be higher in patients with a poor outcome. Also, we hypothesised that miRNA abundance will correlate with kidney injury in urinary exosomes. miR-122 concentrations were significantly higher (275-fold increase, p<0.0001) in the serum of patients with acetaminophen-induced liver injury (n=53) compared to the control cohort (n=25). Serum miR-192 was also significantly higher in the
patient cohort (105-fold increase, p<0.0001). Other non-hepatic serum miRNAs, miR-1 and miR-218 (enriched in heart and brain, respectively) showed no difference between the overdose and control cohorts. Our data show that serum miR-122 significantly correlates with peak ALT levels (r²=0.47; p<0.01) but does not correlate with prothrombin time or serum creatinine. Serum miR-122 levels were 66% higher in patients who had poor outcome but was not statistically significant (p=0.2). In urinary exosomes miR-192 and miR-122 were detectable but did not correlate with kidney function. This work provides the first evidence for the use of miRNAs as novel biomarkers of acute drug-induced liver injury in humans, and is a platform for larger studies to evaluate their use as practical preclinical and clinical biomarkers.

ABSTRACT FINAL ID: 2730 Poster Board #542
TITLE: SAFER AND FASTER EVIDENCE-BASED TRANSLATION (SAFE-T) CONSORTIUM: THE CLINICAL QUALIFICATION OF DRUG-INDUCED VASCULAR INJURY BIOMARKERS

AUTHORS (LAST NAME, FIRST NAME): Lawton, M.¹; Bendjama, K.²
INSTITUTIONS (ALL): 1. Pfizer, Groton, CT, United States. 2. Firalis, Huningue, France.
KEYWORDS: Biomarker, Vascular injury.

ABSTRACT BODY: Drug-induced vascular injury (DIVI) is a common histopathologic observation in animals during safety assessment studies. However, the clinical relevance of preclinical DIVI is unknown. Furthermore, because of a lack of qualified biomarkers to monitor this potential finding in humans, compounds that cause preclinical DIVI are often not advanced into clinical studies. To address the lack of clinically relevant safety biomarkers, a European-based partnership called the SAFE-T Consortium was formed from 20 participants from the pharmaceutical industry, small-medium enterprises, academic institutions and clinical units. The consortium aims at qualifying safety biomarkers for renal, hepatic and vascular drug-induced injuries. Based on the hypothesis that similar histopathologic profiles are likely to present overlapping biomarker signatures, the SAFE-T vascular injury group has designed and initiated clinical qualification studies using surrogate populations to get blood samples from patients that present pathologic features similar to the ones observed in preclinical DIVI, particularly fibrinoid necrosis of the media and perivascular inflammation. Assays are being developed for 38 candidate biomarkers that were selected to detect endothelial and smooth muscle injury, as well as inflammation, which are common morphologic features of preclinical DIVI. Detailed assay performance data (e.g. linearity, recovery, LLOD, etc) that have been generated for 25 of the 38 candidate markers will be presented. Where possible, the same biomarkers will also be tested in preclinical animal models of DIVI and other vascular injuries to ensure their translatability; this will be accomplished in collaboration with the US-based Predictive Safety Testing Consortium (PSTC). Data from two such studies demonstrate the importance of using multiple compounds and a panel of biomarkers. For example, the inflammatory markers TIMP1 and α2-macroglobulin respond strongly in rats treated with a PDE4 inhibitor, CI-1044, but not in rats treated with fenoldopam. Data from these studies will also be presented.

ABSTRACT FINAL ID: 2731 Poster Board #543
TITLE: DI-PALMITOYL-PHOSPHATIDYLCHOLINE (DPPC) AS A BIOMARKER OF PULMONARY SURFACTANT HOMEOSTASIS

AUTHORS (LAST NAME, FIRST NAME): Mineo, Anthony¹; Abid, Sadia²; Papov, Vladimir²; Phillips, Jonathan¹
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KEYWORDS: Mass Spectrometry, phospholipid, Biomarker.

ABSTRACT BODY: Pulmonary surfactant contributes to managing inflammation in the lung and is considered an important part of mediating disease. Although the role surfactant plays in disease is not clear, tight regulation of surfactant homeostasis by pulmonary macrophages and alveolar epithelial cells is well characterized. As a result, surfactant associated proteins, such as SP-D and KL-6, are gaining interest as biomarker candidates for diseases such as active interstitial pneumonia and chronic obstructive pulmonary disease (COPD). While proteins make up
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

approximately 10% of surfactant material, phospholipids account for the remaining 90%. Specifically, Di-palmitoyl-phosphatidylcholine (DPPC) is the principal molecule found in lung surfactant, making up 40% of total phospholipid content. Here we propose and test DPPC as a candidate biomarker of pulmonary surfactant homeostasis. GM-CSF knockout mice were used as a model of misregulated surfactant homeostasis. GM-CSF deficient mice exhibit a surfactant accumulating phenotype. A quantitative LC/MS/MS assay for DPPC was developed. Lavage fluid (BALF), plasma and urine samples were collected from eight month old knockout, wildtype and heterozygous mice. Total phospholipids were extracted and DPPC was quantitated against a calibration curve derived from spike-recovery measurements from each biological matrix. DPPC levels were markedly increased in knockout BALF (10-16 fold) as compared to wildtype and heterozygous. In contrast to BALF levels, plasma DPPC was not significantly different across genotypes. Urine DPPC content was below limits of detection for all genotypes. Additional evidence suggests that solubility of DPPC may limit proportional increases in peripheral biofluids. Taken together, these data indicate DPPC is a sensitive and specific biomarker of pulmonary surfactant homeostasis when direct access to lung samples is feasible.

ABSTRACT FINAL ID: 2732 Poster Board #544
TITLE: IDENTIFICATION OF VOCS BIOMARKER BY ANALYSIS OF MICROARRAY DATA

AUTHORS (LAST NAME, FIRST NAME): An, Yu Ri; Oh, Moon-Ju; Kim, Seung Jun; Youn, Jong-Pil; Han, Jeong; Kim, Youn-Jung; Ryu, Jae-Chun; Kang, Byeong-Chul; Hwang, Seung Yong


KEYWORDS: VOCs biomarker, toxicogenomics, microarray.

ABSTRACT BODY: Toxicogenomics through microarray technology offers a large-scale detection and quantification of mRNA transcripts regarding toxicity that is related to alterations in mRNA stability or gene regulation. In this study, we intended to identify the common biomarker of VOCs (volatile organic compounds) in the human cell line and rat model by HazChem array (HazChem Human array and HazChem Rat array). Utilizing the Agilent custom array platform, HazChem array spotted VOCs, PAHs (polycyclic aromatic hydrocarbons), and POPs (persistence organic pollutants)-dependent expression genes in several toxicogenomic studies. In this study, we treated benzene, toluene, and o-xylene to human promyelocytic leukemia (HL-60) cells. Dichloromethane, ethylbenzene, and trichloroethylene were treated to Sprague Dawley (SD) rats. Then, we discover commonly expressed genes by comparing gene expression profiles each other using GeneSpring GX V11.0.1 software. From gene expression data, 82 and 145 genes were commonly expressed in the human and rat respectively. Among those genes, fourteen genes - AQP1, BCL2A1, BTG1, CFI, HMOX1, KRT14, ME1, PAMIP1, PSEN2, PTGER2, PTGER4, SERPINE1, TNFRSF9, XBPI present in homologue between human and rat. Also, some of fourteen genes were repeated in other studies regarding VOCs toxicity. Finally, this study represents the signature of exposure of VOCs. Moreover, finding of these homologue genes could be the potential biomarker of VOCs.

ABSTRACT FINAL ID: 2733 Poster Board #545
TITLE: TOXICOGENOMIC STUDY OF PESTICIDES THROUGH MICRONRA MICROARRAY ANALYSIS

AUTHORS (LAST NAME, FIRST NAME): Oh, Moon-Ju; An, Yu Ri; Kim, Seung Jun; Youn, Jong-Pil; Han, Jeong; Kim, Youn-Jung; Ryu, Jae-Chun; Kang, Byeong-Chul; Hwang, Seung Yong


KEYWORDS: microRNA, pesticide, toxicogenomics.
**ABSTRACT BODY:** MicroRNAs are small single-stranded RNAs possessing the reverse complement of another protein-coding gene's mRNA transcript. In addition, miRNA (microRNA) has been recognized to play an important role in various diseases and in cellular and molecular responses to toxicants. Pesticides are mainly chemical substances to destroy pest but have drawbacks resulting potential toxicity to human health. In the present study, we investigated differential expression of miRNAs in response to three pesticides (bisphenol A, myclobutanil, and triadimefon). Human hepatoma cell line (HepG2) was treated to above pesticides for 3h and 48h. Cytotoxicity analysis was performed to get LC20 values and miRNA based microarray was carried out using Agilent human miRNA v13 array. At early exposure (3h), two miRNAs were differentially expressed, where miR-365 was up-regulated and miR-431* was down-regulated for each of three pesticides. At late exposure (48h), seventeen miRNA significantly expressed. Among those, miR-19b, miR-20a, miR-29a, miR-1274a, and miR-1274b were up-regulated and let-7b, miR-130a*, miR-196b, miR-299-5p, miR-335*, miR-337-3p, miR-449b*, miR-519e, miR-520a-3p, miR-520d-3p, and miR-924 were down regulated. Overall, this study shows an array of potential biomarker regarding above pesticides. Furthermore, these expressed miRNA against pesticides could be the foundation to develop miRNA based toxic biomarker library, that can predict environmental toxicity.

**ABSTRACT FINAL ID:** 2734 Poster Board #546

**TITLE:** METABONOMICS EVALUATION OF URINE FROM SD RATS DOSED WITH ACETAMINOPHEN USING UPLC/MS AND NMR

**AUTHORS (LAST NAME, FIRST NAME):** Sun, Jinchun; Schnackenberg, Laura; Ando, Yosuke; Pence, Lisa; Yang, Xi; Greenhaw, James; Bhattacharyya, Sudeepa; Salminen, Willie; Mendrick, Donna L.; Beger, Richard D.


**KEYWORDS:** Metabonomics, Acetaminophen, Hepatotoxicity.

**ABSTRACT BODY:** Urinary metabolic perturbations associated with acetaminophen-induced hepatotoxicity were investigated using NMR- and LC/MS-based metabolomics approaches. Single low (100 mg/kg) and high (1250 mg/kg) doses of acetaminophen (APAP) were orally administered to male SD rats in groups of 5. Urine samples from 6, 24 and 72 hours and 7 days after the administration were collected for each rat and monitored by NMR and UPLC/MS. The PCA clustering of NMR and UPLC/MS spectra at each time point were well associated with that the measured clinical chemistry and histopathology showed more liver injury at 24 h. Some changed metabolites are involved in the tryptophan and phenylalanine pathways, including tryptophan, phenylalanine, and xanthurenic acid. The increases of some short chain fatty acids and decreases of some metabolites (ascorbic acid, benzene-diol sulfate, syringic acid sulfate and vanillic acid sulfate) involved with free radical scavenging strongly indicated that APAP may cause oxidative stress, which can affect fatty acid oxidation and lower the reducing agents in the biosystem. In addition, many of the metabolic changes observed may be related to dietary changes in the animals that received the high dose of APAP; for instance, proline betaine decreased after the high dose of APAP was administered. Further, traditional clinic biomarkers uric acid and creatinine were increased in the high-dose group at 24 h after dosing, which highly correlated with histopathology data. In conclusion, our data strongly supported that APAP treatment can cause disrupt fatty acid oxidation.
ABSTRACT FINAL ID: 2735 Poster Board #547
TITLE: HISTOPATHOLOGY METHODS IN BIOMARKER QUALIFICATION

AUTHORS (LAST NAME, FIRST NAME): Zhang, Jun¹; Rouse, Rodney L.¹
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KEYWORDS: Biomarkers, Histopathology, Methods.

ABSTRACT BODY: Qualification and use of tissue injury biomarkers are increasingly important components of regulatory science and the drug development process. Initial attempts to qualify biomarkers through the FDA were based on kidney tissue injury data generated using standard histopathology methods for drug safety assessment. In a significant number of cases, biomarker elevations could be detected in the absence of histopathological changes. One proposed explanation is that knowledge of treatment groups and time points introduces potential bias, particularly at low levels of injury where an accurate assessment of biomarker performance may be most important. To characterize this potential issue, we have undertaken a series of studies to measure the sensitivity of routine histopathology methods and the impact of knowledge bias on lesion scoring in non-clinical studies. Samples were selected from multiple studies featuring varying types and degrees of kidney injury in drug-treated and disease model rats as well as spontaneous changes in control rats. Slides were prepared by an independent contract laboratory using standard paraffin embedding and 5 micron sections. Four government employed pathologists, with different backgrounds of practice, were identified to participate in a virtual working group. Eight sets of digital slide images were hosted on a server where they could be accessed remotely by each study participant. Image sets were to be read blinded or unblinded with a minimum 30 day wash-out period between reads. Interim results indicate that interpretation of biomarker performance using histopathology endpoints can be significantly impacted by the methodology used in scoring, knowledge bias and variability between pathologists. Our evaluation has begun to quantify intra- and inter-pathologist variability present in scoring tissue injury for biomarker qualification and the impact of knowledge bias in documenting biomarker performance. The results indicate the importance of assuring that potential sources of knowledge bias and/or issues of pathologist variability have been identified and addressed in the assessment of biomarker performance.

ABSTRACT FINAL ID: 2736 Poster Board #217
TITLE: DYSREGULATION OF THE INNATE IMMUNE AND INFLAMMATORY RESPONSES TO LIPOPOLYSACCHARIDE (LPS) AND INTERLEUKIN-1β (IL-1β) BY V₂O₅ IN MOUSE LUNG EPITHELIAL CELLS

AUTHORS (LAST NAME, FIRST NAME): Ryan, Lisa K.¹; Simko, Katie¹; Barrett, Leonie¹; Schwartz, Kyell D.¹; Diamond, Gill²
INSTITUTIONS (ALL): 1. Medicine, Public Health Research Institute, NJ Medical School, UMDNJ, Newark, NJ, United States. 2. Oral Biology, New Jersey Dental School-UMDNJ, Newark, NJ, United States.

KEYWORDS: defensin, vanadium, epithelial cells.

ABSTRACT BODY: Low levels of V₂O₅ can suppress β-defensin-2 responses in a dose-dependent manner and can modulate inflammatory cytokine responses in bovine and human airway epithelial cells. We hypothesized that mouse airway epithelial cells would respond similarly. Differentiated primary epithelial cells derived from tracheas (TEC) of C57BL/6 mice (WT) and mice lacking the β-defensin-1 gene (KO) and a mouse alveolar type II epithelial cell line (MLE15) were used to model inflammatory and innate immune responses following exposure to V₂O₅. Triplicate cultures were treated with 10 µg/ml V₂O₅ for 6 hr, washed, then stimulated with medium alone, 100 ng/ml LPS or 100 ng/ml IL-1β for 24 hr. QRT-PCR assessed gene expression. In WT and KO mouse TEC, LPS and IL-1β increased gene expression of mBD-3 and mBD-14. V₂O₅ inhibited the induction of mBD-3 and mBD-14 in WT TEC by IL-1β, but not by LPS. V₂O₅ enhanced LPS-induced mBD-3 but had no effect on LPS-induced mBD-14 in WT TEC. In KO TEC, V₂O₅ did not inhibit IL-1β induction of mBD-3 and mBD-14. V₂O₅ alone induced mBD-3 and -14 in WT TEC, but only mBD-14 in KO TEC. In MLE15 cells, no mBD-3 was made. LPS had no effect on mBD-14, but basal levels of mBD-14 were inhibited by V₂O₅ in MLE15 cells. In MLE15 cells, KC mRNA levels were increased by LPS but not IL-1β. Both LPS and IL-1β increased CCL2 and IL-6 levels. V₂O₅ inhibited basal levels of KC gene expression, but did not prevent the full induction of KC, CCL2 or IL-6 by LPS or of CCL2.
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

and IL-6 by IL-1β in MLE15 cells. Thus, the mouse TEC response is similar to human primary airway epithelium with respect to V̇O₂, suppression of IL-1β-induced β-defensins, whereas MLE15 cells do not resemble the response of their human A549 cell line counterparts. Supported by ARRA funding (NIEHS, NIAID) to K. Simko (Livingston High School, Livingston, NJ), L. Barrett (Carnegie-Mellon) & K. Schwartz (Rutgers).

ABSTRACT FINAL ID: 2737 Poster Board #218
TITLE: TCDD-INDUCED ATTENUATION OF LIVER REGENERATION REQUIRES NATURAL KILLER CELLS

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KEYWORDS: TCDD, Liver Regeneration, Natural Killer Cells.

ABSTRACT BODY: 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a persistent environmental contaminant that elicits toxicity by activating the aryl hydrocarbon receptor. The toxic effects associated with TCDD exposure include immunotoxicity, enhanced inflammation, and dysregulated cell cycle control. We have previously shown that exposure to TCDD suppresses hepatocyte proliferation in a mouse model of liver regeneration induced by 70% partial hepatectomy (PH). Based on recent reports that liver regeneration is negatively regulated by activated natural killer (NK) cells, we hypothesized that TCDD treatment attenuates liver regeneration by enhancing NK cell activation. Mice were treated with TCDD (20 μg/kg) or control one day prior to surgical PH. Lymphocytes were collected from the spleen and remnant liver 36 and 42 hr later and analyzed by flow cytometry. Exposure to TCDD had no effect on the total number of splenic or hepatic NK cells, but it elicited a three-fold increase in the number of hepatic NK cells expressing CD69, a lymphocyte activation marker. Similarly, numbers of CD69+ T cells and NKT cells were twice as high in the regenerating liver of TCDD-treated mice, as compared to vehicle-treated mice. To determine the relevance of NK cells to the TCDD-mediated suppression of liver regeneration, mice were treated with an anti-asialo-GM-1 antibody to deplete NK cells prior to TCDD administration and surgical PH. Hepatocyte proliferation in the remnant liver was measured using bromodeoxyuridine incorporation. Whereas TCDD treatment suppressed hepatocyte proliferation by approximately 80% in control mice, it failed to suppress proliferation in mice depleted of NK cells. Hence, NK cells are required for TCDD-induced attenuation of liver regeneration. Increased CD69 expression indicates that TCDD treatment may enhance the activation of NK cells during liver regeneration. It is possible that increased NK cell activation in TCDD-treated mice may lead to increased IFNy production or cytolytic activity, both of which are implicated in negative regulation of liver regeneration.

ABSTRACT FINAL ID: 2738 Poster Board #219
TITLE: HEMATOXICITY OF MUNITIONS ENVIRONMENTAL DEGRADATE MNX AND LOUISIANA SWEET CRUDE OIL IN RATS

AUTHORS (LAST NAME, FIRST NAME): Jaligama, Sridhar; El Sayed, K. A.; Perkins, E. J.; Meyer, S. A.
KEYWORDS: Myelosuppression, crude oil, hexahydro-1,3,5-trinitro-1,3,5-triazine, RDX.

ABSTRACT BODY: We have shown that hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), nitroreduced, environmental degradation product of munition RDX, causes a delayed-onset neutropenia after a single oral exposure. That bone marrow stem cells are targeted is evidenced by loss of colony-forming units for myeloid (CFU-GMs) lineages at 7d post-exposure to MNX (NOAEL 24 mg/kg) [Kale et al. Toxicologist 2007;96(5-1):35]. Other myelosuppressants include 7,12-dimethylbenz(a)anthracene and benzene, constituents of crude oil such as that released from the recent BP oil spill. One epidemiological study has associated crude oil exposure with “fever” [Janjua,et al. BMC Public Health, 2006;6:84], a possible consequence of immunosuppression. Myelosuppression has been described in oil-exposed wildlife [Kim et al. J Toxicol Environ Health A, 2001;62:97]. Hence, we hypothesized that exposure to crude oil, like MNX, would result in myelosuppression in the rat. Female Sprague-Dawley rats were orally gavaged with Louisiana sweet crude oil (ONTA, Inc., Toronto, Canada; 2 daily doses of 2.5 and 5 ml/kg), as well as oil constituent benzene (0.5
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

ml/kg), and MNX. After 48 h, rats were euthanized, blood was taken for hematology and clinical pathology, and nucleated bone marrow cells from femurs were assayed for CFU-GMs. Findings were that treatment with oil resulted in dose-dependent increases in relative liver weight and blood neutrophils. Serum alkaline phosphatase, but not alanine aminotransferase, was increased and CFU-GMs were decreased at the highest oil dose. Benzene did not affect the above parameters, but did decrease relative spleen weight. MNX did not affect CFU-GMs at 48 h post-treatment. In conclusion, our studies indicated that acute exposure to high doses of crude oil is myelosuppressive. The rapid onset of effect suggests differences in mechanism of MNX- and oil-induced myelosuppression. Support: DoD (CDMRP), US Army Corps of Engineers and LA Board of Reagents.

ABSTRACT FINAL ID: 2739 Poster Board #220
TITLE: IMPACT OF ENDOTOXIN ON IGE RESPONSES TO PROTEINS: STRAIN COMPARISONS

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KEYWORDS: Food allergy, IgE, adjuvant.

ABSTRACT BODY: Assessment of the potential allergenicity (IgE-inducing properties) of novel proteins is an important issue. We have shown previously that the measurement of specific IgE antibody production induced by systemic (intraperitoneal; ip) exposure of BALB/c strain mice to a range of proteins correlates with allergenic potential. One factor which may impact on antibody responses is the endotoxin content of the protein, given that exogenous endotoxin acts as an adjuvant for IgE responses. We have now explored antibody responses to proteins in BALB/c (high IgE responder) and C3HHeJ (endotoxin-resistant/mutant Toll-like receptor [TLR] 4) strain mice. Animals (n=5) were immunized with protein by ip injection on days 0 and 7. Seven days later, serum samples were analyzed for specific IgE antibody by homologous passive cutaneous anaphylaxis assay and for IgG, IgG1 and IgG2a by ELISA. Endogenous endotoxin content (endotoxin units [EU]/mg) was measured by Limulus Amboecyte Lysate assay. Mice were exposed to various doses (0.1 to 1%) of D-ribose, 1,5-diphosphate carboxylase (RUBISCO) from spinach, a negative control protein that is believed to be non-allergenic and which contains high levels of endotoxin (3885 EU/mg). In both mouse strains treatment with RUBISCO provoked relatively vigorous IgE responses and IgG antibody, of both IgG1 and IgG2a subclasses. In addition, C3HHeJ mice were immunized with the known food allergen ovalbumin (OVA) alone and in the presence of equivalent amounts of exogenous endotoxin (20 μg R515 lipopolysaccharide [LPS]) to that received in 1% RUBISCO. Not only did inclusion of LPS have an adjuvant effect on IgG (IgG1 and IgG2a) antibody responses, but IgE titers were also increased. These data demonstrate that the potential adjuvant effect of endogenous endotoxin must be taken into consideration when interpreting IgE antibody responses to novel proteins. Furthermore, despite having a defective TLR4, LPS has an adjuvant effect in C3HHeJ mice, presumably through the lipid A portion of the molecule, such that the use of this strain will not obviate effects of endogenous endotoxin.

ABSTRACT FINAL ID: 2740 Poster Board #221
TITLE: EVALUATION OF THE NON-RADIOACTIVE ENDPOINTS OF LLNA BY APPLYING MODERATE AND WEAK SENSITIZERS

AUTHORS (LAST NAME, FIRST NAME): Karakaya, Asuman; Ulker, Ozge C.; Ates, Ilker; Atak, Aysegul
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KEYWORDS: Local lymph node assay, Non radioactive endpoints, Cytokine production.

ABSTRACT BODY: In the present study, we aimed to verify the non-radioactive endpoints of LLNA (5-bromo-2’-deoxyuridine (BrdU) ex vivo incorporation and cytokine releases from the isolated auricular lymph node cells) by applying a moderate sensitizer cinnamal and a weak sensitizer eugenol to the 8-12-wk-old female BALB/c mice topically. Here, we used a strong sensitizer, 2,4-dinitrochlorobenzene (DNCB) as positive control. DNCB, cinnamal and eugenol in
ABSTRACT FINAL ID: 2741 Poster Board #223
TITLE: EXPOSURE-BASED SCREENING ASSESSMENT FOR CHEMICAL FOOD SAFETY CONCERNS

AUTHORS (LAST NAME, FIRST NAME): Tran, Nga L.; Barraj, Leila M.
KEYWORDS: risk ranking, TOR/TCC, dietary exposure.

ABSTRACT BODY: Risk ranking or screening relies on traditional risk assessment framework of hazard characterization, dose-response, exposure assessment and risk characterization. The Threshold of Regulation/Threshold of Toxicological Concern (TOR/TCC), which establishes an exposure limit that is unlikely to be associated with an adverse health effect based on structural similarities to well-studied chemicals, is well-established approach for hazard identification and dose response when toxicity data are limited or lacking. In order to be used for risk screening, a TOR/TCC must be coupled with exposure estimates (i.e., food consumption and contaminant level data). For screening purposes, estimates of chemical concentration and food consumption can be derived from several readily available sources with varying degrees of confidence and appropriateness given the temporal and spatial conditions of anticipated exposure. A stepwise approach to dietary exposure estimation for TOR/TCC-based risk screening enables safety decisions to be made rapidly with an acceptable degree of confidence. The initial exposure assessment can rely on conservative assumptions and deliberate overestimates of variable contributing to exposure. If a highly conservative estimate of exposure is below the TOR/TCC, no additional, more refined assessment is necessary. Confidence in a ranking scheme depends on the quality and appropriateness of the supporting information. Such confidence can only be obtained when the sources and levels of uncertainty in the underlying information and their impact on the risk ranking are understood and communicated in straight-forward and transparent manner. This paper presents these concepts using examples of contaminant scenarios and readily available food consumption estimates from FCID, TDS, and NHANES.
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

ABSTRACT FINAL ID: 2742 Poster Board #224
TITLE: SCIENCE TO ADDRESS TEXANS' HEALTH

AUTHORS (LAST NAME, FIRST NAME): Kaden, Debra A.¹; Hendler, E.²; Bruhl, R.³; Li, W.³; Sarnat, S.⁴; Olaguer, E.⁵; Guven, B.⁶; Zielinska, B.⁶; Fujita, E.⁶; Beskid, C.²
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KEYWORDS: air pollution, air toxins, exposure.

ABSTRACT BODY: Texans living near sources of air pollutant want to understand resulting risks. High quality research is required to provide the public and policymakers information to understand resulting public health implications. (1) Community exposures from Barnett Shale oil and gas production. Non-methane volatile organic compounds (NM VOC) were examined near Barnett Shale gas well sources, finding >90% ethane, propane, n-butane, iso-butane, iso-pentane, and n-pentane. Benzene, toluene, and xylenes were ~0.1 – 0.2% of NM VOC emissions. There was a steep, spatial decrease in concentrations from close to emission source (~17m) to the next downwind site (~67m), approaching background at ~100m. A saturation monitoring campaign at a nearby residential community found average speciated VOC concentrations generally <1 ppb. This study was a pilot study and findings need to be confirmed in other seasons and locations. (2) Exposures and health effects in asthmatic children in schools near major roadways. Indoor/outdoor pollutants were measured at 4 El Paso elementary schools with health measures (13 wks) from asthmatic children at 2 schools: one (n=20) with high traffic exposure; one (n=18) with low traffic. Air pollutants were monitored at 2 additional schools to characterize traffic-related patterns in the region. Strong spatial gradients in the measured air pollutants were found. Regional pollution was dominated by coarse PM. Association with respiratory health will be discussed. (3) A web-based searchable data tool integrating air toxics exposure measures. A publicly accessible GIS-based database was created integrating data on emissions, ambient concentrations, and indoor/outdoor/personal air toxics exposures (see http://aero.harc.edu). Data from 2 studies were used: “Relationships of Indoor, Outdoor, and Personal Air” and the “Houston Exposure to Air Toxics Study.” This tool may help policymakers, researchers, and the public better understand population exposures to air toxics.

ABSTRACT FINAL ID: 2743 Poster Board #225
TITLE: EFFECTS OF CIGARETTE MENTHOL LEVELS ON CYTOTOXICITY AND GENOTOXICITY IN CELL CULTURES AND RESPIRATORY PATTERN IN MICE AFTER EXPOSURE TO MAINSTREAM SMOKE

AUTHORS (LAST NAME, FIRST NAME): Meng, Ryan¹; Oxford, Tessa L.¹; Thomas, Berta L.¹; Hitchman, A. K.¹; Pierce, Judy T.¹; Anderson, Gregory M.¹; Mellinger, Kathy H.¹; Kerzee, Kevin¹; Harbo, Sam J.¹
KEYWORDS: Menthol, Smoke, Toxicity.

ABSTRACT BODY: These studies were performed to evaluate the in vitro cytotoxic and genotoxic effects and the effects on respiratory physiology of mice after exposure to mainstream smoke (MSS) from reference and mentholated cigarettes. Cigarettes with menthol levels of 0.14% to 0.63% were generated by direct vapor deposition using temporal control. The menthol cigarettes were identical to the reference cigarettes (used as control for comparison) except for the menthol levels. MSS was generated from the mentholated and reference cigarettes using a Borgwaldt-KC SM85 rotary smoking machine under ISO conditions. Smoke particle size, menthol and nicotine levels, aldehydes, and 1,3-butadiene levels were determined in MSS. Chinese Hamster Ovary cells were exposed to 5-6 concentrations of MSS to evaluate cytotoxicity using the neutral red uptake assay and genotoxicity using the sister-chromatid exchange (SCE) assay. Female C57BL/6 mice were exposed to MSS for one hour at a target concentration of 150 µg/L wet total particulate matter. Tidal volume, respiratory rate, and minute volume (MV) were measured in 5 mice/group using whole body plethysmography. Menthol transfer efficiencies to MSS were between 12% and 21%. Menthol levels did not affect MSS stability, aerosol particle size, aldehydes, and 1,3-butadiene levels. For the in vitro results, there were no
significant differences in smoke LC50 and SCE rates between menthol and non-menthol MSS. For the mouse study, the MV was increased by 12% and 19% in mice exposed to mentholated MSS at the 0.14% and 0.22% levels, respectively, compared to the reference cigarette group. These results indicate menthol levels in cigarette did not significantly alter the cytotoxicity and genotoxicity of MSS. However, given that there was an increase in MV, further studies are needed to elucidate the impact of menthol levels on the respiratory system using a larger sample size and repeated smoke exposures in experimental animals and humans.

ABSTRACT FINAL ID: 2744 Poster Board #226
TITLE: PROVISIONAL ADVISORY LEVELS (PALS) FOR VANADIUM PENTOXIDE

AUTHORS (LAST NAME, FIRST NAME): Glass, Dana F.1; McClanahan, Mark2; Gardner, Donald3; Adesina, Femi4
KEYWORDS: Vanadium Pentoxide, Risk Assessment.

ABSTRACT BODY: PAL values developed for hazardous materials by the U.S. EPA represent general public emergency exposure limits for oral and inhalation exposures corresponding to three different severity levels (1, 2, and 3) for 24-hour, 30-day, 90-day, and 2-year durations. PAL 1 represents the threshold for mild effects; PAL 2 represents the threshold for serious, irreversible or escape-impairing effects; PAL 3 represents the threshold for lethal effects. PALS have not been promulgated nor have they been formally issued as regulatory guidance. They are intended to be used at the discretion of risk managers in emergency situations when site specific risk assessments are not available. Application of PAL protocols has been performed for vanadium pentoxide to estimate oral and inhalation exposure limits, as experimental data permit. Vanadium pentoxide is a solid that is used as a chemical intermediate. With exposure, vanadium pentoxide appears to be a direct contact irritant to the surface of entry with limited absorption by the gastrointestinal tract and high absorption by the lungs. While clearance from the lungs is rapid, some vanadium pentoxide can be absorbed into the bone. Limited animal and no human data were available for oral exposure. Oral PAL 1, 2, and 3 values are not recommended due to insufficient data (NR), 5.9 and 8.9 mg/L for 24-hours. There were insufficient data for developing any oral PAL values for 30-days, 90-days or 2-years. Inhalation PAL 1 values were 0.0033 mg/m3 for 24-hours, 30-days and 90-days with no value derived for 2-years. Inhalation PAL 2 values were 0.033 mg/m3 for 24-hours, 0.012 mg/m3 for 30- and 90-days and 0.001 mg/m3 for 2-years. PAL 3 values were 0.27 mg/m3 for 24-hours, 0.048 mg/m3 for 30- and 90-days and 0.0029 mg/m3 for 2-years. PAL values were approved by the Expert Consultation Panel for PALS April 2010.

ABSTRACT FINAL ID: 2745 Poster Board #227
TITLE: ENDOTOXIN: ACUTE ORAL TOXICITY STUDY IN MICE

AUTHORS (LAST NAME, FIRST NAME): Harper, Marc1; Carpenter, Carol2; Klocke, Donald J.3; Delaney, Bryan1
KEYWORDS: Lipopolysaccharide, Acute, Mouse.

ABSTRACT BODY: A single dose of endotoxin from gram negative bacteria was administered to groups of male and female CD-1 mice (n = 5/group) at doses up to 1,000,000 endotoxin units (EU) per mouse by oral gavage. The mice were observed for mortality, body weight effects, and clinical signs for the following 14 days after which they were sacrificed for gross organ necropsy. All mice survived until the scheduled sacrifice, no clinical signs of toxicity were observed, no test substance-related body weight losses occurred and no gross lesions were present at necropsy. Under the conditions of this study, administration of endotoxin to mice at a dose of up to 1,000,000 EU/mouse produced no evidence of toxicity.
ABSTRACT FINAL ID: 2746 Poster Board #228
TITLE: A MODE OF ACTION CELL PROLIFERATION STUDY IN MALE WISTAR RATS AFTER ADMINISTRATION OF IMAZALIL VIA THE DIET FOR 1, 2, 7, 14, OR 28 DAYS.

AUTHORS (LAST NAME, FIRST NAME): Mertens, Jozef J. 1; Parker, George A. 1; Recio, Leslie 2; Holloway, Brian 3; Goodwine, William R. 4; Singh, Pramila 5; Venkatesh, Kris 5; Piccirillo, Vincent J. 6


KEYWORDS: liver, cell proliferation, Tumorigenesis.

ABSTRACT BODY: Imazalil is a nongenotoxic microsomal enzyme inducer that, like phenobarbital (PB), causes liver tumors in Wistar rats following dietary exposure. In the current study the tumorigenic mode of action (MOA) for imazalil was investigated and compared to that of PB. Imazalil was administered via the diet for 1, 2, 6, 14, or 28 days to male Crl:WI(Han) rats at concentrations of 0, 200, 1200, and 2400 ppm. A positive control group received 1200 ppm of phenobarbital (PB) in the diet. Five animals/group were euthanized at each interval. Liver effects of imazalil observed in the 1200 and 2400 ppm groups consisted of differences in cellular staining affinity (increased cytoplasmic homogeneity) as early as study day 1, higher liver weights at study days 14 (2400 ppm) and 28 (1200 and 2400 ppm) and dose-dependent higher CYP2B1/2 and UGT1A activities (1200 and 2400 ppm). PB induced similar findings occurring earlier and with a greater magnitude than those seen with imazalil. PB, unlike imazalil, led to higher hepatic BrdU incorporation, higher ALT and SDH levels, and single-cell hepatocellular necrosis. These differences are a reflection of potency differences between PB and imazalil. Evaluation of apoptosis (caspase immunohistochemistry [IHC]) and oxidative stress (4-hydroxy-2-nonenal IHC) showed no effects for either imazalil or PB indicating that these are not relevant MOAs for induction of liver tumors by imazalil. Quantitative Reverse Transcriptase PCR showed dose dependent induction of mRNA levels of cyp2b1, cyp3a1, cyp3a2, gadd45b in the liver of imazalil and PB treated rats. These results support induction of PB response signature genes as a key event in the MOA for imazalil-mediated liver tumors in rats.

ABSTRACT FINAL ID: 2747 Poster Board #229
TITLE: APPLICATION OF THE TTC CONCEPT TO UNKNOWN SUBSTANCES FOUND IN ANALYSIS OF FOODS

AUTHORS (LAST NAME, FIRST NAME): Hollnagel, Heli M. 1; Koster, Sander 2; Boobis, Alan 3; Carlander, David 4; Cubberly, Richard 5; Richling, Elke 5; Wildemann, Tanja 5; Würtzen, Gunna 6; Galli, Corrado L. 9

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KEYWORDS: Threshold of Toxicological Concern (TTC), TTC, safety assessment.

ABSTRACT BODY: Unknown substances, not previously observed, are frequently detected in foods by quality control laboratories. In many cases, the assessment of these ‘new’ substances requires additional chemical analysis for their identification prior to assessing their risk. This identification procedure can be time-consuming, expensive and in some instances difficult. Furthermore, in many cases, no toxicological information will be available for one or more of the identified substances. Therefore, there is a need to develop new pragmatic tools for the assessment of the potential toxicity of substances with unknown identity to avoid delays in their risk assessment. Hence, the ‘ILSI Europe expert group on the application of the threshold of toxicological concern (TTC) to unexpected peaks found in food’ was established to explore whether the TTC concept may enable a more pragmatic risk assessment of unknown substances that were not previously detected in food. A tiered approach is introduced that uses expert judgment on the source of the food, information on the analytical techniques used, the dietary consumption of food sources containing the
unknown substance and quantitative information on the unknown substance to assess the safety to the consumer using the TTC. Following this tiered approach, higher TTC thresholds of up to 90 µg/day may be applied to unknown substances under certain conditions. Key to the approach is the ability to conclude with sufficient certainty that the non-identified or partially identified substances do not belong to any of the classes exempt from the TTC concept. Application of higher thresholds than 0.15 µg/day requires exclusion of alerts for genotoxicity for the assessed substances.

**ABSTRACT FINAL ID:** 2748 Poster Board #230  
**TITLE:** CHALLENGES IN BIOCOMPATIBILITY TESTING OF MEDICAL DEVICES

**AUTHORS (LAST NAME, FIRST NAME):** Goud, Niranjan S.; Reverdy, Edward E.

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**KEYWORDS:** Medical Devices.

**ABSTRACT BODY:** In general, there are well defined in vitro and in vivo methods to assess the biocompatibility of medical devices. But it is always a challenge to explain test failures and their relevance to patient safety. Two examples below provide the basis for an investigative approach using chemical characterization and risk assessment. The first case illustrates the failure of in vitro cytotoxicity assay for a urology stent. The device is coated with an antimicrobial agent. Therefore its concentration was measured in the test article extract as part of failure investigation. The results showed higher levels of the chemical than a patient would have been exposed to in normal clinical use. Repeating the assay by diluting cell culture extract to physiological levels, the cytotoxicity assay has passed. The failure of Ames genotoxic assay can also be explained by the bacteriostatic effect of the agent. A second case describes the role of device coatings and its effect on test system. Certain devices such as balloon catheters are sometimes coated with a cocktail of chemicals to aid in the lubricity and easy insertion into blood vessels. In one such case, a hydrophilic coated device has failed partial thromboplastin time (PTT) assay but passed other routine biocompatibility assays (in vitro cytotoxicity, rabbit irritation, guinea pig sensitization, acute systemic toxicity in mice, in vitro hemolysis and complement activation). Extensive chemical analysis of device extract by GC-MS revealed traces of carbowax. A literature review revealed that carbowax can sequester calcium ions from extra cellular fluid. This explains the role of carbowax in the failure of PTT by interfering with the availability of calcium ions in the in vitro assay. However, the same device when tested in the in vivo dog thrombosis model revealed no evidence of thrombi formation or blood clots showing limitation of in vitro assays compared to in vivo methods. In summary chemical characterization and risk assessment play a critical role in routine biocompatibility testing and safety evaluation of medical devices.

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**ABSTRACT FINAL ID:** 2749 Poster Board #231  
**TITLE:** HUMAN HEALTH HAZARDS OF EXPOSURE TO NEW TECHNOLOGY DIESEL EXHAUST (NTDE)

**AUTHORS (LAST NAME, FIRST NAME):** Hesterberg, Thomas W.; Long, Christopher M.; Sax, Sonia N.; Lapin, Charles A.; Bunn, William B.; Valberg, Peter A.; McClellan, Roger O.

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**KEYWORDS:** Exhaust, Particulate Matter, Hazard.

**ABSTRACT BODY:** Diesel engines have continually improved over the decades and are widely used in commerce due to efficient fuel use. Early Diesel engines had low emissions of carbon monoxide and high emissions of nitrogen oxides (NOx) and particulate matter (PM). These characteristics of Traditional Diesel Exhaust (TDE) stimulated research to evaluate health effects of TDE. By the mid-1980s, consensus developed: (a) TDE PM contained numerous complex organic molecules, (b) extracts of TDE PM were mutagenic, (c) lifetime exposures of rats to high concentrations of TDE caused lung cancer and pulmonary disease, and (d) epidemiological studies provided inconclusive evidence for TDE causing cancer. These findings lead to stringent regulations to limit Diesel engine emissions which stimulated improvements in engine technology, exhaust after-treatment, fuel, and control systems. The New Technology Diesel Exhaust (NTDE) is compared in this paper to TDE emissions. The concentrations of PM and associated chemicals in
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

NTDE are on the order of 1% or less of that found in TDE. Exposure of laboratory animals (up to 3 months) and human volunteers (several hours) to NTDE did not produce effects at exhaust dilutions similar to those at which TDE produced marked effects. Evaluation of chemical and toxicological characterization data indicate that NTDE has a very low human health hazard potential, a determination which has informed policy decisions to accelerate the replacement of old engines with new Diesel technology. These new scientific findings indicate that any future evaluation of hazards of exhaust from Diesel engines should separately consider TDE versus NTDE. The use of toxicological information to inform revolutionary advances in Diesel engine technology serves as an example of the role of toxicological findings in guiding improvements in technologies that are vital to modern Society.

**ABSTRACT FINAL ID:** 2750 Poster Board #233
**TITLE:** IN VIVO AND IN VITRO EXPOSURE TO NICKEL INDUCES CHANGES IN GLOBAL LEVELS OF H3K4ME3 AND H3K9ACETYL EPIGENETIC MARKS IN PERIPHERAL BLOOD LYMPHOCYTES

**AUTHORS (LAST NAME, FIRST NAME):** Arita, Adriana1; Wu, Fen1; Chervona, Yana1; Kluz, Thomas1; Kiok, Kathrin1; Sun, Hong1; Qingshan, Qu1; Costa, Max1

**INSTITUTIONS (ALL):** 1. New York University, New York, NY, United States.

**KEYWORDS:** nickel, epigenetics, carcinogenesis.

**ABSTRACT BODY:** Nickel (Ni) is of great environmental concern since occupational exposure to Ni has been linked to lung and nasal cancers. However, the precise mechanism(s) of Ni carcinogenesis remains unclear. Here, we have carried out the first study to examine global levels of histone tail epigenetic marks in the peripheral blood lymphocytes of a human population exposed occupationally to Ni. In this study, we have utilized an ELISA method developed in our lab to measure the levels of various histone tail modifications. We have found a statistically significant increase in (p=0.004 (CI=95%)) in the global levels of H3K4me3, based on creatinine-adjusted urinary Ni concentration, in the peripheral blood lymphocytes of 45 workers of a nickel refinery in Jinchang, China. Additionally, a significant decrease in global levels of H3K9acetyl (p=0.017 (CI=95%)) epigenetic mark was also observed in this same population. To further study the effects of Ni on human lymphocytes we have found that a 24 hr in vitro exposure of primary lymphocytes to NiCl2 induces an increase in global levels of H3K4me3 epigenetic mark using both western blot and ELISA methods. Affymetrix HG-U133A_2 arrays found 40% of genes upregulated and 60% of genes downregulated more than 2 fold in primary lymphocytes treated in vitro with NiCl2. Mapping of the H3K4me3 mark in the genome of primary lymphocytes after in vitro treatment with NiCl2 using ChiPseq technology, identified 10165 genes with a peak fold change greater than 2. Comparison between genecip and ChiPseq data found that 72% of genes upregulated in the genechip were also covered by ChiPseq data, including VEGFA, IFI44L, and PPFIA4. In this study we have undertaken a whole genome approach in order to further understand the epigenetic mechanism(s) of nickel compounds in human lymphocytes both in vivo and in vitro and we have identified possible candidate biomarkers for Ni exposure and effect.

**ABSTRACT FINAL ID:** 2751 Poster Board #234
**TITLE:** EFFECT OF ARSENIC ON HISTONE TAIL MODIFICATIONS AND GENE EXPRESSION

**AUTHORS (LAST NAME, FIRST NAME):** Chervona, Yana1; Arita, Adriana1; Wu, Fen1; Sun, Hong1; Kiok, Kathrine1; Tseng, Hsiang-Chi1; Kluz, Thomas1; Gamble, Mary2; Costa, Max1

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**KEYWORDS:** arsenic, epigenetics, histone tail modifications.

**ABSTRACT BODY:** Naturally occurring inorganic arsenic has been implicated in the development of human skin, lung, liver and prostate cancers. Although, its role as a carcinogen is widely accepted, the mechanisms of arsenic induced malignancies remain unclear. One potential mechanism of carcinogenesis may be rooted in arsenic’s ability to alter epigenetic homeostasis. We have previously shown that a 24 hour treatment of A549 cells with NaAsO2 causes global increases in H3K9 di and H3K4 tri methylation and that a 7 day treatment increases and preserves H3K4 tri methylation for at least one week. Now, we have also shown that a 24 hour arsenic treatment of Burkett’s lymphoma BL41 cells, a
lymphocyte cell line, also increases H3K9 acetylation, H3K9 di, H3K27 and H3K4 tri methylation. However, 6 day and/or 1 month treatments decrease H3K9 acetylation and H3K4 tri methylation, while H3K9 di and H3K27 tri methylation remain increased at certain doses. We also found that in samples of peripheral mononuclear cells collected from individuals exposed to inorganic arsenic in their drinking water (N=22) there is a statistically significant dose dependent increase in global H3K9 di methylation (p=0.0275), while H3K9 acetylation and H3K27 tri methylation are increased at lower arsenic doses (p=0.0165 and p=0.0452) and possibly exhibit a biphasic pattern. In addition, we also found that in vitro exposure of primary lymphocytes to arsenic causes changes in histone gene expression with the expression of H3 and H4 being increased in primary lymphocytes, A549, HCT79 and BL41 cell lines, after a 48 hour exposure to NaAsO2. The changes in the global levels of the epigenetic marks were measured using a novel ELISA method and western blots, while gene expression changes were measured using genechip human gene 1.0 ST arrays and RT-PCR. To the best of our knowledge, this study is the first in its class show epigenetic modifications as a potential mechanism of arsenic induced carcinogenesis.

ABSTRACT FINAL ID: 2752 Poster Board #235

TITLE: A SMALL MOLECULE INHIBITOR OF THE TGF B TYPE I RECEPTOR SUPPRESSES UVB-INDUCED MOUSE SKIN CARCINOGENESIS

AUTHORS (LAST NAME, FIRST NAME): Ravindran, Anand; Mohammed, Javed; Glick, Adam B.

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KEYWORDS: Skin Cancer, Solar UV, TGF beta.

ABSTRACT BODY: Ultraviolet radiation acting both as a mutagen and promoting agent is the major cause of nonmelanoma skin cancer in people. Transforming Growth Factor (TGF β) represents an important growth regulatory cytokine in the skin microenvironment with critical roles in modulating tumor formation and progression. In mouse models of chemically induced skin carcinogenesis, genetic manipulation of the TGF β1 pathway shows that it has both tumor-protective and tumor-promoting function depending on the specific stage of tumor development but a role for this pathway in UV induced skin cancer has not been established. Here we show that UVB rapidly induces activation of the TGF β pathway in mouse skin as measured by phosphorylation of Smad2 and Smad3, direct targets of the TGF β type I receptor kinase (ALK5). This phosphorylation was blocked by the topical application of SB431542, a small molecule inhibitor of ALK5. In a chronic UV carcinogenesis study, topical treatment with SB431542 3X per week significantly suppressed the average tumor number per mouse, consistent with a promoting role for TGF β in UV carcinogenesis. Since UV has direct effects on the immune responses in the skin that are linked to tumor formation we analyzed whether inhibition of TGF β signaling pathway with SB431542 altered UV-induced changes in T cell phenotype using flow cytometry. In mouse skin exposed to UV for 2 weeks there was a significant increase in IFNγ secreting CD4+ T cells that was blocked by SB431542. Similarly, in UV-induced skin tumors that developed in mice treated with SB431542 there was a significant reduction in the total level of tumor infiltrating CD4+ and CD8+ T lymphocytes compared to vehicle treated tumors, and a decrease in the IFNγ+ CD4+ and CD8+ T cells. These results indicate that TGF β signaling plays an important role in promoting tumor formation in response to chronic UVB, and this in part is mediated through the ability of TGF β to bring in tumor promoting IFNγ secreting T lymphocytes.
ABSTRACT FINAL ID: 2753 Poster Board #236
TITLE: AKT1 INFLUENCES 7,12-DIMETHYLBENZ[A]ANTHRACENE (DMBA)-INDUCED MAMMARY GLAND HYPERPLASIA

AUTHORS (LAST NAME, FIRST NAME): Pietruska, Jodie R.; Larocca, Jessica; Hixon, Mary L.
INSTITUTIONS (ALL): 1. Pathology & Laboratory Medicine, Brown University, Providence, RI, United States.
KEYWORDS: Akt, mammary, hyperplasia.

ABSTRACT BODY: The Akt family of protein kinases functions in multiple cellular processes, including proliferation, differentiation, and apoptosis, as well as in carcinogenesis. Although the three Akt isoforms (Akt1, Akt2, Akt3) share sequence and functional similarity, they may have nonredundant roles in both physiological and pathological processes. Akt1 is involved in lactation and involution in the mammary gland, and is implicated in oncogene-driven mammary carcinogenesis. We hypothesized that Akt1 modulates tumorigenesis induced by the polycyclic aromatic hydrocarbon 7,12-dimethylbenz[a]anthracene (DMBA), a known carcinogen, and one of numerous toxicants present in cigarette smoke. Using a transgenic mouse model of Akt1 deficiency, we compared the effects of a single dose of DMBA (30mg/kg) administered at postnatal day (PND) 70 on mammary gland hyperplasia in Akt1-WT and Akt1-KO mice. Animals were sacrificed at PND 214, PND 365, or when moribund, and inguinal mammary glands were dissected for whole mount and histological analyses. Compared to untreated Akt1-WT mice, mammary glands from Akt1-KO mice exhibited increased numbers of terminal end buds and a higher percentage of terminal end buds compared to total buds by PND 50 and PND 70, suggesting a more immature phenotype. By PND 365 in DMBM-exposed mice, Akt1-KO mammary glands exhibited abnormal regression, whereas Akt1-WT mammary glands exhibited hyperplastic alveolar nodules. Loss of Akt1 significantly reduced DMBA-induced mammary hyperplasia in Zone C at PND 214 and PND 365 compared to Akt1-WT mice (p<0.01). Thus far, these studies suggest that in this transgenic model of Akt1 deficiency, loss of Akt1 is protective against DMBA-induced mammary hyperplasia. In order to extend these observations, we are currently addressing the role of Akt1 in cell proliferation and survival following DMBA exposure in an in vitro system. Using retroviral-based shRNA targeting Akt1, we are evaluating whether silencing of Akt1 inhibits proliferation and survival in human breast epithelial cells following DMBA exposure.

