These abstracts also are available via the SOT Event App and the Online Planner. All late-breaking abstracts are presented on Thursday, March 31, 8:30 am–11:30 am.
Preface

This issue is devoted to the abstracts of the Late-Breaking Poster Sessions of the 61st Annual Meeting of the Society of Toxicology, held at the San Diego Convention Center, San Diego, California, March 27–31, 2022.

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### Thursday, March 31, Poster Session by Location

<table>
<thead>
<tr>
<th>SESSION TITLE</th>
<th>ABSTRACT #s</th>
<th>POSTER BOARD #s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late-Breaking 1: Metals; Neurotoxicity; Respiratory Toxicology</td>
<td>5000–5019</td>
<td>P101–P120</td>
</tr>
<tr>
<td>Late-Breaking 2: Animal Models; Alternatives to Animal Models; Clinical Toxicology; Nanotoxicology</td>
<td>5020–5036</td>
<td>P123–P139</td>
</tr>
<tr>
<td>Late-Breaking 3: Exposure/Risk/Safety Assessment</td>
<td>5037–5054</td>
<td>P141–P158</td>
</tr>
<tr>
<td>Late-Breaking 4: Biological Modeling; Cardiovascular Toxicology; Computational Toxicology; Emerging Technologies</td>
<td>5055–5073</td>
<td>P161–P179</td>
</tr>
<tr>
<td>Late-Breaking 5: Carcinogenesis; Food Safety; Regulation/Policy; Reproductive and Developmental Toxicology</td>
<td>5074–5093</td>
<td>P182–P201</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>TOPIC</th>
<th>ABSTRACT #s</th>
<th>BOARD #s</th>
<th>SESSION TITLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air Pollution/Respiratory Toxicology</td>
<td>5000–5008</td>
<td>P101–P109</td>
<td>Late-Breaking 1</td>
</tr>
<tr>
<td>Alternatives to Mammalian Models</td>
<td>5023–5028</td>
<td>P126–P131</td>
<td>Late-Breaking 2</td>
</tr>
<tr>
<td>Animal Models</td>
<td>5020–5022</td>
<td>P123–P125</td>
<td>Late-Breaking 2</td>
</tr>
<tr>
<td>Biological Modeling</td>
<td>5056–5057</td>
<td>P162–P163</td>
<td>Late-Breaking 4</td>
</tr>
<tr>
<td>Biotransformation/Cytochrome P450</td>
<td>5068–5069</td>
<td>P174–P175</td>
<td>Late-Breaking 4</td>
</tr>
<tr>
<td>Carcinogenesis</td>
<td>5081–5084</td>
<td>P189–P192</td>
<td>Late-Breaking 5</td>
</tr>
<tr>
<td>Cardiovascular Toxicology/Hemodynamics</td>
<td>5070–5071</td>
<td>P176–P177</td>
<td>Late-Breaking 4</td>
</tr>
<tr>
<td>Clinical and Translational Toxicology</td>
<td>5033–5034</td>
<td>P136–P137</td>
<td>Late-Breaking 2</td>
</tr>
<tr>
<td>Computational Toxicology and Data Integration</td>
<td>5059–5064</td>
<td>P165–P170</td>
<td>Late-Breaking 4</td>
</tr>
<tr>
<td>Drugs of Abuse</td>
<td>5051–5054</td>
<td>P155–P158</td>
<td>Late-Breaking 3</td>
</tr>
<tr>
<td>Ecotoxicology</td>
<td>5018–5019</td>
<td>P119–P120</td>
<td>Late-Breaking 1</td>
</tr>
<tr>
<td>Emerging Technologies</td>
<td>5065–5067</td>
<td>P171–P173</td>
<td>Late-Breaking 4</td>
</tr>
<tr>
<td>TOPIC</td>
<td>ABSTRACT #s</td>
<td>BOARD #s</td>
<td>SESSION TITLE</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>----------------</td>
<td>--------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Epidemiology and Public Health</td>
<td>5089–5091</td>
<td>P197–P199</td>
<td>Late-Breaking 5</td>
</tr>
<tr>
<td>Exposure Assessment/Biomonitoring</td>
<td>5037–5038</td>
<td>P141–P142</td>
<td>Late-Breaking 3</td>
</tr>
<tr>
<td>Food Safety/Nutrition</td>
<td>5085–5087</td>
<td>P193–P195</td>
<td>Late-Breaking 5</td>
</tr>
<tr>
<td>Metals</td>
<td>5015–5017</td>
<td>P116–P118</td>
<td>Late-Breaking 1</td>
</tr>
<tr>
<td>Nanotoxicology, In Vivo</td>
<td>5029–5031</td>
<td>P132–P134</td>
<td>Late-Breaking 2</td>
</tr>
<tr>
<td>Neurotoxicity</td>
<td>5009–5013</td>
<td>P110–P114</td>
<td>Late-Breaking 1</td>
</tr>
<tr>
<td>Regulation/Policy</td>
<td>5092–5093</td>
<td>P200–P201</td>
<td>Late-Breaking 5</td>
</tr>
<tr>
<td>Reproductive/Developmental Toxicology</td>
<td>5074–5078</td>
<td>P182–P186</td>
<td>Late-Breaking 5</td>
</tr>
<tr>
<td>Risk Assessment</td>
<td>5039–5042</td>
<td>P143–P146</td>
<td>Late-Breaking 3</td>
</tr>
<tr>
<td>Safety Assessment: Non-pharmaceutical</td>
<td>5043–5046</td>
<td>P147–P150</td>
<td>Late-Breaking 3</td>
</tr>
<tr>
<td>Safety Assessment: Pharmaceutical</td>
<td>5047–5050</td>
<td>P151–P154</td>
<td>Late-Breaking 3</td>
</tr>
</tbody>
</table>
ABSTRACT NUMBER: 5000    Poster Board Number: P101
TITLE: Effect of Combustion Particle Shape on Biological Responses in a Co-culture of Human Lung (A549) and Macrophage (THP-1) Cells