ABSTRACT FINAL ID: 2754 Poster Board #237
TITLE: LOCALIZED EXPOSURE TO DDT CONGENERS INFLUENCES MAMMARY GENE EXPRESSION

AUTHORS (LAST NAME, FIRST NAME): Johnson, Nakpangi A.; Meng, W. S.; Witt-Enderby, P. A.; Foster, W. G.; Davis, V. L.
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KEYWORDS: Breast Cancer, DDT, Mixture.

ABSTRACT BODY: In young women, early DDT exposure was linked to an increased risk of breast cancer. Similarly, localized, prepubertal exposure to p,p DDE in MMTV-neu female mice accelerated mammary tumor onset, suggesting that DDT stored in breast fat influences tumorigenesis. To investigate potential mechanisms for the shorter latency, gene expression was tested in adult mammary glands to determine if p,p DDE actions were dose-dependent; were related to its antiandrogenic activity; or were modified by an estrogenic isomer, o,p DDE, or o,p DDT, the congener linked to higher BC risk. Also, to determine if p,p DDE induced systemic effects, expression of cytokines with known cancer promoting or inhibiting properties were tested in splenic leukocytes. Despite localized delivery with slow release pellets implanted in each mammary gland, real-time RT-PCR showed that p,p DDE increased expression of TGFβ1 and decreased IL10 in splenic leukocytes; plus, for IL10, p,p DDE acted like the antiandrogen hydroxyxiflutamide. In mammary tissue, elevated expression of differentiation markers, keratin 18 (krt18) and casein gamma (csn1s2a) and an interferon-regulated gene (ifi44) was observed when treated with 5μg p,p DDE vs. control pellets. In the tumor study, this dose also shortened tumor onset and resulted in p,p DDE levels of 1800 μg/kg in the mammary gland. In mixtures, a 2:1 ratio of p,p DDE:o,p DDE (5:2.5 μg) stimulated expression of csn1s2a similar to 5 μg p,p DDE; whereas, 2.5μg o,p DDE suppressed its expression. A 1:1 ratio of p,p DDE:o,p DDE (2.5μg each) altered the expression of csn1s2a,
2011 Society of Toxicology 50th Anniversary Meeting  
Late-Breaking Abstracts

if44, and krt18 to resemble the 5µg doses. Dose-dependent p,p DDE effects were also observed, such as krt18 had stronger responses at the lower doses, 2.5 and 0.5 µg. Collectively, p,p DDE had varied actions that depend on the gene, dose, and/or other congeners that may be important for assessing BC risk with early DDT exposure. Immune gene changes induced by p,p DDE outside the mammary gland and its antiandrogen activity may also contribute to hastening tumor development.

ABSTRACT FINAL ID: 2755 Poster Board #238  
TITLE: EFFECT OF FORMALDEHYDE ON THE EXPANSION OF ERYTHROID PROGENITOR CELLS FROM CIRCULATING PERIPHERAL BLOOD

AUTHORS (LAST NAME, FIRST NAME): Li, Xiyi¹; Ji, Zhiying¹; Mutter-Rottmayr, Liz¹; Smith, Martyn T.¹; Zhang, Luoping¹  
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KEYWORDS: Formaldehyde, erythroid progenitor cells, myeloid leukemia.

ABSTRACT BODY: The association of formaldehyde (FA) and myeloid leukemia has been recently reaffirmed by IARC. Hematopoietic stem/progenitor cells (HSC/HPC), present at low numbers in circulating blood, are potential targets of FA hemotoxicity. Previously, we showed that FA treatment decreased the colony forming from the myeloid HSC/HPC in circulating blood. To explore the effects of FA exposure on erythroid lineage development, we recently developed a simple, reproducible and sample-saving culture method to expand erythroid progenitor cells (EPC) from peripheral blood without mobilization. Cell counts and viability were measured by hemocytometer and the trypan blue exclusion assay. EPC surface markers, CD36, CD235a and CD71, were detected with specific monoclonal antibodies by flow cytometry. Our results showed that the number of EPCs and the expression of CD36, CD235a and CD71 were increased dramatically after a 10-day culture, indicating effective expansion. We then examined EPC expansion from 5 x 10^5 peripheral blood mononuclear cells following treatment with 50-100 µM FA for 10 days. In addition to cell number and surface markers, apoptosis and necrosis were analyzed by Annexin-V/PI staining, and cell cycle was determined by DNA content analysis. We found that EPC expansion was suppressed by 100 µM FA, compared with untreated controls, but the pattern of EPC markers was not significantly changed. Further testing revealed that the suppression was not due to increased apoptosis or necrosis, or cell cycle arrest. However, survived cells tend to more resistant to the following cytokines withdrawal and slightly more EPCs entered into cell cycle (from Go/G1 to G2/M and S phase) at 100 µM FA. These results suggest that FA may modulate the growth of HSC/HPC in circulating blood. This may be a potential mechanism of FA-induced leukemia.

ABSTRACT FINAL ID: 2756 Poster Board #239  
TITLE: GENE EXPRESSION PROFILES OF FOUR NON-GENOTOXIC RENAL CARCINOGENS IN RAT KIDNEY CELLS IN VITRO

AUTHORS (LAST NAME, FIRST NAME): Bloch, Kate¹; Evans, Andrew¹; van Delft, Joost²; Lock, Edward A.¹  
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KEYWORDS: nongenotoxic carcinogenesis, in vitro carcinogenicity, transcriptomics.

ABSTRACT BODY: This study has used genome-wide transcriptomic analysis, to identify genes that may predict the carcinogenic potential of non-genotoxic (NGTX) chemicals to the kidney. Normal Rat Kidney cells (NRK-52E) were exposed individually to 4 NGTX rat renal carcinogens at an IC10 concentration for 72h. The chemicals were; Ochratoxin A (OTA), 0.5µM; Monuron (M), 250µM; Chlorothalonil (C),1.1µM or S-1,2-dichlorovinylcysteine (DCVC), 3µM. Control cells were exposed to 0.1% DMSO the same as treated cells. Cells were exposed for 6h, 24h and 72h in triplicate and RNA isolated and quality checked. Microarray analysis used Affymetrix rat GeneChips 230, 2.0. Analysis of gene changes used GeneSpring GX11 software. Data was normalised using a GC-RMA algorithm and statistical analysis between control and treated cells conducted using a >2-fold threshold change with p <0.001. DAVID website was used for Kegg pathway analysis With C, 174 genes were altered after 6h,324 after 24h and 317 at 72h. With OTA 170 genes were altered at 6h, 1,057 at 24h and 723 after 72h. In contrast, only 6 genes were altered after M exposure for 6h, 24 genes after 24h and 123 genes after 72h. With DCVC no differentially expressed genes were found at 6h, 1 at 24h and
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

1,448 at 72h. Some major pathways affected were steroid biosynthesis, down regulated 24h after C and OTA; terpenoid backbone biosynthesis down regulated after 6h with M and 24h with C. Other pathways affected include cell cycle (C,6h; DCVC,72h, OTA,24h); cell adhesion (C, 6h and 72h); pathways in cancer 24h after OTA and 72h after DCVC and C exposure; aminoacyl t-RNA synthesis, 72h after DCVC; p53 signalling 24h after OTA,72h after DCVC and proteolysis, 24h and 72h after C. These findings indicate that NRK-52E cells are able to detect some of the carcinogenic events associated with NGTX carcinogens in vitro. No commonly deregulated genes were observed 6, 24 or 72h after the 4 NGTX carcinogens. Supported by a grant EU 6th framework on Carcinogenomics.

ABSTRACT FINAL ID: 2757 Poster Board #240
TITLE: THE AUTOMATED FADU ASSAY, A HIGH-THROUGHPUT IN VITRO METHOD FOR EARLY SCREENING OF CLASTOGENICITY

AUTHORS (LAST NAME, FIRST NAME): Buerkle, Alexander; Eltze, Tobias; Dressler, Dirk; Bernhardt, Jürgen; von Scheven, Gudrun; Hirsch, Cordula; Moreno-Villanueva, Maria


KEYWORDS: DNA strand breaks, alkaline unwinding, automation.

ABSTRACT BODY: Genotoxicity tests are essential to identify compounds that have a potential to act as tumor initiators. As there is no single test capable of detecting all types of genotoxic effects, a battery of in vivo and in vitro tests are necessary to determine the genotoxicity of a compound. However, the specificity and sensitivity of current assays have been criticized since they report a high percentage of false positive results. We have previously described an automated method for measuring DNA strand breaks induced in human peripheral blood mononuclear cells and cultured human cell lines by ionizing radiation (Moreno-Villanueva M et al., BMC Biotechnol. 2009;9:39). Here we describe detection of DNA strand break formation by automated FADU in cells treated with several chemical compounds known to induce DNA strand breaks by different mechanisms. Additionally, we also tested several toxic compounds that are known not to induce DNA strand breaks, which is perfectly mirrored by our results. The main advantages of the automated FADU assay are high reproducibility and sensitivity, high-throughput, easy handling, speed, and low cost.

ABSTRACT FINAL ID: 2758 Poster Board #241
TITLE: QUANTITATIVE METHOD FOR THE DETECTION OF BENZO[A]PYRENE-DNA ADDUCTS AND 8-OXO-DG IN ATLANTIC KILLIFISH

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KEYWORDS: benzo[a]pyrene, DNA adducts, 8-oxo-dG.

ABSTRACT BODY: The Atlantic Wood Industries Superfund site on the Elizabeth River (ER) is contaminated with high levels of benzo[a]pyrene (BaP). Although BaP is known carcinogen, a population of Atlantic killifish (Fundulus heteroclitus) inhabits the ER and shows resistance to BaP-mediated liver cancer. ER killifish are suspected to have increased oxidative stress defenses which may be related to the observed BaP resistance. Here, we have developed an LC-MS/MS-SRM method to simultaneously and quantitatively monitor BaP DNA adducts and the oxidative stress adduct, 8-Hydroxy-2′-Deoxyguanosine (8-oxo-dG). BaP metabolism to benzo[a]pyrene diol epoxide (BPDE) is considered the main pathway of BaP-mediated carcinogenesis since BPDE readily reacts with DNA to form BPDE-DNA adducts. The reaction of BPDE with 2′-deoxyguanosine forms four stereoisomers which, with our method, can be individually analyzed to determine stereo-specific differences in adduct formation. Quantitative analysis of BPDE-dG
isomers and 8-oxo-dG was performed on an Acquity UPLC coupled to a TSQ-Quantum Ultra triple-quadrupole mass analyzer. Separation was performed on a Waters C8 BEH 1.7µm column. The mobile phase consisted of 0.1% formic acid in water and 100% acetonitrile. At a flow rate of 50 µL/min, baseline separation of 8-oxo-dG and the four BPDE-dG stereoisomers was achieved with a LOD of 25 fmols on column for 8-oxo and an average LOD of 30 fmols for each of the stereoisomers. The LOQ was 50 fmols and 60 fmols for 8-oxo and the BPDE-dG stereoisomers, respectively. This method will be used to measure BPDE-dG and 8-oxo levels in liver DNA from wild caught killifish from the ER and a non-contaminated reference site. In addition, these adducts will also be measured in killifish acutely exposed to BaP to determine the kinetics of adduct formation and DNA repair.

**ABSTRACT FINAL ID:** 2759 Poster Board #242

**TITLE:** COMPARING GENOTOXIC DAMAGE MEASURED BY COMET AND MICRONUCLEUS ASSAYS USING HIGH CONTENT ANALYSIS

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**KEYWORDS:** High Content Analysis, Comet Assay, Micronucleus Assay.

**ABSTRACT BODY:** Genotoxicity refers to damage of genetic material which results in loss of cellular function, genetic integrity and mutations. Measuring and preventing genotoxicity are major concerns in the pharmaceutical industry. The “comet assay”, or single cell electrophoresis, is a widely accepted and simple method to measure the genotoxic potential of compounds. Another widely-used technique used for measuring the genotoxic potential of chemicals is the micronucleus assay, which is a rare event assay. Imaging methods are generally used to visualize formation of micronuclei or comets due to genotoxicity, but analysis of these images is done mostly manually or by semi-automated tools that are time-consuming and may generate inconsistent data. High content analysis (HCA) is a quantitative automated imaging tool that is gaining greater acceptance in assessing toxicity and genotoxicity of compounds. In this study, we used HCA to compare the clastogenicity of DNA-damaging agents such as Mitomycin-C and 9-aminoacridine (9-AA) in CHO-K1 cells, by evaluating overall micronuclear frequency. We then applied the comet assay to assess the genotoxic potential of the same compounds by evaluating features such as Olive tail moment, tail moment, tail extent, tail area, comet length and % tail DNA. By applying these two methods, we quantitatively compared the genotoxicity potential of various other compounds in a small screen. In CHO-K1 cells, Olive tail moment and micronucleus frequency were used as overall measurements of genotoxicity to help rank the DNA damage potential. Our results show that Mitomycin-C concentrations >500 µM/ml caused gross DNA damage to be seen in the comet assay; likewise it generated a moderate frequency of micronuclei. Order of potency at 100µM concentration ranked 9-AA > Mitomycin-C > Tacrine > control, for tail olive moment. The results show that using high content analysis enables us to rapidly and consistently compare and contrast genotoxic damage to cells as measured via comet and micronucleus assays.

**ABSTRACT FINAL ID:** 2760 Poster Board #243

**TITLE:** CATALASE PLAYS AN IMPORTANT ROLE IN A GENOTOXIC PATHWAY OF METHYLATED ARSENICALS

**AUTHORS (LAST NAME, FIRST NAME):** Muniz Ortiz, Jorge G.; Tennant, Alan H.; Kligerman, Andrew

**INSTITUTIONS (ALL):** 1. NHEERL, US EPA, Research Triangle Park, NC, United States.

**KEYWORDS:** Oxidative Stress, Arsenic, Genotoxicity.

**ABSTRACT BODY:** Arsenic is a common contaminant of drinking water in many parts of the world. Consumption of arsenic-contaminated drinking water has been implicated in both cancerous and non-cancerous health conditions. However, the pathways that lead to arsenic-induced health conditions have not been clearly defined. Previous work in our laboratory demonstrated a key protective role for superoxide dismutase in primary mouse lymphocytes exposed to methylated arsenicals. Here we determine whether catalase (Cat) is also essential in protecting cells against methylated arsenical exposure. Taken together these studies aim to identify an ultimate reactive oxygen species (ROS;
ABSTRACT FINAL ID: 2761 Poster Board #244
TITLE: USING A HIGH CONTENT MICRONUCLEUS ASSAY TO IDENTIFY CLASTOGENS AND ANEUGENS

AUTHORS (LAST NAME, FIRST NAME): Miller, Donna M.; Peters, Amy M.; Haskins, Jeffrey R.
KEYWORDS: Genotoxicity, Genotoxicity - Mechanism of action, High Content Imaging.

ABSTRACT BODY: The Micronucleus (MN) Assay was developed to identify genotoxic agents by measuring the frequency of micronucleus formation in dividing cells. The MN assay is a widely used tool in toxicology testing to detect micronuclei that arise from aneugenic and clastogenic compounds. However, the assay alone cannot differentiate between these two mechanisms of action (MOAs). Aneugens (aneuploidy-inducing substances) cause missegregation of whole chromosomes. Clastogens directly damage DNA resulting in chromosomal aberrations and often acentric chromosome fragments. Understanding the MOA is important due to the fact that aneuploidy and structural aberrations can cause very different and specific phenotypes. Classification of the MOA is helpful in determining the potential risk of a substance to human populations. In this study, we investigated methods of determining the MOA by using automated image analysis of the MN assay with a centromere-specific antibody to measure the proportion of centromere positive MN in A549 cells. A549 cells were treated with compounds in a dose-responsive manner. Cytokinesis was then blocked (via cytochalasin B) and after additional incubation and staining with a cellular marker, cells were fixed, permeabilized, stained and imaged. Aneugenic compounds tested (nocodazole, colchicine and vinblastine) showed greater than 2-fold increase in centromere positive MN over the untreated control, while clastogenic compounds (mitomycin C, cytarabine and chlorambucil) had no statistically significant changes in centromere positive MN relative to the untreated control. The results of this pilot study indicate that an automated method of classifying genotoxic compounds is possible.

ABSTRACT FINAL ID: 2762 Poster Board #245
TITLE: UTILIZATION OF HIGH THROUGHPUT YEAST IN VITRO GENOTOXICITY ASSAY FOR DRUG DISCOVERY AND CORRELATION WITH THE IN VITRO MICRONUCLEUS ASSAY

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KEYWORDS: Genotoxicity, In vitro MN assay, Yeast dual luciferase assay.

ABSTRACT BODY: Eliminating potential genotoxicity liabilities during lead optimization of drug discovery is critical to successful pharmaceutical development. In this study we evaluated a yeast based high throughput genotoxicity screen and compared it to the in vitro micronucleus (MN) assay. Yeast (YPH499) doubly transfected with the RAD51 promoter linked to a Firefly luciferase reporter and with the 3- phosphoglycerate kinase (PGK1) promoter linked to Renilla luciferase reporter were seeded to achieve an OD = 0.3 in 96-well plates. Test compounds, with and without S9, were added to the yeast suspensions, incubated for approximately 18 hours, and then evaluated for both genotoxicity...
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

(induction of Firefly luciferase) and for cytotoxicity (induction of Renilla luciferase). Compounds in the yeast assay were considered genotoxic positive if there was a dose-dependent increase of Firefly luciferase levels ≥ 1.6x at concentrations around 50% cytotoxicity, and weakly positive if the response was 1.3 to 1.6x, relative to the vehicle control (DMSO). When evaluated in the yeast assay, the clastogens, Mitomycin C (-S9) and Cyclophosphamide (+S9), resulted in genotoxic positive responses in the range of 0.5 to 2.5 mM and 0.5 to 5 mM, respectively. This correlated with in vitro MN data in CHO-K1 cells, which produced positive results in roughly similar concentration ranges. The aneugens, colchicine and vinblastine, were also positive in the yeast genotoxicity assay, but at concentrations > 1000X higher than in the MN assay. In general, there was a good concordance between this yeast assay and in vitro micronucleus assays in detecting genotoxicity. Additionally this yeast assay has been previously shown to correlate with Ames positive mutagens as well. Therefore, this high throughput yeast genotoxicity screen could allow for “triaging” of compounds during lead optimization.

ABSTRACT FINAL ID: 2763 Poster Board #246
TITLE: GENOTOXICITY OF THE GAS/VAPOR PHASE OF CIGARETTE MAINSTREAM SMOKE IN THE MOUSE LYMPHOMA TK ASSAY (MLA)

AUTHORS (LAST NAME, FIRST NAME): Wittke, Sandra1; Trelles-Sticken, Edgar2
KEYWORDS: MLA, cigarette smoke, gas/vapor phase.

ABSTRACT BODY: The in vitro mammalian mutagenicity of cigarette smoke by means of the mouse lymphoma TK assay (MLA) is generally assessed by assaying the particulate phase only (Schramke et al., 2006). In order to generate a comprehensive and thus more relevant image of cigarette-smoke-related mammalian mutagenicity in vitro we extended this approach to the water-soluble fraction of the gas/vapor phase (GVP). The GVP of mainstream smoke from the Reference Cigarette 3R4F was assayed in the microtiter plate version (Cole et al., 1986) of the MLA with and without metabolic activation (S9), after 4 and 24 hours of treatment time and 2 and 3 days of expression time. Dose-response: a clear-cut positive dose-response relationship was seen regardless of activation conditions, treatment time, and expression time. Variability of mutant frequency: for the 24-h treatment time (without S9, 2 days expression), mean repeatability was 16% and mean reproducibility was 26%; for the 4-h treatment time (with and without S9, 3 days expression), mean repeatability was 12% and mean reproducibility was 15%. Variability of 1/C3B values: for the 24-h treatment time (without S9, 2 days expression), repeatability was 6% and reproducibility was 8%; for the 4-h treatment time (with and without S9, 3 days expression), mean repeatability was 3% and mean reproducibility was 5%. Thus, the results showed that the MLA is sensitive for the detection of the in vitro mammalian mutagenicity of the gas/vapor phase of cigarette mainstream smoke.

ABSTRACT FINAL ID: 2764 Poster Board #247
TITLE: DAMAGE RECOGNITION AND SIGNALING BY MRE11-RAD50-NBS1-ATM AND TFIIH-CAK KINASE COMPLEXES CONTROL BIOLOGICAL OUTCOMES OF DNA DAMAGE

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KEYWORDS: DNA Damage.

ABSTRACT BODY: DNA ends at breaks, replication forks and telomeres are often critically controlled for repair and integrity by a single trimeric complex of Mre11-Rad50-Nbs1 (MRN) dimers. MRN heterohexamer acts in key sensing, signaling, regulation, and effector responses to DNA double-strand breaks including ATM activation, homologous recombinational repair, microhomology-mediated end joining and, in some organisms, non-homologous end joining. Our results suggest that this is possible because each MRN subunit can exist in three or more distinct states; thus, the
trimer of MRN dimers can exist in a stunning 63 or 216 states, a number that expands further when post-translational modifications are considered. MRN can therefore act as a molecular computer that effectively assesses optimal responses and signals pathway choice based upon its states as set by cell status and the nature of the DNA damage. Similarly, diverse lesions that distort double helical DNA are repaired by nucleotide excision repair (NER) orchestrated by conformations of the TFIIH complex enzymes: the XBP and XPD helicases and CAK kinase. Combined with mapping of XP patient mutations, detailed structural analyses provide a framework for integrating and unifying the rich biochemical and cellular information that has accumulated over nearly forty years of study. This integration resolves puzzles regarding XP helicase functions and indicates that XP helicase positions and activities within TFIIH detect and verify damage, select damaged strand for incision, and coordinate repair with transcription and cell cycle through CAK signaling. This concept that allosteric changes coordinate repair with replication, transcription, and cell cycle by coupling conformations to kinase activity provides opportunities to develop master keys to cell biology and possible therapeutic intervention.

ABSTRACT FINAL ID: 2765 Poster Board #248
TITLE: EXPRESSION LEVEL OF miR-34A CORRELATES WITH MUTAGENICITY OF X-RAY IN HUMAN LYMPHOBLASTOID CELLS WITH DIFFERENT P53 STATUS

AUTHORS (LAST NAME, FIRST NAME): Chen, Xinxrong; Yan, Jian; Chen, Tao
KEYWORDS: miR-34a, mutation, P53.

ABSTRACT BODY: TK6, WTK1 and NH32 are human B lymphoblast cell lines derived from the same lymphoid progenitor, differing in tumor suppressor gene P53 status: TK6 has wild-type P53 genes, NH32 has a null mutation of one P53 allele, and WTK1 has a mutant form of P53. Previous studies showed that TK6 and NH32 cells exhibited similar spontaneous and X-ray-induced mutant frequencies in spite of their different P53 status and were much less mutable than WTK1 cells (x50 difference). It is unclear why NH32 and WTK1 cell lines, both of which have lost the function of P53, have such a big difference in their response to mutation induction. To elucidate the possible mechanisms involved in the different mutagenic responses among the three cell lines, we investigated the cell lines for their expression levels of miR-34a, a tumor suppressor microRNA that is a direct transcriptional target of P53. The expression level of miR-34a in the untreated and X-ray-treated cells was measured using real-time RT-PCR. There were similar basal expression levels of miR-34a in TK6 and NH32 cells. Both of these cell lines had about 10-fold higher miR-34a basal expression than the WTK1 cells. The expression level in the three cell lines correlates well with their mutability: higher levels of miR-34a corresponds with less mutable cells. X-ray treatment increased miR-34a expression in TK6 cells but not in NH32 and WTK1 cells, suggesting that up-regulation of miR-34a by X-ray treatment requires the presence of wild type P53 genes. These results suggest that miR-34a might play a critical role in suppression of mutation induction. Although miR-34a is regulated by P53, its expression level appears to be more closely related to cell mutability than the expression level of normal P53 gene.

ABSTRACT FINAL ID: 2766 Poster Board #837
TITLE: EARLY SAFETY & EFFICACY ASSESSMENT OF ORB0001 FOR CHRONIC MYELOID LEUKEMIA TREATMENT

AUTHORS (LAST NAME, FIRST NAME): Loget, Olivier M.
INSTITUTIONS (ALL): 1. Management, OriBase Pharma, Montpellier, France.
KEYWORDS: Oncology, Chronic Myeloid Leukemia, Chemoresistance.

ABSTRACT BODY: Imatinib (Gleevec®) is standard of care for chronic myeloid leukemia (CML), partly due to its high affinity for tyrosine kinases Bcr-Abl. Discontinuation due to intolerance/resistance is necessary in up to 30 % of patients. According to Src kinase role in imatinib-resistance, several dual Src & Abl inhibitors, including bosutinib, bafetinib & INNO-406 were developed. These are more potent than imatinib in inhibiting Bcr-Abl & able of inhibiting most of known Bcr-Abl mutants except mutation T315I, involved in 15-20 % Gleevec resistant patients. Approved 2nd-line CML drugs were recently approved for 1st-line indication: nilotinib (Tassigna®) & multitargeted kinase inhibitor dasatinib
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

(Sprycel®). There are not yet clinically approved T315I inhibitors. Alternative approaches for T315I mutant inhibition (15-20 % of clinically observed mutants) are of pharmaceutical interest. We designed Abl inhibitors by molecular modeling/combinatorial chemistry techniques. Acting as dual inhibitors of 2 oncogenic enzymes (Bcr-Abl/Src), series of 7-azaindole inhibitors was optimized using docking studies/structure-activity relationships to enhance affinity toward Abl kinase domain T315I mutant. We studied these inhibitors blocking normal & T315I resistant CML (drug candidate ORB0001) with first in vitro (CYP inhibition) & in vivo (xenografts in mice) toxicological results. In vitro: kinase assays: ORB0001, 100x more active than Gleevec, is a potent inhibitor of T315I resistant form of Bcr-Abl, which is not the case of Sprycel or Tasigna ORB0001 blocks normal & T315I resistant CML proliferation. ADME/Tox Results: There is no inhibition toward CYP, which is not the case of Gleevec, Sprycel or Tasigna. In vivo: ORB0001 stops tumor proliferation (50% Gleevec dose) the mice tolerate the drug. The body weight was less affected than with Gleevec. In conclusion, ORB0001, initially expected to cover half of patients failing either of Sprycel or Tasigna treatments, developed by HitFast Discovery® in less than 24 months, is a drug candidate blocking normal & T315I resistant CML, without inhibiting CYP or affecting body weight.

ABSTRACT FINAL ID: 2767 Poster Board #838
TITLE: PROPOSED MECHANISM OF ACTION FOR TOXICITY IN N-ACETYLCYSTEINE OVERDOSE

AUTHORS (LAST NAME, FIRST NAME): Hentz, Karyn¹; Lamb, James¹
KEYWORDS: N-Acetylcysteine, Overdose, Toxicity mechanism.

ABSTRACT BODY: N-acetylcysteine (NAC) is an antidote for acetaminophen poisoning, which is administered to reduce the likelihood of acetaminophen-induced liver toxicity. On hospital admission and determination of the acetaminophen plasma concentration, NAC may be administered to the patient. In cases where the patient is nauseous or has been vomiting, intravenous administration (IV) is recommended. Several cases of severe overdosing with IV NAC (over 10 times the recommended dose) have been reported in the literature. In the most severe cases, patients have developed seizures, intercranial hypertension, and some patients died. Based on a review of case reports, acetaminophen toxicity, and NAC toxicity, a proposed mechanism for the observed toxicity in these cases of NAC overdose was developed. In the brain, the balance of neurotransmitters is critical to normal function and elevated levels of certain amino acids, including glutamate and cysteine, can induce toxicity. NAC is metabolized to cysteine which can be oxidized to cystine; cystine is then taken up by cells through an exchange system with glutamate. The cystine-glutamate exchange system, called X⁻⁻, is responsible for regulating extra- and intracellular levels of these amino acids. The pharmacological effects of NAC for the treatment of addictive behaviors are mediated by the cysteine-glutamate exchange system. Furthermore, glutamate toxicity is associated with status epilepticus and neuronal death. Therefore, it is hypothesized that extreme extracellular levels of glutamate are the eventual cause of injury in patients significantly overdosed with NAC.

ABSTRACT FINAL ID: 2768 Poster Board #839
TITLE: IDENTIFICATION OF POLYMORPHISMS IN GENES OF THE IMMUNE SYSTEM IN CYMONOLGUS MACAQUES

AUTHORS (LAST NAME, FIRST NAME): Wu, Hong¹; Adkins, Karissa K.¹
INSTITUTIONS (ALL): 1. Investigative Toxicology, DSRD, Pfizer Inc, Groton, CT, United States.
KEYWORDS: polymorphism, cynomolgus macaques.

ABSTRACT BODY: Cynomolgus macaques (Macaca fascicularis) are a standard non-rodent species used preclinically for evaluating the toxicity of therapeutic drug candidates. They are especially important for evaluation of biological therapeutics such as monoclonal antibodies which often do not have pharmacological activity in rodent species. Toxicological findings in cynomolgus macaques with biological therapeutics are often highly variable, and there is usually not a clear dose-response relationship observed, making it difficult to understand and explain the findings. One potential factor that may be contributing to the observed variability is genetic diversity. Limited genetic information is publically available on the cynomolgus macaque genome, but there is little doubt that like humans and other species, it
ABSTRACT FINAL ID: 2769 Poster Board #841
TITLE: BLINDED IDENTIFICATION OF CLINICAL DRUG-INDUCED QT PROLONGATION EMPLOYING DISTRIBUTION-BASED QT ANALYSIS

AUTHORS (LAST NAME, FIRST NAME): Holzgrefe, Henry1; Morrison, Royce1
INSTITUTIONS (ALL): 1. Toxicology, Charles River Laboratories, Reno, NV, United States.
KEYWORDS: ECG and QT prolongation, QTc, Torsade de pointes.

ABSTRACT BODY: Accurate clinical assessment of QT interval prolongation, the accepted biomarker for Torsade de pointes liability, remains difficult and quite expensive. We have proposed that accurate assessment of drug-induced QT interval changes can be reliably and inexpensively accomplished through automated, distribution-based QT analysis (QTca) of continuous digital ECG data. In confirmation of this method, it was employed to blindly identify the treatment assignments from randomly assigned clinical subjects’ baseline, post-placebo, and post-moxifloxacin (a “positive control” drug known to prolong QT) ECG data. Continuous digital ECGs (24 h, 500 Hz; Mortara Surveyor) were analyzed employing pattern recognition software (ECG PRO, ver. 5.0, Data Sciences Intl.). In a subset of 14 subjects randomly selected from a larger 3-way crossover clinical study, 24 h ECG data acquired at baseline and after placebo and positive control exposures were analyzed, identifying 78±3% of QRST complexes as valid for analysis across all treatment groups. For each subject, the baseline raw QT values were normally distributed, spanning a range of 50-75 ms for each discrete RR interval, confirming characteristic rate-independent raw QT variability in man. Individual exponential QTca rate-correction coefficients ranged from 0.160 to 0.482. QTca values were derived for all valid QRST complexes; for each sequential 10-min post-dose interval mean QTca and DD QTca (double delta; predose baseline-adjusted change in QT versus time-matched placebo) values were derived. QTca analysis was robust throughout the 24 h acquisition period, including times corresponding to procedures (meals, phlebotomy, etc) eliciting 50-200 ms changes in RR. Blinded DD QTca analysis correctly identified 13/14 moxifloxacin and 27/28 null administrations. After moxifloxacin, unblinded DD QTca exceeded 10 ms from 1-10 h, and the lower bound of 95% CI excluded zero from 1-4 h. These data suggest improved power over standard DD QTc analysis and potential universal applicability for preclinical and clinical assessment of drug-induced QT interval changes.
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

ABSTRACT FINAL ID: 2770 Poster Board #842
TITLE: AN INVESTIGATION INTO BEHAVIOUR, LEARNING AND MEMORY ASSESSMENTS IN THE JUVENILE GÖTTINGEN MINIPIG TREATED WITH HALOPERIDOL, D-AMPHETAMINE, OR SCOPOLAMINE

AUTHORS (LAST NAME, FIRST NAME): Manton, Jason C.1
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KEYWORDS: Göttingen minipig, Behaviour, Learning and Memory.

ABSTRACT BODY: Twenty-one naïve juvenile Göttingen minipigs were used to investigate techniques for behaviour, learning and memory assessments in relation to treatment with haloperidol, d-amphetamine, and scopolamine. These substances were selected to impair normal responses in order to assess the selectivity and sensitivity of each technique for potential use on regulatory safety evaluation studies. An adjusted holeboard test (4 of 16 holes baited) was used to assess cognitive performance after administration of scopolamine. Results showed that the time taken to complete the test was increased for animals dosed with Scopolamine when compared with the concurrent controls; however, there were no discernible differences between the Scopolamine treated animals and concurrent controls for the number of re-visits to baited holes (Working Memory) and to unbaited holes (Reference Memory). A 10 minute open field test was used to assess behavioural response of each animal in a testing arena after administration of either haloperidol or d-amphetamine. Haloperidol and d-amphetamine produced marked changes in motor behaviour and decreased explorative behaviour, consistent with responses documented in the literature. A clear distinction was determinable between the behavioural profiles of these compounds and the concurrent controls. In conclusion, this investigation indicated that the design of the 10 minute open field test was capable of detecting behavioural changes in juvenile Göttingen minipigs treated with haloperidol or d-amphetamine, however, further investigations are required to assess the suitability of the adjusted holeboard test for learning and memory assessments in the juvenile Göttingen minipig treated with Scopolamine.

ABSTRACT FINAL ID: 2771 Poster Board #843
TITLE: LACOSAMIDE DOES NOT ALTER BONE DENSITOMETRY PARAMETERS IN JUVENILE DOGS

AUTHORS (LAST NAME, FIRST NAME): Cornet, Miranda1; Léonard, Michèle2
KEYWORDS: bone mineral density, anti-epileptic drugs, juvenile dog.

ABSTRACT BODY: Rationale: Long-term use of antiepileptic drugs (AEDs) such as phenytoin, carbamazepine, sodium valproate or phenobarbital has been associated with reduced bone density possibly leading to an increased risk of osteoporotic fractures. Animal models have been shown to be good predictors of the human situation in identifying bone effects. In the present study, the potential effect of lacosamide, a newer AED, on bone quality was assessed in juvenile dogs. Design/Methods: Dogs (7 8 weeks old) were treated orally (gelatin capsules, lacosamide 0, 3, 10 and 25 mg/kg once daily and 25 mg/kg bid) for 33 weeks and a subset of animals was allowed to recover for 4 weeks. Due to subsiding signs of toxicity, the 25 mg/kg dose (once daily and bid) was increased to 30 mg/kg from test week 2 onwards and to 35 mg/kg from test day 60 onwards. Bone mineral content, area and density were analyzed for the tibia (regions of interest: total tibia, proximal and distal 25% of the tibia and midshaft 50%) and the lumbar vertebrae L3-L4 by dual energy X-ray absorptiometry (DEXA). Results: After 33 weeks of oral lacosamide treatment with up to 70 mg/kg/day (35 mg/kg bid), there were no treatment-related adverse effects on bone mineral content, area or density in either male or female dogs, at either the lumbar vertebrae or the tibia. There were also no treatment-related effects on serum calcium levels, alkaline phosphatase activity or bone (femur) histopathology. The No-Observed-Adverse-Effect-Level in this juvenile dog toxicity study was defined as 10 mg/kg/day based on clinical signs (including tonic convulsions and emesis) observed at 35 and 70 mg/kg/day. Conclusion: Lacosamide daily treatment of juvenile dogs for 33 weeks at doses up to 70 mg/kg/day did not alter bone mineral densitometry parameters. The exposure at this
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

dose level corresponds to exposure at clinical dose levels. This favorable profile may be one of many key factors to
consider when choosing a treatment for epileptic children.

ABSTRACT FINAL ID: 2772 Poster Board #844
TITLE: ASSESSMENT OF PLACENTAL TRANSFER AND THE EFFECT ON EMBRYO-FETAL DEVELOPMENT OF A
HUMANIZED MONOCLONAL ANTIBODY TARGETING LYMPHOTOXIN-ALPHA IN NON-HUMAN PRIMATES

AUTHORS (LAST NAME, FIRST NAME): Wang, Hong1; Schuetz, Chris1; Arima, Akihiro2; Woods, Cynthia1; Xiao, Jim1; Iyer, Suhas1; Gelzleichter, Thomas1; Cain, Gary1
KEYWORDS: non-human primates, placental transfer of monoclonal antibody,Embryo-Fetal Development.

ABSTRACT BODY: BACKGROUND: A humanized monoclonal antibody targeting lymphotoxin alpha (anti-LTα) is
currently in clinical development for treatment of rheumatoid arthritis. Anti-LTα depletes LTα-expressing lymphocytes
and blocks the binding of surface-bound LTαβ2 to LTβR, and blocks secreted LTα3 binding to TNFR. Anti-LTα binds to
LTα in humans and non-human primates (NHP) with similar affinity, but does not bind LTα in rodent species.
METHODS: The effects of anti-LTα on embryo-fetal development were assessed in cynomolgus monkeys. Pregnant
animals received an IV loading dose on gestation day 20 (GD20), followed by SC doses on GD22, GD27, and weekly
thereafter until GD132. The doses were (IV loading/SC) 0/0, 2.5/5, 7.5/15, and 25/50 mg/kg. Pregnancies were terminated
by Cesarean (C)-section on GD139 to evaluate the effect on fetal development, including the lymphoid organs. An
additional group of 4 high-dose fetuses were collected via C-section on GD50 to evaluate placental transfer of the
antibody during the first trimester. RESULTS: No maternal toxicities were observed. A mild increase in peripheral
lymphocytes was present in maternal animals, consistent with the pharmacologic effect of anti-LTα. Placental transfer
was detected as early as GD50 with the fetal serum concentration of ~2.5 mcg/mL, which was ~0.7-0.8% of the maternal
serum drug concentration. Despite the presence of pharmacologically relevant fetal serum concentrations in the first
trimester, there were no anti-LTα related abortions or embryo-fetal deaths, and no adverse findings in external,
visceral, or skeletal evaluation in any dose group. CONCLUSIONS: No maternal or developmental toxicities were
present following administration of anti-LTα from GD20 to 132 in cynomolgus monkeys. The level of fetal serum anti-
LTα on GD50 demonstrated placental transfer of therapeutic antibody during the first trimester in NHP at levels that
could be potentially pharmacologically or toxicologically relevant.

ABSTRACT FINAL ID: 2773 Poster Board #845
TITLE: TEST ARTICLE BIODISTRIBUTION – IMMUNOHISTOCHEMICAL STUDIES (DEVELOPMENT OF IN VIVO
BIODISTRIBUTION STUDIES FOR ANTIBODY-, PEPTIDE-, OR NUCLEOTIDE-BASED TEST ARTICLES)

AUTHORS (LAST NAME, FIRST NAME): Price-Schiavi, Shari A.1; Rojko, Jennifer L.1
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States.
KEYWORDS: biodistribution, monoclonal antibody, endogenous immunoglobulin.

ABSTRACT BODY: Biodistribution studies are typically used to localize test articles within tissues and/or organs.
However, standard methods for biodistribution studies do not provide information about actual cellular and/or
subcellular localization of a given test article. Immunohistochemical methods can be useful for biodistribution studies
of antibody- peptide-, or nucleotide-based test articles as well as small molecule drugs as long as there is an
appropriate detection reagent available. Thus, immunohistochemistry-based biodistribution studies might be useful
for cellular and subcellular localization of test articles within tissues. In addition, these types of studies can be used to
determine whether or not a given test article is present at the site of a lesion, and additional staining can be performed
to determine if the lesion involves an immune or inflammatory response (e.g., formation/deposition of immune
complexes). However, special consideration should be taken during interpretation of biodistribution studies for
monoclonal antibodies as biodistribution of these test articles must be interpreted in the context of the endogenous
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

IgG pool. Therefore, a clear understanding of immunoglobulin transport and clearance is necessary to facilitate meaningful interpretation of these types of studies.

**ABSTRACT FINAL ID:** 2774 Poster Board #846
**TITLE:** PREDICTIVE TOXICOLOGY METHODS TO IMPROVE COMPOUND DESIGN

**AUTHORS (LAST NAME, FIRST NAME):** Bell, Alexander R.; Swallow, Steve; Boyer, Scott; Graham, Mark; Haddrick, Malcolm; Engkvist, Ola; Garside, Helen; Noeske, Tobias; Rolf, Mike; Warner, Dan; Griffen, Ed; Cook, David; Roberts, Ruth; Hammond, Tim

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**KEYWORDS:** Predictive toxicology, Discovery toxicology, Med Chem.

**ABSTRACT BODY:** Pharmaceutical compound design is a multi-factorial process in which properties of pharmacology, DMPK, physicochemical nature and safety are balanced to provide an optimum for therapeutic purpose. Despite this integrated approach, many compounds still fail due to a range of unmanageable or unwanted toxicities. AZ have adopted a deeper collaboration across scientific disciplines with the aim minimizing the incorporation of substructures or pharmacophores that are known to be or suspected to be involved in causing toxicity. A cross functional, network of Medicinal Chemists, Toxicologists, Safety Pharmacologists and Computational Toxicologists are implementing three approaches to aid chemistry design: 1) Structure-Activity Relationship (SAR) Alerts to toxicophore substructures are presented to chemists within AZ’s compound design environment, providing awareness of associated hazards prior to compounds synthesis. 2) Pharmacophore profiling uses an in house developed tool called Plato that allows chemists to identify overall structural similarity to compounds with known or suspected toxicity problems. 3) Compound screening in cellular and molecular assays to highlight toxic potential and develop primitive SAR around novel toxicophore structures and with additional iterations of design and testing refine the SAR into a QSAR. Compounds containing side chains of 2-fluoroethyl have the potential to be metabolized to fluoroacetate, an inhibitor of the TCA cycle. Our systems alert to this toxicophore and thus enabling us to directly to avoid it in compound design. Similarly, compounds associated with histamine H3 hits can be avoided in rational design when pharmacophore similarity profiling is undertaken. Primitive SAR around of Cinnoline derived compounds binding to AhR has been developed and this was used to design compound with high EC50 for AhR activation. Together these approaches work towards reducing attrition due to safety liabilities.

**ABSTRACT FINAL ID:** 2775 Poster Board #847
**TITLE:** REDUCED BIOACCESSIBILITY OF PSEUDOEPHEDRINE DURING OVERDOSE CONDITIONS WHEN TREATED WITH ACTIVATED CHARCOAL AS DETERMINED BY THE TIM-1 SYSTEM; A COMPUTER CONTROLLED, IN VITRO, GASTROINTESTINAL MODEL

**AUTHORS (LAST NAME, FIRST NAME):** Lyng, Eric E.; Hetsco, Lucy; Harrington, Bonnie; Havenaar, Rob; Vick, Andy; Sagartz, John

**INSTITUTIONS (ALL):** 1. Seventh Wave Laboratories, Chesterfield, MO, United States. 2. College of Veterinary Medicine, The Ohio State University, Columbus, OH, United States. 3. TNO, Zeist, Netherlands.

**KEYWORDS:** safety, overdose, gastrointestinal.

**ABSTRACT BODY:** Minimizing drug absorption following accidental overdose is a critical component of the clinical management of toxicity. Inducing emesis and/or oral administration of activated charcoal are common therapeutic approaches in such cases. However, there are few experimental methods that have been able to predict the time course over which activated charcoal may have an optimal impact on reducing systemic exposure and resultant toxicity. In the present study, we utilized a computer controlled, in vitro gastrointestinal model which mimics physiological parameters of gastrointestinal function to explore the ability of activated charcoal to reduce the bioaccessible fraction of pseudoephedrine following simulated acute overdose. The data generated from the TIM-1 represents the amount of dissolved drug available for absorption in the stomach and small intestine. The TIM-1
received water and a 600 mg dose of pseudoephedrine, 2.5-fold greater than the 24 hour maximum suggested dose, followed by activated charcoal in a small amount of water at 30, 60, 90, or 120 minutes post dose. Sample collections from the simulated jejunum, ileum, and ileal efflux were taken every 30 minutes for four hours. Those samples were then analyzed for pseudoephedrine concentration levels using LC/MS/MS. The data generated from the TIM-1 demonstrated approximate 4.2, 2.0, and 1.5-fold lower cumulative bioaccessibility (p<0.05) of pseudoephedrine when activated charcoal was administered at 30, 60, or 90 minutes post dose, respectively. This would predict that the administration of activated charcoal following a pseudoephedrine overdose would result in a meaningful reduction in systemic exposure and potential toxicity. Thus, the TIM-1 system may have a role in the assessment of therapeutic intervention aimed at reducing systemic exposure following accidental drug overdose.

**ABSTRACT FINAL ID:** 2776 Poster Board #848  
**TITLE:** IMPACT AND FREQUENCY OF DIFFERENT TOXICITIES DURING CLINICAL DEVELOPMENT AND THERAPEUTIC USE IN 2010

**AUTHORS (LAST NAME, FIRST NAME):** Redfern, William S.; Valentín, Jean-Pierre; Hammond, Tim G.

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**KEYWORDS:** attrition, target organ toxicity, pharmaceutical development.

**ABSTRACT BODY:** Preclinical safety assessment resources are finite and, ideally, need to be deployed according to the impact and prevalence of various organ toxicities/toxicity domains. This requires reliable, current data on drug-induced toxicities across the range of organs/domains. Such data will also reveal if we are making progress in reducing attrition. We collated information throughout 2010 across the following categories of toxicity: cardiovascular (CV), nervous system (N), respiratory (RES), gastrointestinal (GI), renal (REN), hepatotoxicity (HEP), musculoskeletal (MSK), haematology/bone marrow (H/BM), immunotox/photosensitivity (IMT/PTS), reprotox (RTX), carcinogenicity (CNG) and metabolic/endocrine (MET/END). ‘Drug therapy’ = drug + formulation + indication. 1. Clinical development (Phase II/III) stopped/abandoned (based on 18 candidate drug therapies): N;CV (22%) > HEP;N;RES;MET/END (6%); 2. Non-approval (12 candidate drug therapies): N (33%) > CNG (25%) > CV;RES;RTX;MET/END (9%); 3. Litigation by patients (11 drug therapies): MSK (36%) > N;MET/END (18%) > CV;RTX;CNG (9%). 4. Withdrawal from sale (8 marketed drug therapies): CV (75%) > HEP;N (25%) > RES;RTX;CNG (13%). Overall, in 2010 the top 3 causes of safety-related attrition in clinical development, marketing approval, and clinical use involved the nervous system, cardiovascular system and/or hepatotoxicity. However, the candidate drugs most recently assessed preclinically are those in the first list above (Phase II/III failures), where other toxicities (notably, IMT/PTS) also contribute to failure. These reflect the shortfalls/blind spots of relatively recent preclinical safety assessment activity, and are disappointingly similar to the causes of attrition during clinical development reported a decade earlier ( Olson et al., 2000). This implies we must improve preclinical detection and decision-making regarding toxicities in these (and other) organ systems. References: DIA Daily, Jan-Dec 2010; Olson H et al. (2000) Reg Tox Pharmacol 32, 56–67.

**ABSTRACT FINAL ID:** 2777 Poster Board #101  
**TITLE:** IN VITRO BIOLOGICAL RESPONSES TO 1D AND 2D NANOMATERIALS: CARBON NANOTUBES AND GRAPHENE

**AUTHORS (LAST NAME, FIRST NAME):** Sanchez, Vanesa C.; Creighton, Megan 2; Hurt, Robert H.; Kane, Agnes B.

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**KEYWORDS:** Graphene, Macrophage, 3D culture.

**ABSTRACT BODY:** The geometry of carbon nanomaterials ranges from spherical (as particles), to fibrous (as one-dimensional nanotubes), and to sheet-like (as two-dimensional graphene) forms. It is unknown whether 2D carbon nanomaterials have the capacity to induce inflammatory responses similar to those induced by multi-walled carbon nanotubes (MWCNTs). Macrophages play a central role in the development and maintenance of chronic inflammation, granulomas and fibrosis. Recently, we have shown that 3D in vitro cultures of primary macrophages, after exposure to MWCNTs but not carbon black (CB) particles, recapitulate the mophological features and biomarkers of granulomas.
observed in in vivo studies. By adapting the monocytic THP-1 cell line to this model, we show similar results to those previous, making this model a favorable alternative to in vivo assays. CB, MWCNTs and few-layer graphene (FLG) samples of various layer number (3 – 20) and lateral dimension (500 nm – 25 μm) were characterized by high-resolution transmission electron microscopy. Spent cell culture media analysis shows that these materials absorb pyridoxine HCl and nicotinic acid in a dose-dependent manner. Folic acid, riboflavin and thiamine HCl depletion was material specific. Scanning electron microscopy, light and fluorescence confocal microscopy with polarized light were used to evaluate cell-material interaction, internalization and morphological changes after exposure to nanomaterials. Macrophages internalize CB particles, MWCNT and FLG up to 5 μm in lateral dimension; however, while they do not internalize the larger FLG, they do tightly adhere to their surfaces. Exposure to sublethal doses of MWCNT and FLG, but not CB particles, induce stable macrophage aggregation resembling epithelioid granulomas. For all materials evaluated, time and dose dependent decrease in cell viability was induced at concentrations above 5 μg/ml.

ABSTRACT: Nanomaterials (NMs) have been suspected to induce toxicity in biological systems mainly due to their small size as well as their specific physico-chemical properties. This study presents a development and validation of a high throughput (HTS) genome-wide screen that could be applied to study the mechanisms of adverse effects of NMs on Escherichia coli cells. We used a set of 4000 E.coli single-gene mutants in HTS growth inhibition assay to seek for genes that if absent, result in altered sensitivity towards the nanomaterials. Cationic (amino-functionalized) polystyrene (PS-NH2) with 62 nm original size was selected as a model nanomaterial. This NM appeared to induce toxicity due to both its charge and size – neither non-charged polystyrene particles with similar size nor cationic polystyrene particles with larger size were toxic to E.coli. IC50 of the PS-NH2 62 nm NM was 158 mg/L, IC5 was 108 and IC90 190 mg/L. Individual deletion of 17 non-essential genes resulted in more resistant bacterial phenotype towards PS-NH2 NM while deletion of 208 genes resulted in increased sensitivity. Most of the genes whose absence increased the resistance were associated with amino acid and carbohydrate transport and metabolism. In contrast, the loss of genes in lipopolysaccharides biosynthesis pathway, the absence of a subset of outer membrane transport channels and defects in ubiquinone and menaquinone biosynthetic pathways resulted in most sensitive phenotypes towards PS-NH2 NM. Deletion of a similar set of genes has been associated with a sensitive phenotype also towards cationic peptide in an earlier study. Thus, the adverse effects of PS-NH2 NM on E.coli cells were likely caused by its cationic nature and appeared specific to bacterial cell wall, the integrity of which was essential to cope with the cationic NM-induced stress. The work was supported by the NSF and the EPA under Cooperative Agreement Number EF 0830117 and ESF program Mobilitas.

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KEYWORDS: nanoparticles, single-walled carbon nanotubes, toll-like receptors.
ABSTRACT BODY: The physicochemical properties that make engineered nanoparticles (ENPs) appealing for use in pharmaceuticals, biomedical and product enhancement applications may also modulate immune defense responses in humans. Specifically, recognition of ENPs by Toll-Like Receptors (TLRs) may trigger an innate immune response through the Nuclear Factor-kappa B (NF-kB) transcription factor pathway. To test the capacity of ENPs to activate this pathway in vitro, two Human Embryonic Kidney cell lines (HEK293) were employed; one expressing TLR 2 (HEK293-TLR2) and the other devoid of TLRs (HEK293-WT). The ability of two types of SWNT, carboxy-functionalized and non-functionalized, in the range of 25-100 μg/ml, to transcriptionally activate a NF-kB reporter gene was tested. Interestingly, our results indicate that carboxy-functionalized SWNT did not stimulate NF-kB. However, pristine unmodified SWNT enhanced reporter gene activity, resulting in a 17-fold increase in NF-kB activation in HEK-TLR2 cells. Morphology, surface charge, and aggregate formation using Transmission Electron Microscopy, Zeta PALS, and Dynamic Light Scattering revealed similar results for both types of SWNT. However, measurements using time dependent static light scattering discovered significant differences in the aggregate structure between the SWNT where carboxy-functionalized SWNT revealed a substantially higher fractal dimension indicating a much denser aggregate structure compared to pristine SWNT. These differences in aggregate structure may be important factors in generating immunological responses from materials with physicochemical characteristics that are otherwise similar.

ABSTRACT FINAL ID: 2780 Poster Board #104
TITLE: SIO2 NANO PARTICLES ACTIVATE IMMUNE DENDRITIC CELLS

AUTHORS (LAST NAME, FIRST NAME): Pallardy, Marc J.; Barillet, Sabrina; Nhim, Cathy; Kerdine-Romer, Saadia
KEYWORDS: immunotoxicology, silica nanoparticles, dendritic cells.

ABSTRACT BODY: Due to their unique physical and chemical characteristics, nanoparticles (NPs) are now one of the leading technologies. As a non-metal oxide, silica (silicon dioxide, SIO2) NPs have found extensive applications in industry and biomedicine. Aiming at evaluating their toxicological impact, we asked the question of SIO2 NPs acting as immune adjuvants. Experiments were carried out on dendritic cells (DCs). DCs have a central role in initiating an adaptative immune response with naïve T-cells when antigens and “danger signals” are concomitantly present within their surrounding environment. Under these conditions, DCs undergo a maturation process leading to phenotypic modifications. We therefore choose to investigate whether SIO2 NPs have an impact on DCs maturation. Primary cultures of both human monocyte derived DCs (MoDCs) and murine bone-marrow DCs (BMDCs) were exposed to 100 nm SIO2 particles. Particle size and zeta potential were characterized using photon correlation spectroscopy (PCS) and zetametry, respectively. After 24 h, NPs internalization, cell viability and markers of dendritic cell maturation were studied. Using fluorescent SIO2 NPs, microscopic observations revealed that NPs were found within the DCs after 24 h of exposure. Cytotoxicity evaluation indicated that both DCs types showed about 20 % cell death after 24 h of exposure to 100 μg/mL SIO2 NPs. Experiments dedicated to the study of phenotypic changes were therefore carried out at this subtoxic concentration. Results showed that both murine and human DCs undergo a maturation process after SIO2 NPs exposure as evidenced by a significant upregulation of maturation markers at their surface (CD40, CD86, CCR7, CD83) as well as by a significant release of cytokines in the culture medium (IL-6, IL-8, IL-10, TNF, CCL5, CXCL10). Our results suggest that SIO2 NPs exposure may have an impact on the immune system function through the maturation of human and murine DCs. Further experiments will be carried out to better understand underlying signaling pathways involved in such a maturation process.
ABSTRACT FINAL ID: 2781 Poster Board #105

TITLE: POLYMER-COATED QUANTUM DOTS ELICIT A PRO-INFLAMMATORY RESPONSE IN PRIMARY HUMAN HEPATOCYTE CULTURES.

AUTHORS (LAST NAME, FIRST NAME): Smith, Wesley E.; Brownell, Jessica L.; Afsharinejad, Zahra; Hu, Xiaoge; Gao, Xiaohu; Polyak, Stephen J.; Eaton, David L.; Kavanagh, Terrance J.

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KEYWORDS: quantum dot, hepatotoxicology, immunotoxicology.

ABSTRACT BODY: Quantum dot nanoparticles (Qdots) have unique ‘tunable’ fluorescent properties and other characteristics of potential value in cancer diagnosis and treatment. The core structure of some semiconductor quantum dots is composed of heavy metals (Hg, Pb, Cd) known to be toxic. In an attempt to mitigate this toxicity and improve biocompatibility, various types of surface coatings have been employed. We have shown that Qdots coated with poly(maleic anhydride-alt-1-tetradecene), tri-n-octylphosphineoxide (PMAT-TOPO) are sequestered in primary human hepatocytes in a dose-dependent manner with no obvious effect on cell viability (MTT) or glutathione levels after 24 hrs of exposure. While this coating appears to protect hepatocytes from metal-induced toxicity, the effect of these Qdots on hepatocyte function has not been fully characterized. To determine if PMAT-TOPO-coated Qdots are capable of inducing a pro-inflammatory response, primary human hepatocyte cultures were treated over a range of doses (2.5-40nM) for 24 hrs. Supernatants were collected from treated cultures and analyzed for levels of IL-8, TNF-α, and CCL4. IL-8 and CCL4, which are both considered chemoattractant cytokines, were elevated by 4-fold and 20-fold, whereas TNF-α levels were not affected. Gene expression analysis confirmed the lack of response to TNF-α. These results suggest that PMAT-TOPO Qdots may promote a recruitment of immune cells to the liver. While it is encouraging that the PMAT-TOPO coating prevents metal-induced toxicity to hepatocytes these Qdots will likely perturb immunological function when administered in vivo.

ABSTRACT FINAL ID: 2782 Poster Board #106

TITLE: DIFFERENTIAL TOXICITY FOLLOWING IN VITRO EXPOSURE TO MANGANESE OXIDE NANOPARTICLES AND IONIC MANGANESE COMPOUNDS

AUTHORS (LAST NAME, FIRST NAME): Jordan, Jacqueline A.; Hasak, Stephen; Wang, Yonggang; Shepherd, Kennie; Kim, Hye Mi; Li, Yingjie; Pennell, Kurt; Miller, Gary W.

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KEYWORDS: nanotoxicology, neurodegenerative, Parkinson's.

ABSTRACT BODY: The role of metal oxide nanoparticles in the development of neurodegenerative disorders is unknown. In this study, we compared the toxicity of manufactured manganese oxide nanoparticles (Mn2O3) to ionic manganese species. Our interest in these particles is based on the fact that experimental studies have shown a positive link between manganese exposure and a Parkinson's disease-like syndrome called manganism. The dispersion of these compounds was confirmed using the CytoViva dark-field microscopy system. Cell viability, induction of oxidative stress, apoptosis, and activation of caspase-3 protein levels were determined using HEK293 cells exposed to increasing concentration of Mn2O3 nanoparticles (up to 100 µg/ml) for up to 24 h. These data were compared to the same concentrations of manganese chloride (MnCl2) and manganese acetate (MnAc). The overall results suggest a dose-dependent increase in cytotoxicity and oxidative stress following exposure to Mn2O3 nanoparticles. At 1-10 µg/ml dose of Mn2O3, we observed a significant increase in caspase 3 expression and the number of apoptotic cells in comparison to untreated, MnCl2-treated or MnAc-treated HEK cells. In summary, the Mn2O3 nanoparticles were more toxic and induced a greater level of oxidative stress at the same comparable doses of ionic manganese. In conclusion, studies examining the overall toxicity of metal oxide nanoparticles and their ability to generate oxidative stress in cultured
neuronal cells may be important in understanding a potential link between metal oxide nanoparticles exposure and the development of various neurological diseases in humans.

**ABSTRACT FINAL ID:** 2783 Poster Board #107  
**TITLE:** CELLULAR EFFECTS OF NANOSILVER IN HUMAN MACROPHAGES: UPTAKE, OXIDATIVE STRESS, LIPID ALTERATIONS AND FUNCTIONAL IMPAIRMENT

**AUTHORS (LAST NAME, FIRST NAME):** Haase, Andrea¹; Tentschert, Jutta¹; Graf, Philipp²; Mantion, Alexandre³; Draude, Felix⁴; Jungnickel, Harald⁴; Galla, Sebastian⁴; Plendl, Johanna⁵; Arlinghaus, Heinrich F.⁴; Thuenemann, Andreas F.⁴; Taubert, Andreas⁵; Luch, Andreas¹

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**KEYWORDS:** silver nanoparticles, protein carbonyls, TOF-SIMS.

**ABSTRACT BODY:** Silver nanoparticles (SNP) belong to the most commercialized nanoparticles. Here we monitored the biological effects of SNP of different sizes (20 nm, 40 nm) and coatings (citrate, peptide) in human macrophages in vitro. We used THP-1 derived macrophages as a model because of their particle-clearing role in vivo. The cellular uptake was analyzed by confocal Raman microscopy, TEM or Laser postionization secondary neutral mass spectrometry (Laser-SNMS). Cellular responses upon SNP treatment were studied by time-of-flight secondary ion mass spectrometry (TOF-SIMS) and several biological endpoints were evaluated, i.e., cytotoxicity, protein carbonyl formation and induction of heme oxygenase-1 (HO-1). Toxicity of SNP was dependent on exposure time, dose and particle coating. Nanogold proved mainly inert. All kinds of nanoparticles were efficiently taken up by cells. Aggregates and single particles could be detected throughout whole cells, including nuclei and lysosomes. With TOF-SIMS and Laser-SNMS we visualized intracellular SNP and detected significant changes in the membrane lipid pattern indicating oxidative stress and fluidity changes. We measured strong induction of HO-1 and formation of protein carbonyls with different time patterns. Each type of SNP induced a characteristic carbonylation pattern as resolved by 2D gel electrophoresis. SNP but not nanogold significantly affected the phagocytic activity of macrophages. Some of the particle-mediated effects could be reversed depending on the time and doses applied. Conclusion: SNP exert adverse effects in human macrophages already at subcytotoxic doses. Different kinds of SNP induce distinguishable effects at cellular and biochemical levels.

**ABSTRACT FINAL ID:** 2784 Poster Board #108  
**TITLE:** INTRATRACHEAL INSTILLATION OF NANOCERIA INDUCE SYSTEMIC TOXICITY IN RATS

**AUTHORS (LAST NAME, FIRST NAME):** Nalabotu, Siva krishna¹; Ma, Jane²; Triest, William³; Kolli, Madhukar¹; Wehner, Paulette¹; Blough, Eric³

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**KEYWORDS:** Cerium oxide nanoparticle toxicity.

**ABSTRACT BODY:** Nanotechnology is a broad interdisciplinary field that is centered on the use of materials that range in size from 1-100 nm. The extensive use of nanomaterials in various sectors such as electronics, consumer goods, transportation and health industries poses an increased risk of exposure to both humans and the environment. Cerium oxide nanoparticles are shown to scavenge reactive oxygen species thereby they are proposed to use for the treatment of cardiovascular disease, neuronal injury and radiation induced damage. However, the systemic toxicity after cerium oxide nanoparticles exposure is not well understood. Herein, we investigate if intratracheal instillation of nanoceria is associated with alterations in blood biochemistry and histopathology of liver, kidney, spleen, and heart.
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

Compared to control animals, cerium oxide instillation increased serum ALT levels [Saline control: 58.29±10.73 vs. CeO2 7.0 mg/kg: 150.50±94.46; p<0.05], reduced albumin levels [Control 4.17±0.17 vs. CeO2 7.0 mg/kg: 3.54±1.14] and diminished the sodium-potassium ratio [Control: 25.78±1.98 vs. CeO2 7.0mg/kg: 22.78±2.54; p<0.05]. An analysis of the blood lipid profile indicated a reduction in the triglyceride levels [Control: 142.86±53.0 vs. CeO2 7.0 mg/kg: 93.14±22.33; p<0.05]. Compared to control animals, animals exposed to cerium oxide exhibited a reduction in liver weight [Control: 14.55±0.57 vs. CeO2 7.0 mg/kg: 12.50±0.54; p<0.05] and dose dependent alterations (hydropic degeneration, enlargement of the hepatocytes, sinusoidal dilatation and the accumulation of granular bodies) in liver histology. No gross histopathological alterations were observed in the kidney, spleen and heart. Taken together, these data suggest that cerium oxide nanoparticles may exit the lung after deposition producing toxicological effects in the liver.

**ABSTRACT FINAL ID:** 2785 Poster Board #109

**TITLE:** TIME-COURSE DETERMINATION OF CELLULAR STRESS RESPONSES ELICITED BY ENGINEERED NANOMATERIALS

**AUTHORS (LAST NAME, FIRST NAME):** Prasad, Raju Y.; Blackman, Carl F.; DeMarini, David M.; Simmons, Steven O.

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**KEYWORDS:** Nanotoxicology, Pathways, Inflammation.

**ABSTRACT BODY:** Engineered nanomaterials are being incorporated continuously into consumer products, resulting in increased human exposures. The study of engineered nanomaterials has focused largely on oxidative stress and inflammation endpoints without further investigating potential pathways. Here we examine time-sensitive biological response pathways affected by engineered nanomaterials using a battery of stable luciferase-reporter cell lines in HepG2 cells. We measured the activation of three key stress responsive transcription factors: NFkB, Nrf2, and AP-1 by exposure to 6 titanium dioxide nanomaterials (nano-TiO2) with rutile, anatase, and rutile/anatase crystal structures, and 2 cerium oxide nanomaterials (nano-CeO2) from various manufacturers. Exposure concentrations ranged from 1-100 µg/ml per nanomaterial at 6 and 24 h. Cytotoxicity was measured in parallel using the MTT assay. Dynamic light scattering was used to determine the size and zeta potential of the nanomaterials in medium. Our results show that there were significant changes in transcriptional activation at concentrations as low as 1 µg/ml. The 10 nm anatase nano-TiO2 elicited the highest effect, a ~2.5 fold increase in NFkB transcriptional activation at 100 µg/ml after 24 h. Nrf2 showed transcriptional activation by one nano-CeO2, showing ~1.5 fold activation at 100 µg/ml after 24 h. AP1 elicited a ~1.3 fold increase in anatase/rutile nano-TiO2 at 1 µg/ml after 24 h. Both anatase/rutile nano-TiO2 were cytotoxic at 100 µg/ml after 24 h. Our results demonstrate the potential for engineered nanomaterials to elicit cellular stress responses through the NFkB and Nrf2 pathways. [This is an abstract or proposed presentation and does not necessarily reflect EPA policy.]
ABSTRACT FINAL ID: 2786 Poster Board #110
TITLE: VIABILITY AND REPRODUCTIVE TOXICITY OF SILVER NANOPARTICLE EXPOSURE ARE REMEDIATED BY VITAMIN C IN DROSOPHILA

AUTHORS (LAST NAME, FIRST NAME): Posgai, Ryan T. 1,2; Hussain, Saber M. 1; Rowe, John J. 1; Nielsen, Mark G. 1
KEYWORDS: nanosilver, Drosophila, developmental toxicity.

ABSTRACT BODY: Nanoparticle (NP) toxicity research has primarily focused on acute exposures both in vitro and in vivo. Few in vivo studies on chronic lifetime effects of NPs exposure are available. In this study, the in vivo toxicity of silver and titanium oxide nanoparticles on reproduction, developmental rate and viability was tested in Drosophila melanogaster. Ingestion of nanosilver during the larval stage of the lifecycle (50 larvae/treatment) showed major, dose-dependent effects on survivorship to pupal stage: LD50 10nm polysaccharide coated = 30µg/mL; LD50 10nm uncoated = 25µg/mL; LD50 60nm polysaccharide coated = 60µg/mL; LD50 60nm uncoated = 45µg/mL. Conversely, titanium oxide has no effect on fly life history, and serves to verify the ability of our model to discriminate among NPs in their toxicity. High dose nanosilver ingestion resulted in cuticular and melanization defects in adult flies surviving nanosilver treatment during the larval stage. This “white” adult phenotype is characterized by a soft, unpigmented cuticle. Lower doses of nanosilver were sufficient to disrupt reproduction compared to viability; reproductive ED50 (a dose that results in 50% of the mating success of control lines) for uncoated 60nm silver was about half of LD50, between 15-20µg/mL. We also demonstrate reversal of nanosilver toxicity through diet supplementation with vitamin C. Larvae growing on 30µg/mL 60nm uncoated silver supplemented with 50mM vitamin C or vitamin C palmitate showed a greater than twofold increase in survivorship compared to flies reared on nanosilver alone, and a 7-fold increase in mating success. Vitamin C also rescued cuticular and pigmentation defects in nanosilver-fed flies. This corroborates previous results that silver toxicity results from oxidative stress, and provides a potential antioxidant-based strategy for the development of prophylactics to nanosilver exposure.

ABSTRACT FINAL ID: 2787 Poster Board #111
TITLE: DIFFERENTIAL INTERFERENCE WITH CLINICAL CHEMISTRY ASSAYS BY GOLD NANORODS AND GOLD AND SILICA NANOSPHERES

AUTHORS (LAST NAME, FIRST NAME): Carpinone, P. L. 1; Hinkley, G. K. 2; Barber, D. S. 2; Munson, J. W. 2; Powers, K. W. 1; Roberts, S. M. 2
KEYWORDS: nanoparticle interference, clinical chemistry assay, gold nanoparticles.

ABSTRACT BODY: Nanomaterials have been shown to cause interference with several standard toxicological assays, potentially leading to erroneous conclusions regarding biological effects. As part of an in vivo study of PEG-coated gold nanorods in mice, nanorods were added to reference serum in concentrations relevant to those observed in mice, and the results for a standard battery of clinical chemistry parameters were compared with those from serum without nanorods. The PEG-coated gold nanorods produced concentration-dependent interference with measurements of calcium, magnesium, and especially total bilirubin. Comparisons were then made with PEG-coated gold and silica nanospheres at approximately equal concentrations in terms of surface area per ml serum. Gold nanospheres caused interference with measurements of total protein, glucose and triglyceride, while silica nanospheres caused interference only for the total bilirubin assay. Spectral properties of the nanomaterials accounted for some, but not all, of the interferences. Removal of the particles from serum by centrifugation prior to assay resolved most of the interferences, but increased the extent of error in some measurements. Further study of the effect of PEG-coated gold nanorods on total bilirubin measurement found varying effects depending upon the assay method, ranging from failure to detect bilirubin to values more than 10-times the actual concentration. We conclude that PEG-coated gold
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

and silica nanoparticles can interfere with standard clinical chemistry tests in ways that vary depending upon the material, its shape, and the specific assay methodology employed. Assay interferences by nanomaterials cannot always be predicted, underscoring the need to verify that nanomaterials under study do not interfere with methods used to evaluate potential biological effects. Supported by the Center for NanoBio Sensors, State of Florida Centers of Excellence.

**ABSTRACT FINAL ID:** 2788 Poster Board #201
**TITLE:** NEUROTOXICITY IN THE LPS DOSAGE IN THE MOUSE SUBSTANTIA NIGRA

**AUTHORS (LAST NAME, FIRST NAME):** Atsuko, Ishii; Tanaka, Sachiko; Ohtaki, Hirokazu; Numazawa, Satoshi; Shioda, Seiji; Yoshida, Takemi

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**KEYWORDS:** neurodegenerative, LPS, microglia.

**ABSTRACT BODY:** We have shown that direct Lipopolysaccharide (LPS) administration into substantia nigra (SN) produced neurodegeneration, looks like to neurodegenerative disease. Parkinson’s disease (PD) is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the SN and gradual worsening of motor symptoms. However, the neurodegenerating mechanism of PD is not well known. Therefore, we have designed the animal model of PD via activation of microglia and examined the neurotoxicological mechanism that was related to inflammatory cytokine and interaction of the microglia. Lipopolysaccharide (LPS, 5 μg/2 μL/injection) was stereotaxically injected into the SN by daily for 5 days. CD11b immunopositive cells in the SN were resulted in the increase of microglia and produced morphological changes. In real time RT-PCR, the gene expression of CD11b, galectin-3 and P2Y6 were increased, P2Y12 were decreased. It showed that microglia was increasing, and phagocytosis and chemotaxis were increasing. Although it seemed significantly loss of tyrosine hydroxylase (TH) gene expression, neuronal cell death in TH positive neuron determined by using Fluoro Jade-B was not observed at 6 hr after the final injection. But, Animal behaviors that assessed by the Rotarod test and wire hanging test were caused motor functional decline. Next, we used IL-1 α/β KO mice and TNF α KO mice to examine microglia-neuron interaction. In the gene expression of TH, although TNF α KO mice was significantly decreased, IL-1 α/β KO mice was not changed. And the exercise function turned worse in wild every progress in time, but was not generated in IL-1 α/β KO mice. These results suggest that LPS-induced activation of microglia causes functional change in the brain such as behavioral impairment without cell death. It seems that IL-1 α/β is extremely related to neurodegeneration with the microglia activation.

**ABSTRACT FINAL ID:** 2789 Poster Board #202
**TITLE:** INFLUENCE OF NEUROTOXIC METALS ON THE EARLY STAGES OF HUMAN NEURONAL DEVELOPMENT

**AUTHORS (LAST NAME, FIRST NAME):** Aboud, Asad A.1; Tidball, Andrew M.1,2; Neely, M. Diana1,5; Litt, Michael1; Hong, Charles C.3,4; Ess, Kevin C.1,5; Bowman, Aaron B.1,2

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**KEYWORDS:** Neurodegenerative diseases, Metal toxicity, Human development.

**ABSTRACT BODY:** Gene-environment interactions are a well-established theme in the pathophysiology of neurodegenerative diseases. Characterizing the nature of these interactions and identifying the main players are important goals. While extensive research has been done using animal models, it is uncertain how applicable these results are to understanding human environmental and genetic risk factors for disease. In addition, the nature of these interactions during early human CNS development has not been clearly elucidated. Our lab has succeeded in making
induced pluripotent stem cells from patients with well-defined genetic mutations associated with neurodegeneration. In this study we used stem cells from patients with PARK2 mutations, TSC1 mutations, chromosome 21 and 12 trisomy, and normal controls to investigate the toxicity of copper, methylmercury, manganese, and cadmium on early stage of neuronal progenitors. We were able to demonstrate increased vulnerability of PARK2 mutated neuroprogenitor cells to copper and cadmium compared to cells from the healthy control. This finding demonstrates the utility of our models to study the gene-environment interactions in a patient-specific manner. Furthermore, it opens the door to developing high throughput screening for the specific gene interactions with a wide array of toxicants and other environmental agents. Support from NIH RO1 ES016931, NIH P30 ES000267, Doris Duke Research Foundation and Peterson Foundation for Parkinsons.

ABSTRACT FINAL ID: 2790 Poster Board #203
TITLE: DIFFERENTIAL VULNERABILITY IN THE NIGROSTRIATAL DOPAMINE SYSTEM TO INFLAMMATION AND PARAQUAT

AUTHORS (LAST NAME, FIRST NAME): Frasca, Joseph M.¹; O'Banion, K.¹; Cory-Slechta, D.¹
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KEYWORDS: Parkinsons Disease, Paraquat, Neurodegeneration.

ABSTRACT BODY: Higher levels of inflammatory mediators such as interleukin 1β (IL-1β) found in the nigrostriatal (NS) system of Parkinson’s disease (PD) patients and animal models may contribute to associated dopamine (DA) cell death. This study sought to determine whether inflammation at the DA terminal (striatum) or cell body (substantia nigra; SN) conferred greater sensitivity to the parquat (PQ)-induced PD phenotype, using wild type (WT) vs. IL-1βXAT transgenic (GFAP promoter) male mice. IL-1β activation via unilateral FIV-Cre mediated excision was followed by 2x/week PQ injections for 6 weeks. In SN groups, IL-1β activation per se increased locomotor activity; PQ reduced activity levels in IL-1β-activated, but not WT mice, consistent with synergistic PQ and inflammation effects. PQ did not reduce tyrosine hydroxylase positive (TH+) cell numbers in either WT or IL-1β-activated SN groups. However, leukocyte infiltration was observed only in SN-activated PQ mice. These findings demonstrate synergistic interactions between neuroinflammation and PQ in SN. Unlike SN, motor behavior was not influenced by inflammation per se in striatally-activated mice, and PQ comparably reduced locomotor activity in both WT and activated mice. Also in contrast to SN, striatal inflammation was sufficient to cause nigral TH+ cell loss. A further modest loss of DA neurons in PQ-treated striatally-activated mice relative to PQ-treated WT mice was not statistically significant. Collectively, these findings suggest enhanced vulnerability of striatum relative to SN to inflammation and subsequent TH+ cell loss, and raise the question of whether SN-activated leukocyte infiltration enhanced by PQ was protective against SN TH+ cell loss. Motor function consequences were observed in both regions, with enhancement of PQ-induced reductions by activation observed only in SN. Characterization of the immune response in the striatum and SN may implicate mechanisms behind the differential vulnerability and the interaction between PQ and neuroinflammation. Supported by T32 ES07026 and ES012712 to DCS.

ABSTRACT FINAL ID: 2791 Poster Board #204
TITLE: THE NFKB P50 RADICAL AND NEUROTOXIC MICROGLIAL ACTIVATION

AUTHORS (LAST NAME, FIRST NAME): Block, Michelle L.¹; Taetzsch, Thomas¹; Levesque, Shannon¹; Duke, Laura¹
KEYWORDS: Microglia, Oxidative stress, Neurotoxicology.

ABSTRACT BODY: Reactive oxygen species (ROS) are key mediators of microglia-mediated dopaminergic (DA) neurotoxicity. However, the precise mechanisms through which ROS change microglia to a neurotoxic phenotype are poorly understood. Using immuno-spin trapping (IS), we began to explore the potential targets (protein radicals) in microglia modified by ROS to result in an enhanced pro-inflammatory response. Primary microglia cultures activated with diverse triggers (10 ng/ml LPS, 250 nM α synuclein, & 0.5μM paraquat) at concentrations previously shown to be neurotoxic to DA neurons through microglial activation resulted in increased levels of total protein radicals, as
Oxidative damage is recognized as a key pathophysiological mechanism contributing to the neurodegenerative process in Parkinson’s disease (PD). We recently identified that protein kinase D1 (PKD1) is activated at early stages of oxidative stress in dopaminergic neuronal cells to initiate a compensatory protective response. In order to further understand the molecular mechanisms underlying PKD1 activation during oxidative insults, we examined signal transduction events that regulate PKD1 activation in neurotoxicity models of PD. Parkinsonian specific neurotoxicants 6-OHDA and MPP⁺ at 10 to 300μM concentrations induced an increase in S744/S748 activation loop phosphorylation in a time- dependent manner in dopaminergic N27 cells and primary mesencephalic neurons. Also, the C-terminus PKD1-S916 phosphorylation preceeded the activation loop phosphorylation in dopaminergic neurons. A PKD1<sup>S916A</sup> mutant attenuated PKD1 activation loop phosphorylation, indicating that the C-terminal phosphorylation is required for activation of PKD1. RNAi knockdown of PKCδ prevented PKD1-S916 phosphorylation and PKD1 activation, demonstrating that PKCδ serves as the upstream regulator of PKD1. Further, PKD1 was also activated in the substantia nigra of a MPTP-treated mouse model, as well as in human PD patients. Interestingly, overexpression of the constitutive active PKD1<sup>S744E/S748E</sup> mutant protected dopaminergic cells from oxidative insult, while the inactive PKD1<sup>S916A</sup> mutant exacerbated cell death. Furthermore, 50 μM kbNB 142-70, the allosteric PKD1 inhibitor, blocked PKD1 S744/S748 and S916 phosphorylation and exacerbated toxicity caused by H₂O₂, 6-OHDA and MPP⁺, confirming the prosurvival function of PKD1 in dopaminergic neurons. Collectively, our translational signal transduction approach modulating the novel PKD1 kinase could offer a novel and promising neuroprotective strategy against the neurotoxic damage associated with PD. (supported by NIH grants ES10586 & NS NS065167).
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

**ABSTRACT FINAL ID: 2793 Poster Board #206**

**TITLE:** PHARMACOKINETICS AND TOLERABILITY OF AN ANTISENSE OLIGONUCLEOTIDE ADMINISTERED AS AN INTRATHECAL LUMBAR BOLUS INJECTION IN MONKEY

**AUTHORS (LAST NAME, FIRST NAME):** Fey, Robert A.; Norris, Daniel A.; Zeller, Jillynne R.; Boyd, Robert B.; Henry, Scott P.

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**KEYWORDS:** phosphorothioate oligonucleotide, Intrathecal (IT) injection, SMA.

**ABSTRACT BODY:** The pharmacokinetics of ISIS 500837, a uniform 2'-O-methoxymethyl phosphorothioate (2'-MOE) oligonucleotide which specifically modulates splicing of survival of motor neuron 1 (SMN 1), was evaluated in adult and juvenile cynomolgus monkeys following intrathecal (IT) lumbar bolus injection. In adults, time-specified plasma, cerebrospinal fluid (CSF) and tissue samples were evaluated following a 24-hour infusion at total dose of 3 mg and subsequent escalating bolus doses of 1, 3 and 10 mg. The 24-hour infusion was included to compare exposure following bolus injection to continuous IT infusion, the route used in previous studies of similar oligonucleotides. Juvenile monkeys received single or multiple doses IT bolus doses of 0.3 and 1.5 mg. The multiple dose regimen included once-weekly injections for the first 2 weeks with subsequent doses administered every 2 weeks thereafter for a total of 4 doses. Plasma and CSF pharmacokinetics demonstrated dose-dependent and dose-proportional exposure during and following bolus injection. Consistent with the route of administration, peak CSF and plasma concentrations were higher following bolus injection than 24-hr infusion. Highest tissue concentrations were observed in the lumbar spinal cord consistent with the site of injection, but pharmacologically relevant concentrations were measurable in the cervical spinal cord and some regions of the brain. Higher concentrations were consistently observed following multiple doses and generally increased with total dose administered. Immunolocalization of oligonucleotide indicated good uptake in neuronal cells throughout the spinal cord. Systemic tissue (kidney and liver) concentrations were measurable, but low compared to the lumbar spinal cord, consistent with the intrathecal route of administration but also demonstrating the ultimate fate of drug via transfer to the system circulation and elimination by the kidney. Tolerability in CNS was good at the doses and durations examined.


**ABSTRACT FINAL ID: 2794 Poster Board #207**

**TITLE:** FYN KINASE ACTIVATION CONTRIBUTES TO MPTP-INDUCED DOPAMINERGIC NEUROTOXICITY: RELEVANCE TO THE PATHOGENESIS OF PARKINSON'S DISEASE

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**KEYWORDS:** Fyn kinase, Parkinson's disease, MPTP model.

**ABSTRACT BODY:** Parkinson's disease is a movement disorder characterized by selective degeneration of nigral dopaminergic neurons, however the molecular mechanisms underlying loss of dopaminergic neurons is unknown. Previously we showed that Fyn tyrosine kinase (FYN), is rapidly activated and modulates the proapoptotic kinase PKCδ during a variety of neurotoxic insults, including H2O2 and dieldrin in dopaminergic neuronal cells. Here we report that FYN acts as an upstream regulator of PKCδ by phosphorylating the tyrosine-311 (Y311) phosphorylation site, which serves as a priming event to PKCδ proteolytic activation. Parkinsonian toxicant MPP⁺ (300μM) activated FYN in dopaminergic N27 neuronal cells. Interestingly, siRNA knockdown of Fyn attenuated MPP⁺-induced caspase-3 activation, PKCδ-Y311 phosphorylation, proteolytic activation and DNA fragmentation, suggesting FYN is required for PKCδ dependent apoptotic cascade. Further, attenuation of FYN activity using 5μM Tyrosine Specific Kinase Inhibitor blocked dopaminergic neurons from MPP⁺-induced degeneration in mouse primary mesencephalic culture. Furthermore, primary mesencephalic neurons from FYN knockout (Fyn⁻⁰⁻) mice were significantly protected against
**2011 Society of Toxicology 50th Anniversary Meeting**

**Late-Breaking Abstracts**

MPP⁺-induced dopaminergic neuronal cell loss. Moreover, FYN was activated in MPTP-treated animals (18 mg/kg x 4 doses at 2h intervals) within 6 h of the neurotoxic insult. Fyn⁺ mice were significantly protected from MPTP-induced PKC8-Y311 phosphorylation. Also, MPTP-induced neurobehavioral deficits and neurochemical depletion were significantly blocked in Fyn⁺ mice. Immunohistochemical analysis revealed a dramatic protection of nigral dopaminergic degeneration, as well as striatal dopaminergic nerve terminal damage in Fyn⁺ mice. Collectively, our results unveil a novel mechanism in which FYN plays an obligitory function in the nigral dopaminergic degeneration induced during neurotoxic insults (NIH grants ES10586 & NS065167)

**ABSTRACT FINAL ID:** 2795 Poster Board #208

**TITLE:** NEUROPROTECTIVE EFFECTS OF GAMMA-TOCOPHEROL-RICH MIXED TOCOPHEROLS IN ANIMAL MODELS OF PARKINSON’S DISEASE

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**KEYWORDs:** tocopherol, parkinson’s.

**ABSTRACT BODY:** Parkinson’s disease (PD) is a progressive neurodegenerative disorder that affects more than 1.5 million people in the United States alone. An abundance of evidence indicates that oxidative stress leads to the loss of the dopaminergic neurons. Vitamin E (α-tocopherol, aT) has been tested both in animal models of PD as well as in human clinical trials, but with equivocal results. Although aT is the biologically most active form of vitamin E, gamma-tocopherol (gT) is the most abundant form found in the U.S. diet and is also a superior antioxidant and anti-inflammatory agent compared to aT. Thus, to determine the efficacy of gT-rich mixed tocopherols to protect against dopaminergic cell death, animals were pre-treated for 2 months with either a 0.3% mixed tocopherol diet (containing about 60% gT) or control diet (0.002% aT) and subsequently treated with either saline or 10mg/kg paraquat (PQ) + 30 mg/kg menab (MB) twice a week for 6 weeks or 20 mg/kg MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) everyday for 5 days. Both MPTP and PQ+MB exposure caused a significant decrease in striatal dopamine (DA) and metabolite levels in mice on the control diet. However, mice on the mixed tocopherol diet treated with either PQ+MB or MPTP were significantly protected against decreases in striatal DA and metabolite levels. The expression of the redox sensitive transcription factor Nrf2, and its associated phase II detoxifying, antioxidant enzymes were determined by qRT-PCR. In MPTP treated mice, those on the mixed tocopherol diet had significantly increased mRNA expression of Nrf2 (63%), NADPH-quinone oxidoreductase-1 (NQO1) (80%), and the glutathione-S-transferases GSTM1, GSTP1, and GSTA4 in the ventral midbrain compared to mice on control diet. This data suggests that a diet enriched with a gT-rich mixture of tocopherols may prove useful in protecting dopaminergic neurons against oxidative stress generated by exposure to dopaminergic neurotoxicants.

**ABSTRACT FINAL ID:** 2796 Poster Board #209

**TITLE:** EVALUATION OF THE EFFECTS OF INFLAMMATORY CYTOKINES ON DRUG-INDUCED LIVER INJURY (DILI) IN HUMAN HEPATOCYTES

**AUTHORS (LAST NAME, FIRST NAME):** Li, Albert P.¹; Doshi, Utkarsh¹; Yang, Qian¹

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**KEYWORDS:** Drug induced liver injury, human hepatocytes, cytokines.

**ABSTRACT BODY:** In our laboratory, primary human hepatocyte cultures were used as an in vitro experimental system to study the role of inflammation in DILI. Plateable cryopreserved human hepatocytes cultured on collagen-coated plates and overlaid with Matrigel (sandwich culture) were used for the study. Time- and concentration-dependent induction of C-reactive protein (CRP), suppressor of cytokine signaling (SOCS3), and LPS binding protein (LBP) gene expression were observed with IL-6, demonstrating that the hepatocytes had intact cytokine receptors and corresponding downstream pathways. IL-6 was found also to down-regulate CYP3A4 gene expression, consistent with the known correlation of inflammation and decreased drug metabolizing enzyme activities in vivo. The cytotoxicity of troglitazone was evaluated in human hepatocytes in the presence and absence of 5 ng/mL of interleukins (IL-1b, IL-2,
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

IL-4, IL-6, IL-8, IL-10) and interferons (IFNa, IFNg). Dose-dependent decrease in viability (based on ATP content) and increase in oxidative stress (based on HOMX-1 gene expression) was observed for troglitazone. Co-treatment with cytokines did not cause significant increase in troglitazone cytotoxicity. Unexpectedly, an apparent protective effect was observed using both endpoints. Our results suggest that the inflammatory cytokines may be protective for acute liver injuries, with a possible mechanism of protection being the decreased metabolism of troglitazone to hepatotoxic metabolites.

ABSTRACT FINAL ID: 2797 Poster Board #210
TITLE: ADIPOSE TISSUE LIPIN 1 HYPOMORPHIC MICE HAVE ABNORMALITIES IN ADIPOCYTE MORPHOLOGY, INFLAMMATION, AND LIPID METABOLISM

AUTHORS (LAST NAME, FIRST NAME): Mitra, Mayurranjan S.; Chen, Zhouj; Brunt, Elizabeth; Finck, Brian N.
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KEYWORDS: inflammation, Fatty liver, Insulin resistance.

ABSTRACT BODY: Lipin 1 is a bifunctional intracellular protein that regulates metabolism by acting as a coregulator of DNA-bound transcription factors and also dephosphorylates phosphatidic acid to form diacylglycerol [phosphatidic acid phosphohydrolase (PAP) activity]. We sought to evaluate the role of lipin 1 in regulating adipose tissue metabolism by generating mice with deletion of exon 3 (encoding the translational start site) and exon 4 of the Lpin1 gene in these tissues by using Cre-loxP methodology. However, after crossing Lpin1flox/flox mice with mice expressing Cre-recombinase under the control of the adiponectin (Adn; adipose-specific) promoters, it was determined that a bona fide, but truncated, lipin 1 protein lacking the N-terminal 115 amino acids was expressed through an intact alternative translational start site. The truncated protein lacked PAP enzyme activity, but the transcriptional regulatory activity was not affected, indicating a hypomorph Lpin1 allele (Lpin1hyp/hyp mice). Adn-Lpin1hyp/hyp mice exhibited moderately-reduced adipose tissue mass, lower triglyceride, diglyceride, and higher free fatty acid in the adipose tissue. Histologic examination of adipose tissue revealed the adipocytes containing multilocular lipid droplets in Adn-Lpin1hyp/hyp mice. Gene expression of cytokines and macrophage chemo-attractants (CD68, IL6, F4/80, and osteopontin) was induced in these adipocytes. Furthermore, MAC3 staining of the adipose tissue suggested macrophage infiltration. Adn-Lpin1hyp/hyp mice also exhibited neonatal fatty liver, and Adn-Lpin1hyp/hyp adults had diminished circulating adiponectin concentration with multiple indications of systemic insulin resistance. In summary, loss of lipin 1-mediated PAP activity in adipose tissue causes a lean, but insulin-resistant, phenotype with abnormalities in adipocyte morphology, inflammation, and lipid metabolism.

ABSTRACT FINAL ID: 2798 Poster Board #211
TITLE: ENDOCRINE DISRUPTING CHEMICALS FROM COSMETICS IN NORWEGIAN 10-YEAR-OLD CHILDREN

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KEYWORDS: Endocrine disrupting chemicals, Epidemiology.

ABSTRACT BODY: Cosmetics and body-care products contain endocrine disrupting chemicals such as phthalates and parabens. Exposure to these compounds should be minimized in children and through puberty when the production and balance of sex-specific hormones are of particular importance. As part of the Environment and Childhood Asthma study, follow-up, in Oslo, Norway, concentrations of phthalate metabolites and parabens were measured (CDC, Atlanta) in urine samples collected in 2002-2004 from 200 10-year-old children (48% girls). The cosmetic additives propyl- and methylparabens and metabolites of the low-molecular weight phthalates, di-n-butyl (DnBP), diethyl (DEP), benzyl-butyl (BBzP) and di-isobutyl (DiBP) were detected in 100%, and butylparaben in 63% of the samples, respectively. The concentrations of all these parabens and phthalate metabolites were significantly higher in girls compared to boys (all p<0.05), and overall higher than previous reports for comparable age-groups from the US NHANES population. The
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

estimated daily intake of DnBP was 6.4, 15.3 μg/kg bw/day (median, 95th percentile), and above the tolerable daily intake (TDI=10 μg/kg bw/day) defined by the European Food Safety Authority (EFSA) in 15% of the children. The TDI for DEP (5 mg/kg bw/day) and BBzP (500 μg/kg bw/day) were not exceeded (no TDI allocated for DiBP). Urinary concentrations of propyl- and methylparabens were significantly increased in girls with atopic eczema (both p=0.03), but not among boys. Urinary concentrations of the phthalate metabolites were not significantly related to eczema. These results may be caused by girls with eczema using more body lotions/ointments than boys with eczema, alternatively a higher absorption of parabens through the skin in girls with eczema. The difference between boys and girls in urinary concentration of the low-molecular weight phthalate metabolites could point to higher use of body-care products by girls already at 10 years of age and/or exposure to other sources of phthalates which are less common among the 10-year-old boys.

ABSTRACT FINAL ID: 2799 Poster Board #212
TITLE: MICROBIOLOGICAL SURVEILLANCE OF LARGE MACAQUE COLONIES

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INSTITUTIONS (ALL): 1. Covance Research Products, Alice, TX, United States.
KEYWORDS: Microbiology, Macaque, Colonies.

ABSTRACT BODY: Understanding the health status of animals at the onset of biomedical research projects is imperative to a successful outcome. Covance Research Products, Alice, Texas, is an AAALAC accredited facility for rhesus and cynomolgus macaques. As part of our health surveillance program, fecal specimens from our population of macaques are tested for the presence of Salmonella, Shigella and intestinal parasites in addition to other bacteriological screens. Tracking the incidence of possible pathogens is important, not only to ensure the animal’s health; but also, to guarantee the delivery of healthy nonhuman primates to the biomedical research community. All testing conducted as part of this project was accomplished at our on-site microbiology lab under the supervision of licensed microbiologists. In 2010, 9,212 clinically healthy macaques were screened for the presence of Shigella. Fecal samples were collected by deep rectal swabs, and plated on Hekeion Enteric agar. 575 macaques cultured positive for Shigella reflecting a 6% overall incidence of Shigella. Only 4% of macaques hospitalized in 2010 for clinical enteritis and screened for Shigella cultured positive. In 2010, 8,368 macaques were screened for parasites using a formalin/ethyl acetate sedimentation/concentration technique during CDC regulated quarantine. 12 samples were found to contain parasites, with 8 of those being Trichuris. This reflects a 0.14% presence of parasites. Similar data from 2008 and 2009 showed overall parasite incidences of 0.12% in 2008, and 0.11% in 2009, respectively. Results from microbiological testing in 2010 reflect low prevalence of Shigella in both healthy macaques as well in clinical cases of diarrhea at our facility. Overall, our microbiological surveillance program has proved vital to understanding the health status of the animals in our care.

ABSTRACT FINAL ID: 2800 Poster Board #213
TITLE: NOVEL PROTEOMIC APPROACHES IN DETERMINING EARLY SIGNALING RESPONSE TO ENVIRONMENTAL TOXICANTS

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KEYWORDS: proteomics, SILAC, cell signaling.

ABSTRACT BODY: Identifying early signaling responses of mammalian cells to environmental contaminants is vital in determining the underlying mechanisms of toxicity. We developed two novel strategies that capitalize on the latest technologies of proteomics to investigate changes in the general proteome and phospho-specific proteome in a rat liver oval-like epithelial cell line (WB-F344). Each strategy used “Stable Isotopes Labeling with Amino Acids in Cell Culture (SILAC)” that allowed for an accurate quantification of the proteins. Strategy-1 used 2-dimensional ZOOM®
isolectric fractionation (2D-ZOOM) to separate proteins that were extracted from the membrane and cytosolic fraction of the control vs. toxicant to identify any proteomic changes. This data was then used to identify the proteins to be assessed by mass spectrometry on a 2d-ZOOM gel loaded with proteins from the SILAC experiment (same treatment except that the toxicant treated cells were grown on SILAC medium with proteins extracted and combined from the control vs. toxicant and loaded onto one gel). The second approach used TiO2 enrichment of phosphopeptides from the SILAC experiment and then used multidimensional separation coupled with liquid chromatography tandem mass spectrometry (LC-MS/MS) for identifying changes in the phospho-proteome. We used an active (inhibits gap junction, activate MAPKs and induce arachidonate release) and an inactive isomer of methylanthracene to identify proteomic changes. The few phosphoproteins identified in the first more general approach were also identified in the second approach which resulted in the identification of hundreds of phosphoproteins. Support: NIEHS grant #R01 ES013268-01A2 to upham.

ABSTRACT FINAL ID: 2801 Poster Board #214
TITLE: GENOMIC CHANGES IN HUMAN PRIMARY UROEPITHELIAL CELLS FOLLOWING EXPOSURES TO ARSENIC MIXTURES.

AUTHORS (LAST NAME, FIRST NAME): Yager, Jan W.; Clewell, Harvey J.; Efremenko, Alina; Black, Michael; Thomas, Rusty S.; Wagner, Hilary; Wilga, Paul C.; McKim, James M.; Arnold, Lora L.; Gill, Gary; Gentry, Robin
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KEYWORDS: arsenic, carcinogenicity, transcriptomics.

ABSTRACT BODY: Gene expression changes in human primary uroepithelial cells were evaluated following exposures to total arsenic concentrations spanning more than two orders of magnitude in order to elucidate the dose-response for the effects of arsenic treatment on cell signal pathways potentially associated with carcinogenesis, as well as to identify candidate biomarkers of arsenic effects. Cells were treated in culture for 24 hours with mixtures of inorganic arsenic and its metabolites at relative proportions (1:1:4) typically observed in the urine of individuals exposed to inorganic arsenic in drinking water. Two series of in vitro exposures were conducted: one with arsenite and the pentavalent methylated metabolites and a second with arsenite and the trivalent methylated metabolites. In both cases, principal component analysis indicated that the variation across individuals was much greater than the changes in expression elicited by arsenic treatment. When an analysis was conducted across all specimens, common gene expression changes were only observed at total arsenic concentrations above 1 μM, and were related to the oxidative stress response (e.g., HMOX1, NQO2). In contrast, gene expression responses from single individuals were observed at total arsenic concentrations below 0.1 micromolar, and were more diverse. Gene expression changes at the lower concentrations were primarily related to inflammation, epithelial-to-mesenchymal transition (EMT), apoptosis/survival, and cell cycle control. Human inter-individual variability in gene expression is large compared to the effects of arsenic exposures at environmentally relevant concentrations. A suite of genomic biomarkers will be required to broadly assess effects of arsenic in exposed populations.
ABSTRACT FINAL ID: 2802 Poster Board #215
TITLE: INVOLVEMENT OF MIR-122 IN THE PHENOBARBITAL-MEDIATED REGULATION OF GENE EXPRESSION

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KEYWORDS: miR-122, phenobarbital, P450.

ABSTRACT BODY: [Purpose] miR-122 is a miRNA that predominantly expresses in the liver and plays a important role in the hepatic function, including regulation of lipid metabolism. It has been recently reported that AMP-activated protein kinase (AMPK) is activated in the liver of miR-122 knockdown mice. We and others have already demonstrated that phenobarbital-mediated activation of AMPK is involved in nuclear translocation of CAR and thus induces transactivation of the cyp2b10 enhancer. In the preliminary experiments using an Agilent miRNA microarray platform, we have observed that phenobarbital induces a decrease in miR-122 levels in the liver of mice. These results intimate that miR-122 is involved in phenobarbital-mediated AMPK activation. In the present study, we examined whether miR-122 is involved in the phenobarbital-regulated gene expression. [Methods] Total RNA was extracted from liver of male C3H/HeN mice treated with phenobarbital (100 mg/kg, ip.) for 1, 4, 12 or 24 hr and subjected to determine the level of miR-122 by real time RT-PCR using a TaqMan probe. HepG2 cell were treated with PB (upto 1 mM) for 1 to 24 hr and thereafter harvested to determine miR-122 levels and AMPK. [Results and Discussion] Phenobarbital induced significant decrease in miR-122 levels, which was observed as early as 1 hr, reached a trough of 70% of the control level 4 hr and returned to the basal level 24 hr after the injection. Activated AMPK levels, as determined by the phosphorylated protein, increased as early as 1 hr and returned to the basal level 24 hr after injection. These results indicate that phenobarbital induces changes of miR-122 level and activated status of AMPK in an inversely correlated manner. PB also produced similar pattern of miR-122 and activated AMPK in HepG2 cells. [Conclusions] The obtained results from the present study suggest that phenobarbital-induced down-regulation of miR-122 could be involved in AMPK activation and CAR-mediated cyp2b10 transactivation.

ABSTRACT FINAL ID: 2803 Poster Board #216
TITLE: ESTROGEN EXACERBATES GENE EXPRESSION ALTERATIONS RELATED TO CIGARETTE SMOKE EXPOSURE

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KEYWORDS: Cigarette Smoke Condensate, 17-beta Estradiol.