KEYWORDS: Particulates; Apoptosis; Cytochrome P450; Aerosol shape, Atmospheric aging, ALI exposure

ABSTRACT: The atmospheric aging of combustion particles alters both their chemical composition and shape. Fresh combustion particles are fiber-like, and as they age in the atmosphere, they become more compact and spherical. Previous studies with fresh and aged combustion particles have linked the changing chemical composition to the observed differences in toxicological response. However, little is known about the contribution of difference in shape in atmospherically aged particles to their toxicological response, possibly due to the difficulty in resolving the two properties (composition and morphology) that change simultaneously. This study altered the shape of lab-generated combustion particles’ shape without affecting the chemical composition, from fiber-like to a more compact spherical shape using a water condensation-evaporation method. Using an electrostatic field-based air-liquid exposure (ALI) chamber, the two shapes were exposed to a co-culture of human airway epithelial (A549) and differentiated human monocyte (THP-1) cells at ALI conditions. For the same mass dose, both shapes were ingested by cells and induced a pro-inflammatory response (IL-8 and TNFα) and enhanced CYP1A1 gene expression compared to air controls. The more compact spherical particles (representative of atmospherically aged particles) induced more early apoptosis and release of TNFα compared to the more fiber-like particles. The result suggests a contribution of morphology to the increased toxicity of aged particles.

ABSTRACT NUMBER: 5001    Poster Board Number: P102
TITLE: Source-Specific Particle Matter Chemical Characteristics Influences RAW 264.7 Macrophage Immune Function and Cell Cycle Progression


KEYWORDS: Macrophage; Cell Culture; In Vitro and Alternatives

ABSTRACT: Ambient and household air pollution is a major health problem worldwide, contributing to cardiopulmonary diseases and shorter life expectancies. Studies focusing on source-specific particulate matter (PM) have demonstrated that particle chemistry differences can moderate cellular processes that promote disease. Macrophages are the first line of cellular defenses in the lung that initiate innate immune responses against foreign invaders and regulate tissue inflammation. Macrophages release cytokines and activate oxidative stress pathways when exposed to PM in vitro which can overwhelm normal antioxidant capacity and impair immune cell function. The goal of this study is to measure the oxidative potential (OP) of biomass PM generated from wood and dung combustion using traditional
Indian cookstoves and determine whether or not cells exposed to these particles are more susceptible to immunomodulation compared to cells exposed to urban PM (NIST standard reference material 1648a). We hypothesized that highly oxidative samples will inhibit normal immune defenses such as respiratory burst and will compromise cell cycle progression. PM extracts were used for dithiothreitol (DTT) oxidative potential assays. In-vitro exposures (4 hr exposures to 50µg/ml of PM) of cultured RAW 264.7 cells were used to assess cytotoxicity, superoxide burst capacity, and cell cycle changes. The cell-free DTT assays show that urban PM has a higher OP than biomass PM. The rate of DTT consumed was variable among the different biomass PM tested. Cell cycle aberrations measured with flow cytometry were compared against vehicle treated cells (VTC). VTC have 45% of cells in G0/G1 state and dung combustion exposed cells ranged from 46-49% in G0/G1 phase while urban PM treated cells increased to 60% of cells in G0/G1 phase. This suggests that exposure to PM with higher OP increased the % of cells in cell cycle arrest or quiescent state. Apoptosis and cell death following in vitro exposures was measured using an Annexin V flow cytometry kit; there were negligible cell death. All cells showed reduced superoxide burst capacity even 12-hours following a single 4-hour PM exposure (National Diagnostics Diogenes chemiluminescent superoxide enhancer kit). These findings indicate that source-specific characteristics can induce sub-lethal immunomodulatory damage and induce cell cycle arrest and quiescence, which may contribute to disease-promoting mechanisms.