ABSTRACT BODY: Rationale: Cigarette smoke-induced lung cancer is a disease driven by complex innate and exogenous factors. Among the many factors contributing to lung cancer risk, gender has recently been examined as a modulating co-factor. Previous work conducted in our laboratory has suggested that female mice have an innate increased risk for lung cancer, which may be attributed to estrogen levels. In particular, female gender and cigarette smoke-induced lung cancer were linked to a loci on the distal end of mouse chromosome 10. This region contains cyclin dependent kinase 4 (CDK4), signal transducer and activator of transcription 6 (STAT6), STAT2, interferon-γ (IFNG), and glioma-associated oncogene homolog 1 (GLI1), all of which have been implicated in human lung cancer. This study investigates alterations in gene expression due to estrogen and cigarette smoke exposure. Methods: A human bronchial epithelial cell line (BEAS-2B) was cultured in DMEM supplemented with 10% FBS and 1% Pen/Strep. Three treatment groups were established and cells were exposed for 24 hrs to either 100 nM 17β-estradiol (E2) alone, E2 with 10 μg/ml cigarette smoke condensate (CSC), or CSC alone. RNA was isolated from BEAS-2B cells using RNeasy Mini kit (Qiagen) and mRNA expression levels were quantified by real-time PCR using the One-Step Quantitech SYBR Green real-time PCR kit (Qiagen). Results: Gene expression changes were observed in all three treatment groups. Of particular interest, there was a decrease in CDK4 expression when cells were treated with E2 alone but an increase when treated with both E2 and CSC, and this increase was greater than in cells treated with CSC alone. Similarly, increases in expression of STAT2 and STAT6 by CSC alone were augmented by combined treatment with E2 and CSC. Conclusion: Exposure to estrogen exacerbated gene expression changes related to CSC exposure. Furthermore, estrogen treatment resulted in gene expression alterations in genes specifically implicated in human lung cancer.
TITLE: MODULATION OF PROSTATE CANCER CELL PROLIFERATION AND GENE EXPRESSION BY DIETARY OMEGA-3 FATTY ACIDS

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KEYWORDS: omega-3 fatty acids, prostate cancer, gene expression.

ABSTRACT BODY: Prevention studies have demonstrated decreased incidence of prostate cancer in patients with diets containing higher concentrations of the marine omega-3 polyunsaturated fatty acids (n-3 PUFA) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Much less is known about the non-marine n-3 PUFA alpha linolenic acid (ALA). To study which n-3 PUFA are more effective in cancer prevention, and whether mechanisms of action are conserved between them, we tested DHA, EPA and ALA on the androgen independent human prostate cancer cell line PC-3. Prostate cancer cell lines were treated with 100 μM of DHA, EPA, or ALA and changes in cell proliferation and gene expression were examined. Different trends of inhibition of PC-3 proliferation were observed for the three n-3 PUFA. Numbers of DHA-treated cells dropped rapidly while EPA inhibited proliferation more gradually. ALA also inhibited PC-3 proliferation, though more slowly and to a lesser extent. All n-3 PUFA decreased fatty acid synthase (FASN) mRNA, and its regulator steryl response element binding protein 1c (SREBP-1c) mRNA to 50% of control levels. Fish oils DHA and EPA increased gene expression of the pro-apoptotic protein Activating Transcription Factor 3 (ATF3) mRNA by 15-fold and 3-fold relative to control, respectively. ALA did not induce a significant change in ATF3 levels. All three FA decreased expression of macrophage chemotactic factor 1 (MCP-1), an autocrine prostate cancer growth factor. The
decrease in MCP-1 mRNA was most significant with DHA, followed by EPA and ALA. DHA produced the most pronounced inhibition of PC-3 proliferation and the largest changes in gene expression. We conclude that, while some mechanisms of cancer cell inhibition are conserved among n-3 PUFA, the extent, magnitude, and duration of transcriptional changes vary.

ABSTRACT FINAL ID: 2806 Poster Board #219
TITLE: RAPID, NON-GENOMIC SIGNALING IN PROSTATE CANCER CELLS VIA MEMBRANE ESTROGEN RECEPTORS

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KEYWORDS: Non-Genomic, Prostate Cancer, Membrane Estrogen Receptor.

ABSTRACT BODY: Men with prostate cancer are often administered the pharmaceutical estrogen diethylstilbestrol (DES). DES’s effects are attributed to negative feedback of the hypothalamic-pituitary-testicular (HPT) axis, which decreases the production of endogenous androgens required for prostate growth. While that describes an indirect mechanism of estrogens on prostate growth, this study evaluates a direct, non-genomic mechanism, as mediated through membrane estrogen receptors (mERs). Our lab has shown the involvement of mERs in activating rapid cellular signaling cascades. Based on these observations, we hypothesized that prostate tumors behave similarly. The androgen-dependant prostate cancer cell line LAPC-4, was studied for the presence of mERs alpha (α), beta (β), and GPER, using a fixed cell-based plate immunoassay developed in our lab. All three receptor forms were identified both in the cell and on the membrane. E₂ and DES may initiate non-genomic responses via multiple pathways, but we began our studies by examining the activation of mitogen-activated protein kinases (MAPKs) which are involved in cellular responses controlling cell number (cell proliferation and cell death). Extracellular signal-response kinase (ERK) was activated as soon as one minute after E₂ treatment. Low doses of E₂ (10⁻¹⁴-10⁻¹² M) caused continued ERK activation at 10 minutes, while higher doses caused a strong deactivation. E₂ caused a deactivation of c-Jun N-terminal kinase (JNK) after 5 minutes of treatment, with higher doses (10⁻¹⁰-10⁻⁶ M) showing the strongest deactivation. DES treatment caused a deactivation of ERK as rapidly as 1 minute. At 5 minutes, high concentrations (10⁻¹⁰-10⁻⁶ M) deactivated ERK further. Based on these results, we conclude that E₂ and DES can elicit rapid, non-genomic cellular responses across a broad range of concentrations. Studies to determine which mERs are responsible for these signaling cascades are ongoing.

ABSTRACT FINAL ID: 2807 Poster Board #220
TITLE: IDENTIFICATION OF GENE MARKERS FOR ACTIVATION OF THE NUCLEAR RECEPTOR PREGNANE X RECEPTOR

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KEYWORDS: PXR, Biomarkers, Liver.

ABSTRACT BODY: Many environmentally-relevant chemicals and drugs activate the nuclear receptor pregnane X receptor (PXR). Activation of PXR in the mouse liver can lead to increases in liver weight in part through increased hepatocyte replication. Identification of genes that are accurate and specific predictors of PXR activation would be useful in screens to assess potential chemical toxicity and underlying mechanisms. We identified PXR-dependent genes by comparison of differential gene expression in the livers between wild-type and PXR-null mice treated with pregnenolone 16alpha-carbinonitrile (PCN), a model PXR agonist in rodents. Using Affymetrix full-genome mouse arrays, 68% of the genes altered by a 4-day exposure to 400 mg/kg of PCN were completely PXR-dependent and included many involved in DNA replication and the cell cycle. Ingenuity-derived pathways common in both strains included Nrf2 and FXR activation, indicating that PCN activates other transcription factors in addition to PXR. Using the same chip type and identical bioinformatic procedures, we identified putative PXR signature genes by comparing the
ABSTRACT FINAL ID: 2808 Poster Board #221

TITLE: EGF RECEPTOR PHOSPHORYLATION BY AN AQUEOUS EXTRACT OF HOGBARN DUST IS INDEPENDENT OF ITS PROTEASE ACTIVITY

AUTHORS (LAST NAME, FIRST NAME): Dodmane, Puttappa R.; Heires, Art J.; Schulte, Nancy A.; Romberger, Debra J.; Toews, Myron L.

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KEYWORDS: EGF receptor, hogbarn dust, lung inflammation.

ABSTRACT BODY: Workers exposed to dust inside swine confinement facilities can develop chronic inflammatory lung disease characterized by increased levels of proinflammatory cytokines IL-6 and IL-8. An aqueous extract of this hogbarn dust (HDE) increases IL-6 and IL-8 release and phosphorylates epidermal growth factor receptors (EGFRs) in cultured airway epithelial cells. This EGFR phosphorylation mediates about half of the HDE-induced cytokine release. Inhibitors of cellular matrix metalloproteases (MMPs) did not prevent EGFR phosphorylation or cytokine release. HDE contains multiple proteases, and protease inhibitor treatment of HDE markedly reduced cytokine release. Porcine pancreatic elastase (PPE) was able to stimulate IL-6 and IL-8 release also. Thus we hypothesized that proteases present in HDE would mediate EGFR phosphorylation by releasing cellular EGFR-like ligands. HDE was treated with two serine protease inhibitors or with SPIC (Sigma protease inhibitor cocktail). Beas-2B human bronchial epithelial cells were exposed to protease inhibitor-treated or untreated HDE or porcine pancreatic elastase (PPE) for 15 min or 18 h. Cells were lysed and EGFR phosphorylation was quantified. Serine protease inhibitors did not prevent HDE-induced EGFR phosphorylation either at 15 min or 18 h. SPIC, which contains multiple varieties of protease inhibitors, was also ineffective. These same inhibitors do reduce HDE-stimulated cytokine release. PPE failed to stimulate EGFR phosphorylation, even though it did stimulate cytokine release. The failure of protease inhibitors to prevent HDE-induced EGFR phosphorylation suggests that proteolytic activity of HDE does not mediate EGFR phosphorylation. Since neither transactivation by cellular MMPs or proteases in HDE mediate EGFR phosphorylation, we are testing whether HDE may contain EGF-like ligands that can directly phosphorylate EGFRs.

ABSTRACT FINAL ID: 2809 Poster Board #222

TITLE: IDENTIFICATION OF A DIRECT ROLE FOR MITOCHONDRIA IN THE TOXICITY OF RIBOTOXIC STRESS INDUCING TRICHOThECENES

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KEYWORDS: Trichothecene, Mitochondria, Ribotoxic stress.
ABSTRACT BODY: Trichothecenes are protein synthesis inhibitors that trigger a ribotoxic stress response leading to rapid activation of MAPKs, induction of proinflammatory responses, and cell death. These foodborne toxins include deoxynivalenol (DON), diacetoxyscirpenol (DAS), T-2, and trichotecin (Tcin). Identifying the molecular mechanisms underlying trichothecene toxicity is vital to understanding trichothecene toxicosis. To identify the genes that confer susceptibility to trichothecenes, we carried out a genome-wide screen of the 4720 yeast knockouts (YKOs) for increased resistance to Tcin. We identified mitochondria as a key target with 60% of the resistant YKOs associated with mitochondrial function (morphology, ribosome structure, genome maintenance). Growth of trichothecene-treated yeast cells was inhibited in non-fermentable media, which requires functional mitochondria, while cells devoid of mitochondria showed no such inhibition. [35S]-methionine incorporation of yeast cells treated with lower concentrations of trichothecenes showed ~50% inhibition of mitochondrial translation, but not total translation. When isolated yeast mitochondria were treated with 4μM and 8μM Tcin, [35S]-methionine incorporation decreased by 40% and 78%, respectively. Trichothecene-treated yeast cells were stained for mitochondrial membrane potential (Rh123), ROS generation (DCFH-DA) and cell death (PI) and analyzed using flow cytometry. Median fluorescence intensities for all three markers increased when yeast cells were treated with trichothecenes. These results demonstrate a direct role for mitochondria in trichothecene toxicity. Mitochondrial translation is inhibited by trichothecenes. Furthermore, flow cytometry results indicate that trichothecenes trigger ROS generation resulting in hyperpolarization of the mitochondrial membrane, which eventually leads to cell death.

ABSTRACT FINAL ID: 2810 Poster Board #223

TITLE: INTRACELLULAR TRAFFICKING OF RICIN A CHAIN AND ITS RELATIONSHIP TO CYTOTOXICITY AND DEPURINATION

AUTHORS (LAST NAME, FIRST NAME): Yan, Qing1; Li, Xiaoping1; Tumer, Nilgun1


KEYWORDS: Ricin, Intracellular trafficking, Cytotoxicity.

ABSTRACT BODY: Ricin, extracted from the castor bean (Ricinus communis), is a type 2 ribosome-inactivating protein, consisting of a catalytic A chain (RTA) and a cell binding B chain (RTB). RTA specifically depurinates an adenine residue from a universally conserved α-sarcin/ricin loop in 28S rRNA and inhibits protein synthesis. Pre-RTA has a 35-residue signal peptide at the N-terminus, followed by a 267-residue mature RTA. The relationship between RTA transport, ribosome depurination and cytotoxicity are crucial to our understanding of the mechanism of ricin intoxication in vivo. In this study, we used Saccharomyces cerevisiae as a model to track the intracellular trafficking of C-terminal EGFP tagged RTA (RTA:EGFP). We verified that RTA:EGFP was toxic and enzymatically active. The pre-RTA:EGFP, containing the signal peptide, localized to the ER first and then to the vacuole. Our previous study identified several non-toxic mutants with different depurination levels. By labeling these mutants with the EGFP tag, we observed that the pre and mature form of G212E and P95L-E145K had the same localization as the wild type RTA. Most interestingly, the pre and mature form of P250L-A253V, whose double mutations were both localized at the C-terminal hydrophobic domain, were trapped in the ER. These results demonstrated that the C-terminal hydrophobic domain was critical for RTA to get out of the ER. Compared to the mature RTA, pre-RTA had lower cytotoxicity, indicating that the cytotoxicity of RTA was affected by its trafficking. We examined the kinetics of depurination in vivo and in vitro and found that the depurination activity of wild type RTA was higher than that of the mutants, although some of the mutants could depurinate at a higher level than the wild type in vivo. These results suggested that the cytotoxicity of RTA was associated with the depurination rate rather than the depurination level. We demonstrated that the signal sequence of pre-RTA directed it to the ER first and then to the vacuole, and the C-terminal hydrophobic domain was critical for RTA to get out of the ER.
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

ABSTRACT FINAL ID: 2811 Poster Board #224
TITLE: PALMITATE-INDUCED A BIPHASIC CELL DEATH IS RELATED TO ENDOPLASMIC RETICULUM STRESS AND DOWN-REGULATION OF AKT SIGNALING PATHWAY

AUTHORS (LAST NAME, FIRST NAME): Yin, Xia1,2
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KEYWORDS: palmitate, cell apoptosis, H9c2.

ABSTRACT BODY: Cell death induced by lipid accumulation in the heart may contribute to cardiac complication of obesity and type 2 diabetes, but its mechanisms remain elusive. In the present study, we used H9c2 cardiac cell line and treated the cells in vitro with palmitate at 62.5 nM for 3 to 15 hours. Cell death was examined by activation of caspase-3 and -12 as well as CHOP by Western blotting assay. Endoplasmic reticulum (ER) stress associated molecules including GRP78 and ATF4 were examined for their expressions with Western blotting assay, and Akt cell survival signaling was examined by Western blotting assay for the total and phosphorylated Akt and GSK-3β. In addition, we also did Western blotting of Akt negative regulator Drosophila tribbles homologue 3 (Trib3). Results showed that caspase-3 activation was significantly evident at the 3 hours after exposure to palmitate, no significant change from 6 to 9 hours, but significantly increased again from 12 to 15 hours. H9c2 cells exposed to palmitate for 3 hours also showed a significant induction of GRP78 expression and caspase-12 activation without significant changes of other measurements. However, CHOP and ATF4 expression was increased in the cells exposed to palmitate for 9 to 15 hours, along with an increase in TRIB3 expression from 12 to 15 hours. Akt phosphorylation was significantly down-regulated in the cells exposed to palmitate in a time-dependent manner from 6 to 15 hours, while phosphorylation of GSK-3β was up-regulated at the cells exposed to palmitate for 15 hours. Therefore, these results showed that there is a biphasic cell death in the palmitate-treated cardiac cells, reflected by the activation of caspase-3. ER stress-associated cell death was induced at the early phase around 3 hours after palmitate treatment, shown by the increased expression of GRP78 and caspase-12 activation while Akt-related cell survival pathway was down-regulated at the late phase around 12 - 15 hours, which may be related to the up-regulation of Akt’s negative regulator TRIB3.

ABSTRACT FINAL ID: 2812 Poster Board #537
TITLE: IN VITRO PHYSICOCHEMICAL PROPERTIES AND IN VIVO PHARMACOKINETICS OF H6CAHA, A NOVEL HYDROXAMATE BASED HISTONE DEACETYLASE INHIBITOR

AUTHORS (LAST NAME, FIRST NAME): Konsoula, Zacharoula1; Jung, Mira1
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KEYWORDS: pharmacokinetics, hystone deacetylase inhibitors, physicochemical.

ABSTRACT BODY: Structurally diverse histone deacetylase (HDAC) inhibitors have emerged as a new class of anticancer agents that hinder the proliferation of cancer cells in vitro and in vivo and induce cell cycle arrest, terminal differentiation, and/or apoptosis. As such, identifying stable and potent HDAC inhibitors is an important focus for translational research. The assessment of drug like properties is an important phase in the selection of lead compounds for preclinical investigation and for the design of more potent and less toxic compounds. H6CAHA, a novel hydroxamate based HDAC inhibitor, preferentially inhibits the growth of cancer than nonmalignant cells. In this study, the in vitro permeability, solubility, lipophilicity properties as well as in vivo plasma pharmacokinetics of H6CAHA were assessed. H6CAHA exhibited high lipophilicity (logD = 2.8) and good solubility under conditions simulating the acidic environment of the stomach (pH 1.2) and the neutral environment of the small intestine (pH 7.4). The Caco-2 studies revealed that H6CAHA is permeable compound with apparent permeability coefficient (Papp) in the apical to basolateral direction of 6.33 x 10^-6 cm/s. Plasma pharmacokinetics in nude mice showed that H6CAHA reached peak plasma concentrations within 2 h and exhibited a half-life of 11.17 ± 0.87 h, maximum plasma concentration of 6.88 ± 0.71 µM, and area under the curve of 8.08 ± 0.91 µM x h. Pharmacodynamic studies revealed that H6CAHA induced
ABSTRACT FINAL ID: 2813 Poster Board #539

TITLE: SUBSTRATE SELECTIVITY OF HETEROLOGOUSLY EXPRESSED ZEBRAFISH CYP1C1, CYP1C2 AND CYP1D1 WITH XENOBIOTIC, STEROID, AND INDICATOR SUBSTRATES

AUTHORS (LAST NAME, FIRST NAME): Stegeman, John J.; Urban, Philippe; Goldstone, Jared V.; Pompon, Denis


KEYWORDS: cytochrome P450, zebrafish, PAH.

ABSTRACT BODY: Zebrafish (Danio rerio), an important model in developmental toxicology, express five CYP enzymes, CYPs 1A, 1B1, 1C1, 1C2 and 1D1. Zebrafish CYP1A, CYP1B1 and both CYP1Cs are inducible by AH receptor agonists while CYP1D1 is not. Tissue and developmental expression patterns suggest different roles for these CYPs. We focused on assessing the substrate space of CYP1C1, CYP1C2 and CYP1D1, novel enzymes that are poorly known. CYP1s were expressed in W(R) yeast engineered to over-express P450 reductase, optimizing CYP activity. Amounts of recombinant protein were assessed by CO-reduced difference spectra. Catalytic functions were characterized by high-throughput analyses with substituted resorufins and an array of potential endobiotic and xenobiotic substrates. Substrates examined included 10 polycyclic aromatic hydrocarbons (naphthalene, phenanthrene, chrysene, fluorene, benzo[a]pyrene, anthracene, fluoranthene, 9-methylanthracene, 9-phenylanthracene, 9-vinylanthracene), 9 steroids (testosterone, progesterone, RU486, corteloxone, corticosterone, cortisone, nooaktone, 21-hydroxyprogesterone, and nile red), and multiple flavonoids and fluorescent substrates. Many of the substrates have multiple possible sites of oxidation, yielding data for 100 separate activities with the various substrates. The information was used to identify diverging catalytic capacities and selectivities. CYP1C2 clustered with CYP1D1 for activities with PAH substrates, contrary to phylogeny, while with steroids the CYP1Cs cluster together and CYP1D1 was an outlier, in agreement with phylogeny. Modeling and docking studies are consistent with the selectivity with substrates examined in silico as well. The results indicate that the novel CYP1s could contribute substantially to toxicological and physiological processes in cells and organs where they are prominently expressed. (NIH R01ES015912 and P42ES007381, and CNRS)

ABSTRACT FINAL ID: 2814 Poster Board #540

TITLE: NOVEL DUAL-PHASE UPLC-MS/MS ASSAY FOR PROFILING ENANTIOMERIC HYDROXYWARFARINS AND WARFARIN IN HUMAN PLASMA

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KEYWORDS: UPLC-MS/MS, Metabolic Profiling, Chiral.

ABSTRACT BODY: Coumadin (R/S-warfarin) is prescribed for over ~20 million Americans. Although highly efficacious, successful anticoagulation depends on maintaining drug levels within a narrow therapeutic range. This goal is complicated by large inter-individual variability in patient response, which has been attributed to diversity in drug metabolism. Evidence shows that R and S-warfarin have different therapeutic activities and metabolism. The use of warfarin metabolites as biomarkers has been hampered by the inability to quantify the individual enantiomers. To address this limitation, we combined phenyl based reverse phase chromatography and chiral phase chromatography with quantitation by tandem mass spectrometry. The resulting dual-phase UPLC-MS/MS method was made possible with UPLC technology which produces narrow peaks suitable for transferring to a second column. The method
ABSTRACT FINAL ID: 2815 Poster Board #541
TITLE: SEX‐SPECIFIC GENE REGULATION OF DRUG METABOLIZING ENZYMES DEDUCED BY AHR/ARNT TRANSCRIPTIONAL COORDINATION DURING THE RAT LIFE CYCLE

AUTHORS (LAST NAME, FIRST NAME): Ge, Yun1; Kwekel, Joshua1; Han, Tao1; Desai, Varsha1; Fuscoe, James1
KEYWORDS: hepatic drug metabolism enzyme, transcriptional coordination, rat.

ABSTRACT BODY: Aryl hydrocarbon receptor (Ahr) and Ahr nuclear translocator (Arnt) are transcriptional regulators that control toxin metabolism and response to estrogen receptor (Esr) functions. In the current study, we hypothesized that mRNA abundance of genes encoding hepatic sex specific drug metabolism enzymes (DMEs) in the Ahr/Arnt associated pathway would be correlated to each other in a coordinated manner throughout the rat life cycle and that gene regulation could be deduced by a pattern correlation analysis. The aim of the study was to use a hepatic whole genome expression dataset from male and female rats of 2, 5, 6, 8, 15, 21, 52, 78, and 104 weeks of age and focus analysis on 34 DME gene expression patterns in the Ahr/Arnt associated signaling pathway. We observed that the sex factor captured the highest amount of variability (F(1,1) = 23.14) while the age factor contributed the second greatest amount of variability (F(8,1) = 13.69) in two‐way ANOVA for these 34 DME genes. Interestingly, sex differences were demonstrated in some DME genes whose expression patterns were correlated to that of Arnt. In females, correlation coefficients between Arnt and Cyp1a2, Cyp2e1, Maoa, Maob, Nqo1, Ugt1a1, Ugt2b were 0.597, 0.507, 0.653, 0.66, 0.674, 0.7, 0.562, respectively. In males, correlation coefficients between Arnt and Ahr, Cyp11a1, Ptgs2 were 0.508, 0.545, 0.626, respectively. The gene expression pattern of Arnt was correlated with those of transcription factor specificity protein 1 (Sp1), peroxisome proliferator‐activated receptor (Pparg), and Esr in both sexes over the life cycle (correlation coefficient r > 0.5). Although Ahr/Arnt signaling is regulated by Esr and Sp1, which was inferred by pattern correlation and is supported in the literature, potential additional, novel mechanisms of Ahr/Arnt transcription regulation by Pparg were deduced. These findings suggest that sex differences in regulation of genes encoding DMEs in the Ahr/Arnt‐related signaling pathway may be influenced by transcription factors Esr, Sp1 and Pparg.
ABSTRACT FINAL ID: 2816 Poster Board #542
TITLE: PENTA-ESTER PRODRUG HEPATIC AND INTESTINAL METABOLISM IN HUMAN, BEAGLE DOG AND SPRAGUE DAWLEY RAT

AUTHORS (LAST NAME, FIRST NAME): Pacyniak, Erik K; Leed, Marina G.; Sadgrove, Matthew P; Jay, Michael
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KEYWORDS: Ester prodrug, Drug metabolism, Decoration.

ABSTRACT BODY: An injectable formulation of the Ca and Zn trisodium salts of diethylenetriamine pentaacetic acid (DTPA) is currently the only FDA approved therapy for radionuclide contamination. However, in a mass casualty situation, i.v. administration of treatment is not optimal. A DTPA pentaethyl ester, referred to as C2E5, is being developed as an orally bioavailable prodrug for use as a radionuclide decorporation agent. Orally administered C2E5 has shown improved 241Am decorporation in rat models. The cleavage of the five esters of C2E5 results in the formation of four intermediates (C2E4, C2E3, C2E2, C2E1) and the product DTPA. While carboxylesterases have been implicated in this conversion process, the exact mechanism of has yet to be investigated. Therefore, in the present study, S9 intestinal and hepatic fractions were used to test the hypothesis that carboxylesterases are responsible for metabolism of 50 μM [14C]C2E5 in humans, Beagles and Sprague Dawley rats. The metabolic profile was evaluated using radiomeric HPLC analysis. Hydrolysis of C2E5 in intestinal fractions was detected in only humans and rats. Hepatic C2E5 metabolite formation occurred in a species dependent manner. C2E4 was the primary metabolite identified in both human and Beagle with C2E3 in rat. Interestingly, the hydrolysis of C2E5 in both fractions did not proceed further than C2E3 suggesting a potential novel mechanism for penta-ester metabolism by carboxylesterases. Taken together, the results of the current study clearly demonstrates hydrolysis of C2E5 by both intestinal and hepatic S9 fractions in human, Beagle and rat by carboxylesterases.

ABSTRACT FINAL ID: 2817 Poster Board #543
TITLE: CELL BASED MEASUREMENTS OF OXIDATIVE STRESS WITH A NEW FAR RED ROS SENSOR: APPLICATIONS FOR MULTIPLEXED EVALUATION OF CELL HEALTH

AUTHORS (LAST NAME, FIRST NAME): Mandavilli, Bhaskar S.; Yan, Michelle; Wu, Yexin; Kang, Hee Chol; Gee, Kyle; Janes, Michael S.

KEYWORDS: Oxidative stress, Apoptosis, Far red probe.

ABSTRACT BODY: Oxidative stress results from an imbalance between production of reactive oxygen species (ROS) and the ability of cells to scavenge them. ROS play an important role in the progression of several diseases including inflammation, atherosclerosis, aging and age-related degenerative disorders. Oxidative stress can be caused by many different pathways, intrinsic and extrinsic, mediated either by mitochondrial respiration or by membrane-bound NADPH oxidases. Given that imaging enables multiplex analysis, localization and quantitation of different parameters related to cytotoxicity and cell death in the same cell, detection of ROS by conventional fluorescence microscopy or by high content imaging is advantageous over other techniques. Here, we describe a very sensitive ROS probe to measure oxidative stress in cells by fluorescence microscopy. The peak fluorescence emission of this probe is approximately 665 nm, rendering it particularly useful in multiplex applications with green fluorescent probes. This probe was used to evaluate ROS generated by various agents including lipopolysachharide, menadione, angiotensin II, and nefazodone in several different live cell models. The ROS probe, in combination with probes for mitochondrial membrane potential, plasma membrane permeability, or caspase activation, was used to effectively differentiate hepatotoxic compounds from non-toxic compounds, such as nefazodone and rosiglitazone, respectively. We demonstrate here the effectiveness of a new infrared-emitting ROS sensor in multi-parametric measurements of oxidative stress and other aspects of cell health.
**ABSTRACT FINAL ID:** 2818 Poster Board #544

**TITLE:** Γ-GLUTAMYLCYSTEINE (GGC) INHIBITION OF OXIDATIVE STRESS IN HUMAN ENDOTHELIAL CELLS

**AUTHORS (LAST NAME, FIRST NAME):** Nakamura, Yukiko K.¹; Dubick, M. A.²; Omaye, Stanley T.³


**KEYWORDS:** Glutathione, lipid peroxidation, TBARS.

**ABSTRACT BODY:** γ-Glutamylcysteine (GGC) is a precursor of glutathione (GSH), the most abundant cellular reducing compound. Unlike GSH, GGC uptake is not limited by plasma membrane or blood brain barrier; a therapeutic benefit. We investigated concentration effects of GGC (0, 50, 100, 1000 μmol/L, 37°C, 5% CO₂, 24 hr-treatments) on GSH synthesis and oxidative stress in human umbilical vein endothelial cells (HUVEC). All test samples were replicated (n=4). GSH levels (0.66 to 0.41 fold, p<0.01) decreased in a concentration-dependent manner, compared with the control. PPARγ DNA binding levels increased (1.37 to 1.72 fold, p<0.005); however, NF-κB p65 DNA binding (0.62 to 0.72 fold, p<0.005), thiobarbituric acid reactive substances (TBARS) (0.27 to 0.29 fold, p<0.005), and 8-epi-PGF2α (0.76 fold, p<0.01) levels decreased in a concentration-dependent manner. GSH levels were correlated with PPARγ DNA binding levels (p<0.05) and demonstrated + correlation trends with NF-κB p65 DNA binding, 8-epi-PGF2α, and TBARS levels. In addition, our computational search of transcription factor binding sites showed a putative binding site of NF-κB (5'-GGGRNNYYCC-3') at -3444 bp in the promoter region of glutathione synthetase (GSS). Interestingly, the decrease in GSS protein levels was found for all GGC concentrations tested (0.93 to 0.85 fold, p<0.005) compared with the control, but with no changes in GSS mRNA levels. GSS protein levels were + correlated with GSH levels (p<0.01), - correlated with PPARγDNA binding levels (p<0.05), and exhibited + correlation trends with NF-κB p65 DNA binding, 8-epi-PGF2α, and TBARS levels. Feedback inhibition of GSS expression can occur with high concentrations of GGC; however, it is likely that NF-κB p50/p65 deactivation is the consequence of GGC inhibition of oxidation. Thus, we speculate that GGC may be a novel intra-/intercellular therapeutic dipeptide for oxidative stress-related injuries and diseases.

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**ABSTRACT FINAL ID:** 2819 Poster Board #545

**TITLE:** HIGHLY ACTIVE ANTIRETROVIRAL THERAPY DRUG COMBINATION INDUCES OXIDATIVE STRESS AND MITOCHONDRIAL DYSFUNCTION IN IMMORTALIZED HUMAN BLOOD-BRAIN BARRIER ENDOTHELIAL CELLS

**AUTHORS (LAST NAME, FIRST NAME):** Manda, Kalyan¹; Banerjee, Atryee¹; Ercal, Nuran¹; Banks, William A.²

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**KEYWORDS:** Blood-brain barrier, HAART, Oxidative stress.

**ABSTRACT BODY:** The era of highly active antiretroviral therapy (HAART) has controlled AIDS and its related disorders considerably; however, prevalence of HIV-1-associated neurocognitive disorders (HAND) has been on the rise in the post-HAART era. In view of these developments, we investigated whether a HAART drug combination of 3'-Azido-2', 3'-deoxythymidine (AZT) and Indinavir (IDV) can alter the functionality of the blood-brain barrier (BBB) endothelial cells, thereby exacerbating this condition. Viability of hCMEC/D3 cells (in vitro model of BBB) that were exposed to these drugs was significantly reduced after 72 hr treatment, in a dose-dependent manner. Reactive oxygen species (ROS) were highly elevated after the exposure, indicating that mechanisms that induce oxidative stress were involved. Measures of oxidative stress parameters, such as glutathione (GSH) and malondialdehyde (MDA), were found to be altered in the treated groups. Loss of mitochondrial membrane potential (Ψm), as assessed with fluorescent microscopy and decreased levels of ATP, indicated that cytoxicity was mediated through mitochondrial dysfunction. Furthermore, AZT + IDV treatment caused apoptosis in endothelial cells, as assessed by the expression of cytochrome c and procaspase-3 proteins. Pretreatment with thiol antioxidant N-acetylcysteine amide (NACA) reversed some of the pro-oxidant effects of AZT-IDV. Results from our in vitro studies indicate that the AZT + IDV combination may affect the BBB in HIV infected individuals treated with HAART drugs.
ABSTRACT FINAL ID: 2820 Poster Board #546
TITLE: GSH/GSSG RATIO: RAPID LUMINESCENT ASSAY TO MEASURE OXIDATIVE CHANGES IN CELLS GROWN IN TISSUE CULTURE PLATES

AUTHORS (LAST NAME, FIRST NAME): Shultz, John1; Sobol, Mary1; Cali, James1; Murphy, Nancy1; Zhou, Wenhui1
KEYWORDS: ROS detection, GSH/GSSG Ratio, Cell Culture.

ABSTRACT BODY: A wide variety of compounds can induce intracellular formation of reactive oxygen species (ROS) and the resulting cellular damage can eventually lead to apoptosis or necrosis. Measuring the ratio of total to oxidized glutathione (GSH/GSSG ratio) is a way to determine how such compounds alter the redox potential in the cell. A new method is presented for rapid determination of the GSH/GSSG ratio in mammalian cells in multi-well tissue culture plates. The method is based on the formation of luciferin from a glutathione S-transferase catalyzed reaction using GSH and a luciferin precursor. The method utilizes a cell lysate generated directly in the wells of the cell culture plate and does not require protein removal steps. In the reaction to measure total glutathione, DTT is added to convert all the glutathione in the reaction to the reduced form. In the reaction to measure oxidized glutathione (GSSG), free sulfhydryl groups are inactivated with NEM leaving the GSSG intact. The inactivation step is followed by a reducing step that converts the GSSG to GSH for quantification in the luminescent reaction. Since the reaction of free sulfhydryl groups takes place immediately upon cell lysis, additional formation of GSSG from air oxidation of GSH is prevented. The assay takes less than two hours from the time a plate of cells is ready for measurement and can be performed using less than 1000 HeLa cells per well. The performance of the method is demonstrated by following the effects of compounds such as menadione, cumene peroxide and chlorodinitrobenzene on the levels of GSH and GSSG in HeLa and Hep G2 cells. This is a faster, easier, more sensitive assay for identification of compounds that alter the GSH/GSSG ratio.

ABSTRACT FINAL ID: 2821 Poster Board #547
TITLE: EXAMINING THE ROLE OF SUPEROXIDE DISMUTASE ON PEROXYNITRITE FORMATION AND TYROSINE NITRATION USING A FLUORESCENT PROBE SPECIFIC FOR PEROXYNITRITE

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KEYWORDS: peroxynitrite, superoxide dismutase, nitric oxide.

ABSTRACT BODY: Copper,zinc superoxide dismutase (Cu,Zn-SOD) plays a critical role in preventing cellular oxidative injury by scavenging superoxide radicals. However, CuZn-SOD can also catalyze protein nitration by peroxynitrite, a strong oxidant derived from the reaction between nitric oxide (NO) and superoxide. This paradoxical function of CuZn-SOD has been illustrated in cases of acetaminophen toxicity where tyrosine nitration was greatly diminished in the livers of CuZn-SOD-knockout mice (JH Zhu, et al 2008). The recent development of a novel boronate-based fluorogenic probe specific for peroxynitrite (J Zielonka, et al 2010) enabled investigation toward the role for CuZn-SOD in the chemistry of peroxynitrite formation and tyrosine nitration. Equimolar flux of NO and superoxide via SIN-1 decomposition showed a continuous increase in fluorescence of the oxidized probe product, consistent with peroxynitrite formation. The addition of up to 5 uM CuZn-SOD only partially reduced peroxynitrite formation, because NO levels rose to higher steady-state concentrations that out competed SOD for superoxide. The decreased rates of peroxynitrite formation are consistent with peroxynitrite reacting with CuZn-SOD to generate other reactive nitrogen species that could increase nitration. Additionally, the continuous flux of NO and superoxide via SIN-1 dramatically increased the decay of NO following a bolus addition of 667 nM NO (t1/2 = 1200 vs. 13 seconds), suggesting the reaction of NO with nitrogen dioxide (NO2) that could result in nitrosation. Through the use of simultaneous measurement of
ABSTRACT FINAL ID: 2822 Poster Board #901
TITLE: IDENTIFICATION OF THERAPEUTIC COMBINATIONS TO TREAT CUTANEOUS SULFUR MUSTARD INJURY

AUTHORS (LAST NAME, FIRST NAME): Dillman, James F.; Plahovinsak, Jennifer L.; Reid, Frances; Phillips, Christopher S.; Mitcheltree, Larry; Graham, John S.
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KEYWORDS: sulfur mustard, vesicant, inflammation.

ABSTRACT BODY: Cutaneous sulfur mustard (SM) exposure results in severe wounds that are slow to heal. Currently no therapeutics are specifically approved to treat exposure. Recent work has focused on two FDA-approved anti-inflammatory drugs: the steroid clobetasol propionate and the non-steroidal anti-inflammatory diclofenac sodium. Anesthetized weanling pigs were exposed on the abdomen to neat liquid SM to generate superficial or deep dermal burns. When exposed sites were treated topically with both clobetasol and diclofenac, improved healing was observed. The treatments improved healing of SM lesions as the frequency and duration of treatment increased; however the duration of treatment was observed to be more critical for healing than either the frequency or onset of application. To more precisely define the molecular mechanism of SM-induced inflammation, we performed gene expression profiling with histopathology scoring of SM-exposed pig skin 1h, 2h, 4h, 24h, 48h, 72h, 7d, 14d or 21d post-exposure. Gene expression profiles were correlated with histopathology scores to identify pathways significantly correlated with histopathology endpoints. Pathways linked to inflammation were highly correlated with clinical histopathology endpoints assessed through 72h, including epidermal necrosis and microvesication. Pathways linked to inflammation were also highly correlated with 7-21d total histopathology scores. Canonical pathway analysis identified inflammatory cytokine signaling cascades responsive to SM and identified FDA-approved anti-cytokine drugs for testing in vivo. The addition of an anti-cytokine drug to the combination of clobetsol and diclofenac had a positive effect on treatment efficacy as determined by clinical observations and histopathology. Thus correlating histopathology observations with gene expression profiles can help guide selection of therapeutics for the development of SM medical countermeasures.

ABSTRACT FINAL ID: 2823 Poster Board #902
TITLE: DEVELOPING A HAEMOSTATIC DECONTAMINANT FOR SULPHUR MUSTARD; AN IN VIVO FEASIBILITY STUDY

AUTHORS (LAST NAME, FIRST NAME): Hall, Charlotte; Lydon, Helen; Chipman, Kevin; Chilcott, Robert; Graham, John
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KEYWORDS: Sulphur Mustard, Skin, Biophysical measurements.

ABSTRACT BODY: Haemorrhage due to trauma remains one of the leading causes of potentially preventable death for civilians and military personnel. Sulphur mustard (HD) still presents a significant dermal hazard. Under certain circumstances, haemorrhaging wounds could become contaminated with toxic chemicals such as HD. At present there is currently no specific medical countermeasure for treatment of such injuries. The aim of this project is to assess the suitability of commercial-off-the-shelf (COTS) haemostats, as a decontaminant for contaminated wounds. The criteria for decontaminating haemostats would include; (i) retain their haemostatic efficacy in the presence of HD, (ii) reduce the penetration of HD through intact and damaged skin and (iii) halt or reduce lesion development. Following in vitro down-selection the best performing haemostatic product was evaluated in an in vivo damaged skin swine model. Haemostatic product efficacy at reducing lesion development against a neat liquid 14C-HD challenge was evaluated.
using a range of biophysical measurements including; skin reflectance spectrometry, Laser Doppler imaging, trans-epidermal water loss, tissue viability imaging and infrared thermography, as well as histological assessment and 14C-HD dose distribution. Radiometric analysis has shown a significant reduction in the amount of 14C-HD available for dermal or systemic toxicity following treatment with the haemostat. Biophysical measurements have shown a qualitative reduction in lesion pathology following treatment with the haemostat. Thus COTS haemostats may show promise as haemostatic decontaminants and provide a useful medical countermeasure for both civilian and military personnel.

**ABSTRACT FINAL ID:** 2824 Poster Board #903
**TITLE:** DIET COMPOSITION EXACERBATES OR ATTENUATES SOMAN TOXICITY IN RATS: IMPLIED METABOLIC CONTROL OF NERVE AGENT TOXICITY

**AUTHORS (LAST NAME, FIRST NAME):** Myers, Todd M. ; Langston, Jeffrey L.

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**KEYWORDS:** organophosphorus nerve agent, diet composition, neurotoxicity.

**ABSTRACT BODY:** To evaluate the role of diet composition on nerve agent toxicity, rats were fed four distinct diets ad libitum for 28 d prior to challenge with 110 μg/kg (1.0 LD50, sc) soman. The four diets used were a standard rodent diet, a choline-enriched diet, a glucose-enriched diet, and a ketogenic diet. Body weight was recorded throughout the study. Toxic signs and survival were evaluated at key times for up to 72 h following soman exposure. Additionally, acquisition of discriminated shuttlebox avoidance performance was characterized beginning 24 h after soman challenge and across the next 8 d (six behavioral sessions). Prior to exposure, body weight was highest in the standard diet group and lowest in the ketogenic diet group. Upon exposure, differences in soman toxicity as a function of diet became apparent within the first hour, with mortality in the glucose-enriched diet group reaching 80% and exceeding all other groups (in which mortality ranged from 0 – 6%). At 72 h after exposure, mortality was 100% in the glucose-enriched diet group, and survival approximated 50% in the standard and choline-enriched diet groups, but equaled 87% in the ketogenic diet group. Body weight loss was significantly reduced in the ketogenic and choline-enriched diet groups, relative to the standard diet group. At 1 and 4 h after exposure, rats in the ketogenic diet group had significantly lower toxic sign scores than all other groups. The ketogenic diet group performed significantly better than the standard diet group on two measures of active avoidance performance. The exacerbated soman toxicity observed in the glucose-enriched diet group coupled with the attenuated soman toxicity observed in the ketogenic diet group implicates glucose availability in the toxic effects of soman. This increased glucose availability may enhance acetylcholine synthesis and/or utilization, thereby exacerbating peripheral and central soman toxicity.

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**ABSTRACT FINAL ID:** 2825 Poster Board #904
**TITLE:** DIET COMPOSITION MODIFIES THE TOXICITY OF REPEATED SOMAN EXPOSURE

**AUTHORS (LAST NAME, FIRST NAME):** Langston, Jeffrey L. ; Myers, Todd M.

**INSTITUTIONS (ALL):** 1. Neurobehavioral Toxicology, USAMRICD, Aberdeen proving Ground, MD, United States.

**KEYWORDS:** organophosphorus compounds, diet composition, neurotoxicity.

**ABSTRACT BODY:** The current investigation was undertaken to examine the influence of diet on the toxic effects of repeated sublethal doses of soman. Rats were fed one of four diets (standard, choline-enriched, glucose-enriched, or ketogenic) for four weeks prior to and throughout a repeated soman dosing and recovery regimen. Soman exposures occurred across a three-week period. During Week 1, animals received three consecutive daily doses of 0.4 LD50 soman. In the second and third weeks of exposure, animals received four and five consecutive daily doses of 0.5 LD50 soman, respectively. Week 4 constituted a post-exposure recovery evaluation. Throughout the experiment, neurobehavioral function was assessed by discriminated avoidance performance. Survival and body weight changes were recorded daily. Differences in toxicity as a function of diet composition became apparent during the first week. Specifically, rats fed the glucose-enriched diet showed pronounced intoxication during Week 1, resulting in imperfect survival, weight loss, and deteriorated avoidance performance relative to all other
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

ABSTRACT FINAL ID: 2826 Poster Board #905
TITLE: TRICLOSAN INCREASES RESISTANCE TO CHLORINATION IN URBAN ENVIRONMENTAL WATER SAMPLES

AUTHORS (LAST NAME, FIRST NAME): Blackmon, Sherry L.; DelPrete, Jodi L.; Roling, Jonathan A.
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KEYWORDS: antimicrobial, drinking water, bacterial resistance.

ABSTRACT BODY: Triclosan is an antimicrobial agent added to a variety of medical and consumer-care products such as soaps, deodorants, toothpastes, and cleaning supplies. The environmental fate of this widely used product is in the wastewater effluent that is discharged into our rivers. Triclosan is an aromatic hydrocarbon with three potentially bioavailable chlorine atoms that may dissociate from the molecule, thereby exposing the bacteria to free chlorine. Therefore, bacterial exposure to triclosan could lead to chlorine-resistant bacteria. These bacteria may then survive chlorination, the standard method used to disinfect our municipal water supply. As a result, our drinking water may become more contaminated with viable bacteria and pose increased risk for human illness. To analyze whether triclosan leads to increased chlorine tolerance, water samples were obtained downstream from a wastewater treatment plant (WWTP) in Bridgewater, MA and reference virgin stream (VS) in Monroe, MA. The VS site is in a rural area of western MA with no local anthropogenic chemical exposure. Bacteria from WWTP and VS water were isolated and exposed to triclosan (either 0.001 or 0.05mg/mL), and then exposed to chlorine (0.05mg/mL). When comparing chlorine tolerance before and after the triclosan challenge, 33% of all bacterial strains (112/336) increased chlorine resistance after triclosan exposure. The bacteria’s prior exposure also influenced chlorine resistance. Fifty-two percent of the WWTP bacterial strains (87/168) increased chlorine resistance after a triclosan challenge. Meanwhile, only 15% of the VS bacterial strains (26/168) increased chlorine resistance after a triclosan challenge. These results suggest triclosan could potentially allow bacteria to survive chlorination of our drinking water supply and pose greater risk to human health.

ABSTRACT FINAL ID: 2827 Poster Board #906
TITLE: SHORT-TERM EXPOSURE OF FATHEAD MINNOWS TO MUNICIPAL WASTEWATER EFFLUENT INHIBITS THE CANONICAL WNT SIGNALING PATHWAY IN THE LIVER

AUTHORS (LAST NAME, FIRST NAME): Arstikaitis, Jennifer¹; Gagné, François²; Cyr, Daniel¹
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KEYWORDS: Wastewater effluent, microarray, fathead minnow.

ABSTRACT BODY: Municipal wastewater effluent can negatively impact its receiving environment. In the St. Lawrence River, male fish living downstream from Montreal exhibit increased hepatic vitellogenin, intersex, delayed spermatogenesis and decreased sperm functions. Some of these effects may be passed on to humans that eat contaminated fish. Few studies have examined the consequences of genome-wide effects associated with municipal effluent exposure in fish to decipher the mechanisms by which these alter physiological processes. The objective of this study was to identify hepatic cellular signaling pathways altered in Fathead Minnows (FHM) following exposure to
municipal wastewater effluent. Immature FHM were exposed for 21 days to either 0% (Control) or 20% municipal effluent, the highest concentration in the St. Lawrence River. Hepatic RNA was extracted and used to hybridize a 15K gene FHM microarray (Ecoarray). A total of 309 genes were differentially expressed following exposure to wastewater effluent. Of those, 118 were up-regulated and 191 down-regulated. Altered genes grouped according to function indicate effects on various signaling pathways, apoptosis, immune responses, and cellular metabolism. Various components of the canonical Wnt pathway, including KREMEN1, Frizzled, DACT1, and DVL2, were dramatically down-regulated. Several genes of the non-canonical Wnt pathway, such as Wnt4, LRP6, and PPP2R5E (canonical Wnt pathway inhibitors) were increased. This is the first report of altered Wnt signaling following exposure to wastewater effluent. Alterations in the Wnt canonical pathway can cause developmental malformations, cancer, and are implicated in many other pathologies. Whether or not these effects may passed on to humans or other animals who eat fish from the St. Lawrence remains unknown. Supported by Environment Canada, Canadian Water Network, and NSERC.

ABSTRACT FINAL ID: 2828 Poster Board #907
TITLE: GLUTATHIONE BIOAVAILABILITY IN HEALTHY HUMANS: A RANDOMIZED CONTROL TRIAL

AUTHORS (LAST NAME, FIRST NAME): Allen, Jason1,2; Bradley, Ryan1,2

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KEYWORDS: glutathione, oxidative stress, human.

ABSTRACT BODY: Background: Increasing clinical and epidemiological evidence demonstrates low GSH status in numerous human diseases. While oral delivery of GSH would be ideal for ease of administration, systemic absorption has not been conclusively established. Methods: We conducted a randomized, double-blind, placebo-controlled trial in forty healthy adult volunteers to determine the effect of oral GSH supplementation (L-glutathione, 500 mg twice daily/four weeks), on systemic markers of oxidative stress. Primary outcome measures included change in ELISA-measured urinary 8-epi-PGF2alpha (F2-isoP) and urinary 8-hydroxy-2’-deoxyguanosine (8-OHdG), each standardized by creatinine excretion. Changes in erythrocyte GSH concentrations, including reduced glutathione (GSH), oxidized glutathione (GSSG), and their ratio (GSH:GSSG), were measured by LC/MS/MS, and evaluated as secondary outcome measures. ANOVA was used to evaluate the significance of between group differences for all outcome measures. Results: There were no between-group differences in oxidative stress biomarkers at baseline. Changes in creatinine-standardized F2isoP (ng/mg creatinine) (0.0 +/- 0.1 vs. 0.0 +/- 0.1, p=0.38) and 8-OHdG (ug/g creatinine) (-0.2 +/- 3.3 vs. 1.0 +/- 3.2, p=0.27) were not statistically significant at 4 weeks. Similarly, neither reduced, oxidized, or GSH:GSSG ratio measures of glutathione status were impacted by oral supplementation. Conclusions: Although well-powered to detect differences between GH and placebo on primary endpoints, we did not observe significant changes in this clinical trial of oral GSH supplementation in healthy adults. Our findings suggest that, in the absence of conditions of known increased oxidative stress, oral GSH supplementation does not impact systemic redox status following four weeks of supplementation. Future research on the potential of oral GSH supplementation should focus on conditions of increased oxidative stress.

ABSTRACT FINAL ID: 2829 Poster Board #908
TITLE: HEPATOPROTECTIVE AND IN VIVO ANTIOXIDANT ACTIVITIES OF ETHANOLIC EXTRACT OF WHOLE FRUIT OF LAGENARIA BREVIIFLORA (BENTH) ROBERTY

AUTHORS (LAST NAME, FIRST NAME): Saba, Adebowale B.1; Omowunmi, Onakoya1; Ademola, Oyagbemi A.1


KEYWORDS: Lagenaria breviflora, oxidative stress, hepatoprotective.

ABSTRACT BODY: This study was designed to investigate the hepatoprotective and in vivo antioxidant effects of ethanolic extract of whole fruit of Lagenaria breviflora (LB) in experimental animals. Protective action of LB extract was evaluated using animal model of carbon tetrachloride (CCl4)-induced hepatotoxicity. Forty nine (49) Wistar albino rats were divided into seven groups of seven. Group I represented control group; Group II, the hepatotoxic group and
were given CCl4 only (1.5ml/kg b.w/ i.p.); Groups III-VI received different doses of the plant extract and CCl4. Group VII received Garcinia biffavonone (GBI) as reference hepatoprotective drug. Liver marker enzymes and markers of oxidative stress were assayed in serum. LB brought about a significant (p<0.05) decrease in the activities of all these enzymes. There was significant (p<0.05) increased in Malondialdehyde (MDA) and hydrogen peroxide (H2O2) generation in serum of CCl4-treated rats (group II) while the serum glutathione (GSH) level crashed significantly. Pretreatment with LB extract led to significant (P<0.05) increase in serum GSH and significant (P<0.05) reduction in MDA and H2O2 generation. The activities of marker enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) together with bilirubin, creatinine and blood urea nitrogen (BUN) were increased (p<0.05) significantly in CCl4 treated rats (group II). LB pre-treatment led to the restoration of these high levels to near normal. In conclusion, the study suggested that the treatment with LB extract enhanced the recovery from CCl4 induced hepatic damage and oxidative stress via its antioxidant and hepatoprotective properties.

ABSTRACT FINAL ID: 2830 Poster Board #909
TITLE: MC-252 CRUDE OIL WEATHERING FROM SOURCE TO POTENTIAL WORKER AND COMMUNITY RECEPTORS

AUTHORS (LAST NAME, FIRST NAME): Nony, Paul A. ; Millner, Glenn; Nye, Alan; Goad, Phillip; Kind, John
KEYWORDS: Gulf of Mexico, Crude oil, VOCs.

ABSTRACT BODY: The 2010 MC-252 well blowout in the Gulf of Mexico resulted in marine and shoreline crude oil impacts at isolated areas from Texas to Florida. Crude oil that was not recovered near the source or dispersed floated for days to weeks before reaching shore and was subject to a variety of degradative processes referred to collectively as “weathering”. To determine the potential human health impacts of airborne MC-252 oil constituents, air samples were collected at source control vessels above the well head, on vessels performing oil cleanup operations in near shore waters, and in community locations along the Gulf Coast. Samples were analyzed for volatile organic compounds (VOCs) using EPA TO-15, including reporting of tentatively identified compounds (TICs). Crude oil-associated chemicals frequently identified in samples collected over oil-impacted waters near the well head (n=6) included cyclohexane (5.0-372 ppbv), ethyl benzene (1.6-69.1 ppbv), 4-ethyl toluene (7.8-131 ppbv), n-heptane (10-302 ppbv), toluene (4.6-99 ppbv), 1,2,4-trimethylbenzene (3.0-145 ppbv), m,p-xylene (3.7-237 ppbv), o-xylene (1.5-109 ppbv), n-hexane (10.2-300 ppbv), and total hydrocarbons as gas (645-15200 ppbv). Benzene was only positively identified in one sample at a concentration of 6.4 ppbv. Results revealed that the levels of these VOCs were much lower or not detected in near shore and onshore samples (n=1830 or greater) compared to samples collected near the source. Our results are consistent with the results of chemical analyses of weathered MC-252 crude oil demonstrating an absence of lower molecular weight hydrocarbons in weathered oil. These data indicate that the effects of weathering decreased or eliminated the airborne chemical vapors associated with the MC-252 crude oil as it migrated away from the source toward shore. Thus, health risks due to crude oil vapors at the point of exposure were negligible for near shore and onshore workers and residents in the Gulf communities.

ABSTRACT FINAL ID: 2831 Poster Board #910
TITLE: RISK COMMUNICATION TO WORKERS AND THE PUBLIC DURING THE DEEPWATER HORIZON (DWH) OIL SPILL

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KEYWORDS: risk communication, BP oil spill.

ABSTRACT BODY: Communicating with workers and the public regarding the potential for health effects from oil and dispersants and the precautions taken to protect worker and public health was a vital part of the DWH oil spill
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

response. The following approach was used by personnel at the Houma Incident Command Post to understand and address stakeholder concerns: 1) Research the specific nature of health concerns expressed in media reports, discussions with workers and community representatives; 2) Gather relevant information on MC 252 oil weathering, dispersants, dispersed oil and dispersant monitoring, sample locations, analysis and results, and dispersant operations; 3) Develop factual information using photos, posters and summary-level information in handouts to describe MC252 oil, visual images of MC252 oil weathering, how dispersants work, and the locations where dispersants were being sprayed; 4) Gather information on the health effects of dispersant ingredients published in the scientific literature, and the use of dispersant ingredients in common consumer products; 5) Summarize safety precautions taken to protect workers and the public; 6) Provide input to and review of dispersant-related communications materials developed by the Joint Information Center in Houma; and 7) Dialogue with stakeholders who attended the community expositions (“expos”) that were held in Louisiana coastal parishes to provide factual information and help correct common misunderstandings. Feedback from the public indicated that the information provided was well understood and that the community expo format for information delivery was effective and provided an opportunity for engaging stakeholders in a dialog that allowed them to express their concerns and receive answers. In addition, face-to-face communication and training with groups of response workers was an effective means of providing information, addressing concerns, and responding to possible worker exposure incidents.

ABSTRACT FINAL ID: 2832 Poster Board #911
TITLE: 28 DAY REPEATED DOSE TOXICITY TEST ON AN IN VITRO CELL MODEL

AUTHORS (LAST NAME, FIRST NAME): Huang, Song; Constant, Samuel; CaulFuty, Mireille; Bonfante, Rosy; Monachino, Melany; Frauenfelder, Rebecca; Wiszniewski, Ludovic
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KEYWORDS: In vitro inhalation Toxicity, MuclAir, Repeated dose Toxicity Testing.

ABSTRACT BODY: In attempt to transpose the OECD TG412 Guideline to an in vitro cell model, we performed a series of repeated dose toxicity tests on an in vitro cell model of the human airway epithelium (MuclAir™) using several reference chemical compounds. MuclAir™ is not only morphologically and functionally differentiated, but also stays at a homeostatic state for more than one year. It exhibits a gene expression profile of CYPs similar to that of native airway tissues. Four chemical compounds, Ammonium Hexachloro Platinate (IV), Formaldehyde, Cadmium Chloride and Ammonium Hydroxide, were applied 6 hours per day on the apical surface on a 5-day per week basis for a period of 28 days. 8 different concentrations of each compound have been tested. The toxicity of the compounds was monitored by several endpoints: the trans-epithelial electric resistance (TEER), Alamar Blue test, cilia-beating, morphological and histological analysis. Expression of relevant CYPs, such as 1A1, 2A13, 2A6, 2B6, 2C8-1, 2F1 and 2S1 has also been analyzed by quantitative PCR. The NOAELs for each compound has been determined: 10 mM for Formaldehyde, 1 mM for Ammonium Hexachloro Platinate (IV), 10 mM for Ammonium Hydroxide, and 0.01 mM for Cadmium Chloride. The expression of CYPs were differentially modulated: the expression of 1A1 and 2C8-1 were increased by Formaldehyde treatment; 2B6 level was decreased. Our results demonstrated, for the first time, it is possible to transpose the OECD TG412 Guideline to an in vitro cell model.

ABSTRACT FINAL ID: 2833 Poster Board #912
TITLE: EFFECTS OF INTRATRACHEAL INSTILLATION OF TIRE AND ROAD WEAR PARTICLES (TRWP) AND TREAD PARTICLES (TP) ON INFLAMMATION AND CYTOTOXICITY IN RAT LUNG: A COMPARATIVE TOXICITY STUDY

AUTHORS (LAST NAME, FIRST NAME): Kreider, Marisa; Panko, Julie M.; McDonald, Jacob D.; McAtee, Britt L.; Finley, Brent L.; Seagrace, JeanClare
KEYWORDS: particulate matter, tire, inflammation.
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

ABSTRACT BODY: Recent literature has implicated a role for tire and road wear particles (TRWP) in cardiopulmonary morbidities and mortalities associated with ambient particulate matter (PM) exposures. In this study, we compared the response to TRWP and tire tread particles (TP) to other particle types, including respirable titanium dioxide (TiO2), a relatively non-toxic particle; respirable silica, a relatively inflammogenic particle; and diesel exhaust particles, known to induce inflammation and oxidative stress and found in ambient PM. Rats were intratracheally instilled with 1 or 2 mg of size-fractionated TP or TRWP or 1 mg of one of the comparative particle types suspended in 0.1% Tween-80 in phosphate buffered saline (PBS) and sacrificed at 24 hours or 7 days post-instillation. Bronchoalveolar lavage fluid (BALF) was analyzed for changes in markers of inflammation (cell differential profile and cytokine expression), and cytotoxicity (total protein, lactate dehydrogenase, alkaline phosphatase); lung tissue homogenates were analyzed for markers of oxidative stress (hemeoxygenase-1 and thiobarbituric acid reactive substances [TBARS]). For nearly every marker of inflammation and cytotoxicity, the response to TP or TRWP was not statistically different than the relevant controls, whereas equivalent doses of both diesel exhaust particles and crystalline silica produced significant increases in markers of both inflammation (i.e. influx of inflammatory cells and increase of inflammatory cytokines in BALF) and cytotoxicity (i.e. increases in LDH activity and total protein levels in BALF). These results indicate that tire wear particles are less potent inducers of adverse effects associated with exposure to ambient PM, compared to other constituents of ambient PM.

ABSTRACT FINAL ID: 2834 Poster Board #913
TITLE: NOVEL METHODS TO ASSESS GENE EXPRESSION IN NASAL EPITHELIUM COLLECTED FROM CONSCIOUS CYNOMOLGUS MONKEYS

AUTHORS (LAST NAME, FIRST NAME): Dalmas, Deidre A. 1; Drobotz, Lita S. 1; Hughes, Steve 2; Moore, Eric 1; Frazier, Ken S. 1; Thomas, Heath C. 1; Scicchitano, Marshall S. 1

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KEYWORDS: Intranasal gene expression, Cynomolgus monkey, Immunostimulation.

ABSTRACT BODY: Intranasal administration of test article in preclinical studies is performed when it is an intended clinical route. Gene expression analysis of nasal epithelium in animals or patients can be used to assess pharmacodynamic activity (PD) and/or toxicological responses following intranasal dosing of therapeutic agents. Since monkeys are considered the best predictor of immunostimulation and induction of IFN response in man, methods to assess PD and potential toxicological responses in the local nasal environment of cynomolgus monkeys were developed. Using a Rhino-Pro curette, nasal epithelium was collected from the mid-inferior portion of the nasal turbinate of conscious monkeys and RNA was isolated using optimized methods. Five nanograms of RNA was amplified, analyzed using GeneChip Rhesus Macaque Arrays and real-time RT-PCR (TaqMan), and the presence and intra/interanimal variability of expression of selected genes were assessed. On average, 7 to 30 ng of intact Total RNA was isolated from each sample. Standard array quality metrics indicated array performance (RawQ = 0.69, Scaling Factor = 1.9, % Present = 60%, β-Actin 3′/5′ ratio = 4.1 and GAPDH 3′/5′ ratio = 2.8) was optimal. GeneChip and TaqMan analysis of select housekeeping genes (HBP1, GAPDH, TBP1, β-Actin), epithelial markers (EMP1, cytokeratins 6A, 16, 8, 10, 4) and known IFN responsive genes showed relatively stable expression in nasal epithelial samples with minimal intra/interanimal variability. This demonstrates the ability to evaluate gene expression from nasal epithelial samples collected from conscious cynomolgus monkeys which can be used to define local nasal pharmacodynamic activity, assist in evaluation of PD biomarkers, allow for assessment of local (nasal) vs systemic cytokine response and help align preclinical to clinical models.
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

ABSTRACT FINAL ID: 2835 Poster Board #914
TITLE: LUNG FUNCTION AND PATHOGENESIS OF BRONCHIOLITIS OBLITERANS IN RATS EXPOSED TO 2,3-PENTANEDIONE

AUTHORS (LAST NAME, FIRST NAME): Morgan, D. L.; Price, H. C.; Johnson, C. L.; Jokinen, M. P.; Gwinn, W. M.; Flake, G. P.
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KEYWORDS: 2,3-pentanedione, bronchiolitis obliterans, inhalation exposure.

ABSTRACT BODY: 2,3-Pentanedione (PD) is a vicinal diketone used as a replacement for diacetyl in artificial butter flavorings. PD was recently shown to cause bronchiolitis obliterans (BO) in rats after 12 exposures to 200 ppm. The objectives of this study were to characterize airway lesions at early time points, to determine if early lesions progress to fibrosis after exposure is terminated, to determine if fibrosis progresses after termination of exposure, and to determine if lung function is impaired. Male, Wistar-Han rats were exposed to air or 200 ppm PD 6hr/day, 5 days/wk for 1, 3, or 7 exposures. On the morning following each time point the lungs were harvested from control rats (4) and half the exposed rats (6) for histopathology. The remaining exposed rats (6/timepoint) were held without additional exposure and lungs harvested on day 17. Additional rats received 12 exposures to air or 200 ppm PD. After the last exposure lung function was evaluated in half the rats and then the lungs were harvested (day 17). The surviving rats were held for another 2 wk without exposure before evaluating lung function and harvesting lungs (day 30). With the exception of minimal bronchial necrosis in 1 rat, neither bronchial epithelial necrosis, nor fibrosis of bronchi, bronchioles, or alveolar walls occurred after 1 or 3 exposures, even when animals were held for 2 wks prior to sacrifice. Bronchial intraluminal and intramural fibrosis were observed immediately after 7 exposures, and when held for an additional wk after exposure. When rats were subjected to 12 exposures and then held an additional 2 wks, fibrosis was also observed in the alveoli. Airway resistance was 8-fold greater on day 30 compared to day 17 after 12 exposures to PD. Furthermore, dynamic and static lung compliance were decreased by 76% and 46%, respectively, suggesting that lung function progressively worsens after terminating exposure. This study provides important information needed to understand the pathogenesis of BO and to identify potential treatment strategies.

ABSTRACT FINAL ID: 2836 Poster Board #915
TITLE: COAGULATION PARAMETER DIFFERENCES BETWEEN Macaca mulatta AND Macaca fascicularis.

AUTHORS (LAST NAME, FIRST NAME): Waller, Donald P.; McGeehan, Elizabeth; Dubach, Jean; Hoppensteadt, Debra A.; Fareed, Jawad
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KEYWORDS: coagulation, non-human primates, macaque.

ABSTRACT BODY: Macaca mulatta (rhesus) is used extensively for anticoagulant therapy development. Availability, cost and size has led to an increased use of M. fascicularis (cynomolgus) for testing. We observed genetic diversity among macaques from different sources and wanted to compare coagulation responses to examine potential interspecific disparities. The coagulation cascade is evaluated by clotting tests such as prothrombin time (PT), activated partial thromboplastin time (aPTT), Anti-Xa clotting method (Heptest), and thrombin time (TT). Serine protease inhibitors (Anti-Xa, Anti-IIa, and ATIII) target trypsin-like serine proteases including thrombin and plasmin. Interactions with these inhibitors are also used to assess potency and efficacy. Blood was drawn, transferred to tubes with 3.2% sodium citrate, centrifuged to obtain plasma and stored at -70°C. Clotting tests performed included PT, aPTT, Heptest and TT assays using fibrometers. Commercially available chromogenic substrate assays for Anti-Xa and Anti-IIa were also performed in the presence of heparin (H) and Enoxaparin (E). Pooled samples were also serially diluted and percentage of antithrombin determined. The PT and TT activities were higher for cynomolgus vs rhesus whereas aPTT and Heptest were lower. Similar interspecies responses for aPTT were observed with increasing concentrations of H; with E differences were observed at higher concentrations. The rhesus demonstrated a stronger inhibition of Anti-IIa
activity when supplemented with H, however with E, the differences in inhibition was reduced between species. Similar responses were observed for Anti-Xa for both species and drugs. ATIII activity was higher in cynomolagus at greater than 50% dilution. The data show striking differences for coagulation parameters and responses to heparins between the two species of macaques. A clear understanding of the differences in these species is essential when interpreting past data and assessing new anticoagulants for efficacy, safety and pharmacokinetics.

ABSTRACT FINAL ID: 2837 Poster Board #916
TITLE: BISPHENOL A ACTIVATES HUMAN PXR AND INCREASES FOAM-CELL FORMATION IN PXR-HUMANIZED APOLIPOPROTEIN E DEFICIENT MICE

AUTHORS (LAST NAME, FIRST NAME): Zhou, Changcheng1; Sui, Yipeng1; Rios-Pilier, Jennifer1; Ai, Ni2; Kholodovych, Владыслав3; Welsh, William J.2


KEYWORDS: Bisphenol A, pregnane X receptor (PXR), Atherosclerosis.

ABSTRACT BODY: Human exposure to bisphenol A (BPA), a base chemical used extensively in polycarbonate plastics in many consumer products, is thought to be ubiquitous. Recent studies found that higher BPA exposure is consistently associated with cardiovascular disease in the general adult US population. However, the mechanisms responsible for these associations remain unknown. We have previously reported that BPA can activate the nuclear receptor pregnane X receptor (PXR), which, in turn, acts as a xenobiotic sensor to regulate xenobiotic metabolism and exhibits considerable differences in its pharmacology across species. Very recently, we found that chronic activation of PXR increased atherosclerosis in atherosclerosis-prone apolipoprotein E deficient mice (ApoE−/−) and this PXR ligand-mediated pro-atherogenic effect was abolished in PXR−/−ApoE−/− double knockout mice. We also found that BPA is a potent human PXR activator but has no effects on mouse PXR activity. We have elucidated specific key intermolecular interactions between BPA and human PXR by in silico docking studies and identified several BPA analogs as human PXR activators. In order to study the effect of BPA exposure on atherosclerosis, we generated PXR-humanized ApoE−/− mice (huPXR−/−ApoE−/−, ApoE knockout mice with the human PXR transgene in place of mouse PXR) that can respond to human PXR ligands. Feeding huPXR−/−ApoE−/− mice a modified AIN76a diet containing 50 mg/kg BPA can efficiently activate PXR and upregulate its target gene expression. BPA feeding promoted lipid uptake and accumulation in the macrophages of huPXR−/−ApoE−/− mice, which might relate to the PXR-mediated CD36 upregulation. Our results suggest that chronic activation of human PXR may contribute to BPA-elicited atherogenesis and the choice of animal model is paramount in predicting the human risk assessment of BPA.

ABSTRACT FINAL ID: 2838 Poster Board #917
TITLE: ASSESSMENT OF NON-INVASIVE TELEMETRIC BLOOD PRESSURE AND ELECTROCARDIOGRAM MEASUREMENT IN CONSCIOUS DOGS

AUTHORS (LAST NAME, FIRST NAME): McMahon, Nick1; Ward, Gemma1; Milliken, Phil1; Aylott, Mike2; Patel, Bela1

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KEYWORDS: cardiovascular drug safety, Telemetry, Non-invasive blood pressure.

ABSTRACT BODY: Arterial blood pressure is widely used to monitor for adverse effects on haemodynamic status, yet current recording methods require surgical intervention or restraint. The objective of this study was to evaluate the veracity of a non-invasive telemetry (NI) system for detecting changes in blood pressure (BP), heart rate (HR) and electrocardiogram (ECG) parameters in response to vehicle, L-NAME or minoxidil given to freely moving beagle dogs. Data from the NI system (EMKA Technologies, France) oscillometric tail cuff method was compared to data from an invasive telemetry (IT) system (Data Sciences International, USA). All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals. Ambulatory BP and ECG data were simultaneously acquired using the NI and IT.
systems for 2h predose and 24h post dose in male dogs administered p.o. vehicle (0.5% (w/v) aqueous methylcellulose containing 0.1% (w/v) Tween 80; n=5), 1mg/kg minoxidil (n=4) or 10mg/kg L-NAME (n=5) on separate days. Hourly averages were calculated for mean, systolic, and diastolic BP, pulse pressure (PP), heart rate, RR, PR, QRS, QT and QTcL interval for both methods. For NI and IT systems, statistically significant reductions in BP and PP and increases in heart rate, with associated ECG interval changes were apparent with minoxidil. For IT system statistically significant increases in BP and PP were apparent with L-NAME, changes were apparent using the NI system, however, were of shorter duration and reduced magnitude. For both systems statistically significant decreases in heart rate, with associated changes in ECG intervals, were apparent with L-NAME. In conclusion, this preliminary study shows that NI telemetry is able to accurately detect depressor effects in freely moving dogs and that further work is required to assess method sensitivity for detection of pressor effects prior to use in regulatory dog toxicology studies.

ABSTRACT FINAL ID: 2839 Poster Board #918
TITLE: ORGANOTYPIC IN VITRO HUMAN EPITHELIAL MODELS WITH ENGINEERED TOXICOLOGIC REPORTER FUNCTIONS

AUTHORS (LAST NAME, FIRST NAME): Hayden, Patrick J.; Jackson, George R.; Stolper, Gina; Armento, Alexander; Bolmarcich, Jennifer; Kandarova, Helena; Klausner, Mitchell


KEYWORDS: Organotypic models, Reporter assay, NFkB.

ABSTRACT BODY: 3D organotypic in vitro human epithelial models including skin (e.g. EpiDerm, EpiDerm-FT) and airway (e.g. EpiAirway, EpiAirway-FT) are important advances over traditional monolayer cell culture models. For toxicology applications, these models provide more realistic, in vivo-like structure, barrier properties, metabolic functions and dosing capabilities. In the current poster we describe development of organotypic human epithelial models with the added feature of engineered toxicologic reporter functions. Early passage normal human epidermal keratinocytes, dermal fibroblasts, tracheobronchial epithelial cells and airway fibroblasts were transduced with lentiviral vectors containing NFkB reporters linked to either GFP or luciferase, and puromycin resistance elements. Stably transduced cells were selected by puromycin resistance, expanded several passages and cryopreserved to produce large pools of reporter-expressing cells. Reporter-expressing cells were then utilized to produce organotypic skin and airway epithelial models. Organotypic structure and barrier properties of models produced from reporter-expressing cells were found to be similar to models produced from untransduced cells, as determined by histological assessment of H&E stained paraffin sections, and barrier assessment by transepithelial electrical resistance and/or resistance to TX-100 penetration. NFkB reporters linked to either GFP or luciferase were found to be activated about 5-fold above background by treatment of the organotypic models with TNFα. GFP was detected in formalin fixed paraffin sections by epifluorescence microscopy. GFP could also be quantified after extraction from the models by microfluorimetry. Luciferase activity in tissue extracts was quantified with a microplate luminometer. Production of models containing other reporters of toxicological significance (e.g. for DNA damage, oxidative stress, heavy metal stress, ER stress, etc.) by the same process will provide a suite of human epithelial reporter models that can be utilized to provide mechanistic toxicity screening assays.
ABSTRACT FINAL ID: 2840 Poster Board #919
TITLE: A REVIEW OF HEPATOTOXICITY SCREENING IN EARLY DRUG DISCOVERY USING THE ZEBRAFISH MODEL

AUTHORS (LAST NAME, FIRST NAME): Hill, Adrian J.; Jones, Matt; Dodd, Andrew; Diekmann, Heike
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KEYWORDS: hepatotoxicity, zebrafish, alternative animal model.

ABSTRACT BODY: Toxicity remains one of the most prominent causes of candidate drug failure. Commonly identified during late stage animal testing or even post marketing, there is an increasing drive in the pharmaceutical industry to reduce this expensive attrition by integrating new predictive assays much earlier in discovery. Although well established in the field of developmental and genetic research for the past 40 years, the zebrafish vertebrate model has only been used for drug testing over the past decade. Due to numerous advantages over other in vivo screens, such as an in vitro-like assay format requiring only single milligrams of test compound to obtain a swift, full dose response readout, the zebrafish has gained increasing popularity. In particular, zebrafish larvae are now being utilised regularly as a model for the evaluation of hepatotoxicity and the results of over 100 blinded test compounds taken from several studies are presented herein. Larvae were exposed post-embryogenesis for 48 h using a standardised range of aqueous concentrations and assessed for aberrant morphology. The phenotypic readouts included chemically-induced alterations to hepatic tissue, hepatomegaly and an assessment for yolk retention as a measure of hepatic function. A bioanalytical measurement of larval tissue and dosing medium was also performed to allow correlation of any observed toxic phenotype with the actual larval body burden. It also helped to rule out possible false-negatives due to poor uptake and to identify false-positives due to overt toxicity attributable to overdosing of compound. When compared to mammalian data, the zebrafish in vivo hepatotoxicity model provided a positive predictive value (PPV) of 94.7%, a sensitivity of 87.1% and a specificity of 90.9%. As such, this hepatotoxicity model continues to show great potential as an early screening tool to improve decision making in drug discovery.

ABSTRACT FINAL ID: 2841 Poster Board #920
TITLE: HUMANIZING Π-CLASS GLUTATHIONE S-TRANSFERASE REGULATION IN A MOUSE MODEL ALTERS LIVER TOXICITY IN RESPONSE TO ACETAMINOPHEN OVERDOSE

AUTHORS (LAST NAME, FIRST NAME): Biswal, Debika; Vaughan, Matthew; Castagna, Nicole; Hicks, Jessica; Netto, George; DeMarzo, Angelo; Speed, Traci; Reichert, Zachary; Kwabi-Addo, Bernard; Henderson, Colin; Wolf, Roland; Yegnasubramanian, Srinivasan; Nelson, William
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KEYWORDS: GST-pi, humanized mouse, acetaminophen.

ABSTRACT BODY: Π-class Glutathione S-transferases (GSTs) metabolize drugs and xenobiotics and are known to be critical determinants of liver response to drugs and toxins. Despite high protein sequence homology, expression of Π-class GSTs varies significantly between species, making extrapolation from preclinical toxicology studies in mice to humans difficult. To more faithfully model the contribution of Π-class GSTs to human liver toxicity, we introduced the human GSTP1 locus (hGSTP1), with its exons, introns, and flanking sequences, into the germline of mice carrying disrupted mouse Gstp (mGstp) genes. In the resultant humanized GSTP1 mouse strain (hGSTP1+mGSTp1/-/-) genotype), Π-class GSTs were regulated differently than in wildtype mice. Immunohistochemistry revealed that human-like expression patterns were established in both the liver and the prostate. The human patterns of hGSTP1 transgene regulation were accompanied by human patterns of DNA methylation, with bisulfite genomic sequencing revealing establishment of an unmethylated CpG island sequence encompassing the gene promoter. When overdosed with acetaminophen, [hGSTP1+;mGstp1/-/-] mice showed limited liver damage that was intermediate relative to wildtype mice and [hGSTP1--;mGstp1/-/-] mice, as assessed by histopathologic examination and measurement of serum markers.
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

of liver toxicity. By recapitulating both human π-class GST expression and methylation patterns, [hGSTP+mGSTP1/2/-] mice may better model human xenobiotic toxicity. This new “humanized” hGSTP1 transgenic mouse strain model may be valuable not only for preclinical toxicity analyses, but for more faithfully modeling other human disease processes such as carcinogenesis in mice.

ABSTRACT FINAL ID: 2842 Poster Board #921
TITLE: NEEDLE-FREE INJECTIONS VIA THE SUBCUTANEOUS ROUTE IN RATS USING THE BIOJECT DEVICE (AN ALTERNATIVE TO DOSING PRE-CLINICAL SPECIES)

AUTHORS (LAST NAME, FIRST NAME): Ruppert, Gregory¹; Hadd, Sarah M.¹; Stout, Richard²; Hargreaves, Denise¹; Coffey, Craig¹; Frantz, Stephen²
INSTITUTIONS (ALL): 1. MPI Research, Mattawan, MI, United States. 2. Bioject, Tualatin, OR, United States.
KEYWORDS: subcutaneous injection, Rodent.

ABSTRACT BODY: The industry standard for subcutaneous and other parenteral routes of drug administration has historically involved the use of a needle and syringe system. However, there are many drawbacks to needle usage in pre-clinical and clinical settings such as: passage of infectious diseases, potential fear of needles, and accidental needle sticks. Alternatively, needle-free injection systems (NFIS) provide an empowering technology that work by forcing liquid medications at high speed through a tiny orifice held against the skin. This creates a fine stream of high-pressure fluid penetrating the skin and depositing medication in the tissue beneath in a fraction of a second. The novel technology of NFIS has been used recently in pre-clinical and clinical research but has not been previously used in rodents via all routes of injection: intradermal, intramuscular, and subcutaneous. In a recent two-phase study conducted to evaluate and characterize the acute toxicity and estimate the maximum tolerated dose following a single subcutaneous dose, and evaluate the toxicity and toxicokinetics of the test article following 7 days of repeat subcutaneous dosing in CD® [Crl:CD®(SD)] rats, NFIS was used as an alternative to the traditional needle and syringe system. The Bioject device had not been previously used for subcutaneous injection in rats and the poster will describe the different trials used to determine the best technique for administration. Several techniques were employed to determine the ideal method to deliver the test article formulation in the subcutaneous space consistently and accurately that would result in predictable and repeatable result. The technique used on study involved “tenting” the skin with the injection taking place perpendicular to the animal.

ABSTRACT FINAL ID: 2843 Poster Board #922
TITLE: PLASMA MICRO-SAMPLING IN A 6-MONTH TOXICOLOGY MOUSE STUDY MAKES SATELLITE ANIMALS REDUNDANT – ANIMAL WELFARE, SCIENCE AND PRODUCTIVITY – IT’S A WIN, WIN, WIN!

AUTHORS (LAST NAME, FIRST NAME): Königsson, Kristian¹; Malmgren, Birgitta¹; Eriksson, Marie¹; Bergh, Susanne²; Jonsson, Ove³
KEYWORDS: 3Rs, Bioanalysis, Alternative Animal Model.

ABSTRACT BODY: The potential for all new blood microsampling methods is high when it comes to refinement, reduction of animal use and scientific gain. In a recent 6-month repeat dose toxicity study in mice a novel method for blood microsampling and plasma collection was used for bioanalysis and toxicokinetics (TK). The amount of blood collected was 35 µL per sample (<2% of total blood volume in the mouse). In addition to TK, blood samples were collected for metabolite investigation (MIST) as well as clinical pathology. The design of the study included 160 mice (4 dose groups + control). No satellite animals were used in the study! All mice were sampled once each on 5 different occasions. TK was analysed based on composite sampling. In total 800 blood samples were scheduled – no samples were lost! For the TK/MIST analysis, the mice were sampled by puncturing the lateral tail vein. Blood was collected with a 35 µL, EDTA coated, haematocrit tube that was plugged with wax in one end. After centrifugation the tube was cut to isolate plasma. An exact volume of plasma (8 or 4 µL) was transferred from the haematocrit tube to a sample tube
ABSTRACT FINAL ID: 2844 Poster Board #923
TITLE: RABBIT VAPS IDEAL FOR STUDIES UTILIZING ABSL2/ABSL3/ABSL4 AGENTS

AUTHORS (LAST NAME, FIRST NAME): Fleener, Sarah
KEYWORDS: VAP, rabbit, safety.

ABSTRACT BODY: The New Zealand White (NZW) rabbit is an industry standard animal model for utilization in research studies. Repeated or serial blood collection can be difficult to perform due to the limitations, accessibility and viability of available injection or venous access sites. In addition, infusion studies with continuous delivery of a select agent can be difficult to apply for the same reasons. Performing these procedures creates a safety hazard to employees. Covance Research Products tested several methods of placements to compare accessibility, patency and chronic utilization of vascular access ports for serial blood collection and infusion studies. Twelve animals were selected for jugular cannulation and 12 animals were selected for femoral cannulation. The jugular cannulated animals were implanted with a 5 french catheter attached to a port placed subcutaneously located posterior to the right scapula. The femoral cannulated animals were implanted with a 3.5 french catheter attached to a port placed subcutaneously located posterior to the right scapula. After multiple patency checks and evaluating blood flow/collection from the port, it was concluded that a properly placed catheter in the jugular or femoral vein will produce equal results in utilizing the catheter for drug delivery or blood collection. The same results for jugular or femoral cannulation were also found in non-human primate models. Jugular access ports in the canine model also provided sustained access for delivery or collection. Vascular Access Ports are an essential tool for infusion or serial blood collection to reduce or minimize technician risk associated with biohazardous agents. The enhanced safety provided to staff is critical in the workplace environment.

ABSTRACT FINAL ID: 2845 Poster Board #924
TITLE: ANALYSIS OF BODY WEIGHT DIVERGENCE IN GROUP-HOUSED RODENTS

AUTHORS (LAST NAME, FIRST NAME): Ng, Sheung P.; DeLorme, Michael P.; Anand, Satheesh S.; Loveless, Scott E.
INSTITUTIONS (ALL): 1. DuPont Haskell Global Centers for Health & Environmental Sciences, Newark, DE, United States.
KEYWORDS: Body weight divergence, Group housing, Rodents.

ABSTRACT BODY: Body weight is an important toxicological end-point in various research studies. Principles of animal husbandry, described in the Guide for the Care and Use of Laboratory animals from NIH, recommended social animals should be housed in groups rather than individually. Since rodents have territorial behavior, concerns have been raised if dominant animals restrict food access of their cage-mates and thus impede the detection of any body weight change/loss due to test material treatment. Therefore, body weight and food consumption data were collected from 3 studies: (1) a 90-day inhalation study exposing rats (n=20/sex/exposure) at 0, 500, 1500, and 10000 ppm test material, (2) a 90-day gavage study dosing rats (n=10/sex/exposure) at 0, 100, 300, and 1000 mg/kg test material, and (3) a 28-day housing study with no exposure (n=10 rats/sex and n=10 female mice). These data were analyzed for each cage and exposure group to evaluate body weight gain and percent body weight difference (between cage-mates) with time. Results showed that percent body weight difference (per cage) varied over time. Although the percent body weight
ABSTRACT FINAL ID: 2846 Poster Board #925
TITLE: CONFIRMATION OF SEXUAL MATURITY FOR FEMALE CYNOMOLGUS MACAQUES

AUTHORS (LAST NAME, FIRST NAME): Armstrong, Karyn1; Derfler, Kurt1

INSTITUTIONS (ALL): 1. Covance Research Products, Alice, TX, United States.

KEYWORDS: Sexual, Maturity, Macaques.

ABSTRACT BODY: Significant research has been conducted on confirmation of sexual maturity in male non-human primates (NHPs) with emphasis on age, body weight, testes volume, etc. Less information is currently available in the literature for female NHPs. Determining sexual maturity for females continues to be debated, however, the current definition for 100% confirmation of sexual maturity used at Covance is: two menstrual bleedings (assessed from vaginal swabs) of at least 2 days duration, separated by at least 20 days. Alternative methods of determining sexual maturity in female NHPs were compared, but did not reach the same level of confirmation. Efficiencies, rates of confirmations and risks will be discussed.

ABSTRACT FINAL ID: 2847 Poster Board #926
TITLE: ADIPOSY IN YOUNG RATS EXPOSED PERINATALLY TO BISPHENOL A

AUTHORS (LAST NAME, FIRST NAME): Weiss, Bernard1; Jacob, Mathews2; Kennedy, Scott3; Jones, Brian4; Bachmann, Katherine4; Stahlhut, Richard6

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KEYWORDS: Bisphenol A, Magnetic Resonance, Endocrine disruptor.

ABSTRACT BODY: Exposure to the plasticizer Bisphenol A (BPA) is associated with a variety of biological outcomes. Some of these indicate it to be a risk factor for obesity, leading to its classification as an obesogen. In addition to promoting excessive weight gain, it inhibits adiponectin, alters adipocyte differentiation, and induces other metabolic aberrations. Portions of the current study, undertaken to explore sexually dimorphic neurobehavioral endpoints, include both simple and complex measures designed to reflect obesogenic properties. One of its indices is based on the decomposition of fat and water by magnetic resonance imaging. BPA was administered to female Long-Evans rats in cookies containing the following doses: 0, 10, 100, 1000, and 10,000 mcg/kg dissolved in corn oil. The rats were dosed from prenatal day 10 to postnatal day 10. One male and one female from each litter was euthanized on postnatal day 21 and the carcasses frozen. For MR imaging, the carcasses were thawed and assayed on a 3T MRI scanner using a novel analysis algorithm and novel MR spectroscopic imaging pulse sequences developed as a non-invasive scheme to provide high-resolution maps of the spatial distribution and chemical composition of lipids in animal models. Comparisons of the fat and water images reveal that all BPA doses produced increases in fat intensity, with the greatest increase seen at the highest dose. These data indicate that, at least at some ages, perinatal BPA exposure may alter the amounts and spatial distribution of lipid deposition. (Research supported by NIEHS grant 1RC2ES018736 and Center grant ES01247).
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

**ABSTRACT FINAL ID:** 2848 Poster Board #927

**TITLE:** EVALUATION OF ENDOCRINE DISRUPTORS USING GFP-TAGGED RECEPTORS AND HIGH CONTENT ANALYSIS.

**AUTHORS (LAST NAME, FIRST NAME):** Lewis, Raymond; Peters, Amy; Haskins, Jeff; Pietila, Martin; Fedorov, Yuriy

**INSTITUTIONS (ALL):** 1. Life Sciences Research - Cellomics, Thermo Fisher Scientific, Pittsburgh, PA, United States.

**KEYWORDS:** Endocrine disruptor, High Content Image Analysis, screening assay.

**ABSTRACT BODY:** Endocrine disruptors are compounds that mimic estrogen or androgen steroid activity and affect the endocrine system by altering hormone function. Environmental endocrine disruptors have been linked to numerous adverse health effects and reproductive problems in both humans and wildlife. The advent of complex chemical library production in addition to a large catalog of existing compounds necessitates a high throughput procedure to assess endocrine activity and potential environmental impact. GFP-tagged steroid receptors such as estrogen receptor alpha (ERα) and beta (ERβ) form nuclear foci in response to stimulation that can be easily imaged and quantitated by high content analysis (HCA), thereby establishing a high-throughput assay for endocrine disruptors. In this study, we demonstrate the potential of using GFP-tagged steroid receptor cell lines to screen for endocrine disruptors. Cells were treated with a negative control (acetaminophen) or test compounds including natural and synthetic hormones, industrial chemicals, and phytoestrogens. Following fixation and nuclear counterstaining, plates were scanned and quantitated using automated image analysis to evaluate nuclear GFP foci count, area, and intensity as well as overall nuclear morphology on a cell-by-cell basis. For ER-activating compounds, a dose-dependent response was observed for nuclear foci formation with reproducible EC50 values, with no response observed for acetaminophen. Rank order potency demonstrated differential effects of individual chemicals for each cell line and delineated distinct steps in the receptor activation pathway. Diethylstilbestol treatment resulted in equivalent EC50 values for nuclear foci formation in both ER lines (~2nM) while 17α-ethynylestradiol induced nuclear foci at a lower concentration in ERα cells compared to ERβ (EC50 of ~0.2nM and ~3 nM, respectively). These data suggests that GFP-receptor cell lines can be utilized with automated HCA to evaluate chemicals as potential endocrine disruptors.

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**ABSTRACT FINAL ID:** 2849 Poster Board #928

**TITLE:** FLUORIDE IN DRINKING WATER DETERIORATES RENAL DAMAGE IN ICR-DERIVED GLOMERULONEPHRITIS (ICGN) MICE

**AUTHORS (LAST NAME, FIRST NAME):** Hosokawa, Mayuko; Asakawa, Hideo; Sugaya, Chiemi; Tsunoda, Masashi; Itai, Kazuyoshi; Kodama, Yukio; Sugita-Konishi, Yoshiko; Takata, Ayako; Yokoyama, Kazuhiro; Aizawa, Yoshiharu

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**KEYWORDS:** Fluoride, Kidney, Glomerulonephritis.

**ABSTRACT BODY:** Fluoride (F) in drinking water at 150 ppm increased blood urea nitrogen (BUN) of ICR-derived glomerulonephritis (ICGN) mice in our previous study. The mean relative liver weight of the 150 ppm ICGN mice was significantly lower than that of the controls. The objective of this study is to evaluate the effects of F on the liver and kidney of ICGN mice by laboratory tests and pathological changes in the kidney. F was administered at 0, 25, 50, 100, and 150 ppm in drinking water for 4 wk to the ICGN mice and ICR mice at 0 and 150 ppm. Blood was sampled from the tail artery of each mouse and BUN, creatinine, GOT, and GPT in the serum were determined. The mean values of the indexes on the last day were calculated. When a mouse died, the sample from the day closest to the death was assigned. The pathological changes in the kidney were examined after PAS staining. All of the ICGN mice in the 150 ppm group and 1 of 7 in the 100 ppm group died before the end of wk 4, but no ICR mice died. The mean value of the body weight in the 150 ppm group was significantly lower than those in the other ICGN groups. The mean values of relative organ weight of liver or kidney in the 100 and 150 ppm groups were significantly lower than those in the control. The mean values of BUN, creatinine and GPT in the 150 ppm group were significantly higher than those in the
control. The thickness of the glomerular capillary wall and increased mesangial matrix in the kidney were prominent among the F-treated ICGN mice. Glomerulosclerosis and dilation of urinary tubules were also observed more often in the F-treated mice. This suggests that F exacerbates renal dysfunction and induces liver toxicity in ICGN mice.

**ABSTRACT FINAL ID:** 2850 Poster Board #929

**TITLE:** IDENTIFICATION OF METABOLOMICS BIOMARKERS FOR EARLY DETECTION OF CISPLATIN-INDUCED NEPHROTOXICITY

**AUTHORS (LAST NAME, FIRST NAME):** Lim, Hyun Jung; Lee, Young Ju; De, Umasankal; Ahn, Il Young; Bae, Jung Yun; Kwon, Mi Jung; Lee, Byung Mu; Kim, Hyung Sik

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**KEYWORDS:** Metabolomics, Cisplatin, Kidney toxicity.

**ABSTRACT BODY:** Cisplatin is major antineoplastic drug for the treatment of solid tumors. Although cisplatin significantly induced acute kidney injury (AKI), cellular and molecular mechanisms responsible for cisplatin-induced nephrotoxicity to renal tubular epithelial cells are not well understood. Metabolomics has been proved to be a highly successful approach that is capable of detecting metabolic changes under different pathophysiological procedures. To discover biochemical biomarkers useful for early identification of cisplatin-mediated nephrotoxicity, metabolomic experiments were performed in HK-2 cells treated with cisplatin by 1H-Nuclear Magnetic Resonance (NMR)-based spectroscopy. Furthermore, cisplatin-induced metabolites pathway were also confirmed by the expression of related proteins. In the present study, cisplatin reduced the viability in a dose-dependent manner (IC50, 5.4 μM) in HK-2 cells after 48 h. For evaluation of metabolites by cisplatin, HK-2 cells were treated with cisplatin (10 μM) for 6, 12, 24, and 48 h without serum. A number of metabolites were changed in HK-2 cells lysates and media, respectively. Among them, N-acetylasparate, creatine, uracil proline, and acetate levels were reduced in HK-2 cell lysates, but lactate, acetate, and glutamate levels were increased in media of HK-2 cells. In particular, acetate level was significantly affected by cisplatin in both HK-2 cell lysis and media. As expected acetyl-CoA Synthetase (AceCS2) and Sirt3 expression levels were markedly decreased in HK-2 cells treated with cisplatin. These data suggest that acetate-involved metabolic pathway may be useful biomarkers of the detection of cisplatin-mediated kidney injury. Our future efforts will be focused on the validation of those potential biomarkers, and expansion of the test panel of nephrotoxicity to include other mechanisms of action.

**ABSTRACT FINAL ID:** 2851 Poster Board #930

**TITLE:** PROTECTION AGAINST FAS-INDUCED FULMINANT HEPATIC FAILURE IN LIVER SPECIFIC INTEGRIN LINKED KINASE KNOCKOUT MICE

**AUTHORS (LAST NAME, FIRST NAME):** Donthamsetty, Shashi; Mars, Wendy; Wu, Cary; Michalopoulos, George

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**KEYWORDS:** apoptosis, liver failure, integrin.

**ABSTRACT BODY:** This study investigates the role of Integrin linked kinase in Fas-induced fulminant hepatic failure. WT mice and ILK KO mice were challenged with a lethal dose of Jo-2. There was 100% mortality in the WT mice as compared to 80% in the KO mice. We also found that hepatocyte specific ILK KO mice (Integrin linked kinase) died much later than WT mice after challenge with a lethal dose of Fas agonist Jo-2. At sublethal dose of Jo-2, all KO mice survived with minimal apoptosis whereas WT mice developed extensive apoptosis and liver Injury leading to 30% mortality due to liver failure at 12 h. Proteins known to be associated with cell survival/death were differentially expressed in the 2 groups. ILK KO mice showed downregulation of proapoptotic genes and upregulation of antiapoptotic genes. Mechanistic insights revealed that survival pathways like Akt, ERK1/2, and NFKb signaling were upregulated in the IKL KO mice. Inhibition of Akt and ERK1/2 signaling led to a slight increase in the susceptibility of ILK KO hepatocytes to Jo-2-induced apoptosis. These studies suggest that knocking down ILK from the liver protects hepatocytes against Jo-2 induced apoptosis by upregulating survival pathways.
ABSTRACT FINAL ID: 2852 Poster Board #931
TITLE: REGULATION OF ANGIOTENSINOGEN GENE AFTER ETHANOL IN HEPATOCYTES

AUTHORS (LAST NAME, FIRST NAME): Ansari, Rais A.; Clark, Michelle A.
INSTITUTIONS (ALL): 1. Pharmaceutical Sciences, Nova Southeastern University, Fort Lauderdale, FL, United States.
KEYWORDS: Alcohol, Toxicity, Hypertension.

ABSTRACT BODY: Chronic alcohol usage causes liver steatosis, liver cirrhosis, hepatitis and increased blood pressure. A direct link between alcohol usage & binge drinking and hypertension is well documented. The mechanism of alcohol induced hypertension is not fully established. Besides various proposed mechanisms of alcohol mediated hypertension, renin angiotensin system (RAS) has also been implicated. The active vasopressor octapeptide, angiotensin-II (Ang-II) is formed from the precursor, angiotensinogen (AGT) by action of renin, the rate limiting enzyme in the process. The product of this reaction, Ang I is further cleaved by angiotensin-I converting enzyme into Ang II. The concentration of AGT in blood is less than the Michaelis-Menten constant (Km) of renin. Therefore, an increase in blood AGT level leads to increased processing by renin which ultimately leads to an increased level of Ang-II. Variations in blood pressure are linked to changes in Ang-II levels which are further linked to AGT level. Studies with human hypertensive subjects and human-AGT transgenic models have proven that circulating AGT levels correlate to blood pressure. Therefore, studying the regulation of hAGT biosynthesis by liver and extra hepatic organs becomes imperative to address the blood pressure problems created by alcohol abuse. Hepatocytes (HepG2 and Hep3B) were exposed to 0, 25, 50, 100, 150 and 200 mM ethanol for 7 days. The amount of AGT secreted after 7days exposure from these hepatocytes were determined by Western blotting. The secretion of AGT was increased with ethanol treatment. The increase in AGT secretion from Hep3B was observed at 25 and 50 mM while in HepG2 at 150 and 200 mM ethanol concentration. The possible mechanism of transcriptional regulation of increased AGT secretions from these hepatocytes after ethanol exposure will be discussed.

ABSTRACT FINAL ID: 2853 Poster Board #932
TITLE: IMBALANCED BILE ACID COMPOSITION IN LIVER CONTRIBUTES TO BILE ACID HEPATOTOXICITY IN MICE

AUTHORS (LAST NAME, FIRST NAME): Song, Peizhen; Klaassen, Curtis
INSTITUTIONS (ALL): 1. Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS, United States.
KEYWORDS: Bile Acids, Hepatotoxicity, liver.

ABSTRACT BODY: Bile acid (BA) feeding has been used to study BA signaling and toxicity in vivo. However, the concentration of BAs in feed that is non-hepatotoxic is not well defined in mice. Therefore, the purpose of this study was to determine the non-hepatotoxic and hepatotoxic concentrations of five BAs in mouse feed, as well as the effect of feeding mice individual BAs on BA concentrations and BA composition in liver. Mice were fed five individual BAs, cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA), and ursodeoxycholic acid (UDCA) at 0.01, 0.03, 0.1, 0.3, 1.0, or 3.0% in their diets for 7 days. The data showed: 1) LCA, DCA, and CDCA at 0.3% in the diet could be fed without inducing lethality, whereas, CA could be fed at 1.0% and UDCA at 1.0 and 3.0% in the diets could be fed without lethality. 2) Using serum ALT activity levels as the index of hepatotoxicity, LCA produced hepatotoxicity at 0.03, DCA at 0.1%, and CDCA and CA at 0.3% in the diet. UDCA at 0.3% in the diet might be hepatotoxic because serum BA concentrations were increased, but the serum ALT did not increase. 3) Feeding BAs at hepatotoxic doses did not increase the total BA concentrations in liver, however it altered liver BA composition by increasing the percentage of the fed BAs, and decreasing the percentage of muricholic acids. In conclusion, it appears that an imbalance in BA composition in liver contributes to BA hepatotoxicity. Thus, to study the physiological or pharmacological functions of each BA, non-hepatotoxic concentrations of BAs in the feed should be used, namely, CA, CDCA, and UDCA at 0.1% or lower, DCA at 0.03% or lower, and LCA at 0.01% or lower.
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

ABSTRACT FINAL ID: 2854 Poster Board #933
TITLE: SPECIFIC MOLECULAR MECHANISMS OF TGFβ2 SIGNALING ARE DISRUPTED BY ARSENIC, BLOCKING CARDIAC EPITHELIAL TO MESENCYHMAL TRANSITION

AUTHORS (LAST NAME, FIRST NAME): Allison, Patrick B.; Camenisch, Todd
INSTITUTIONS (ALL): 1. Pharmacology and Toxicology, University of Arizona, Tucson, AZ, United States.
KEYWORDS: Arsenic, Cardiac, EMT.