ABSTRACT NUMBER: 5002  Poster Board Number: P103
TITLE: Inhalation of Iron Nanoparticles and Sex-Dependent Brain Volumetric Changes Measured by 3D Magnetic Resonance Imaging and Light Sheet Microscopy
AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: E. Marvin1, K. Hornburg2, K. Conrad1, A. Merrill1, D. Chalupa1, R. Gelein1, G. Oberdorster1, A. Hall3, G. Johnson2, D. Cory-Slechta1, and M. Sobolewski1. 1University of Rochester Medical Center, Rochester, NY; 2Duke University, Durham, NC; and 3LifeCanvas Technologies, Cambridge, MA.

KEYWORDS: Nanoparticles; Neurotoxicity; Metals; Inhalation Toxicology; Iron Oxide

ABSTRACT: Iron (Fe) is a frequent contaminant of air pollution particulate matter. While Fe is requisite to brain development and function, excess Fe is neurotoxic and mechanisms to eliminate Fe from brain are not evident. A recent study (Luglio et al., 2021) reported extremely high Fe concentrations in PM2.5 in subway stations in the northeastern U.S. To assess the impact of such exposures (averaging 200 ug/m³) on brain, adult mice were exposed to ultrafine Fe oxide at 100 ug/m3 for 2 hr/day for 20 days. Subsequently, MR histology was performed on post-mortem specimens collected 48 hr post exposure using a 7T/Agilent MRI system designed to acquire 3D whole brain images @ 200,000 X the resolution of a clinical scanner. Images were acquired with the brain in the skull to limit geometric distortion. Automated processing pipelines delineated 180 subregions in each hemisphere, determined their volumes and measured the scalar diffusion properties (axial diffusivity (AD), radial diffusivity (RD) and fractional anisotropy (FA) - measures of tissue cytoarchitecture. Volumetric changes were sex-dependent. In Fe-exposed males, significant volumetric reductions were found in the trigeminal nerve, medial and lateral amygdalar nuclei, optic tract and chiasm and anterior olfactory nucleus, while in Fe-exposed females, reductions were found in the medial geniculate complex, the inferior olivary complex, nucleus of the solitary tract, tuberal nucleus, posterior commissure and medullary reticular zone. In both sexes, measures of diffusivity (white matter microstructure) identified olfactory bulb and hippocampal...
reductions in males, whereas increases were seen in olfactory bulb in females. In summary, these findings are consistent with olfactory and trigeminal nerve uptake and translocation of inhaled Fe ultrafine particles into brain with early targets including olfactory bulb, amygdala, brain stem and hippocampus. Supported by R35 ES031689-01A1, R01 ES032260 and P30 ES001247.

ABSTRACT NUMBER: 5003 Poster Board Number: P104
TITLE: Sex Differences in the Lung Microbiome in a Mouse Model of Allergic Asthma

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: R. Alford, and P. Silveyra. Indiana University Bloomington, Bloomington, IN.