ABSTRACT BODY: The epithelial to mesenchymal transition (EMT) governing the formation of structures of the heart is a complex and highly regulated program that is essential in the formation of cardiac mesenchyme of the endocardial cushion, valves and septum. EMT disruption by separate genetic knockout of Vascular Endothelial Growth Factor (VEGF) and Hyaluronan Synthase-2 (Has-2) in utero results in blocked cushion morphogenesis and a variety of other structural malformations that lead to a lethal phenotype as a result of these heart defects. Atherosclerosis and hypertension also are correlated with environmental arsenic ground water exposure in human populations. Previous studies in our lab have shown that in utero exposure to environmentally relevant levels (50ppb and 100ppb) of Arsenic (AsIII) results in cardiac hypertrophy and delayed valve formation. Utilizing RT-PCR, we demonstrate a significant decrease in expression of Has-2, VEGF, Transforming Growth Factor Beta Receptor III (TBRIII) and other genes necessary for cardiac EMT in a dose dependent fashion. Using immunofluorescent and western blot analysis, we find an elevation of PECAM expression in response to arsenite, conferring more epithelial than mesenchymal cell type. Furthermore, TGFβ2 mediated vimentin expression (a marker for mesenchymal cells) was blocked by arsenite exposure. Utilizing a quantitative Hyaluronan capture assay, we demonstrate that arsenite exposure results in a significant decrease in Hyaluronan production when stimulated with TGFβ2; a novel finding. Using immunofluorescent detection of Smad6 (downstream effector of TGFβ2), we report that Smad6 nuclear translocation and phosphorylation is decreased in arsenite pretreated cardiac epithelial cells. Taken together, these findings show that multiple layers of TGFβ2 signaling are disrupted in response to environmentally relevant levels of arsenic, blocking EMT of the developing heart. (NIHES 04940)

ABSTRACT FINAL ID: 2855 Poster Board #934
TITLE: EFFECTS OF CADMIUM ON N-CADHERIN AND BETA-CATENIN PHOSPHORYLATION IN THE PROXIMAL TUBULE OF RATS

AUTHORS (LAST NAME, FIRST NAME): Schmitz, Matthew; Edwards, Joshua; Prozialeck, Walter
INSTITUTIONS (ALL): 1. Department of Pharmacology, Midwestern University, Downers Grove, IL, United States.
KEYWORDS: cell adhesion, cadmium, Wnt/beta catenin pathway.

ABSTRACT BODY: Cadmium is a toxic metal that is currently ranked 7th on the EPA’s priority list of hazardous substances. The kidney is the critical target organ of chronic low-level cadmium exposure that is common in humans. Recent studies indicate that the N-cadherin/β-catenin complex in epithelial cells of the proximal tubule is an early molecular target of cadmium. However, the specific mechanisms underlying this effect have yet to be elucidated. Since the function of the N-cadherin/β-catenin complex is phosphorylation-dependent, the present studies were undertaken to characterize the effects of cadmium on the phosphorylation status of N-cadherin and β-catenin in the proximal tubule. Male Sprague-Dawley rats were injected with cadmium (0.6 mg Cd/kg, SC, 5 days per week) for 6-12 weeks. Samples of the renal cortex were extracted in tissue protein extraction reagent (20 ml solution/g tissue) with protease and phosphatase inhibitors. Samples were then subjected to polyacrylamide gel electrophoresis and Western blot analyses using antibodies specific to native and phosphorylated N-cadherin and β-catenin. Bands were quantified with Kodak Molecular Imaging software. Cadmium had no effect on total levels of N-cadherin and β-catenin, or phosphorylated forms of β-catenin. However, increased levels of phosphorylated-N-cadherin, at tyrosine 820, were detected at 6, 9, and 12 weeks in tissues from the cadmium treated rats. Samples from cadmium treated animals also contained an additional phospho-tyrosine band (84-120 kDa) at 6 and 9 weeks, and an additional phospho-serine band.
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

(-50 kDa) at 12 weeks. These findings indicate that changes in phosphorylation of tyrosine residues in N-cadherin may be involved in the disruption of cadherin-dependent cell adhesion in the proximal tubule.
Supported by Grant ROI – ES006478

ABSTRACT FINAL ID: 2856 Poster Board #935
TITLE: SKN-1 PLAYS A ROLE IN METHYLMERCURY-INDUCED DOPAMINERGIC NEUROTOXICITY IN CAENORHABDITIS ELEGANS

AUTHORS (LAST NAME, FIRST NAME): Martinez-Finley, Ebany J. 1,2; Ashchner, Michael 1,2
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KEYWORDS: Methylmercury neurotoxicity, C. elegans, skn-1.

ABSTRACT BODY: Mercury (Hg) is a persistent environmental contaminant that exerts its toxic effects on the nervous system through molecular mechanisms that remain unknown. Epidemiological studies have pointed to the contribution of methylmercury (MeHg) to dopamine neuron vulnerability and the predisposition to Parkinson’s disease (PD). Nrf2, a phase II antioxidant transcription factor, has been shown to be involved in MeHg neurotoxicity. Overexpression of Nrf2 inhibits MeHg-mediated cell death and deficits in Nrf2 leaves cells vulnerable to MeHg. We hypothesize that skn-1, the Caenorhabditis elegans (C. elegans) orthologue of mammalian Nrf2, is an important factor in MeHg-induced dopamine neurodegeneration as its expression is shown in dopaminergic neurons. We knocked down skn-1 (skn-1 KO) in C. elegans and exposed both N2 (control) and skn-1 KO worms to 0, 10, 20 and 30µM MeHgCl for 30 minutes or 4 hours following synchronization. Our data suggest that skn-1 KO nematodes (LC50=16µM MeHg) are more sensitive to MeHg than N2 controls (LC50=25µM MeHg). Dopaminergic neuronal morphology was subsequently observed via fluorescent analysis at L1, L4 and adult life stages. Presence of puncta is visible at the 20µM dose in the skn-1 KO nematodes following 30 minute exposure. Longevity and brood size were also assessed.
Decreased longevity was observed at high doses in both N2 and skn-1 KO nematodes. MeHg had a significant effect on brood size at the 20µM dose and decreased nematode size immediately following exposure indicates a developmental delay. We are currently running genetic screens to test the vulnerability of known PD genes to MeHg to delineate the contribution of MeHg to PD. Our data suggest that C. elegans is a valuable model for studying the effects of MeHg on the nervous system, delineating molecular mechanisms of toxicity and determining genetic susceptibility.
Acknowledgements: ES R01 07331.

ABSTRACT FINAL ID: 2857 Poster Board #936
TITLE: THE NEW ORLEANS ANALOG SOIL LEAD MAP: SOIL INTERVENTION AS A STRATEGY FOR LEAD EXPOSURE PREVENTION ON CHILDREN’S PLAYGROUNDS

AUTHORS (LAST NAME, FIRST NAME): Mielke, Howard 1; Covington, Tina P. 2; Mielke, Paul W. 3; Wolman, Fredericka J. 4; Powell, Eric 5; Gonzales, Chris R. 5
KEYWORDS: Lead, Prevention, Children.

ABSTRACT BODY: The feasibility of reducing children's exposure to lead (Pb) polluted soil in New Orleans is tested. Childcare centers (median = 48 children) are often located in former residences. The extent of soil Pb was determined by selecting centers in both the core and outlying areas. The initial 558 mg/kg median soil Pb (range 14-3692 mg/kg) decreased to median 4.1 mg/kg (range 2.2-26.1 mg/kg) after intervention with geotextile covered by 15 cm of river alluvium. Pb loading decreased from a median of 4887 µg/m² (454 µg/ft²) range 603-56,650 µg/m² (56-5263 µg/ft²) to a median of 398 µg/m² (37 µg/ft²) range 86-980 µg/m² (8-91 µg/ft²). Multi-Response Permutation Procedures indicate similar (P-values = 0.160 to 0.231) soil Pb at childcare centers compared with soil Pb of nearby residential communities. Within hours, at a cost of about U.S. $100 (2010) per child, it is feasible to transform exterior play areas at childcare
centers from Pb contaminated to Pb-safe with a large margin of safety and advances an upstream intervention conceptualization about Pb exposure prevention.

ABSTRACT FINAL ID: 2858 Poster Board #937
TITLE: EVALUATION OF RISKS DUE TO CHILDREN’S EXPOSURE TO CADMIUM IN CONSUMER PRODUCTS

AUTHORS (LAST NAME, FIRST NAME): Adams, Rebecca E.; Perez, Angela; Donovan, Brooke; Fillos, Dimitri; Donovan, Ellen
KEYWORDS: cadmium, consumer products, exposure assessment.

ABSTRACT BODY: Recently, there has been concern regarding exposure of children to cadmium (Cd) in consumer products. Exposures occur via direct ingestion of small items such as jewelry or toys, mouthing/sucking on products, or hand-to-mouth contact after handling products. Cadmium is used in pigments, plasticizers, and alloys. Since January 2010, there have been five recalls involving Cd in jewelry and one recall of decorative drinking glasses. To date, consumer product testing has been done by advocacy groups, governmental organizations, trade associations, or individuals using a variety of methods. These reports do not always differentiate between total and soluble Cd, nor do they attempt to perform robust exposure assessments for normal use or worst-case scenarios. An analysis of the available literature was conducted to identify case studies where Cd was detected in children’s consumer products, including glassware, jewelry, and toys. In glassware, wipe sampling results ranged from 0.019 to 0.154 μg Cd/100 cm²/swipe, 6-hr saline extraction results ranged from 0.38 to 2.68 μg Cd per 5 g of glass containing the colored logo, and XRF results ranged from non-detect to over 1000 ppm. In toys, concentrations of total Cd ranged from non-detect to 720 ppm (XRF measurements), while reported measurements of soluble Cd ranged from 0.03 to 0.42 ppm (acid immersion). For jewelry, soluble Cd measured using a 24-hr acid immersion test ranged from 0.72 to 19,362 ppm, while results for the same products using a 6-hr extraction in a saline solution ranged from 0.08 to 495 ppm; these results were compared to Cd content in the same products measured using both XRF and ICP-OES. This analysis also evaluates the correlation between Cd levels measured in certain products using these different analytical methods. The impact of the use of different analytical methods and the underlying exposure assumptions used to determine compliance for a given product are discussed in the context of proposed changes to regulatory standards.

ABSTRACT FINAL ID: 2859 Poster Board #938
TITLE: ALTERATIONS IN MONOAMINERGIC NEURAL SYSTEM IN THE BRAIN OF ADULT MALE MICE BORN TO DAMS PERINATALLY EXPOSED TO 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

AUTHORS (LAST NAME, FIRST NAME): Zhang, Yan; Haijima, Asahi; Hosaka, Ryota; Ling, Wenting; Endo, Toshihiro; Kimura, Eiki; Miyazaki, Wataru; Kakeyama, Masaki; Tohyama, Chiharu
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KEYWORDS: dioxin, monoamine, emotion.

ABSTRACT BODY: Disruption of the formation and/or retention of fear memory was observed in male mice born to dams administered 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Haijima et al., 2010). Emotional dysfunction is known to be accompanied with alteration of monoamine contents in human and experimental animals, suggesting that perinatal exposure to TCDD altered not only memory but also emotional functions. However, how perinatal exposure to TCDD affected brain regions responsible for emotional function remains elusive. Here, we studied whether and how perinatal exposure to TCDD affects basal monoamine contents in brain areas in adult male mouse progeny. Pregnant C57BL/6J mice were administered TCDD by gavage at a dose of 0.6 or 3.0 μg/kg b.w. on gestational day 12.5. After mice progeny reached adulthood, the brain was collected by decapitation and sliced 1 mm sections. Then, specifically designated areas (anterior and posterior cingulate cortex, basolateral amigdala, dorsal and ventral hippocampus, dorsal and median raphe nuclei, ventral tegmental area, substantia nigra, nucleus accumbens, and striatum) were punched out for determination of monoamine contents using a high performance liquid chromatograph, equipped
2011 Society of Toxicology 50th Anniversary Meeting
Late-Breaking Abstracts

with an elecrochemical detector. TCDD at a dose of 0.6 μg/kg b.w. significantly increased dopamine content in the ventral tegmental area and ventral hippocampus compared to control animals. At a dose of 3.0 μg/kg b.w., TCDD significantly increased dopamine content in the ventral hippocampus compared to the control group. These results suggest that perinatal exposure to TCDD affects dopaminergic neurons. In addition, the present results are consistent with our previous finding on deficits in fear memory in mice perinatally exposed to TCDD.

ABSTRACT FINAL ID: 2860 Poster Board #939
TITLE: INDIVIDUAL POLYCHLORINATED BIPHENYL CONGENERS INDUCE SEX SPECIFIC RETINOID TOXICITY PROFILES FOLLOWING ACUTE EXPOSURE

AUTHORS (LAST NAME, FIRST NAME): Heimeier, Rachel A.; Thörnqvist, Per-Ove; Roos, Robert; Schrenk, Dieter; De Boever, Patrick; Schoeters, Greet; Halldin, Krister; Håkansson, Helen
KEYWORDS: PCBs, retinoids, transcriptomics.

ABSTRACT BODY: Polychlorinated biphenyls (PCBs) are persistent environmental contaminants found in human and animal tissues. Emerging evidence indicates that PCBs can disrupt metabolic and/or signaling events in the retinoid system with potential consequences for developmental programming as well as adult life. The retinoid system plays a central role in vertebrate development, growth and metabolism. Thus the effects of PCBs on retinoid signaling will undoubtedly pose a threat to human and wildlife health. Surprisingly, there is limited information on the retinoid pathways affected by PCBs based on molecular analysis in vivo. The objective of this study was to combine exposure studies with genome wide and functional assays to determine the retinoid pathways whose disruption by PCBs may contribute to developmental disorders. We evaluated the effects of single oral exposure to six individual non-dioxin like PCBs 28, 52, 101, 138, 153, or 180, and the dioxin like PCB 126 in the liver of male and female mice 5 days after exposure. Significant increases in animal liver weights were only observed with PCB 126. All PCB congeners were detected in the liver with no significant differences in the levels for males or females. Transcriptomics revealed congener specific effects for males and females, where the molecular profiles differed between PCB 126 and the non-dioxin like PCB congeners, as well as within the group of non-dioxin like PCBs. Several genes and pathways involved in retinoid metabolism and signaling were identified, and HPLC analysis supported significant congener and sex specific changes of hepatic levels of several retinoid forms. Taken together, these data advocate for the existence of sex specific retinoid profiles and pathways induced by individual PCB congeners, which may contribute to physiological modulations of the retinoid system and the increasing dissimilarity towards developmental disorders associated with males and females.

ABSTRACT FINAL ID: 2861 Poster Board #940
TITLE: THE JANUS FACED BISPHENOL A – A STUDY OF PRO-OXIDANT ACTIVITY

AUTHORS (LAST NAME, FIRST NAME): Babu, Sainath; Uppu, Satvika; Raghavamenon, Achuthan C.; Uppu, Rao M.
KEYWORDS: Bisphenol-A, Prooxidant effects, Oxidative stress.

ABSTRACT BODY: Recently, the toxicity of bisphenol-A (BPA) due to low-level exposures has become a major concern. Male albino rats exposed to low levels of BPA were shown to have decreased anti-oxidant enzymes. In order to understand BPA-induced alterations in cellular redox status, we investigated the pro-oxidant effects of BPA and compared with four structurally-related phenolic compounds, viz., 4-tert-butyphenol (TBP), tert-buty-4-hydroxyanisole (BHA), 2,6-di-tert-buty-4-methylphenol (BHT), and Trolox (TRO). The phenolate radicals of BPA, TBP, BHA, BHT and TRO, in situ generated via 1-electron-oxidation of the corresponding phenol in the HRP/H2O2 system, were allowed to react with excess GSH, NADPH, and rifampicin. BPA phenolate radical was found to increase the oxidation of GSH by 9.0±3.39%, while the radicals of TBP and TRO inhibited the oxidation by 10.4±6.86% and 13.5±4.73%,
respectively (values are M±SD; n=3). In the rifampicin assay, higher rates of oxidation were observed with the radicals of BPA (41.6±3.92 μM/min) and TBP (19.7±2.94 μM/min) compared to those of BHT (10.2±0.87 μM/min) and BHA (11.8±0.97 μM/min) and the corresponding control assay (3.8±1.95 μM/min). The oxidation of NADPH (basal rate: 0.2±0.04 μM/min) was also markedly enhanced by the radicals of BPA (23.3±2.51 μM/min) and TBP (12.7±0.28 μM/min), while the radicals of BHT (0.2±0.09 μM/min) and BHA had little or no effect (0.1±0.07 μM/min). Overall, the radicals of BPA and TBP exhibited a mild pro-oxidant activity; BPA was found to be twice more effective consistent with the presence of two phenolic hydroxyl groups. Bisphenol-A per se did not show any free radical scavenging activity in the 1,1-diphenyl-2-picrylhydrazyl assay. It is therefore plausible that BPA favors oxidation reactions in biological systems altering the pro-oxidant/antioxidant balance and causes increased oxidative stress status. Given the recent reports that people can be exposed to high levels of BPA through handling of cash receipts printer paper, our findings are significant and may serve as a forewarning to avoid increases in toxic exposures.
ABSTRACT FINAL ID: 2862 Poster Board #101
TITLE: SPECIES DIFFERENCES OF NUCLEAR RECEPTOR-DEPENDENT DEVELOPMENTAL NEUROTOXICITY: OF MICE AND MEN.

AUTHORS (LAST NAME, FIRST NAME): Fritzsche, Ellen¹;²; Merk, Hans F.; Krutmann, Jean¹; Gassmann, Kathrin¹; Schreiber, Timm¹

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KEYWORDS: neurosphere, Arylhydrocarbon receptor (AhR), PBDE.

Abstract Body: Chemical testing for the identification of hazards for human health bears the well-known problem of species extrapolation. One approach of gaining knowledge about the molecular aspects underlying species differences with regard to chemical sensitivity is the direct comparison of cells derived from different species including humans. Especially for pathologies where not much human epidemiologic data is available, such comparisons are very helpful for extrapolation of animal data to humans. Such a condition is given for developmental neurotoxicity (DNT) where so far only seven compounds are known developmental neurotoxicants for humans. Therefore, we have set up comparative in vitro systems for the development of human (h) and mouse (m) primary neural progenitor cells (NPC) in culture. With this ‘neurosphere assay’ we tested the effects of brominated diphenyl ether (BDE)-99 and Arylhydrocarbon receptor (AhR) agonists/antagonist on neurosphere development. Both, BDE-99 and AhR modulation impaired neurosphere development. However, for BDE-99 hNPCs were much more sensitive than the murine counterparts in disturbing migration and differentiation. These effects are due to a differential expression of thyroid hormone receptors between the species. In contrast, hNPCs were completely insensitive to AhR modulation while mNPC proliferation and migration was affected. This can be explained by a lack of AhR expression in hNPCs as also AhR knockout mNPCs are protected against the adverse effects of AhR modulation. Although basic processes of cell and organ function like brain development are quite similar between mice and men, molecular dissimilarities cause differences in susceptibilities towards chemicals between species as is shown here for chemicals which interact with the thyroid hormone receptor or the AhR. Therefore, knowledge on toxicity pathways for humans vs rodents is a necessity for human hazard identification.

ABSTRACT FINAL ID: 2863 Poster Board #102
TITLE: DESIGN OF A HIGH-THROUGHPUT SCREEN FOR CHEMICALS THAT CAUSE MEIOTIC ANEUPLOIDY

AUTHORS (LAST NAME, FIRST NAME): Allard, Patrick¹; Colaiacovo, Monica¹


KEYWORDS: Aneuploidy, High-throughput screen, C. elegans.

Abstract Body: Abnormal chromosome segregation during meiosis, the process by which haploid sperm and eggs are generated, is a major contributor to aneuploidy and therefore to infertility, miscarriages and birth defects. Although environmental exposure plays a significant role in the etiology of these diseases, there is currently no high-throughput approach for the identification of environmental meiotic disruptors. We propose to use the worm Caenorhabditis elegans in a novel screening strategy for the identification of toxicants altering meiotic chromosome segregation. C. elegans is both a well established meiotic model system that has illuminated our understanding of the genes and pathways governing this process and an emerging animal model used in toxicological studies. Here, we describe a high-throughput method for the identification of environmental aneugenins in C. elegans. We have developed a dual luciferase/GFP reporter that is specifically induced in aneuploid embryos. First, worms are exposed to environmental compounds and screened for the presence of aneuploid embryos via a standard luciferase assay. The positive hits are then validated by direct or automated presence of GFP positive embryos in the worms’ uterus and also by DNA staining for a detailed analysis of the meiotic defects in the germline. In test experiments, we have successfully induced luciferase and GFP expression following exposure to known aneugenic chemicals, such as chemotherapeutic agents,
and also detected the expression of the reporters using an automated set-up, indicating that our system is suitable for high-throughput screens. With this novel screening strategy, we address the need for fast and reliable screening of environmental meiotic toxicants and their involvement in the induction of aneuploidies.

**ABSTRACT FINAL ID:** 2864 Poster Board #103  
**TITLE:** A COMPARATIVE ANALYSIS OF BLOOD SAMPLING TECHNIQUES IN THE RAT  

**AUTHORS (LAST NAME, FIRST NAME):** Mathieu, Cary-Ann; Cahill, Sarah  
**INSTITUTIONS (ALL):** 1. Toxicology, Covance, Harrogate, United Kingdom.  
**KEYWORDS:** Jugular, Vein, Blood.  

**Abstract Body:** Blood sampling is one of the most common procedures performed on laboratory animals as part of scientific research. As an ongoing commitment, Covance, Harrogate, UK identified a need to improve the current blood sampling method in rats from a quality and animal welfare perspective. The aim of the study was to investigate and evaluate sampling from the jugular vein (JV) as a new blood sampling technique at Covance and compare it to the alternative methods available to establish the most appropriate method. There are alternative sites used to sample blood from rats; including the sublingual vein (SV), the orbital sinus (OS) and the lateral caudal vein (LCV), the latter being the preferred method used at Covance. A 22 day study was conducted comparing the LCV, JV, SV and OS as sites of blood sampling whereby animals were bled on several occasions. Throughout the study the following parameters were evaluated; clinical signs, body weight, localised damage at the site of sampling was examined visually, food and water consumption, ophthalmoscopy, haematology and clinical chemistry. All blood samples were visually assessed for haemolysis and clotting. On completion of the last blood sampling, all animals were subjected to a macroscopic examination and selected organs were weighed. The most pertinent findings noted included localised damage of the tail, increase in food consumption, body weight and water consumption in animals sampled from the LCV. Animals sampled from the OS had lens opacities findings. The JV route was the only in-life sampling method that produced no clotted EDTA or trisodium citrate samples in Week 4. When compared to alternative methods on welfare grounds, sampling from the JV using the Covance jugular bleeding technique does not require the animals to be heated (LCV) or anaesthetised (OS). One major benefit is that blood can be taken within one minute of the animal being dosed as a result of the manual restraining, consequently reducing the stress which could potentially affect the physiological state of the animal and variables attributed to the blood parameters.

**ABSTRACT FINAL ID:** 2865 Poster Board #104  
**TITLE:** EVALUATION OF MICROsampling IN PRECLINICAL SAFETY STUDY: TOXICOKINETIC COMPARISON WITH SERIAL BLOOD SAMPLING FROM DIFFERENT SITES IN MOUSE AND RAT  

**AUTHORS (LAST NAME, FIRST NAME):** Zhao, Dong; Wan, Katty; Ruppert, Gregory; Paramadilok, Auratip; Mattis, Charles; Kavetskaia, Olga; El-Shourbagy, Tawakol; Gallenberg, Lori  
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**KEYWORDS:** microsampling, toxicokinetics, preclinical safety.  

**Abstract Body:** Background: Blood microsampling is becoming more commonly utilized in rodent preclinical safety studies in drug development. Microsampling enables serial bleeding from a single animal for PK/TK determination, thereby reducing the animal usage, test article and labor requirements, and data variability that results from inter-animal differences. The purpose of the present study is to investigate the effect of blood sampling site on the pharmacokinetic parameters obtained after a single oral administration of a test article in mice and rats. Methods: The test article (t1/2 = 6 hour) was administered orally to mice or rats, and at each time point, blood samples were concurrently (within 10 minutes) collected from the different sites: (lateral tail vein, saphenous vein, and heart in mice and lateral tail vein, saphenous vein, sublingual vein and jugular vein in rats). Whole blood drug concentrations were measured using liquid chromatography/mass spectrometry and Cmax and area under curve (AUC) were obtained as
parameters for comparison. Results and Conclusion: Comparable AUC values were obtained from the samples collected from different sites in both rats and mice. However, consistent trends indicated that at multiple time points, the drug concentrations with samples from tail vein were the highest whereas concentrations with samples from rat sublingual or mouse saphenous were often 20-30% lower. Based on these results, it is recommended that consistent bleed site should be used when performing serial blood microsampling.

**ABSTRACT FINAL ID:** 2866 Poster Board #105  
**TITLE:** EFFECTS OF TWO STUDY PROCEDURES ON CLINICAL PATHOLOGY DATA IN CYNOMOLGUS MONKEYS: SITE OF COLLECTION AND METHOD OF RESTRAINT

**AUTHORS (LAST NAME, FIRST NAME):** Lynch, Jessica L.¹; Wilcox, Angela ¹; Baxter, Erin¹; Tokuyama, Hisako¹; Elliot, Glenn S.¹

**INSTITUTIONS (ALL):** 1. Charles River, Reno, NV, United States.

**KEYWORDS:** Clinical Pathology, Monkey, blood collection.

**Abstract Body:** In vivo toxicology assessment requires the evaluation of clinical pathology parameters. Alterations in these parameters are often subtle and can occur without histological correlation; therefore, sampling variability must be minimized. The site of collection is reported to affect clinical pathology parameters in BALB/c mice and Sprague Dawley rats; however, less is known whether the site of collection or anesthesia as a method of restraint can alter clinical pathology parameters in cynomolgus monkeys. Blood samples were drawn from the vena cava and femoral vein from 40 cynomolgus monkeys. Most red blood cell and leukocyte parameters were higher when collected from the femoral vein compared to the vena cava, most notably white blood cell, neutrophil, lymphocyte, and monocyte counts. Statistically significant alterations in serum chemistry and coagulation parameters were also noted. A comparison between samples collected from alert and anesthetized (ketamine) cynomolgus monkeys (at least 40 per group) was also conducted. In general, clinical pathology parameters were significantly different in alert versus anesthetized animals. In particular, lymphocyte and reticulocyte counts were increased, and eosinophil counts were decreased in alert animals. The method of collection such as sampling site or presence of anesthesia can influence clinical pathology parameters. These findings underscore the need to standardize sampling procedures during toxicology studies.

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**ABSTRACT FINAL ID:** 2867 Poster Board #106  
**TITLE:** IMMUNOEXPRESSION OF ESTROGEN RECEPTORS ALPHA AND BETA A AND B PROGESTERONE RECEPTOR [PR] AND PROLIFERATING CELL NUCLEAR ANTIGEN [PCNA] IN LEIOMYOMA AND NORMAL MYOMETRIAL TISSUES FROM THE MINIATURE PIG, SUS SCROFA: A NEW FIBROID MODEL

**AUTHORS (LAST NAME, FIRST NAME):** Mozzachio, Kristie¹; Moore, Alicia B.²; Flagler, Norris D.²; Swanson, Cynthia¹; Dixon, Darlene²

**INSTITUTIONS (ALL):** 1. WIL-Biotechnics, LLC, Hillsborough, NC, United States. 2. National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC, United States.

**KEYWORDS:** uterine leiomyoma, miniature pig, steroid receptors.

**Abstract Body:** Uterine leiomyomas occur in miniature pet pigs with a similar frequency to that observed in women, with a positive correlation to advancing age and negative correlation to parity. Clinical signs, gross and histologic features closely resemble leiomyomas (fibroids) in women, and the porcine exhibits an estrus cycle that better approximates that in women compared to current animal models. Although fibroids are hormonally responsive in women, the roles of estrogen and progesterone have not been clarified and current animal models present challenges in data extrapolation to humans. In this study, the expression of ER-α, ER-β and PR was determined in porcine tumors versus matched myometria. Additionally, the PCNA proliferation marker was also evaluated. Immunohistochemistry was performed on formalin-fixed tissues from miniature pigs diagnosed with uterine leiomyoma(s), evaluated by routine light microscopy, and positive-immunostaining quantitated via a computer-assisted technique. Both ER-α/β and
PR were localized to nuclei of normal and neoplastic smooth muscle cells. PR expression was intense and diffuse throughout examined tissues and generally consistent between tumors and matched myometria. Contrarily, ER-α expression was varied among examined tumors, including among multiple tumors in single individuals. ER-β positivity was present at very low percentages, with no remarkable difference between normal and neoplastic samples. PCNA immunostaining was absent in all normal myometria; tumors were variable, ranging from 0-52% positivity. Findings support further investigation into the miniature pet pig as a model of fibroids in women, and establishing a reliable means to stage estrus in the miniature pig is of critical importance in data interpretation of this suspected hormonally responsive neoplasm in the porcine.

**ABSTRACT FINAL ID:** 2868 Poster Board #109  
**TITLE:** HUMAN VARIABILITY IN RESPONSE TO ACUTE RADIATION  
**AUTHORS (LAST NAME, FIRST NAME):** Stricklin, Daniela L.; Pellmar, Terry; Millage, Kyle  
**KEYWORDS:** human variability, acute injury, radiation.

**Abstract Body:** Individual variability can affect response to acute radiation injury, and knowledge of this variability is critical for emergency preparedness planning for radiological incidents. This information can help identify vulnerable sub-populations, provide insight on treatment requirements, and improve casualty estimations. In the clinical setting, understanding human variability to acute radiation exposure is needed for radiation oncology treatment planning. This study sought to identify demographic, genetic, and environmental factors that significantly influence response to acute radiation injury. Information on in utero exposures, gender, age, and co-morbidity status was examined from data from the atomic bombings, radiation accidents, animal experiments, and clinical oncology. Burn and trauma data were examined as surrogate acute injury as needed. Sensitivity to radiation in the fetus has been well documented; increased mortality or malformations result, depending on gestational age. A gender difference in response to acute radiation has been observed; males appear to be more susceptible than females as evidenced by nearly a 10% greater incidence of radiation syndrome among male A-bomb survivors. Trauma data supports male sensitivity to acute injury; increased mortality is observed, apparently due to immunological differences between genders. The limited data available on the effect of age suggests vulnerability in the very young and old due to immunological status and co-morbidities, respectively. Certain genetically susceptible sub-populations demonstrate marked increased sensitivity to radiation exposure. Persons with heritable mutations such as ataxia telangiectasia show dramatically increased mortality after radiation therapy. Interaction of radiation and co-morbid conditions has not been well studied, though burn and trauma surrogate data indicate that co-morbidities negatively impact human response to acute injury. The key factors together with their prevalence provide an indication of the potential impact of human variability in radiation response.
**Abstract Body:** Acrylonitrile is widely used, principally as a monomer in the industrial manufacture of synthetic polymers and as a precursor of acrylamide and acrylic acid. The present study defined a simplified physiologically based pharmacokinetic (PBPK) model for acrylonitrile in humans based on in vitro metabolic parameters determined using liver microsomes, coefficients derived in silico, physiological parameters derived from the literature, and a developed PBPK model in rats. The model basically consists of a chemical absorption compartment, a metabolizing compartment, and a central compartment for acrylonitrile. Evaluation of a rat model was performed by comparisons with experimental pharmacokinetic values from blood and urine obtained from rats in vivo after oral treatment with acrylonitrile (30 mg/kg, a no-observed-adverse-effect level) for 14 days. Elimination rates of acrylonitrile in vitro were established using data from liver microsomes form rats untreated- or treated with acrylonitrile and from pooled human livers. Acrylonitrile was expected to be absorbed and cleared rapidly from the body in PBPK model, as was the case for rats confirmed experimentally in vivo with repeated low-dose treatments. These results indicate that the simplified PBPK model for acrylonitrile is useful for a forward dosimetry approach in humans. The simplified PBPK model of acrylonitrile was developed with a combination of algorithms, in vitro and in vivo experimentation and literature resources. Although this model currently might not predict the details such as cyanoethylene oxide concentrations in terms of its simplicity the utility of this model could be also expanded to the industry researchers and regulatory authorities in risk assessment, instead of complex multi-compartment models which might be rarely performed. In summary, the present study indicates that simplified PBPK modeling for acrylonitrile is useful for a forward dosimetry approach in humans to estimate blood concentrations of acrylonitrile and other related compounds from low chemical doses such as those at the no-observed-adverse-effect level.

**Abstract Body:** Cocaine is a powerful sympathomimetic associated with systemic inflammation, oxidative stress and vascular dysfunction. Its use has been linked to renal disease, rhabdomyolysis, vasoconstriction, acute myocardial ischemia and infarction. Activation of inflammatory and oxidative stress pathways and the onset of renal, cardiovascular, cerebrovascular and other systemic damage have been shown to be mechanistically linked. Sex-based differences in the clinical presentation, diagnosis, and treatment outcomes of renal and cardiac disease have long been recognized. This investigation examined urine specimens for potential differences between the expression of myoglobin, a biomarker associated with cardiovascular damage, inflammation and oxidative stress, and cocaine use for the purpose of determining if gender can play a significant role in toxicological endpoints related to illicit drug exposures. Urine specimens were assayed for cocaine metabolites, creatinine, total protein (BSA), and myoglobin using ELISA and colorimetry. We observed significant differences between male control and male cocaine positive urines for myoglobin. Interestingly, there was no statistically significant difference in females between control and cocaine positive urines. The reason for the differences remains to be elucidated. Differences in the urinary expression of myoglobin may be important in evaluating the gender based effects of cocaine use and may have potential clinical applications which may be related to gender differences in signs and symptoms of cocaine toxicity.
ABSTRACT FINAL ID: 2871 Poster Board #112
TITLE: ACUTE TOXICITY AND BIOTRANSFORMATION OF 2,3,3,3-TETRAFLUOROPROPENE (HFO-1234yf) IN PREGNANT AND NON-PREGNANT RABBITS

AUTHORS (LAST NAME, FIRST NAME): Schmidt, Tobias¹; Bertermann, Rüdiger¹; Rusch, George M.²; Hoffman, Gary³
KEYWORDS: biotransformation, acute inhalation exposure, pregnant rabbit.

Abstract Body: HFO-1234yf is a novel refrigerant intended for use in mobile air conditioning showing a low potential for toxicity in rat and mouse studies with most NOAELs > 10,000 ppm. In a rabbit developmental toxicity study, inhalation exposures to HFO-1234yf caused lethality at exposure levels of 5,500 and 7,500 ppm; no lethality was observed at exposure levels of 4,000 ppm (6h/day, GD 6 through 28).

Since the lethality in pregnant animals may be due to altered biotransformation, this study compared the acute toxicity and biotransformation of HFO-1234yf in male (n = 5), female (n = 5) and pregnant female (n = 6) rabbits. Animals were exposed to 50,000 ppm (male and pregnant female rabbits on GD 12) and to 100,000 ppm (all groups) of HFO-1234yf for one hour. Urine was collected in 12 h intervals for 48 h after the end of the exposure and analyzed by 19F-NMR and by LC/MS-MS. The predominant metabolites were S-(3,3,3-trifluoro-2-hydroxypropanyl)-mercaptolactic acid and N-acetyl-S-(3,3,3-trifluoro-2-hydroxypropyl)-L-cysteine whose signal intensities in 19F-NMR spectra represented more than 70 % (100,000 ppm) of total 19F related signals in all urine samples. Quantification of the HFO-1234yf-mercapturic acid isomers by LC/MS-MS showed no difference in the mean recovery of this metabolite between female (43.11 ± 22.35 µmol) and pregnant female rabbits (44.78 ± 16.48 µmol) excreted in urine within 48 hours.

No lethality and no clinical signs were observed in the exposed animals. With the exception of one as yet unidentified metabolite (15-19% female rabbits and < 9% male rabbits of the five main metabolites), no differences in urinary metabolite pattern or quantity of metabolites excreted were observed between the different groups. The results suggest that lethality of HFO-1234yf in pregnant rabbits after inhalation exposure is unlikely due to changes in biotransformation patterns or capacity of pregnant rabbits.

ABSTRACT FINAL ID: 2872 Poster Board #113
TITLE: KAVA EXTRACT ACTIVATES ARYL HYDROCARBON RECEPTOR (AHR) AND AFFECTS TCDD-ELICITED AHR RESPONSE IN HEPATIC CELLS

AUTHORS (LAST NAME, FIRST NAME): Li, Yan¹; Wu, Qiangen²; Zhang, Suhui¹; Fang, Jia-Long³; Mei, Hu¹; Shi, Leming¹; Guo, Lei²
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KEYWORDS: kava, AhR, CYP1A1.

Abstract Body: Extracts from kava have been used as an anti-anxiety preparation and are continually sold in the U.S. despite their ban in the European Union and Canada because of their suspected liver toxicity. The National Toxicology Program (NTP) initiated short- and long-term studies to characterize the toxicity of kava extracts. Using rat and mouse liver samples collected from the NTP 14-week studies we previously found that kava induced a significant increase of Cytochrome P450 1A1 (CYP1A1). As a follow-up study, here we report our investigation on the activation of the aryl hydrocarbon receptor (AhR) pathway by kava in hepatic Hepa1c1c7 cells and the interaction between kava and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on AhR activation. Kava exhibited a dose-dependent effect on CYP1A1 induction at the level of mRNA expression, protein expression, and enzymatic activity. Using a reporter gene activity assay, we demonstrated that the induction of CYP1A1 was via activation of the AhR signaling pathway. In addition, we also investigated the effects of kava on TCDD-mediated AhR activation. Kava inhibited TCDD-mediated CYP1A1 activity when cells were treated with kava and TCDD simultaneously.
In contrast, kava increased TCDD-elicited CYP1A1 activity when cells were pre-treated with kava before TCDD addition, indicting a complication in kava-TCDD interaction regarding AhR modulation. To the best of our knowledge, this is the first report demonstrating the dual effects of kava on TCDD-mediated CYP1A1 activity. Furthermore, we conducted homology modeling to construct the 3-D structure of the ligand binding domain of AhR and docked six main kavalactones from kava to the homology model. The docking results suggested that methysticin was the most potent CYP1A1 inducer followed by dihydromethysticin among the six kavalactones, an observation that was further experimentally confirmed at the level of mRNA expression, protein expression, and enzymatic activity.

**ABSTRACT FINAL ID:** 2873 Poster Board #114  
**TITLE:** VASCULAR CELL SPECIFIC FUNCTION OF CYTOCHROME P450 1B1

**AUTHORS (LAST NAME, FIRST NAME):** Elmergreen, Tammy L.; Scheef, Elizabeth A.; Sorenson, Christine M.; Sheibani, Nader


**KEYWORDS:** Cytochrome P450 1B1, Retina, Angiogenesis.

**Abstract Body:** Purpose: Cytochrome P450s are expressed in the vasculature and their metabolites from arachidonic acid play a crucial role in the modulation of vascular tone and blood flow. The objective of this study was to establish expression of Cytochrome P450 1B1 (Cyp1B1) in vascular cells, retinal endothelial cells (EC) and pericytes (PC) and to determine the impact of Cyp1B1-deficiency on vascular cell function. Methods: Primary cultures of retinal EC and PC were prepared from Cyp1b1+/+ and Cyp1b1−/− mice. The expression of Cyp1b1 was determined using retinal EC and PC by western blot. Proliferation was determined by counting cell numbers every other day for two weeks. The expression of various vascular cell markers and the production of extracellular matrix (ECM) proteins were determined by western blot. The ability of Cyp1b1+/+ and Cyp1b1−/− EC to undergo capillary morphogenesis in Matrigel was determined. Rates of migration were compared using a transwell migration assay, with or without 2,3',4,5'-Tetramethoxy-stilbene (TMS). The adhesion to various ECM proteins was also determined. Results: The Cyp1b1+/+ retinal EC and PC constitutively expressed Cyp1B1, and Cyp1b1−/− retinal EC and PC lacked expression, as expected. Cyp1b1−/− retinal EC and PC were more proliferative than Cyp1b1+/+ EC and PC. Cyp1b1+/− retinal EC were less migratory and less adherent. In contrast, Cyp1b1−/− retinal PC were more migratory and more adherent. In the absence of Cyp1B1, the production of ECM protein, thrombospondin-2, was increased in both EC and PC. Cyp1b1−/− retinal EC also failed to undergo capillary morphogenesis in Matrigel. Cyp1b1+/+ retinal PC incubated with TMS, a potent and selective inhibitor of Cyp1b1, exhibited increased migration. Conclusion: Cyp1B1 is constitutively expressed in retinal vascular EC and PC and is essential for maintaining their normal proliferative, migratory and adhesive properties. Thus, aberrant expression and/or activity of Cyp1B1 may have significant impact on vascular cell function affecting vascular development and angiogenesis.

**ABSTRACT FINAL ID:** 2874 Poster Board #115  
**TITLE:** ASPIRIN OR VITAMIN D DOES NOT PREVENT COLON TUMORS IN RODENT MODELS OF COLON CANCER

**AUTHORS (LAST NAME, FIRST NAME):** Irving, Amy1,2; Halberg, Richard; Albrecht, Dawn; Plum, Lori; Krentz, Kathleen; Clipson, Linda; Drinkwater, Norman; Amos-Landgraf, James; DeLuca, Hector; Dove, William1,2

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**KEYWORDS:** colon cancer, chemoprevention, animal models.

**Abstract Body:** Colon cancer risk varies greatly around the world, indicating an environmental component to its pathogenesis. Epidemiological studies suggest that vitamin D and aspirin are each associated with a reduced risk of colon cancer. Individuals with lower sunlight exposure, resulting in reduced 25(OH)D serum levels, have increased rates of colon cancer. Some studies have shown that individuals who
take aspirin regularly have a reduced risk of developing colon cancer. However, short-term studies in humans show conflicting efficacy of these compounds. We have used two genetic models of familial colon cancer, the Apc\textsubscript{Min}\textsuperscript{+/-} mouse and the Apc\textsuperscript{Pirc/+} rat, to investigate the effect on colonic tumors of aspirin and 25-hydroxyvitamin D\textsubscript{3} [25(OH)D\textsubscript{3}]. Rats and mice were randomized to one of three test diets: aspirin, 25(OH)D\textsubscript{3} or the combination. Some of the mice and rats were also treated with dextran sodium sulfate, a colon-specific inflammatory agent, to test these agents on tumors with two potentially different etiologies. Longitudinal endoscopic monitoring allowed us to test these compounds both in preventing newly arising colonic tumors and against established tumors. Terminal intestinal tumor counts, as well as serum salicylate, calcium and 25(OH)D\textsubscript{3} measurements, were obtained at study termination. Despite significant increases of the relevant serum levels, aspirin and 25(OH)D\textsubscript{3} singly or in combination, failed to reduce tumor numbers or the number of growing colonic tumors in the Min mouse or the Pirc rat. Notably, a high daily dose of 25(OH)D\textsubscript{3} resulted in a statistically significant increase in the multiplicity of colonic tumors in the Pirc rat. This is a timely contribution to a current public health issue, as reflected in a recent statement by the Institute of Medicine: “Serum [25(OH)D\textsubscript{3}] above 75 nmol/L are not consistently associated with increased benefit... [with] reason for concern at serum 25(OH)D\textsubscript{3} levels above 125 nmol/L.”

ABSTRACT FINAL ID: 2875 Poster Board #116

TITLE: QUANTIFICATION OF INTRA- AND INTEROBSERVER VARIABILITY WITHIN A POOL OF NONCLINICAL ECG ANALYSTS

AUTHORS (LAST NAME, FIRST NAME): Osinski, Mark¹; Schweitz, Karl¹; Pedersen, Jessica¹; Hanke, Nate¹; Kremer, John¹; Miller, Elaine¹; Foley, C. M.¹


KEYWORDS: Six Sigma, Cardiovascular safety, Safety pharmacology.

Abstract Body: The inclusion of cardiovascular (CV) safety end points (e.g., electrocardiograms, ECGs) within nonclinical toxicology studies is increasing. Although sophisticated software algorithms are available for ECG waveform identification/quantification, manual overreading of the data is still required. Drift in an ECG analyst (EA)’s marking of waveforms is inevitable due to the heterogeneity of ECG morphology collected from nonclinical species. Large intra- and interobserver variability (IAOV and IEOV) within and between EAs will compromise the ability to detect the small changes in QT interval duration that can signal potential CV liability. Gage R&R, a type of measurement system analysis (MSA), was used to evaluate the repeatability and reproducibility of ECG interval measurements made by a group of EAs. Ten segments of digital ECG waveforms (30-120 sec duration) were collected via surface electrodes from 8 cynomolgus monkeys. Five contiguous ECG waveforms were selected from each monkey and saved with a coded identifier. Each of 5 EAs adjusted the validation marks on each waveform of each animal’s file using Ponemah software. To estimate IAOV, each analyst adjusted the ECG validation marks on the same files, under blinded conditions, at least one week after the initial ECG evaluation. Gage R&R analysis was completed on PR, QRS, and QT data using Minitab software. The vast majority of measurement variability was due to differences in PR, QRS, and QT values between animals (89, 91, and 98% of total variation, respectively) and not to EA repeatability (4, 4, and 1%) or EA reproducibility (7, 5, and 1%). The standard deviation values for IAOV and IEOV were 2.7 and 3.4 msec (PR), 0.7 and 0.9 msec (QRS), and 2.0 and 1.4 msec (QT). Gage R&R analysis revealed acceptable levels of variation between 5 EAs as well as within a given analyst. This analysis can be used to monitor training effectiveness of new EAs and to evaluate EA drift over time. MSA methods can be important tools for maintaining process excellence in safety pharmacology.
ABSTRACT FINAL ID: 2876 Poster Board #117  
TITLE: TOXIDROME UPDATE  

AUTHORS (LAST NAME, FIRST NAME): Whitmire, Mark2,3; Salem, Harry 1,3  
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KEYWORDS: Toxidromes, CTRA, Medical Mitigation.  

Abstract Body: Presidential Directive -22 (HSPD-22) requires the Chemical Security Analysis Center (CSAC) of the Department of Homeland Security (DHS) to conduct Chemical Threat Risk Assessments (CTRA) on a select number of toxic industrial chemicals (TICs). This probabilistic risk assessment includes the threat, vulnerability, and consequence as well as medical mitigation. There are millions of chemicals that could be used by terrorists to cause mass casualties. Specific antidotes are not available for most of these chemicals. Therefore, Toxidromes were developed to categorize these chemicals according to their routes of exposure, and their clinical signs and symptoms. This is so that the medical community can manage the effects of these chemical exposures more effectively. Previously we had reported on toxidromes using only lethality as the endpoint. Currently we have selected medical endpoints of 3 levels of severity. These are, MILD, MODERATE, and LIFE-THREATENING. Certified toxicologists from CSAC, ECBC, and other certified toxicologists from the medical community have re-evaluated the previous toxidrome classification, and have expanded on the signs and symptoms for these 3 levels of severity, and the medical mitigation for these levels of chemical effects.

ABSTRACT FINAL ID: 2877 Poster Board #118  
TITLE: CASE ULTRA: AN EXPERT SYSTEM FOR IN SILICO PREDICTIVE TOXICOLOGY WITH A NOVEL FRAGMENT BASED ALGORITHM  

AUTHORS (LAST NAME, FIRST NAME): Chakravarti, Suman1; Saiakhov, Roustem D.; Fuller, Matthew1; Klopman, Gilles1  
KEYWORDS: QSAR modeling, expert systems, In Silico Models.  

Abstract Body: Computational techniques fill a large void in the safety assessment of drugs and chemicals by providing the capability of identifying adverse effects in the early stages of product development. They are particularly appealing because they are fast, can screen large number of compounds in a short time and in addition they don’t need the compounds to be physically available. In this respect we are presenting here about the development of a novel algorithm in the form of a computer program called CASE Ultra which falls under the broad category of data driven expert systems and belongs to the MCASE family of in silico toxicology/bioactivity software programs. CASE Ultra advances the state of the art of the MCASE fragment based approach which worked by breaking the molecules of the training set in fragments of predefined number of atoms and predefined branching pattern. Although highly effective and successful, this approach faces problems in identifying activity related substructures that do not fall under a classical fragment category and have more complex structural pattern, e.g. with complex branching or ring pattern. Additionally, as the size of the training set increases (more than 5000 entries) the identified fragments become more prone to statistical anomalies. CASE Ultra uses a proprietary algorithm that solves the problem and identifies substructures that are relevant to activity/toxicity without having any limitation on the size or branching pattern of the identified fragments with an end result of increased efficiency and effectiveness. It proceeds in a completely automated fashion which is a hallmark of all the MCASE expert system algorithms. A major advantage of this development is in dealing with modern drugs with increasing structural complexity. Also, the algorithm is well suited for building expert system models from very large data sets (~50000 chemicals) with significantly reduced statistical anomalies. In this report we will be presenting major features of this new program and some of the results.
Abstract Body: With a view to developing a Systems Biology-based product risk assessment approach, classical toxicology was combined with analysis of gene expression and protein abundance to investigate disease-relevant molecular perturbations at the sites of histopathological changes in an OECD 28-d rat inhalation study. Rats were exposed for 28 d to filtered air (sham), or to a low, medium, or high concentration of cigarette mainstream smoke (MS). Histopathology of respiratory tract sections revealed typical dose-dependent adaptive changes in the airway epithelia and inflammatory responses in the lung. For each rat, respiratory nasal epithelium (RNE) was dissected bilaterally and split for RNA and protein samples. Whole lung cryosections were used for protein samples; respiratory epithelium of the main bronchus and lung parenchyma were separated by Laser Capture Microscopy for RNA samples. Systems profiles of gene expression (Affymetrix microarrays) and principal component analysis (PCA) showed MS dose-dependent numbers of significantly up- or down-regulated genes in all 3 tissues. The differentially regulated genes were related mainly to the expected pathways, e.g., inflammation, oxidative and ER stress, etc. Correlating with histological changes, stress-related responses were more pronounced in RNE, while inflammatory responses were more pronounced in lung parenchyma. Reverse Protein Arrays (RPA) analysis generated systems response profiles for 46 (RNE) and 47 (lung) proteins / protein modifications. PCA showed clear separation of all MS groups from sham for RNE, and of the high MS group from sham for the lung. RNE from the medium and high MS groups differed significantly from sham in the abundance of proteins related to pathways, including the MAPK-stress signaling pathway. Responses were less pronounced in the lung. In conclusion, a correlative evaluation of classical histopathology with molecular patterns (gene expression and RPA) may facilitate a Systems Biology-based product risk assessment approach.

Abstract Body: The chemical and physical complexity of mainstream cigarette smoke (MSS) presents a major challenge in the understanding of smoking-related diseases worldwide. Using epidemiologic studies for the determination of health risks caused by smoking may require smokers to be exposed to the product for several decades. Thus, quantitative risk assessment is a useful tool to predict the chemical hazards due to smoking cigarettes commercially available at present. Toxicological risk assessment principles have recently been applied to rank or prioritize chemical constituents in MSS to inform tobacco product regulation and product standards. In the current study, yields of a selected group of chemical constituents in MSS were quantified in machine-generated smoke from 30 different brands of cigarettes sold in China. Using constituent yields and available dose-response data, a Monte Carlo method was applied to simulate the distributions of incremental lifetime cancer risk (ILCR), hazard quotient (HQ) and margin of exposure (MOE) values for each constituent as appropriate. Exposure parameters used in this analysis were specific to, and representative of, the Chinese population. The use of a Monte Carlo approach for risk assessment in this study may provide a more accurate estimation of the health risks associated with smoking.
Carlo method allows accounting for the variability and the uncertainty inherent to exposure estimation. Consequently, constituents were prioritized according to the three comparative ‘risk’ indices. The constituents that exceeded the values of 10E-4 for ILCR, 1 for HQ and 10,000 for MOE, included acetaldehyde, acrylonitrile, benzene, cadmium, formaldehyde and pyridine. While limitations exist to this methodology in estimating the absolute magnitude of health risk contributed by each MSS constituent, this approach provides a plausible and objective framework for the prioritization of toxicants in cigarette smoke and is valuable in guiding tobacco risk management efforts globally.

**ABSTRACT FINAL ID:** 2880 Poster Board #121

**TITLE:** ACUTE NEURONAL CELL DEATH, AND LONG-TERM COGNITIVE IMPAIRMENTS IN RATS EXPOSED TO THE ENVIRONMENTAL TOXIN BMAA (B-N-METHYLAMINO-L-ALANINE) DURING THE NEONATAL PERIOD

**AUTHORS (LAST NAME, FIRST NAME):** Karlsson, Oskar1; Roman, Erika1; Berg, Anna-Lena2; Brittebo, Eva1


**KEYWORDS:** Cyanobacteria, algal blooming, nonprotein amino acid.

**Abstract Body:** The cyanobacterial neurotoxin β-N-methylamino-L-alanine (BMAA) is considered to have a relatively low neurotoxic potency in adult rodents and the transfer of BMAA to the adult rodent brain appears to be restricted. However, our previous studies in neonatal rodents, demonstrated an efficient transport across the blood-brain barrier and a selective uptake of BMAA in the hippocampus and striatum. We have also reported that exposure to the mixed glutamate receptor agonist BMAA during the neonatal period causes cognitive impairments in adult rats. The aim of this study was to investigate the long-term effects of neonatal BMAA exposure on learning and memory mechanisms and to identify early morphological changes in the neonatal brain. BMAA was injected subcutaneously in rat pups on postnatal days 9–10. BMAA (50 and 200 mg/kg) caused distinct deficits in spatial learning and memory in adult animals but no morphological changes. No impairment of recognition memory was detected, suggesting that neonatal exposure to BMAA preferentially affects neuronal systems that are important for spatial tasks. Histopathological examination revealed early neuronal cell death as determined by TUNEL staining in the hippocampus 24 hours after a high dose (600 mg/kg) of BMAA whereas no changes were observed at lower doses (50 and 200 mg/kg). In addition, there was a low degree of neuronal cell death in the retrosplenial and cingulate cortices, areas that are also important for cognitive function. Taken together, these results indicate that BMAA is a developmental neurotoxin inducing long-term changes in cognitive function. No previous in vivo studies have shown any adverse effects after exposure to BMAA at a dose as low as 50 mg/kg. The risk posed by BMAA as a potential human neurotoxin merits further consideration, particularly if the proposed biomagnifications in the food chain are confirmed.

**ABSTRACT FINAL ID:** 2881 Poster Board #122;

**TITLE:** PHARMACOLOGIC DEVELOPMENTAL TOXICITY IN RATS INDUCED BY AGONISM AT THE FETAL MUSCLE NICOTINIC ACETYLCHELONE RECEPTOR BY X11422208

**AUTHORS (LAST NAME, FIRST NAME):** Rasoulpour, Reza J.1; Ellis-Hutchings, Robert1; Terry, Claire2; Millar, Neil3; Gibb, Alasdair3; Zablotny, Carol1; Marshall, Valerie1; Brooks, Keith1; Andrus, Amanda1; Carney, Edward1; Billington, Richard2

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**KEYWORDS:** Developmental & Reproductive Toxicity.
**Abstract Body:** A new agricultural molecule X11422208 induced fetal effects (primarily forelimb flexure and bent clavicle, plus hindlimb rotation, and convoluted/hydroureter) and neonatal death in rats at high doses; these effects did not occur in rabbits at comparable maternal and fetal blood exposure levels. A series of mode-of-action (MoA) studies were conducted based on the hypothesis that both of the effects in rats had a single MoA associated with X11422208 agonism to the fetal rat muscle nicotinic acetylcholine receptor (nAChR). The studies within this MoA program included a cross-fostering study in rats, a neonatal survival study in rabbits, fetal ((α1)2β1δγ) and adult ((α1)2β1δε) rat and human muscle nAChR in vitro agonism experiments, critical windows of exposure studies in rats, and ex vivo phrenic-nerve hemi-diaphragm contracture experiments in neonatal rats. In summary, these studies demonstrated that X11422208 is an agonist to the fetal, but not adult, rat muscle nAChR and that prolonged agonism at this receptor in fetal/neonatal rat causes sustained striated muscle contracture and concomitant reduced muscle responsiveness to normal nerve stimulation, which are responsible for the fetal effects and neonatal death in the rat. Fetal effects were inducible with as little as one day of exposure at the end of gestation and were rapidly reversible after birth, indicating a pharmacologic as opposed to a teratogenic MoA. In addition, X11422208 was shown to have no agonism at human fetal or adult muscle nAChRs. Taken together, the data demonstrate pharmacologic effects of X11422208 in rats mediated via agonism at the fetal muscle nAChR during late fetal in utero development; these effects did not occur in rabbits and are not relevant to humans.

**ABSTRACT FINAL ID:** 2882 Poster Board #123

**TITLE:** DRIED BLOODSPOT COMPARISON STUDY: COMPARISON OF TWO BLOOD COLLECTION SITES AND TWO BLOOD COLLECTION METHODS AFTER A SINGLE-DOSE OF ACETAMINOPHEN IN MALE RATS

**AUTHORS (LAST NAME, FIRST NAME):** Melich, David1; Stokes, Alan1; Moose, Tammy2; Liv, Chhou3; Parry, Simon4; Barfield, Matthew5; Lovatt, Cerys6; Dopson, Wesley7; Overvold, Carol8; Gade, Sonya9; Evans, Christopher10; Spooner, Neil6

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**KEYWORDS:** Dried Bloodspots, Tail Snip, Acetaminophen.

**Abstract Body:** Dried bloodspot technology has been available for many decades but only in the last 5 years has it been considered for routine bioanalysis of blood samples collected on non-clinical and clinical studies as part of a drug development program. Advantages to using dried bloodspots versus plasma samples include, but are not limited to, less blood volume required, less processing of the samples (e.g., no centrifugation), potentially using less animals, and no storing or shipping frozen samples. The current study compared blood concentrations (AUC0-t and Cmax) of acetaminophen after a single 600 mg/kg dose using two different sampling sites (tail vein versus tail snip) and two different collection methods (3 separate 15 μL end to end EDTA-coated capillary tubes versus an integrated capillary tube/micro container) in male Crl:CD(SD) rats. The objective was to evaluate the reproducibility and identify differences in serial-micro sampling of rats using these methods. After receiving a single oral gavage dose, each rat was sampled at multiple timepoints on the day of dosing and the resultant plasma analyzed for blood acetaminophen concentrations (AUC0-t and Cmax) using LC-MS/MS. The results showed that there were no meaningful differences (i.e., twofold or greater) in blood concentrations of acetaminophen using the different sites or methods. Furthermore, to determine spot to spot variability, comparisons of the acetaminophen blood concentrations obtained after analysis of a duplicate bloodspots from the same bloodspot card were within 12% of the original concentration.
2011 Society of Toxicology 50th Anniversary Meeting
Grace Period Abstracts

ABSTRACT FINAL ID: 2883 Poster Board #124
TITLE: ENDOCRINE DISRUPTOR TESTING IN AMPHIBIANS

AUTHORS (LAST NAME, FIRST NAME): Wood, Eric2; Memmert, Ulrich1; Peither, Armin1; Weber, Klaus1
INSTITUTIONS (ALL): 1. Harlan Laboratories, Itingen, Switzerland. 2. Harlan Laboratories, Shardlow, United Kingdom.
KEYWORDS: Endocrine Disruption, Xenopus laevis, amphibian metamorphosis assay.

Abstract Body: OECD and US-EPA published a new test guideline to endocrine disruptor testing in amphibians in 2009 (OECD Guideline 231 and OPPTS Guideline 890.1100). This amphibian metamorphosis assay (AMA) intends to screen chemicals which may interfere with the normal function of the thyroid system of vertebrates. The OECD test guideline was validated in international inter-laboratory ring tests. The assay starts with tadpoles of the frog species Xenopus laevis at the development stage 51 on the Nieuwkoop and Faber scale for a duration of 21 days. Three test concentrations and control(s) are generally tested, with four replicate test vessels per treatment. After 7 days of exposure, a sub-set of tadpoles from each treatment level is sampled for the measurement of the developmental stage, snout-to-vent length, hind limb length and body wet weight. At termination of the 21-day exposure period, these endpoints are measured again on all remaining tadpoles. A sub-set of tadpoles from each treatment level is fixed (whole-body or dissected) after 21 days for histopathology of the thyroid gland. The test guideline does not request for a positive control or a reference test. However, to ensure the correct performance of this new study type, an intra-laboratory validation study was conducted at Harlan Laboratories under GLP with two relevant chemicals (sodium perchlorate as antagonist and thyroxine T4 as agonist). These chemicals were already tested in the inter-laboratory ring test of the OECD. The results of the developmental stages and of the growth of the hind limbs show very similar effects compared to the mean values of the OECD ring test. The thyroid tissue demonstrates thyroid activity of the two reference items, sodium perchlorate and thyroxine. Treatment related findings in the thyroid glands of tadpoles consist of diffuse follicular hypertrophy induced by both test items without significant differences for the treatment with both test items.

ABSTRACT FINAL ID: 2884 Poster Board #127
TITLE: PRODUCT SUSTAINABILITY: THE ROLE OF CHEMICAL “WATCH” LISTS IN CHEMICAL DESELECTION FROM PRODUCTS MANUFACTURING PROCESSES.

AUTHORS (LAST NAME, FIRST NAME): Cowan, Dallas M.1; Anderle de Sylor, Marianna3; Laura, Lievense3; Michele, Fromowitz2; Slocombe, Andrew3; Perez, Angela3; Paustenbach, Dennis J.3
KEYWORDS: Product Sustainability, Risk Assessment, Restriction List.

Abstract Body: Environmentally sustainable products are designed to remain on the market yet exert minimal or no environmental or human health impact during manufacture, use, and end-of-life. Several drivers exist for deselection of chemicals used in manufacturing including, regulation or threat of regulation, public or media pressure, and findings of actual hazards through chemical product risk assessments. Although more than 50 chemical restriction or substance of concern lists exist, domestically and internationally, generated by various regulatory agencies, NGOs, media entities, and product trade associations, it is not well-known whether such lists are likely to impact product manufacturing such that they pose a lesser hazard to humans or the environment. The purpose of this evaluation was to describe the commercial and toxicological relevance of chemical restriction lists. We collected a representative sample of chemical restriction lists from regulatory, NGO, and trade association websites. We found that the nearly half of the chemicals on these lists were historical contaminants that are largely phased-out from commercial use; however, the remaining half of the chemicals are unregulated and sparse data exist regarding risk to human health. We compiled a list of unregulated chemicals of concern and recommend methods, including individual product risk assessments, for understanding the human health and environmental risks. Overall, the current relevance of some chemical lists as they are applied to product manufacturing, use, and end-of-life is unclear because principles such as dose, potency, and
Abstract Body: Perennial ryegrass is a perennial cool-season grass which has been deliberately infected with the endophytic fungus Neotyphodium lolii, as it confers benefits such as insect resistance, growth enhancement and drought tolerance to the plant, thereby decreasing the use of pesticides, fertilizers and irrigation. Unfortunately, the endophyte exerts some of these benefits through production of lolitrem alkaloids which also cause a tremorgenic neurotoxicity syndrome known as ‘ryegrass stagers’ in cattle and other herbivores when endophyte-infected grasses are grazed or fed as hay. Further, concerns have been raised recently regarding the public health safety of the compound lolitrem B, as it has been detected in the fat of cattle fed lolitrem B-containing perennial ryegrass. Currently, no data exists on the hepatic metabolism of lolitrem B in any animal species. Thus, the fate and metabolism of lolitrem B (m/z 686.4) was studied by LC-MS/MS in cattle liver microsomes from ten steers. An average kinetic rate of 67 nmol lolitrem B/min/mg protein was found. Metabolites produced include: addition of m.w. 2 (m/z 688.4), m.w. 1 (m/z 687.4), and loss of m.w. 83 (m/z 603.4), which increased over 4 hours then sharply declined by 7 hours. A low abundance metabolite of addition of m.w. 16 (m/z 702.4) was also seen. Results indicate oxidation is occurring, but this needs further investigation. To our knowledge, this data represents the first elucidation of lolitrem B metabolites formed in the liver. In establishing the specific compounds produced by bovine liver microsomes, the etiology of this toxin can be defined and appropriate therapies can be developed for treating ryegrass stagers in livestock. Further, lolitrem B metabolites likely to be present in animal by-products available for human consumption can be established, which will allow for further study on potential human toxicity caused by this tremorgenic mycotoxin.
the presence of TiO₂ the potency of DNCB was increased; EC₃ 0.03%. The sub-cutaneous administration of nano-TiO₂ modulates the dermal sensitization potency of DNCB.

ABSTRACT FINAL ID: 2887 Poster Board #130
TITLE: OPTIMIZATION OF THE LOCAL LYMPH NODE ASSAY: BROMODEOXYURIDINE DETECTED BY FLOW CYTOMETRY (LLNA:BrdU-FC) IN BALB/C MICE FOR HYPERSENSITIVITY EVALUATION

AUTHORS (LAST NAME, FIRST NAME): Guo, Tai L.; Germolec, D. R.; McLoughlin, C. E.; Smith, M. J.; White, Jr., K. L.; Auttachao, W.

INSTITUTIONS (ALL): 1. Virginia Commonwealth University, Richmond, VA, United States. 2. NTP, DIR, NIEHS, RTP, NC, United States.

KEYWORDS: Local Lymph Node Assay, Bromodeoxyuridine, Hypersensitivity.

Abstract Body: The objective of this study was to optimize the LLNA:BrdU-FC for hypersensitivity evaluation by comprehensively examining several essential parameters, including route of BrdU administration (i.p. vs. i.v.), BrdU dose-response (2.5, 5, 10 mg/mouse), and BrdU time-course response (administration 6, 24 or 48 hrs prior to study termination). The number and percentage of BrdU+B220+ and BrdU+B220- cells following treatment with the potent sensitizer 2,4-dinitrofluorobenzene (DNFB) were determined to further establish whether the assay primarily measured a T-cell response. In the time-course study, a moderate contact sensitizer, hexyl cinnamaldehyde (HCA), was also evaluated. Female BALB/c mice were treated with vehicle (4:1 acetone:olive oil [AOO]), DNFB, or HCA by application to the dorsa of both ears daily for 3 days. Animals were rested for 2 days before study termination on day 6, when both the right and left auricular lymph nodes from each mouse were removed for flow cytometric analysis. BrdU administration via i.p. injection produced a greater number and percentage of BrdU+ cells and a larger stimulation index (SI: 27 vs. 7 following DNFB treatment) than when administered i.v. Accordingly, the i.p. route was used in subsequent studies. In the dose-response study, results showed that mice treated with 10 mg BrdU produced the greatest SI (SI = 23) following DNFB treatment. In the time-course study, the data indicated that injection of BrdU 24 hours prior to study termination produced the highest SI for both HCA (SI = 3.7) and DNFB (SI = 16.5). In conclusion, we have optimized the LLNA:BrdU-FC to produce the greatest SI (compared to vehicle control) by administering 10 mg BrdU/mouse (i.p.) 24 hrs prior to study termination. Furthermore, our results have indicated that the BrdU is taken up predominately by B220- cells, suggesting that the LLNA:BrdU-FC measures primarily T-cell proliferation, a cell-mediated immune response (Supported by NO1-ES05454).

ABSTRACT FINAL ID: 2888 Poster Board #133
TITLE: TOXICITY AND TOXIKINETIC EVALUATION OF AVI-7100, A PHOSPHORODIAMIDATE MORPHOLINO OLIGOMER WITH SELECTIVELY INTRODUCED POSITIVE CHARGES (PMOplus™) TARGETED TO A HIGHLY CONSERVED REGION OF INFLUENZA A VIRUS.

AUTHORS (LAST NAME, FIRST NAME): Sazani, Peter; Heffernan, Jane; Iversen, Pat


KEYWORDS: Influenza, Antisense Oligonucleotides, Morpholino Oligomers.

Abstract Body: AVI-7100, a phosphorodiamidate morpholino oligomer with selectively introduced positive charges (PMOplus™), is currently in preclinical development for the treatment of seasonal and pandemic influenza infection. This new therapy is targeted to a highly conserved region of the influenza A virus. Seasonal influenza (H₃N₂), along with the recently emergent swine origin influenza virus (SOIV), H₁N₁, are caused by the influenza A virus. In a ferret model of H₁N₁ disease that we established, AVI-7100 was shown to reduce the combined average daily viral titer in nasal wash through peak viral load (days 1 - 3) versus saline control (p=0.0012) and oseltamivir control (p=0.0103) by up to 3.9 log. Microscopic and pathology scores also revealed a benefit only to animals receiving AVI-7100 versus controls. These data show that AVI-7100 is active against a fully virulent and non-adapted strain of pandemic H₁N₁ virus in the ferret model. In our current repeat dose toxicological studies, AVI-7100 was evaluated in rats and cynomolgus monkeys (non-human primate, NHP) following 14 once daily intravenous slow bolus injections, at doses up to 240 and 180
mg/kg/injection, respectively, at termination and following a 14 day recovery period. Clinical observations, mean body weight, organ weights, mean food consumption, microscopic and macroscopic observations, ophthalmoscopic observations, clinical chemistry, hematology and coagulation evaluations were performed for all animals. In addition, urinary cystatin C and quantitative total protein concentrations, investigational biomarkers for nephrotoxicity were measured through the dosing and recovery phases. At all doses tested, including those that are equivalent or greater than the expected clinical dose, AVI-7100 was extremely well tolerated in both species. Histopathological findings related to AVI-7100 were limited to the kidney, and the incidence and intensity of the findings were dose dependent and were reflected in the renal urinary biomarkers tested.

ABSTRACT FINAL ID: 2889 Poster Board #134
TITLE: INCREASED MOBILE ELEMENT-MEDIATED DAMAGE AS A MECHANISM OF HEAVY METAL CARCINOGENESIS

AUTHORS (LAST NAME, FIRST NAME): Engel, Astrid M.; Wagstaff, Bradley J.; Servant, Geraldine; Derbes, Rebecca S.; Deiningher, Prescott L.
INSTITUTIONS (ALL): 1. Epidemiology, Tulane University, New Orleans, LA, United States.
KEYWORDS: heavy metals, LINE-1, Nucleotide excision repair.

Abstract Body: INTRODUCTION: The mobile elements known as Long Interspersed Elements (LINEs) are major sources of insertional mutagenesis in the human genome. LINE-1 (L1) is the only active human non-LTR retrotransposon. Because L1 retrotransposition contributes to human genetic instability, cells have developed mechanisms to control the deleterious insertion of these elements. We have previously shown that several heavy metals are able to stimulate the insertion of L1 elements. We have also shown that ERCC1 inhibits L1 activity and we hypothesize that the nucleotide excision repair (NER) pathway blocks the insertion process. RATIONALE: Because heavy metal exposure is known to inhibit nucleotide excision repair (NER) proteins, we propose that the mechanism of heavy metal stimulation is by inhibiting NER, thus minimizing the inhibitory influence of NER on L1 insertion. METHODS: We evaluated the effects of heavy metal exposure on L1 activity in NER competent and deficient cell lines or cells with targeted inhibition of NER proteins. RESULTS: Cells exposed to heavy metals such as Cd, Hg and Ni stimulate L1 retrotransposition in a dose dependent manner. The resulting increase in L1 activity by Cd and Ni can be competed with Mg(II) and/or Zn(II). Additionally, L1 retrotransposition increases in cells lacking ERCC1, XPF, XPA or XPC activity. In contrast to NER competent cells, treatment with CdCl2 did not increase L1 retrotransposition in the ERCC1 or XPA deficient cell lines. This suggests that one mechanism underlying heavy metal stimulation of L1 is due to the ability to displace essential metal ions required for function, thus reducing the L1 surveillance capability of NER proteins. CONCLUSION: Our data suggest that heavy metals increase L1 activity through the inhibition of the NER pathway. We propose that increase in mobile element-mediated genetic damage may play an important role as an additional mechanism of heavy metal carcinogenesis.

ABSTRACT FINAL ID: 2890 Poster Board #135
TITLE: THE METHYLATED TRIVALENT METABOLITES OF ARSENIC ALTER THE FUNCTION OF PANCREATIC ISLETS

AUTHORS (LAST NAME, FIRST NAME): Douillet, Christelle; Styblo, Miroslav
KEYWORDS: arsenic, diabetes, pancreatic islet.

Abstract Body: Associations between chronic exposure to inorganic arsenic (iAs) and risk of diabetes have been reported in areas with high levels of iAs in drinking water. Consistent with these reports, animals exposed to iAs demonstrated impaired glucose tolerance and altered plasma insulin levels. iAs and its methylated metabolites were detected in the pancreas of As-exposed mice. It has been well established that the methylated trivalent metabolites, methylnarsonite (MAs\textsuperscript{III}) and dimethylnarsonate (DMAs\textsuperscript{III}), are more toxic than iAs. However, no data is available on the effects of these metabolites on β-cell function. Here, we hypothesized that exposure to MAs\textsuperscript{III} or DMAs\textsuperscript{III} inhibits
Abstract Body: Humans are always exposed to low levels of complex mixtures containing metals. Epidemiological studies report that populations exposed to such mixtures show increased incidence rates of cancer. Therefore, chemical interaction is of major concern in the assessment of risk by regulatory agencies. In this study, treatment of Chinese Hamster Ovary (CHO) cells with nickel chloride (1.0 and 5.0 μM), or sodium chromate (0.5, 1.0, and 2.5 μM) induced sister chromatid exchanges (SCE) in a dose dependent fashion. Statistical analysis of the interaction factor, show that the combined treatments of nickel (1.0 and 0.5 μM) with chromium (0.5, 1.0 and 2.5 μM) interacted antagonistically for the induction of SCE. Previously, we reported that nickel with chromium, or with UV light or with X-rays interacted antagonistically for the induction of SCEs and MN (micronuclei) in human peripheral lymphocytes. These observations indicate that heavy metals, such as nickel and chromium, present in complex mixtures, may reduce the response, even in the presence of strong SCE or MN inducers, and may lead, therefore, to an underestimate of chemical exposure as assessed by these assays. Therefore, further studies are a necessity.
myoblasts and myotubes exposed to 0.5 μM iAsIII or MAsIII revealed that the myogenic differentiation increases the cell capacity to methylate these arsenicals. The methylation yields from either iAsIII or MAsIII were ~7-fold greater in the myotube cultures as compared to the myoblast cultures. The greater methylation capacity of myotubes was associated with an increased intracellular retention of the arsenicals. Taken together, these data suggest that the myogenic differentiation stimulates the expression of arsenic (+3 oxidation state) methyltransferase and/or the expression of membrane transporters that mediate the uptake, and thus facilitate the methylation of trivalent arsenicals. In summary, we have found that one of the toxic metabolites of As, DMAAsIII, can interfere with glucose uptake by differentiated skeletal muscle cells in a manner that is consistent with the diabetogenic effects of As exposure.

**ABSTRACT FINAL ID:** 2893 Poster Board -138

**TITLE:** MULTIPLE MOLECULAR TARGET SITES FOR THE ORGANO TinS TRIBUTYL Tin AND TRIPHENYL Tin

**AUTHORS (LAST NAME, FIRST NAME):** Irwin, William A.¹; Doherty, John D.¹

**INSTITUTIONS (ALL):** 1. USEPA, Washington DC, DC, United States.

**KEYWORDS:** tributyltin, triphenyltin, mitochondria.

**ABSTRACT BODY: Abstract Body (Abstract Submission):** Organotins (OT) can have multiple effects on organisms. Some OTs are known to cause imposex or the formation of male sex organs in female gastropods and fish. In mammals, OTs prevent implantation and cause degeneration of the testis, other male structures and decreased spermatogenesis in laboratory animals. The OT pesticides tributyltin (TBT) and triphenyltin (TPhT) are also well known to be immunotoxic. Development of imposex and effects on mammalian reproductive organs implies that TBT and TPhT might have endocrine disruptive properties. Initial studies implicated inhibition of aromatase as the causative effect for imposex, but this theory is challenged for imposex and would not explain all effects in mammals. Chemicals can be endocrine disruptors through several different mechanisms. This presentation will summarize an overview of the toxicity of TBT and TPhT in mammals. The doses in vivo and/or concentrations in vitro will be compared with their reported effects on nuclear receptors, enzymes involved in steroid metabolism, alterations in mRNA and OT interaction with mitochondrial energy production. All these effects are factors that can affect the endocrine function, but some may also affect other physiological functions. Thus, relating the effects on the reproductive systems with the immunotoxicity of TBT and TPhT to a common mechanism is more challenging. Chemically, TBT and TPhT are organic cations existing as weakly ionic-covalently bonded to anions such as chloride or hydroxide. The OTs chemical nature as cations and OT potential to accumulate within cells and within the mitochondria by Nernst kinetics will be described. Data from studies to define mechanisms for immunotoxicity or on the male and/or female reproductive tract will be integrated to identify the targets contributing to the expression of the toxicity of these chemicals. Comments on the need for future research will be presented to establish the path between in vitro and in vivo observations related to the modes of molecular action of TBT and/or TPhT.
ABSTRACT FINAL ID: 2894 Poster Board #139
TITLE: EFFECT OF GOLD NANOROD SURFACE CHEMISTRY ON CELLULAR RESPONSE

AUTHORS (LAST NAME, FIRST NAME): Grabinski, Christin1; Schaeublin, Nicole1; Wijaya, Andy1; D'Couto, Helen1; Baxamusa, Salmaan2; Hamad-Schifferli, Kimberly2; Hussain, Saber M.1
KEYWORDS: Gold nanorods, Surface chemistry, Cytotoxicity.

Abstract Body: Gold Nanorods (GNR) stabilized with cetyltrimethylammonium bromide (CTAB) and GNR functionalized via a ligand exchange method with either thiolated polyethylene glycol (PEG) 5000 or mercaptohexadecanoic acid (MHDA) were investigated for their stability in biological media and subsequent toxicological effects to HaCat cells. The MTS assay demonstrated that GNR-PEG and GNR-MHDA exhibited minimal effects on cell proliferation, whereas GNR-CTAB reduced cell proliferation significantly due to the inherent toxicity of the cationic surfactant to cells. Cell uptake studies performed using transmission electron microscopy (TEM) indicated relatively low uptake for GNR-PEG and high uptake for GNR-MHDA. Reverse transcriptase polymerase chain reaction (RT-PCR) revealed that GNR-PEG did not induce any significant changes in the transcription levels of 84 genes related to stress and toxicity, while GNR-MHDA influenced the transcription levels of several of the genes investigated. The results demonstrate that although cell proliferation was not affected by both particles, there is a significant difference in gene expression in GNR-MHDA exposed cells, suggesting long-term implications for chronic exposure.

ABSTRACT FINAL ID: 2895 Poster Board #140
TITLE: CELLULAR EFFECTS OF RELEASED HMGB1 UPON CARBON NANOTUBE TOXICITY

AUTHORS (LAST NAME, FIRST NAME): Nalvarte, Elisabet L.1; Das, Monisha1; Herndon, Betty1
INSTITUTIONS (ALL): 1. School of Medicine, University of Missouri-Kansas City, Kansas City, MO, United States.
KEYWORDS: single walled carbon nanotube, HMGB1, lung cells.

Abstract Body: An increased potential for human lung exposure to single wall carbon nanotubes (SWCNT) exists, particularly in occupational settings. SWCNTs are known to produce cellular cytotoxicity and lung inflammation in animals, but the mechanisms of these toxicities are incompletely understood. We hypothesize that, on pulmonary cell exposure, the initial cytotoxic effect of SWCNT is the cellular release of damage associated molecular pattern recognition molecules (DAMPs), specifically the high mobility group box 1 (HMGB1) protein. HMGB1 in the extracellular milieu can exert its actions as a ligand for pattern recognition receptor (PRR) activation in pulmonary cells. Single doses of 0.025 mg SWCNT (endotoxin free) dispersed in 0.050 mL pulmonary surfactant were intratracheally instilled into rat airways. Rats were sacrificed 0.5h, 24h, 1 week or 2 weeks later and broncho-alveolar lavage fluid (BALF) collected for measurement of HMGB1 in BALF by ELISA. A positive linear correlation was seen in HMGB1 released vs. cell injury (total protein) in rats exposed to SWCNT for the first week. This response was accompanied by the appearance of inflammatory cells in lung tissue and BALF. HMGB1-containing BALF obtained from rats treated with SWCNT were used to activate PRRs in RAW Blue cells, stably transfected lung macrophage cells expressing the secreted embryonic alkaline phosphatase (SEAP) gene inducible by NF-kB and AP-1 activation. The HMGB1-containing BALF induced significant expression of SEAP in the macrophage cell supernatants, highest with 24h exposure. Control wells with pig HMGB1 protein activated RAW Blue cells in a dose-dependent manner. These results suggest a role for HMGB1 in the cytotoxicity and inflammatory effects of SWCNT through potential activation of cellular PRRs. Research supported in part by Saint Luke’s Hospital Foundation (ID-00022468).
ABSTRACT FINAL ID: 2896 Poster Board #141
TITLE: EFFECT OF NANO SILVER SIZE AND COATINGS ON AGGREGATION AND BIOAVAILABILITY TO LUMBRICULUS VARIEGATUS

AUTHORS (LAST NAME, FIRST NAME): Coleman, Jessica 1; Kennedy, Alan J. 1; Steevens, Jeffery A. 1; Bednar, Anthony J. 1; Williams, Jamma F. 1
INSTITUTIONS (ALL): 1. U.S. Army Engineer Research and Development Center, Vicksburg, MS, United States.
KEYWORDS: nanotoxicology, ecotoxicology, silver.

Abstract Body: As the production and applications of nano silver increase, it is essential to characterize fate, transport, and effects in environmental systems and how factors such as particle size, surface area, and coatings may alter risk. Aquatic ecosystems are the ultimate repository for many contaminants; thus are relevant for study before environmental concentrations of nano silver increase. To assess the fate of nano silver in aquatic environments, we performed a series of dynamic light scattering (DLS) measurements on various nano particle suspensions. Suspensions in serial dilutions of synthetic freshwater media in a range of electrical conductivities (0-280 µS/cm) were created to determine risk on aggregation. Suspensions included a broad range of nano particles to assess implications of size and coatings; tested particles included 30, 1500 nm particles and a 20-30 nm polyvinylpyrrolidone (PVP) coated silver. Particle aggregation in 30 and 1500 nm silver increased with conductivity, and therefore fell out of suspension. Aggregation was minimal in PVP coated silver. Based on freshwater media fate results, we investigated uptake kinetics in the freshwater oligochaete, Lumbriculus variegatus. Lumbriculus were exposed to either nano-silver or AgNO3 spiked sediment (nominally 100 mg/L, n=3) following a two week aging period; time points were assessed at 2, 4, 7, 14, and 28 days. Data obtained through field flow fractionation and ICP-MS indicated an increasing trend in silver uptake over time. Total body burdens were higher in 1500 nm treatments relative to 30 nm treatments early in the course of the exposure; although, 30 nm-28 day time point replicates contained higher body burdens relative to 1500 nm-28 day replicates (4.57 and 3.35 mg/kg, respectively). The present study provides information on the potential for nano particle size and coatings to affect distribution within environmentally relevant waters and impact bioavailability to benthic invertebrates.

ABSTRACT FINAL ID: 2897 Poster Board #142
TITLE: POTASSIUM-REGULATED APOPTOSIS INDUCED BY THE MITOCHONDRIAL PATHWAY IN CULTURED TRIGEMINAL SATELLITE GLIAL CELLS: IMPLICATIONS FOR CHEMOTHERAPY-INDUCED NEUROPATHIES

AUTHORS (LAST NAME, FIRST NAME): Bustamante, Hedie 1; Rogers-Cotrone, Thomas 1; Jortner, Bernard S. 1; Rossmieisl, John 1; Ehrich, Marion F. 1; Klein, Brad 1
KEYWORDS: neuropathic pain, apoptosis, satellite glial cells.

Abstract Body: Toxic neuropathies, including those induced by cancer chemotherapy (CIN), may cause axonal degeneration, segmental demyelination or directly affect cell bodies in the sensory ganglia. Most CIN cases develop intense neuropathic pain (NP). Satellite glial cells (SGC) surrounding neurons in sensory and autonomic ganglia buffer extracellular K+, regulating the excitability of injured neurons. Therefore, they may play a role in the injury-induced transition from acute to chronic NP. Changes in [K]c could lead to apoptosis of SGC, which may be a key component in this transition to chronic NP. This work evaluated changes in [K]c and apoptosis in cultured trigeminal SGC. SGC cultured from trigeminal ganglia of healthy rats were depleted of K+ by hypoosmotic shock (136 mOsm), which was terminated by incubation in isoosmotic PBS with 4 mM bumetadine and 10 mM ouabain. [K]c was measured in 10,000 cells after loading with the K+ fluorescent indicator PBFI-AM. Values were corrected for autofluorescence and the 340/380 ratio was used to indicate [K]c. For apoptosis, cells were incubated for 5 h with either 2 µM staurosporine (mitochondrial pathway) or 20 ng/mL TNF-a (death receptor pathway) and evaluated by flow cytometry. At 2 min, hypoosmotic shock induced a 100% decrease in [K]c relative to the control, without inducing apoptosis. In contrast,
after incubation with 2 uM staurosporine apoptosis increased from 7.02% to 20.35% in K+ depleted cells. In disparity, 20 ng/ml of TNF-a did not increase apoptosis in normal or K+ depleted SGC. Hypoosmotic shock induced [K]c depletion in cultured trigeminal SGC. Moreover, Kc depletion increased staurosporine-induced (mitochondrial pathway) apoptosis. These results suggest that K+ dysregulation in trigeminal SGC may underlie the apoptosis-induced development of NP in CIN.

**ABSTRACT FINAL ID:** 2898 Poster Board #143

**TITLE:** STRUCTURE-SPECIFIC EFFECTS OF THE ORGANOCHLORINE INSECTICIDE DIELDRIN IN A PARKINSON’S DISEASE MODEL

**AUTHORS (LAST NAME, FIRST NAME):** Allen, Erin M.; Florang, Virginia R.; Doorn, Jonathan A.

**INSTITUTIONS (ALL):** 1. Medicinal and Natural Products Chemistry, University of Iowa, Iowa City, IA, United States. **KEYWORDS:** Dieldrin, Parkinson’s Disease.

**Abstract Body:** Parkinson’s disease (PD) is a progressive disorder characterized by the degeneration of dopaminergic neurons in the substantia nigra. This neurodegeneration has been shown to significantly correlate with a number of environmental factors, including pesticide exposure, such as the organochlorine insecticide, dieldrin. This pesticide is ranked one of the twelve most persistent, bioaccumulative and toxic chemicals by the US EPA. Previous studies found an increased concentration of dieldrin in the striatal region of brains of PD patients, and that dieldrin adversely affects a number of cellular processes associated with PD. However, the mechanism responsible for dieldrin-mediated cellular dysfunction and the structural components contributing to its toxicity have not been defined. In order to identify the toxicophore of dieldrin, a structure-activity approach was used, with the toxicity profiles of numerous analogs of dieldrin (including aldrin, endrin, and cis aldrin diol) assessed in differentiated, dopaminergic PC6-3 cells. Cellular assays monitoring mitochondrial activity, cytotoxicity, reactive oxygen species production, and extracellular dopamine metabolites were used. It was determined that aldrin, dieldrin, and cis aldrin diol substantially inhibited mitochondrial activity, as assessed with an MTT assay. An LDH assay was conducted to evaluate compound cytotoxicity, and aldrin and cis aldrin diol were found to be significantly cytotoxic. In addition, all of the compounds tested were found to disrupt dopamine metabolism, indicated by significant changes in the production of downstream metabolites of dopamine. Comparisons of the toxicity profiles for each dieldrin analog indicate a structure-specific effect that will be important for elucidating the mechanisms of dieldrin toxicity as they relate to PD.

**ABSTRACT FINAL ID:** 2899 Poster Board #144

**TITLE:** A MODEL TO EXPLORE THE SPECTRUM OF DIVERSE MICROGLIAL FUNCTIONS WITH INJURY

**AUTHORS (LAST NAME, FIRST NAME):** Harry, G. J.; Kraft, Andrew D.; McPherson, Christopher A.

**INSTITUTIONS (ALL):** 1. Laboratory of Toxicology and Pharmacology, NIEHS, RTP, NC, United States. 2. Curriculum in Toxicology, UNC, Chapel Hill, NC, United States. **KEYWORDS:** Microglia, Heterogeneity, Neuroinflammation.

**Abstract Body:** Microglia are a resident cell of the brain involved in a number of regulatory processes. They respond to multiple types of injury displaying heterogeneity of functional phenotypes, including immune-like responses and phagocytosis. Characterizing how these cells respond to changes in their environment can identify mechanisms associated with an outcome of neuronal support versus neurodegeneration. Using the prototypical hippocampal toxicant, trimethyltin (TMT), we present data with regards to the morphological characteristics and possible functional phenotypes of resident microglia. Macrophage-like characteristics, including process retraction, proliferation, and respiratory burst were demonstrated across hippocampal regions. Process retraction and phagocytosis was observed in areas of neuronal death in the absence of proliferation. With the loss synapses, proliferation of process-bearing microglia was observed in the CA3 pyramidal cell layer possibly contributing to synapse stripping and remodeling. A morphologically different process-bearing phenotype was observed in the spared CA1 region. Ameoboid microglia coincided with neuronal death and uptake of hydroethidine occurred later with the phagocytic respiratory burst. The resident MG response was transient and cells downregulated to a normal phenotype At 72 hrs post-TMT, neuronal
death, MG activation and mRNA levels TNFα and TNF receptors peaked followed by a rapid downregulation to a normal phenotype. Region specific protein expression profiles were examined by mass spectrometry to further characterize the different MG phenotypes. By pinpointing correlations between neuronal survival, microglia phenotype, and specific signaling pathways, the disparate roles of this complex regulatory cell may be better understood. Support: NIEHS Div of Intramural Research Z01 ES101623, ES021164.

ABSTRACT FINAL ID: 2900 Poster Board #145
TITLE: MATERNAL AND EARLY LIFE TRICHLOROETHYLENE EXPOSURE MODULATES GENE EXPRESSION OF CHEMOKINES AND NEUROTROPHINS IN THE BRAIN

AUTHORS (LAST NAME, FIRST NAME): Blossom, Sarah1; Melnyk, Stepan1; James, Jill1
INSTITUTIONS (ALL): 1. Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR, United States.
KEYWORDS: trichloroethylene, neuroimmune, developmental.

Abstract Body: Trichloroethylene (TCE) is an organic solvent and major water pollutant that pollutes the air, water supplies and soil. Low-level TCE exposure is common in non-occupationally exposed individuals and children may be an especially vulnerable population. Notably, it has been reported that ~7% of school-age children (6 to 10y) have detectable blood levels of TCE. Maternal TCE exposure has also become an important public health issue since proinflammatory T cell cytokines were detected in neonates of TCE-exposed mothers. Thus, in view of the ubiquitous TCE exposure, there is an urgent need to understand the health effects associated with low level developmental exposure. This is particularly true since the brain and immune system are immature at birth and thereby highly vulnerable to environmental stress. Continuous maternal and early life exposure to TCE in the drinking water of MRL+/+ mice promoted the production of CD4+ T cell proinflammatory cytokines in postnatal day (PND) 42 juvenile male mice which was associated with adverse neurologic and behavioral effects. Specifically, TCE altered cerebellar redox status and promoted abnormal behavioral effects. New evidence of altered expression of genes important in neuroinflammation and brain growth and neural plasticity was also discovered in these mice. Future studies will further define the specific susceptibility windows required for immunologic, brain-region specific neurologic, and associated behavioral alterations linked with developmental TCE exposure.

ABSTRACT FINAL ID: 2901 Poster Board #146
TITLE: DELETION OR ACTIVATION OF THE AHR BY DIOXIN IMPAIRS HIPPOCAMPAL NEUROGENESIS AND CONTEXTUAL MEMORY IN ADULT MICE

AUTHORS (LAST NAME, FIRST NAME): Latchney, Sarah E.1; Opanashuk, Lisa1
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KEYWORDS: Adult neurogenesis, Hippocampus, Proliferation.

Abstract Body: The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that mediates the toxic effects of dioxin and is believed to have physiological roles in the developing brain. In the adult brain, the AhR is expressed in newborn neural progenitor cells of the hippocampus but its function is unknown. This study tested the hypothesis that the AhR has a physiological role in mediating hippocampal neurogenesis and function, which is disrupted following AhR deletion or activation by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a potent neurotoxicant and high affinity ligand. Studies in unexposed adult AhR+/ mice revealed significant impairments in hippocampal-dependent contextual fear memory, while hippocampal-independent memory remained intact. Using BrdU incorporation to measure cell proliferation, immunohistochemistry (IHC) analysis in young adult AhR+/ mice revealed a 27% reduction in cell birth compared to age-matched wild-types (WT). In older mice (10-months), the reduction in cell birth was more pronounced (52%) in AhR+/ mice compared to 10-month old WTs. AhR+/ mice also exhibited abnormal neuronal differentiation, as assessed by doublecortin (DCX), which was also more pronounced in older animals. Similarly, AhR activation in adult WT mice exposed to 0.5μg/kg TCDD impaired hippocampal-dependent contextual memory, while hippocampal-independent memory remained intact. IHC analysis of TCDD exposed mice revealed a 30%
2011 Society of Toxicology 50th Anniversary Meeting Grace Period Abstracts

reduction in cell proliferation (BrdU+ cells) compared to vehicle controls. Neuronal differentiation, as assessed by DCX and NeuN expression, was also delayed in TCDD exposed mice, while glial differentiation was unaffected. Lastly, examination of the apoptotic marker, cleaved caspase-3, in both TCDD-exposed and AhR−/− animals did not reveal significant cell death compared to their respective controls. Our findings suggest that the AhR has a functional role during hippocampal neurogenesis, which is disrupted following deletion or activation by TCDD, ultimately leading to impaired hippocampal function.

ABSTRACT FINAL ID: 2902 Poster Board #147
TITLE: ABNORMAL EMOTIONAL BEHAVIOR AFTER MATERNAL EXPOSURE TO BISPHENOL A IN MICE DETECTED BY INTELLICAGE

AUTHORS (LAST NAME, FIRST NAME): Miyazaki, Wataru; Kimura, Eiki; Hosaka, Ryota; Sun, Xiance; Endo, Toshihiro; Hajjima, Asahi; Tohyama, Chiharu; Kakeyama, Masaki

INSTITUTIONS (ALL): 1. Laboratory of Environmental Health Sciences, Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.

KEYWORDS: Bisphenol-A, emorional behavior, maternal exposure.

Abstract Body: Many studies have suggested that exposure to low-dose bisphenol A (BPA) during gestation may induce adverse health effects including neurodevelopmental alterations in progeny later in adulthood. However, it is largely unknown whether the low-dose BPA exposure induce abnormal brain functions. Previously, we have developed our own test protocol using IntelliCage (IC), a fully-automated behavioral analysis system which allows us to test up to 16 mice simultaneously in a home-cage environment, to detect not only learning deficits but also emotional abnormality in mouse progeny born to dams exposed to dioxin (Toxicologist, 2010). Here we studied effects of maternal low-dose BPA exposure using IC on the progeny. Pregnant C57BL/6J mice were given orally BPA at a dose of 0, 40, 400 μg/kg b.w. from gestational days 8 through 18, and their male progeny were used for IC test (n=8 for each dose group). A mouse cohort (n=12) was made by selecting mice from each dose group in a counter-balanced manner and accommodated in each IC. The BPA exposure was not found to produce any effects on behavioral inflexibility, perseverative behavior, and social anxiety-like behavior, all of which were found in dioxin exposure. We thus developed a new task protocol in IC addressing impulsivity in mice. In this protocol, after shaping nose-poke responses with a high frequency for a water reward, mice had to wait for appropriate time from the last nose poke to get reward in the task. BPA-exposed progeny tended to have shorter nose poke intervals and smaller number of rewards, suggesting that they developed highly impulsive behavior. Moreover, myelin associated protein 2 in the developing brain of BPA-exposed offspring was significantly higher than that in those of control. In conclusion, the present results indicate that maternal exposure to BPA affects brain development and tend to exhibit impulsive behavior in adulthood.

ABSTRACT FINAL ID: 2903 Poster Board #149

TITLE: INVESTIGATING THE MECHANISMS OF AROMATIC AMINE-INDUCED PROTEIN FREE RADICAL FORMATION BY QSARs: IMPLICATIONS FOR DRUG-INDUCED AGRAINOCYTOSIS

AUTHORS (LAST NAME, FIRST NAME): Siraki, Arno G.; Jiang, Jinjie; Mason, Ronald P.

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KEYWORDS: free radical, QSAR, myeloperoxidase.

Abstract Body: Aromatic amine drugs have been associated with agranulocytosis (neutrophil depletion) for which the mechanism is unknown. We have previously shown that the metabolism of two aromatic amine drugs by human myeloperoxidase (MPO) results in phenyl radical metabolite formation and also in protein free radical formation on MPO. Since the concentration of drug required to produce a maximum signal for MPO protein free radical (MPO•) detection was different for each drug, this prompted us to consider that other aromatic amines may also show varying
Abstract Body: Human studies indicate that exposure to dioxin-like compounds is associated with arthritis. Arthritis, in particular involves the progressive destruction of cartilage matrix by the impaired function of chondrocyte. Since chondrocytes play an essential role in keeping cartilage integrity, apoptosis of chondrocyte is considered a critical event in the progression of arthritis. Recently, it is reported that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induces apoptosis of articular chondrocytes. In this study, protein kinase C (PKC) signaling pathways were investigated to understand the mechanism of TCDD-induced apoptosis. Rabbit articular chondrocytes in culture were exposed to TCDD. Immunoblot analysis revealed that TCDD induced the most significant translocation of PKC-delta among the PKC isoforms tested. The translocation was then blocked by ROS inhibitors (trolox or N-acetyl cysteine), a PKC-δ inhibitor (rottlerin), a caspase-3 inhibitor (z-DEVD-fmk) or an AhR blocker (α-naphthoflavone). TCDD increased caspase-3 activity, the activating enzyme for PKC-δ, and prior treatment with trolox blocked such an increase. These results suggest that the translocation of PKC-δ was mediated by ROS-dependent caspase-3 activity. Pretreatment with rottlerin or trolox dampened TCDD-induced apoptosis of chondrocyte, as determined by TUNEL staining and ELISA. Taken together, this study suggests that ROS generation is an upstream event for TCDD-induced chondrocyte apoptosis and PKC-δ mediates the apoptotic processes through ROS-dependent caspase-3 activation. TCDD also increased levels of matrix metalloproteinase (MMP)-13, a cartilage degrading enzyme, which is controlled by PKC-delta. The result further suggests that PKC-δ may play a key role in the dioxin-mediated apoptosis and subsequent cartilage damage. This study may contribute to understanding the mechanism of joint disease associated with the exposure of dioxin-like compounds and identifying a target for the therapeutic interventions.
2011 Society of Toxicology 50th Anniversary Meeting
Grace Period Abstracts

hydrolysing OPs compounds. The enzymatic activity of PON1 depends on genetic polymorphism present at position 192 (glutamine or arginine). The enzymatic activity of PON1 is an important factor that determines the toxic susceptibility to OPs. In the present work, we characterized the stereospecific hydrolysis of HDCP by human serum samples using chiral chromatography. The 47 human samples were diagnosed to PON1 192 polymorphism. The results demonstrate that all human serum samples studied showed a significant stereospecific Ca2+-dependent hydrolysis of S(-)-HDCP independently of the human serum PON1 isozymes. This study reinforces that the R(+)-HDCP (isomer that inhibited and aging NTE) could be the stereoisomer that induced the neurotoxic effects in vivo due to low level of hydrolysis observed in this study. This work was supported by CONACYT project 106436.

ABSTRACT FINAL ID: 2906 Poster Board #152
TITLE: SUPPORTING BEST PRACTICES IN CROP PROTECTION USING INTEGRATED MODELING APPROACHES

AUTHORS (LAST NAME, FIRST NAME): Singh, Pramila1; Lichtveld, Kim2; Ebersviller, Seth3; Everich, Rob1; Sexton, Ken1
KEYWORDS: drift modeling, estimating exposure, crop protection.

Abstract Body: Models capable of accurately simulating off target movement of applied crop protection products are needed to correctly estimate potential human exposures and the effectiveness of mitigation strategies. This project was designed to help estimate realistic drift parameters and atmospheric transformation rates of the fungicide Folpan using relevant application methods and regional conditions to construct a simulation model. The experimental test system consisted of an environmental chamber and a sprayer unit. Characterizing the sprayer unit and the sprayer output provided further utility of the model. The sprayer was used with different scenarios of mixing concentration, field application rates, and relevant environmental/ atmospheric conditions. Studies were conducted near or after sundown to investigate sprayer droplet behavior in the absence of sunlight with relatively constant temperature and controlled humidity. Sprayer output rate and droplet size distribution were measured and observed as a function of time, temperature and humidity. Droplet deposition and fraction of aerosols available for drift were also measured. The atmospheric degradation rate of folpan was observed relative to organic compounds found in ambient air and found to be relatively non-reactive. These data can be used to estimate degradation loss to aerosols containing the active ingredient folpan under relevant conditions of drift and transport. The results demonstrate a way to estimate realistic human exposure to folpan that is an improvement over currently used air monitoring strategies.

ABSTRACT FINAL ID: 2907 Poster Board #153
TITLE: AMOXILLIN- AND PEFLOXACIN-INDUCED CHOLESTEROGENESIS AND PHOSPHOLIPIDOSIS IN RAT TISSUES

AUTHORS (LAST NAME, FIRST NAME): Ademuyiwa, Oladipo1; Rotimi, Solomon O.2; Ojo, David A.3; Balogun, Elizabeth A.1; Talabi, Olu sola A.4
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KEYWORDS: Phospholipidosis, Cholesterol, Amoxillin and Pefloxacin.

Abstract Body: Dyslipidemia is currently becoming a confounding factor in assessing the safety of new and existing drugs. In order to investigate whether amoxillin and pefloxacin perturb lipid metabolism, rats were treated with therapeutic doses of each antibiotic for 5 and 10 days respectively. Twenty four hours and 5 days after antibiotic withdrawal, blood and other tissues (liver, kidney, brain, heart and spleen) were removed from the animals after an overnight fast and analysed for their lipid contents. Both antibiotics produced various degrees of compartment- specific dyslipidemia in the animals. While plasma and erythrocyte dyslipidemia was characterised by up-regulation of the concentrations of the major lipids (cholesterol, triglycerides, phospholipids and free fatty acids), hepatic and renal dyslipidemia was characterised by cholesterogenesis and phospholipidosis. Splenic dyslipidemia was characterised by cholesterogenesis and decreased phospholipid levels. Cardiac and brain cholesterol was not affected by the
Abstract Body: N-acetyltransferase 1 (NAT1) is a phase II metabolic enzyme responsible for the metabolism of arylamine carcinogens such as 4-aminobiphenyl (ABP). NAT1 metabolism can act either to detoxify or activate arylamines by two different pathways. NAT1-catalyzed N-acetylation serves as a detoxification pathway, while cytochrome P450 hydroxylated arylamines are activated by NAT1-catalyzed O-acetylation. O-acetylation leads to the formation of electrophilic intermediates that can readily form DNA adducts. NAT1*10, a common NAT1 allele, is associated with increased risk for numerous cancers. NAT1*10 is putatively a rapid acetylator and is characterized by two polymorphisms located in the 3′ untranslated region (UTR), one of which disrupts a polyadenylation (polyA) signal. To determine functional effects of NAT1*10 polymorphisms, constructs of NAT1*10 and the referent allele, NAT1*4, in full length NAT1 mRNAs were used. These constructs include the complete 5′-UTR derived from either the NATa (alternative) or NATb (major) promoter, the open reading frame and 888 base pairs of the 3′-UTR. Following transfection into nucleotide excision repair-deficient Chinese hamster ovary cells, NAT1-catalyzed N- and O- acetylation activity, NAT1 protein and mRNA levels were measured and polyA patterns were examined. Following exposure to ABP, hypoxanthine phosphoribosyltransferase mutations and DNA adducts were measured. No differences were observed between NAT1*10 and NAT1*4 in N- or O-acetyltransferase activity, protein, mRNA (p>0.05), or polyA pattern. Following exposure to ABP, no differences in DNA adducts or mutations were observed between NAT1*10 and NAT1*4 transfected CHO cells (p>0.05). These results suggest that acetylation differences observed in persons carrying NAT1*10 alleles cannot be explained by simple effects of the polymorphisms. Supported by USPHS grants CA034627, ES011564, ES014443 and DOD BC083107.
manufacturing, the legal bioavailability assure guidance investigated therefore intended testing, data and we consuming; (e.g. considerations) toxotyping, the Research Information Abstract 

TITLE: LESSONS LEARNED FROM ECHA’S REACH REGULATION THAT COULD HELP US BE BETTER PREPARED FOR U.S. TSCA REFORM

AUTHORS (LAST NAME, FIRST NAME): Lemus, Ranulfo; Jackson, Mark; Heim, Kate; Satter, Cathy; Inhof, Christina

INSTITUTIONS (ALL): 1. ARCADIS US, Cincinnati, OH, United States.

KEYWORDS: REACH, TSCA, CLP.

Abstract Body: In December 2010, US EPA and the European Chemicals Agency (ECHA) announced a partnership intended to promote enhanced technical cooperation on chemical management activities to improve chemical safety. ECHA is the agency that implements the European Union’s chemical management program on Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) and Classification, Labeling, and Packaging (CLP) of substances. The partnership establishes a process for working together on issues of mutual interest, including toxicity testing, the hazard and risk assessment of chemicals, risk management tools, scientific collaboration, and information exchange. One of the major anticipated areas of collaboration will be on the exchange of non-confidential information data (e.g. hazards, uses, substance identification) between ECHA and US EPA, including data collected under REACH. This new agreement between EPA and ECHA could have an impact on the US Toxic Substances Control Act (TSCA) reform which is under legislative discussion. Based on the REACH submissions and CLP notification experiences that we have been involved in over the past few years, we have learned several lessons that can help better prepare us for the challenges that TSCA reform may bring. Among the more important lessons learned are the following: 1) Substance Information Exchange Forum (SIEF) were not formed early enough and communications were difficult; 2) Data sharing and purchasing of toxicological studies are complex and needs appropriate consideration and follow through; 3) The legal considerations for contracts and financial considerations for data sale/Letters of Access are complicated and time consuming; 4) CLP registrations need to be consistent across manufacturers to accurately characterize potential substance hazards; and 5) MSDS generation will be challenging, as CLP and REACH requirements must both be met to assure compliance in different geographies. This understanding of the EU’s chemical management program will help us to navigate and plan our way through the TSCA amendments.

ABSTRACT FINAL ID: 2910 Poster Board #156

TITLE: ANALYSIS OF PROARRHYTHMIC EFFECTS OF VERAPAMIL AND BEPRIDIL USING THE CHRONIC ATRIOVENTRICULAR BLOCK (AVB) MONKEY

AUTHORS (LAST NAME, FIRST NAME): Sugiyama, Atsushi; Sakaguchi, Yasue; Sakamoto, Kengo; Ootani, Mitsutsugu; Wakasa, Yoshio; Morikawa, Hiroshi; Miyazaki, Hiroko; Uematsu, Atsushi; Hombo, Toshiyasu; Nomura, Mamoru

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2011 Society of Toxicology 50th Anniversary Meeting
Grace Period Abstracts

KEYWORDS: Monkey model, Torsades de pointes, QT prolongation.

Abstract Body: Purpose: Both verapamil and bepridil show inhibitory effects in in vitro hERG studies, with IC50 values similar to therapeutically effective plasma concentrations. In contrast, bepridil has been reported to cause QT prolongation and lethal arrhythmia called torsades de pointes (TdP) in clinical studies, while there have been no such reports for verapamil. In this study, the AVB cynomolgus monkey model (Sugiyama A. Br J Pharmacol; 154: 1528-1537, 2008) is used to measure the occurrence of proarrhythmia caused by verapamil and bepridil, in order to evaluate the clinical predictive ability of this model. Method: Verapamil (1.5, 15 & 75 mg/kg, p.o.) and bepridil (10 & 100 mg/kg, p.o.) were administered respectively to a total of 3 animals; 1 male and 2 female AVB model monkeys (64-99 months of age, 32-43 months post surgery). Holter ECG was used to observe any occurrence of QT prolongation, leading to the onset of TdP. Plasma concentrations of verapamil and bepridil were measured by the LC/MS/MS method. Results: The QTcF of verapamil (75 mg/kg) was 385±32 ms prior to administration, increasing to a maximum of 418±44 ms post administration. A tendency for QTcF prolongation was noted, but no TdP was observed. At the Cmax of 383±168 ng/mL, plasma concentrations were 3.2 (2.9-6.6) times therapeutically effective levels. The QTcF of bepridil (100 mg/kg) was 375±31 ms prior to administration, but increased to a maximum of 445±45 ms, a 19% prolongation, post administration. Two (2) cases of TdP were also observed. At the Cmax of 227±110 ng/mL, plasma concentrations were 1.8 (0.8-2.4) times therapeutically effective levels. TdP occurred in the 2 animals whose concentrations went over 2 times this level. Conclusion: The AVB monkey model may provide a more accurate prediction of the proarrhythmic effects that can be expected in humans, even when hERG study results of a compound are positive.

ABSTRACT FINAL ID: 2912 Poster Board #166
TITLE: PFOS AND PFOA: REVIEW OF TOXICOLOGICAL LITERATURE TOWARDS SUPPORT OF A TOXICOLOGICAL REFERENCE VALUE (TRV)

AUTHORS (LAST NAME, FIRST NAME): McAtee, Britt¹; Hitchcock, Kristen¹
KEYWORDS: perfluorinated chemicals, perfluorononanoic acid, perfluorooctane sulfonic acid.

Abstract Body: Perfluorinated compounds (PFCs) are among emerging chemicals of concern. PFCs have been used in many applications including stain resistant carpeting and non-stick cookware. Two common PFCs are perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). Scientific interest in these two chemicals has increased due to their global distribution, and environmental and biological persistence. The number of studies published regarding PFC toxicity has grown exponentially in the past decade. There is concern with both chemicals for possible developmental, metabolic, and immunological effects. PFOA has been implicated as an obesogen, and PFOS has been added to the list of restricted chemicals under the Stockholm Convention on Persistent Organic Pollutants (POPs). Despite this increased interest, few reference levels for health effects exist. As such, we derived and compared toxicological reference values (TRVs) based on various endpoints for PFOS and PFOA. As part of this TRV derivation, we conducted an extensive literature review in order to identify key studies. Next, we used benchmark dose modeling to assess the points of departures from each study. After the benchmark dose (BMD) and benchmark dose lower confidence limit (BMDL) were identified for each study, uncertainty factors were applied in order to calculate potential tolerable daily intakes (TDI). This exercise illustrates not only the usefulness of the BMD approach to deriving TRV but also the need for careful selection of studies.

ABSTRACT FINAL ID: 2913 Poster Board #167
TITLE: PROVISIONAL ADVISORY LEVEL (PAL) DEVELOPMENT FOR 1,2-DICHLOROETHANE

AUTHORS (LAST NAME, FIRST NAME): Wood, Carol S.; Gardner, Don⁰; McClanahan, Mark³; McConnell, Ernest⁴; Adeshina, Femi⁵
Abstract Body: PAL values developed for hazardous materials by the US EPA represent general public emergency exposure limits for oral and inhalation exposures corresponding to three different severity levels (1, 2, and 3) for 24-hr, 30-d, 90-d, and 2-yr durations. PAL 1 represents the threshold for mild effects; PAL 2 represents the threshold for serious, irreversible or escape-imparing effects; PAL 3 represents the threshold for lethal effects. PALs were intended to be used at the discretion of risk managers in emergency situations when site-specific risk assessments are not available. Application of PAL protocols has been performed for 1,2-DCE to estimate oral and inhalation exposure limits, as experimental data permit. Inhalation is readily absorbed, metabolized via P450 and glutathione conjugation, and eliminated in the urine. Saturation of metabolic pathways has been demonstrated after oral exposure in rats. No quantitative exposure data on humans were found so the PAL values were based on experimental animal data. Oral exposure resulted in kidney lesions and decreased survival. Oral PAL 1, 2, and 3 values are 120, 160, and 350 mg/L for 24-hr; 57, 120, and 140 mg/L for 30-/90-d; NR, NR, and 39 mg/L for 2-yr. Inhalation exposure resulted in nasal cavity lesions, decreased body weight and tumors. Inhalation PAL 1 is 0.69 ppm for 24-h/30-d/90-d and 0.24 ppm for 2-yr. Inhalation PAL 2 is 1.1 ppm for 24-h/30-d and 0.95 ppm for 90-d/2-yr. Inhalation PAL 3 is 2.8 ppm for 24-hr, 2.7 ppm for 30-/90-d, and NR for 2-yr.

**Abstract Body:** PAL values developed by the U.S. EPA represent general public emergency exposure limits for oral and inhalation exposures for hazardous materials corresponding to three severity levels and durations of 24 hrs, 30 and 90 d, and 2 yr durations. PAL 1, 2, and 3 severity levels represent the threshold for mild effects, serious/irreversible/escape-imparing effects, and lethal effects, respectively. PALs have not been promulgated nor have they been formally issued as regulatory guidance; they are intended to be used at the discretion of risk managers in emergency situations when site-specific risk assessments are not available. The PAL protocol has been applied to estimate oral and inhalation exposure limits for propylene oxide (PO). PO is an extremely flammable, highly volatile, colorless liquid, with a boiling point of 35°C. PO is a direct alkylating agent that has been shown to alkylate proteins and DNA. In addition, PO acts as an irritant, inducing lacrimation and mucous discharge. Much of the toxicological evidence in animals suggests that PO reacts at the site of entry. Oral exposure causes a gastric response, inhalation produces upper respiratory tract effects, and chronic exposure can lead to cancer at the site of contact. Data were available for deriving oral and inhalation PALs. The 24-h oral PAL 1, 2, and 3 are 350, 700, and 1100 mg/L, respectively; the 30-d PAL 1, 2, and 3 are 210, 420, and 630 mg/L, respectively; and the 2-yr PAL 2 is 15 mg/L. The 24-h inhalation PAL 1, 2, and 3 are 33, 46, and 140 ppm, respectively; the 30-d PAL 1, 2, and 3 are 3.1, 9.4, and 19 ppm, respectively; the 90-d PAL 1 and 2 are 3.1 and 9.4 ppm, respectively; and the 2-yr PAL 1, 2, and 3 are 0.54, 1.8, and 5.4 ppm, respectively. Data were insufficient for derivation of the 90-d oral PAL 1, 2, and 3 and 2-yr oral PAL 1 and 3 and the 90-d inhalation PAL 3. PAL values were based on evaluation of experimental data in humans and animals, and were approved by the Expert Consultation Panel for Provisional Advisory Levels in 2008.
Abstract Body: Soil contamination with semi-volatile chemicals such as PAHs, PCBs, dioxins and furans, and nonvolatile chemicals such as lead and inorganic mercury compounds can occur with airborne emissions. Hazardous waste and brownfield sites can have volatile organics soil contamination as well. Dermal contact with contaminated soil can result in chemical skin absorption. We have developed an approach that estimates part of the variability in dermal exposure and can be used in stochastic risk assessment. There are four variates commonly used in the dermal dose equation: body weight, exposed surface area of skin (SA), soil load on skin (SL), and exposure frequency (EF). Use of high end point estimates for all three of these variables tends to overestimate high end exposure. OEHHA is proposing to use a combined variate, “annual dermal load”, or ADL, which is a composite of the body surface area (BSA) per kg body weight (BW), EF, and SL on skin. The ADL is in units of mg of soil loaded onto skin per kg body weight per year and expressed as: ADL (mg/kg-yr) = (BSA / BW) * SL * SA * EF Distributional data are available for BSA/BW and recently for EF. Only point estimates for soil loading and percent of surface area exposed are available. Point estimates of SL, SA and distributions of BSA/BW and EF were used for a Monte Carlo simulation for ADL. A parametric model was fit to the simulated distribution for ADL that could be used for stochastic estimation of dermal exposure. The mean, 90th and 95th percentiles of the simulated ADL can be used for average and high end point estimates of dermal exposure. ADLs were developed for 5 age groups and 3 climate conditions (cold, mixed and warm climates). Sample mean ADLs generated for adults ranged from 0.7 to 12.2 mg/kg-yr, depending on climate. For children age 2<16 yrs, mean ADLs ranged from 2.8 to 6.4 mg/kg-yr, depending on climate. Measured or modeled estimates of soil concentration can be used with ADL and chemical specific absorption factors to estimate a distribution of dermal exposure, or for average and high end point estimates.

2011 Society of Toxicology 50th Anniversary Meeting
Grace Period Abstracts

ABSTRACT FINAL ID: 2915 Poster Board #169
TITLE: STOCHASTIC APPROACH FOR ESTIMATING DERMAL EXPOSURE.

AUTHORS (LAST NAME, FIRST NAME): Dodge, Daryn1; Arcus, Amy1; Welling, Roberta1; Blaisdell, Robert1
KEYWORDS: dermal risk assessment, risk assessment, dermal exposure assessment.

ABSTRACT FINAL ID: 2916 Poster Board #170
TITLE: EMPIRICAL MODELING PREDICTS DRINKING WATER PERCHLORATE CONCENTRATIONS WITH NO INHIBITORY EFFECT ON THE IODIDE CONTENT OF BREAST MILK

AUTHORS (LAST NAME, FIRST NAME): Goodman, Gay1; Dwyer, Scott D.2
KEYWORDS: perchlorate, iodine, drinking water.

Abstract Body: The results of an empirical modeling analysis predict that a drinking-water perchlorate (DWP) concentration of 49 ppm will produce no decrease in the iodine content of breast milk in lactating women. Part 1: Kirk et al. (2005) obtained DWP and breast milk perchlorate (BMP) measurements for 15 US women; DWP concentrations ranged from <0.5 μg/L (the detection limit) to 2 μg/L. Téllez et al. (2005) measured BMP in 55 women in three Chiléan cities with mean drinking water perchlorate (DWP) concentrations of 0.46, 5.8, and 114 μg/L, respectively. We performed linear regression analysis of BMP on DWP for the data sets separately and combined. For DWP levels <2 μg/L (Kirk data or combined data) there was no significant dependence of BMP on DWP. For all data combined, analysis of the combined data yielded a slope of 0.75 (p <0.0001, SE = 0.08). Part 2: Kirk et al. (2007) measured BMP and recorded dietary intakes of fruit and vegetables for 9 US women. We performed linear regression analysis of the women’s BMP on their average fruit and vegetable intake as a percentage of the daily recommendation (FVDI%) and found a marginally significant linear dependence with a slope of 13.4 (p = 0.9, SE = 6.9). Part 3: We found that the paired iodide and perchlorate concentration data in breast milk reported by Dasgupta et al. (2008), Pearce et al. (2007), Kirk et al. (2005), and Kirk et al. (2007) conform to an inverted U-shaped pattern in which the threshold BMP concentration for inhibition of iodide secretion is >50 μg/L. Results: For DWP >2 μg/L, the dependence of BMP on FVDI...
2011 Society of Toxicology 50th Anniversary Meeting
Grace Period Abstracts

and DWP can be written BMP = (0.75 DWP) + 13.4 (FVDI/0.9), where BMP and DWP are in units of µg/L, FVDI is in units of kg/day, 0.9 kg/day is the recommended intake, and 0.75 and 13.4 are best-fit parameters in the model fits described above. For BMP = 50 µg/L and FVDI = 0.13 kg/day (the average FVDI for women 20-45 years of age) or 0.9 kg/day (the recommended allowance), the model predicts no effect on BMP at DWP levels as high as 64 µg/L or 49 µg/L, respectively.

**ABSTRACT FINAL ID:** 2917 Poster Board #171
**TITLE:** PROVISIONAL ADVISORY LEVELS (PALS) DEVELOPMENT FOR PARAQUAT

**AUTHORS (LAST NAME, FIRST NAME):** Rayner, Jennifer L.; Koller, Loren; Dorman, David; Adeshina, Femi
**INSTITUTIONS (ALL):** 1. Oak Ridge National Laboratory, Oak Ridge, TN, United States. 2. Environmental Health & Toxicology, Corvallis, OR, United States. 3. NCSU, Raleigh, NC, United States. 4. US EPA, Washington, DC, United States.

**KEYWORDS:** Provisional Advisory Level, Pesticide.

**Abstract Body:** PAL values developed for hazardous materials by the US EPA represent general public emergency exposure limits for oral and inhalation exposures corresponding to three different severity levels (1, 2, and 3) for 24-hr, 30-d, 90-d, and 2-yr durations. PAL 1 represents the threshold for mild effects; PAL 2 represents the threshold for serious, irreversible or escape-impairing effects; PAL 3 represents the threshold for lethal effects. PALs have not been promulgated nor have they been formally issued as regulatory guidance. They are intended to be used at the discretion of risk managers in emergency situations when site specific risk assessments are not available. Application of PAL protocols has been performed for paraquat to estimate oral and inhalation exposure limits, as current experimental data permitted. Oral exposure to paraquat results in dyspnea, respiratory damage, and death in multiple species including humans. Oral PAL values for a 24-hr exposure are 11 mg/L (PAL 1), 78 mg/L (PAL 2) and 230 mg/L (PAL 3); 30-d exposure: 3.1 mg/L (PAL 1), 6.3 mg/L (PAL 2), 19 mg/L (PAL 3); 90-d exposure: 1.9 mg/L (PAL 1), 6.1 mg/L (PAL 2), 12 mg/L (PAL 3); and 2-yr exposure: 0.70 mg/L (PAL 1) and 1.6 mg/L (PAL 2). PAL 3 values were not derived for the 2-yr exposures. Inhalation of paraquat causes rapid shallow breathing, pulmonary congestion, hemorrhage, and death in male mice and male and female guinea pigs and rats. Dogs and female rabbits and mice are less affected by inhaled paraquat. Inhalation PAL values for a 24-hr exposure are 0.010 mg/m³ (PAL 1), 0.012 mg/m³ (PAL 2), and 0.019 mg/m³ (PAL 3), and 0.00018 mg/m³ (PAL 1), 0.00011 mg/m³ (PAL 2), and 0.00071 mg/m³ (PAL 3) for a 30-d exposure. Data are insufficient to derive inhalation PAL values for 90-d and 2-yr exposures. PAL values were approved by the Expert Consultation Panel for Provisional Advisory Levels. (This abstract presents PAL values that are subject to change pending further review and new data.)

**ABSTRACT FINAL ID:** 2918 Poster Board #172
**TITLE:** A RISK ASSESSMENT EVALUATION OF NEW TECHNOLOGY DIESEL ENGINE EXHAUST COMPOSITION

**AUTHORS (LAST NAME, FIRST NAME):** Budroe, John D.; Salmon, Andrew G.; Marty, Melanie A.
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**KEYWORDS:** Cancer Risk Assessment, Diesel Exhaust, Complex mixtures.

**Abstract Body:** Diesel engine exhaust (DEE) has been demonstrated to cause several toxic effects in animals and humans. These effects include respiratory, cardiovascular and immune system toxicity. DEE has also been demonstrated to be genotoxic and carcinogenic. IARC and US EPA have described DEE as probably and likely to be carcinogenic to humans, respectively. The California Air Resources Board (CARB) listed particulate matter from diesel-fueled engines as a Toxic Air Contaminant (TAC) in 1998, with a cancer unit risk factor of $3 \times 10^{-4} \, \mu g/m^3$. Since the DEE TAC listing in 1998, diesel engine manufacturers have developed diesel engines (“new technology engines”, or NTE) which produce substantially lower exhaust levels of particulate matter (DEP) and air toxics compared to older engines. Reported reductions in DEP and air toxics vary according to the engine type, fuel, type of emissions controls used, and engine test cycles used to evaluate engine emissions. Experimental data from several NTE engine emissions studies
Abstract Body: The IARC Monographs have been published continuously since 1971. For the 100th Vol. of the programme the evidence on all human carcinogens (Group 1) that have been identified to date has been updated. 147 experts from 28 countries contributed to the Vol. 100 series developed in six meetings from Oct 2008 to Oct 2009: A. Pharmaceuticals, 23 agents; B. Biological agents, 11 agents; C. Metals, particles and fibres, 14 agents; D. Radiation, 14 agents; E. Lifestyle factors, 11 agents; F. Chemicals and related occupations, 34 agents. For each agent evaluations of the evidence in humans and in experimental animals and an overall evaluation of the human carcinogenicity have been developed and tumour sites with sufficient evidence of carcinogenicity as well as tumour sites that are strongly suspected and plausible mechanisms have been identified. The presentation will highlight some mechanistic findings that influenced the overall evaluation. For instance, TCDD was the first agent classified initially in Group 1 based on sufficient evidence in experimental animals and mechanistic considerations, and later confirmed by increased cancer incidence in humans. Like TCDD, 2,3,4,7,8-pentachlorodibenzofuran and 3,3',4,4',5-pentachlorobiphenyl are complete carcinogens in experimental animals with extensive evidence that they act through the same AhR-mediated mechanism. Based on these considerations, the Working Group classified these two chemicals in Group 1. More examples will be presented. This highlights the ability of mechanistic information to provide early, robust evidence of carcinogenicity. Subsequent workshops will synthesize this information for two related publications: 1) Tumour concordance between humans and experimental animals. 2) Mechanisms involved in human carcinogenesis. The objective for these two publications is to facilitate identification of carcinogens based on mechanistic and molecular information in the absence of cancer studies in animals or in humans.
Abstract Body: As a result of former handling practices during the use of trichloroethene (TCE) as a solvent and its relative stability in subsurface soil, TCE is a frequently detected in U.S. groundwater. Despite extensive study in laboratory animals and in human (chiefly occupational) populations, derivation of a cancer slope factor (CSF) representative of potential human health risk remains challenging. While animal and human biological and biochemical data are suggestive of a threshold for cancer, lack of consensus on the mechanism(s) of action complicates implementation of a threshold approach. Epidemiology studies include cohort, case-control, and ecological studies that, taken together, suggest kidney cancer as an appropriate basis for derivation of risk-specific concentrations. Increased incidences have been marginal when present and all studies lack reliable exposure data adequate for quantitative risk estimates. Studies in laboratory animals demonstrate high species, strain, sex, and route of exposure specificity. Cancer typically occurs following cellular damage at high doses, with kidney emerging as a primary target. A dose-response assessment was conducted applying data for kidney tumors (adenomas and carcinomas combined) in laboratory rodents exposed to TCE. Based upon the most plausible mode of action for TCE-induced renal tumors, area under the curve for the TCE metabolite S-(1,2-dichlorovinyl)-L-cysteine (AUC DCVC) in kidney was selected as an internal dose measure. Both linear and nonlinear models of carcinogenicity were considered. Estimated reference doses and concentrations based on the use of a non-linear approach are substantially higher than proposed EPA reference doses based on non-cancer endpoints. Estimated cancer slope factors for both oral and inhalation exposure under a linear low-dose extrapolation approach are substantially lower than the most recent values proposed by US EPA.
have not been promulgated nor have they been formally issued as regulatory guidance, but are intended for use at the discretion of risk managers in emergency situations when site-specific risk assessments are not available. PALs are developed using standard protocols with appropriate human and animal data. Thallium sulfate is a heavy metal that occurs naturally in the soil, water, air, plants, and animals. Thallium was previously used as a depliatory and rodenticide, but large amounts are still released to the environment by combustion of fossil fuels, oil refining, and smelting of ores. Thallium has a long half-life in mammals (up to 30 days), and replaces potassium in key biologic processes. Symptoms of poisoning include abdominal pain, vomiting, constipation, paresthesia, hypertension, muscle wasting, ataxia, skin lesions, and hair loss, the latter being a unique effect of thallium. No appropriate human or animal inhalation studies were available for derivation of inhalation PALs. Oral PALs were derived using human and animal studies, in which exposure was to the thallium sulfate and acetate salts. The derived PAL 1, PAL 2, and PAL 3 values, as mg Tl2SO4/L, were 0.88, 6.3, and 88 mg/L for 24 hours, 0.88, 2.8, and 6.0 mg/L for 30 days, and 0.18, 0.88, and 6.0 mg/L for 90 days. Only a PAL 3 was derived for a 2-year exposure (2.0 mg/L). These values were approved by the Expert Consultation Panel for Provisional Advisory Levels in April 2010.

ABSTRACT FINAL ID: 2923 Poster Board #177

TITLE: PROVISIONAL ADVISORY LEVELS (PAL) DEVELOPMENT FOR PARATHION

AUTHORS (LAST NAME, FIRST NAME): Marshall, Tom1; McClanahan, Mark3; Gardner, Don3; Adesina, Femi4


KEYWORDS: risk assessment, exposure guideline, emergency response.

Abstract Body: PAL values developed for hazardous materials by the US EPA represent general public emergency exposure limits for oral and inhalation exposures corresponding to three different severity levels (1, 2, and 3) for 24-hr, 30-d, 90-d, and 2-yr durations. PAL 1 represents the threshold for mild effects; PAL 2 represents the threshold for serious, irreversible or escape-impairing effects; PAL 3 represents the threshold for lethal effects. PALs have not been promulgated nor have they been formally issued as regulatory guidance. They are intended to be used at the discretion of risk managers in emergency situations when site specific risk assessments are not available. The PAL protocols were applied to estimate oral and inhalation exposure limits for parathion as permitted by experimental data. Parathion is a broad-spectrum organophosphorous insecticide. Acute toxicity and limited chronic effects are attributed to cholinergic-mediated neurotoxicity. Metabolism to paraoxon is necessary for this activity. Quantitative toxicity data on both humans and animals were used to derive oral PAL values. The respective oral PAL 1, 2, and 3 values are 0.30, 1.4, and 2.3 mg/L for 24-hr; 0.30, 0.39, and 2.3 mg/L for both 30 and 90d; 0.12, 0.39, and 1.9 mg/L for 2-yr. Quantitative inhalation data were limited to animal studies of both single and repeated short-term exposures that caused overt cholinergic signs and/or cholinesterase activity inhibition. No values were recommended (NR) for the 90-d/2-yr durations. Respective inhalation PAL 1, 2, and 3 values are NR, 0.070, and 0.21 mg/m3 for 24-hr; 0.000069, 0.0017, and 0.0050 mg/m3 for 30-d.

ABSTRACT FINAL ID: 2924 Poster Board #178

TITLE: EXPOSURE AND HAZARDS OF INORGANIC ARSENIC FROM CONSUMPTION OF RICE AND RICE PRODUCTS

AUTHORS (LAST NAME, FIRST NAME): Zang, Yu1; Tao, Shyy Hwa1; Carrington, Clark D.1; Bolger, P. Michael1


KEYWORDS: Rice, Inorganic Arsenic, Health Risk.

Abstract Body: Inorganic arsenic (iAs) is widely distributed in the environment due to natural and anthropological sources. For the general population, ingestion is the primary route of exposure. Chronic exposure of iAs from drinking water has been associated with various adverse human health effects, including skin lesions, cardiovascular effects and several types of cancer. Besides drinking water, food can be another important source of iAs exposure. The FDA
2011 Society of Toxicology 50th Anniversary Meeting
Grace Period Abstracts

Total Diet Study (TDS) has frequently detected total arsenic in rice/rice product samples. Among food, rice contains relatively high iAs levels and has been demonstrated to be one of the major contributors to total dietary iAs. Understanding the contribution of rice and rice products to total daily iAs exposure is of both scientific and regulatory importance. Using the TDS, and other sources of dietary exposure, we estimated the dietary iAs exposure from rice and rice products. These estimates were in turn compared to biomarker information in the National Health and Nutrition Examination Survey (NHANES). Dietary iAs intake from the consumption of rice and rice products varies by age, consumption patterns, and rice species and sources. In areas where drinking water contamination is minimal, food can be a major contributor of total iAs exposure, especially in populations whose diet includes rice as a staple food. The overall exposure, however, may still be lower than in populations living in areas with high iAs in drinking water. Based on human epidemiological evidence of relative risk a 5% Benchmark Dose Lower Confidence Limit (BMDLo.5) for lung cancer was derived. In addition, estimates of lifetime risk of dietary iAs were generated from the same data. The comparison of the BMDLo.5 to various estimates of iAs dietary exposure, particularly at upper percentile levels, suggests a possible health risk for some populations.

ABSTRACT FINAL ID: 2925 Poster Board #201
TITLE: DEVELOPMENT OF A NOVEL HPLC METHOD FOR THE DETERMINATION OF FLURIDONE IN UNTREATED RAT PLASMA SPIKED WITH FLURIDONE

AUTHORS (LAST NAME, FIRST NAME): Albayati, Zaineb A.; Reddy, Narsimha P.; Reddy, Yerram T.; Crooks, Peter A.
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KEYWORDS: HPLC Method Development, Herbicides, Human toxicity.

Abstract Body: Fluridone (1-Methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone) is a new broad spectrum herbicide widely used to manage aquatic weeds in ponds and lakes. There are no direct food uses for fluridone, however, humans are exposed to water from areas treated with fluridone can be used for the irrigation of crops and pastures. Many studies have shown that fluridone produces acute oral and acute inhalation toxicity and dermal and eye irritation. But data on assay of fluridone in human plasma are lacking. The purpose of our study was to develop a novel high-performance liquid chromatography (HPLC) method for the determination of fluridone in plasma samples from untreated Sprague Dawley rats spiked with fluridone. Method: fluridone was extracted from samples of plasma using acetonitrile, centrifuged and the supernatant dried under nitrogen gas. The residue reconstituted in acetonitrile and injected into HPLC. A wavelength (λ=240 nm) and a flow rate of 0.6 mL/min were used. A volume of 15 μL of analyte was injected into the system. An isocratic mobile phase consisted of water containing 1 mL/L formic acid (mobile phase A) and acetonitrile containing 1 mL/L formic acid (mobile phase B). Results: Retention time of fluridone was 7.06 min and 9.77 min for benzophenone (internal standard). The area under the curve ratio for fluridone was measured relative to that of benzophenone. Significant concentrations of fluridone were observed in plasma. The limit of detection (LOD) was 20 ng/mL, while limit of quantitation (LOQ) was 50 ng/mL with an efficiency of extraction = 0.78 ± 0.004 - 0.83 ± 0.011. This method is convenient to determine the concentration of fluridone in human plasma.

ABSTRACT FINAL ID: 2926 Poster Board #202
TITLE: LOCAL AND SYSTEMIC TOXICITY OF THREE INFLUENZA VACCINES FOLLOWING INTRAMUSCULAR INJECTION TO NEW ZEALAND WHITE RABBITS

AUTHORS (LAST NAME, FIRST NAME): Godin, C. S.; Umlauf, Scott
INSTITUTIONS (ALL): 1. AVANZA Laboratories, Gaithersburg, MD, United States. 2. VaxInnate Corporation, Cranbury, NJ, United States.
KEYWORDS: influenza, vaccine safety, vaccine immunogenicity.
Abstract Body: The purpose of this study was to evaluate the toxicity of three influenza vaccines when administered to rabbits. The three vaccines differed with respect to the position of the influenza HA head with the flagellin fusion partner. Twelve/sex/group received the control and vaccines by IM injection on Study Day (SD) 1, 22, and 43. The vaccines had no effect on mortality, clinical observations, ophthalmology, hematology and urinalysis variables, organ weights, or histopathology. STF2R3.HA1 and STF2R3.2xHA1 had no effect on Draize scores, body weight changes, food consumption, and clinical chemistry. Body temperatures were higher 6 hr after the first dose but were not adverse. On SD 23, there was an increase in mean C-reactive protein values in animals receiving these vaccines. Following administration of the first dose of STF2.HA1 vaccine test article-related erythema and edema were noted but were not adverse. Decreases in absolute body weights and food consumption also occurred in both sexes following the first dose of this vaccine at both dose levels but the effects were not adverse. As with the other two vaccines, STF2R3.HA1 and STF2R3.2xHA1, body temperatures were elevated 6 hr after dosing on SD 1 but were not adverse. Changes in serum globulin and mean albumin/globulin ratios also occurred but were not adverse. There were also test article-related increases of mean fibrinogen and activated partial thromboplastin values, and a decrease in mean prothrombin time in animals that received STF2.HA1 but none of these changes was considered adverse. C-reactive protein also increased following the first administration of this vaccine. Samples collected prior to dosing and on SD 23, 46, and 68 were analyzed in HA-specific and STF2-specific IgG ELISA, as well as hemagglutination inhibition (HAI), and the results suggest that the rabbit model is an appropriate immunological model for all three vaccine test articles. In conclusion, administration of the three vaccines was well tolerated and no adverse effects occurred at the highest administered doses.

ABSTRACT FINAL ID: 2927 Poster Board #203
TITLE: A COMPREHENSIVE ASSESSMENT OF CARDIAC AND RENAL EFFECTS OF DOXORUBICIN IN THE AUTOMATED BLOOD SAMPLING AND TELEMETRY SYSTEM

AUTHORS (LAST NAME, FIRST NAME): Kamendi, Harriet W.; Chen, Yafei; Fang, Chengwei; Bialecki, Russell
KEYWORDS: Cardiovascular, Renal, ABST.

Abstract Body: Doxorubicin is an anthracycline antibiotic that treats various cancers including multiple solid tumours and myelomas. Despite its efficient treatment of cancerous tumours, its therapeutic use is limited due to cardiac and renal toxicity. This study evaluates temporal relationships that may exist between CNS, cardiac or renal toxicity induced by a single dose of 20 mg/kg, i.p. Rats fitted with telemetry implants, were acclimated to an Automated Blood Sampling and Telemetry system for 48 hrs before dosing. EEG, blood pressure and core body temperature were recorded from day 1 of acclimation to 3 days post-dose. Blood and urine samples were collected pre-dose and on day 1 and 3 post-dose. The maximum plasma concentration was 3.0± 0.6 μM (n=6) at sixty minutes post-dose. Doxorubicin decreased and also disrupted the circadian rhythm of mean arterial pressure and temperature. There were no changes observed in average heart rate although variability diminished in most of the treated rats. Although there were no histological lesions observed at the end of the three day study, doxorubicin significantly altered some urinary biomarkers of injury in a time dependent manner. The 400% increase in lipocalin (post-dose vs pre-dose creatinine ratio: 8000± 2500 vs. 32000± 7500, p < 0.05, n=6), and 60% reduction in KIM-1 (post-dose vs pre-dose creatinine ratio: 12.3±1 vs. 4±0.8, p<0.05, n=6) preceded a 26-fold increase (p<0.05, n = 6) in levels of urinary albumin on day 3 post-dose. This study provides a comprehensive and time dependent assessment of the acute effects of doxorubicin in the rat. The study reveals that changes observed in lipocalin and KIM-1 may be used as early markers of toxicity.
Abstract Body: GKAs are being developed for the treatment of Type 2 diabetes. The nonclinical toxicity of four, structurally diverse, GKAs was assessed in rat, dog, and/or monkey studies. GKAs were administered for 4 to 8 weeks, standard endpoints, and time courses for glucose and insulin were assessed. In each study, the low dose was an expected no observed adverse effect level, the high dose was the maximum tolerated dose based on tolerance or hypoglycemia, and the mid dose was chosen to provide a dose-response. All compounds produced varying degrees of hypoglycemia in all species. Brain neuronal necrosis and peripheral neuropathy were observed with most compounds in one or more species and are consistent with literature reports linking hypoglycemia with effects on the nervous system. Coronary arteriopathy was observed at a low frequency in monkey and/or dog. Since this lesion occurred in multiple studies with structurally distinct GKAs these results suggests this finding is related to the pharmacology of these agents. Arteriopathy only occurred at doses that produced severe and prolonged periods of repeated hypoglycemia. The morphological characteristics of the arteriopathy were consistent with that produced by experimental catecholamine administration. We hypothesize that the prolonged periods of hypoglycemia resulted in increased local and/or systemic concentrations of catecholamines via a counter-regulatory and/or stress-related mechanism. This risk can be managed in human clinical studies by careful glucose monitoring and intervention to avoid prolonged episodes of hypoglycemia.

ABSTRACT FINAL ID: 2929 Poster Board #205
TITLE: EVALUATION OF KETAMINE, AMPHETAMINE AND NICOTINE IN RATS TRAINED TO SELF-ADMINISTER COCAINE

AUTHORS (LAST NAME, FIRST NAME): Moser, Paul; Coquelin, Céline; Froger-Colleaux, Christelle; Castagné, Vincent; Froget, Guillaume
KEYWORDS: self-administration, drug abuse potential, Abuse Liability Assessment.

Abstract Body: Detection of abuse liability is becoming an essential component of CNS safety. Self-administration (SA) procedures, in which animals are trained to respond for an infusion of test substance, are widely regarded as the most predictive approach to evaluate abuse liability in humans. Male Sprague-Dawley rats were prepared with i.v. jugular catheters which could be attached to an infusion pump controlled by computer. After recovery, rats were trained to lever-press for i.v. infusions of 0.6 mg/kg cocaine under a Fixed Ratio 10 (FR10) schedule. Daily sessions were 2h long and rats could receive up to 50 infusions. Cocaine SA was rapidly acquired rats taking 30.4±2.9 infusions/ session (average of final 3 sessions at stable infusion rates for n=13). When saline was substituted for cocaine, responding dropped to 4.2±0.4 infusions/session. Subgroups of those rats were then given the possibility to self-administer amphetamine (0.05 mg/kg/inf), ketamine (0.5 mg/kg/inf) or nicotine (0.03 mg/kg/inf). All three substances supported SA, with amphetamine and nicotine achieving steady-state infusion rates from the first session (21.1±3.3, n=7 and 16.6±5.6, n=4, respectively). In contrast, ketamine did not support SA over the first few sessions it was available and only started to stabilize at high rates after 4–5 sessions (26.2±6.0, n=6). These data demonstrate that cocaine SA can detect a broad range of substances with abuse liability, including less overtly stimulant substances such as ketamine but that care must be taken to ensure that sufficient sessions are run to avoid false negatives.
2011 Society of Toxicology 50th Anniversary Meeting
Grace Period Abstracts

ABSTRACT FINAL ID: 2930 Poster Board #206
TITLE: CRF R1 ANTAGONIST BMS-764459 DECREASES BASAL STRESS HORMONE LEVELS IN RATS WITHOUT INHIBITING THEIR ABILITY TO RESPOND TO STRESS

AUTHORS (LAST NAME, FIRST NAME): Van Vleut, Terry R.3; Clark, S.3; Sivarao, D. V.1; Reisinger, A. J.2; Healy, F.1; Lodge, N. J.1; Bronson, J.1; Bounous, D.2; Bunch, R. T.2; Peden, W. M.3; Schilling, B. E.3; Dominick, M. A.3; Sanderson, T. A.3
KEYWORDS: CRF R1, CRH receptor antagonist, stress.

Abstract Body: BMS-764459, a CRF R1 antagonist, was dosed orally to stressed/unstressed rats daily for 4 weeks to evaluate serum CORT and ACTH levels. Groups of 24 (unstressed) to 72 (stressed) male rats were dosed with 0, 10, or 100 mg/kg. A stress protocol of 45 minutes of restraint with three 1 min jets of air sprayed on the dorsum was used to induce stress. Animals were acclimated to handling to reduce basal stress. Trunk blood was collected by decapitation from 8 animals per collection period. Blood was collected before, immediately after, and 60 min after exposure to stress. The first blood collection took place 60 min after dosing (before stress). Blood was also collected from 8 unstressed animals in each dose group at 165 min after dosing. Sampling took place after the first dose, after 1 month of daily dosing, and following a 2-week recovery period. In unstressed rats, 10 and 100 mg/kg BMS-764459 reduced CORT and ACTH levels compared to controls after 1 dose and following 1 month of daily doses. Two weeks after cessation of treatment, there were no differences in serum CORT or ACTH levels in rats that had been treated with 10 or 100 mg/kg BMS-764459 compared to controls. In untreated rats exposed to stress, there was a 50-fold increase in serum CORT compared to pre-stress levels. Mean ACTH levels increased 3- to 20-fold with stress. In rats treated with either 10 or 100 mg/kg of BMS-764459 as a single dose or 1 month of daily doses, there was no difference in serum CORT or ACTH levels from untreated animals following exposure to stress. Collectively, these data indicate that BMS-764459 reduces basal CORT and ACTH levels in rats, but does not interfere with their ability to respond to severe stress. Additionally, basal stress hormone levels return to normal after cessation of treatment for up to 1 month.

ABSTRACT FINAL ID: 2931 Poster Board #207
TITLE: THREE DIMENSIONAL PERFUSED TISSUE CULTURE: A PLATFORM FOR ACUTE AND CHRONIC TOXICOITY TESTING

AUTHORS (LAST NAME, FIRST NAME): Hart, Tim1
KEYWORDS: hepatotoxicity, neurotoxicity, 3D.

Abstract Body: Improving the clinical relevance of in vitro toxicity tests enables potential liabilities to be detected earlier in the drug development process. Culturing cells in three dimensions (3D) provides a more realistic environment that more closely mimics human responses. Here we present a multiwell microfluidic unit for 3D perfused cell culture and testing. The versatility of this platform is demonstrated through the culture of human liver and neuronal tissues and toxicity testing thereof. TissueFlex® microfluidic units used to culture HepG2 cells in an hydrogel scaffold. High cell viability (>80%) and function (urea/ammonia production, phase I/II metabolism) was routinely achieved for more than 14 days. The effects of troglitazone on cell viability and function were assessed using a plurality of assays over 14 days at concentrations of 25mM, 6mM (Cmax for 600mg daily dose) and 3mM. Our results show that in contrast with monolayer cultures, using 3D perfused cultures we can identify chronic toxicity effects at doses that are more consistent with patient plasma concentrations. This 3D liver model could be used to assess chronic toxicity liabilities, replacing the less reliable extrapolation of acute toxicity data. We also explored the controlled differentiation in TissueFlex® of human Nteraz2/clone D1 embryonic carcinoma cells to produce over 4 weeks a 3D co-culture of neurons and astrocytes in Matrigel™ and Hystem™ scaffolds. Stress response and metabolic activity were monitored throughout differentiation. Immunohistological and microscopic analyses show that these 3D neuronal tissue co-cultures are physiologically more representative of the in vivo state than 2D co-cultures. Preliminary toxicological studies suggest that this 3D neuronal model provides a platform for drug and cell testing.
2011 Society of Toxicology 50th Anniversary Meeting
Grace Period Abstracts

ABSTRACT FINAL ID: 2932 Poster Board #208
TITLE: AMPHETAMINE AND METHAMPHETAMINE ARE MUCH MORE POTENT AT INDUCING HYPERTHERMIA AND NEUROTOXICITY THAN METHYLPHENIDATE DURING THE WAKING CYCLE

AUTHORS (LAST NAME, FIRST NAME): Bowyer, John F.1; Divine, Becky L.3; Hanig, Joseph P.4; Doerge, Daniel R.1; Vanlandingham, Michelle M.1; Twaddle, Nathan C.1; Levi, Mark1


KEYWORDS: Stimulants, Neurotoxicity, Hyperthermia.

Abstract Body: Previous studies focusing on amphetamine (AMPH), methamphetamine (METH) and methylphenidate (MPH) neurotoxicity have almost exclusively been conducted in rodents during the light cycle which is when most rodents sleep. The present study compared the effects of AMPH, METH, or MPH treatment on body temperature and neurotoxicity during the waking (dark) cycle of the rat. This was done to more effectively replicate stimulant exposure in aware humans. Four subcutaneous injections, 2 hr between each, were used to administer the stimulants tested. Several doses for each of the three stimulants were chosen to produce plasma levels ranging from slightly above the highest therapeutic levels to those only attained by accidental overdose or intentional abuse in humans. Four doses of ≥2 mg/kg of either AMPH or METH administered during the waking cycle produced hyperthermia, 70% striatal dopamine depletions, and neurodegeneration in the cortex and thalamus. Changes in the vasculature of the ventral amygdala were noted as well. Four doses of ≤0.6 mg/kg MPH did not significantly affect body temperature. During the sleep cycle, administration of four doses of 3 mg/kg AMPH produced lesser increases in body temperature, minimal dopamine depletions and little neurodegeneration. These results show that METH or AMPH are much more potent at producing hyperthermia, neurotoxicity, and death in laboratory animals than MPH. Also, administration during the waking cycle appears to significantly increase the potency AMPH and METH to produce hyperthermia, neurotoxicity and lethality.

ABSTRACT FINAL ID: 2933 Poster Board #209
TITLE: CHEMICAL INJURY-INDUCED NEUROGENESIS: POTENTIAL CONTRIBUTION OF RESIDENT MICROGLIA

AUTHORS (LAST NAME, FIRST NAME): McPherson, Christopher1;2; Harry, Gaylia J.1

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KEYWORDS: Neurogenesis, Hippocampus, Neuroinflammation.

Abstract Body: Adult neurogenesis occurs in the subgranular zone (SGZ) of the hippocampal dentate gyrus generating new dentate granule neurons. This process can be induced with brain injury or chemical insult, suggesting a capacity for “self-repair”. Infiltration of peripheral macrophages with injury can be detrimental to the repair process while an activation of resident microglia may promote neurogenesis. Thus the question arises, what distinguishes beneficial versus adverse effects of inflammation on neurogenic “self-repair”? To address this question, we used the hippocampal toxicant, trimethyltin (TMT; 2.3mg/kg, ip), as a tool to selectively target dentate granule cell death in adolescent CD-1 male mice. Within 48h post-TMT, neuronal death is accompanied by resident microglia activation, and elevations in tumor necrosis factor alpha (TNFα) and interleukin-1α (IL-1α) mRNA levels. Bromodeoxyuridine (BrdU) incorporation identified the peak time of neurogenesis as coinciding with peak of neuroinflammation. BrdU+ cells were transiently in contact with process bearing microglia within the SGZ and inner granule cell layer (GCL). The proliferative response was sufficient to fully repopulate neurons in the GCL and provide functional recovery. Using laser-capture microdissection, SGZs were isolated at 48h post-TMT for qPCR analysis. Key molecules in the IL-1α pathway were induced by TMT exposure. Effects of IL-1α [150pg/ml] were identified in the proliferation and differentiation of hippocampal neural progenitor cells (NPCs) in vitro. These data suggest a role for resident microglia and secreted IL-1α
ABSTRACT FINAL ID: 2934 Poster Board #210

TITLE: INNATE DEFENSE RESPONSE OF MOUSE MULTIPOTENT MESENCHYMAL STROMAL CELLS (MSCS) INHIBITS IN VITRO PROLIFERATION OF ESCHERICHIA COLI

AUTHORS (LAST NAME, FIRST NAME): Gorbunov, Nikolai V.1; Garrison, Bradley R.1; McDaniel, Dennis P.; Ledney, G. David2; Elliott, Thomas B.; Kiang, Juliann C.3

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KEYWORDS: multipotent mesenchymal stromal cells, Escherichia coli, innate response.

Abstract Body: Stromal cells (SC) constitute stromal barriers that support tissue architecture and function and protect against invasion of pathogens. Recent discovery of immunomodulatory function of multipotent mesenchymal stromal cells (MSCs) provides a new insight into the role of stroma in tissue homeostasis and opens a promising cell therapy approach to manage acute sepsis and penetrating wounds. However, the molecular mechanisms underlying MSC action in septic conditions are not clear. This report focuses on in vitro investigations of the response of mouse MSCs to challenge with Escherichia coli. MSCs were obtained from the femoral bone marrow of B6D2F1/J mice. The MSC phenotype and proliferative activity were analyzed with flow cytometry and immunofluorescence imaging. MSCs (3x10^6) were challenged with 0.5x10^6 – 10x10^6 E.coli per ml for 1-5 h and analyzed for (i) colony-forming activities, (ii) production of reactive oxygen (ROS) and nitrogen species, (iii) activation of redox signaling, (iv) up-regulation of NF-kB, iNOS, and autophagy mechanisms, (v) expression of anti-bacterial alpha-defensin 4 (AD4), and (vi) damage to the genomic DNA using TUNEL assay, qRT-PCR, fluorescence confocal imaging, and transmission electron microscopy. The antibacterial defense response of MSCs was characterized by increases in nuclear translocation of NF-kB, induction of iNOS and AD4, and production of ROS and NO that were corroborated with profound (over 50%) inhibition of E.coli proliferation. The extended E.coli/MSC interaction led to the bacterial phagocytosis, oxidative damage and further inactivation via macroautophagy mechanism. The above effects were accompanied by a compensatory antioxidant response of MSCs mediated by nuclear translocation of nuclear factor (erythroid-derived 2)-like 2 and thioredoxin 2. The collective results suggest that MSCs can contribute to the innate response to bacterial infection (Supported by NIH/NIAID Y1-AI-5045-04).