KEYWORDS: Microbiome; Lung; Pulmonary or Respiratory System; Other: Sex Differences

ABSTRACT: Asthma is a chronic inflammatory disease of the airway that leads to compromised lung function and affects more than 300 million patients worldwide. Research shows a clear involvement of environmental components in the induction and exacerbation of asthma. Disparities in sensitivity between men and women to these factors have also been identified; however, it remains unclear whether mediators such as the microbiome contribute to such differences. To understand the mechanisms associated with previously observed sex disparities in allergen-induced asthma, we used a mouse model of house dust mite (HDM)-induced asthma. Our preliminary findings showed sex differences in respiratory mechanics and activation of the immune response following HDM challenge. In the present study, we tested the hypothesis that the lung microbiome promotes different disease phenotypes in mice challenged with HDM. For this, we analyzed the 16S microbiome in DNA extracted from whole lung tissue of male and female C57BL/6 mice exposed to HDM or phosphate buffered saline (PBS) for 5 weeks (n=3/group); finding that males had a higher relative abundance of Firmicutes and other bacteria than females at basal levels, but this pattern was reversed with HDM challenge. In addition, the relative ratio of Firmicutes:Bacteroidetes was similar for both sexes in PBS-treated animals, but was almost twice as high in males than in females (4.2 vs 2.24, respectively) in mice challenged with HDM for 5 weeks. Moreover, females treated with HDM displayed higher bacterial diversity and relative abundance of Proteobacteria when compared to controls, an effect that was not observed in males. We conclude that sex-specific changes in lung microbial communities contribute to the mechanisms underlying differential asthma phenotypes in males vs. females.

ABSTRACT NUMBER: 5004 Poster Board Number: P105
TITLE: Effects of Melatonin and Vaped Melatonin Aerosols on Bronchial Epithelial Cell Mucin Secretion in an In Vitro Model of Asthma


KEYWORDS: Respiratory Toxicology; Toxicity; Acute; Inhalation Toxicology; Melatonin

ABSTRACT: Non-tobacco vaping products designed to deliver aerosols containing vitamins and nutraceuticals, such as melatonin, vitamin B12, and caffeine are growing in popularity. Manufacturers of these products often claim they provide health benefits; however, there is a paucity of data regarding the pulmonary and overall health effects of inhalation exposures. Melatonin vapes are advertised as
safe and effective sleep aids that provide a faster time-to-effect than orally administered formulations. Recent *in vitro* studies using lung cell lines and *in vivo* studies using murine models report that melatonin suppresses expression of proinflammatory cytokines and Mucin 5AC (MUC5AC), a gel-forming mucin that is aberrantly overexpressed in some lung diseases, including asthma and COPD. However, the pulmonary effects of melatonin inhalation in the context of vaping are currently unknown. Here, we assessed the acute toxicity of emissions produced by a commercially-available melatonin vape on an organotypic model of the human lung. Additionally, the ability of melatonin or vaped melatonin aerosols to attenuate mucin hypersecretion in an *in vitro* model of asthma was investigated. Primary human bronchial epithelial cells (HBECs; n=1 donor) were differentiated at an air-liquid interface (ALI) to a mucociliary phenotype. Prior to melatonin exposure, HBECs were treated with IL-13 for 5 days to induce an asthma-like phenotype. Cells were then treated apically with melatonin (100uM) or 40 puffs from a commercial melatonin vape. Cultures were lysed after 24 hours to assess MUC5AC and MUC5B gene expression. Cytotoxicity and inflammatory responses were quantified by measuring lactate dehydrogenase and proinflammatory cytokine levels in the basolateral medium. Exposure of HBECs to apical melatonin decreased IL-13-induced MUC5AC expression by 11.97-fold; however, exposure to vaped melatonin aerosols increased expression by 3.38-fold and decreased MUC5B expression in non-IL-13-treated cells by 0.36-fold. Neither melatonin nor melatonin vape exposures induced cytotoxicity or secretion of IL-8, IL-6, IL-1β, or TNF-α over levels measured in the control. These data demonstrate that acute melatonin treatment reduces mucin levels in a primary HBEC model of asthma. Thus, additional studies investigating the therapeutic potential of inhaled melatonin and melatonin vapes for reducing mucus hypersecretion in lung diseases are warranted.

**ABSTRACT NUMBER:** 5005  
**Poster Board Number:** P106  
**TITLE:** Development and Characterization of Rotary and Rotary-Like Smoke Machine Collection Methods for Heated Tobacco Product Aerosol Sample Collections for *In Vitro* Toxicology Testing  

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** M. Scian, P. Kosachevsky, C. Sovick, H. Maines, K. Brooks, and B. Coffa. *Enthalpy Analytical LLC, Richmond, VA.*

**KEYWORDS:** Tobacco Products; *In Vitro* and Alternatives; HTP, Toxicology, Condensate Collection, Aerosol

**ABSTRACT:** Due to the reduced harm nature of Heated Tobacco Products in comparison to combustible cigarettes, the generation of sufficiently concentrated extract is necessary. Condensate testing in *in vitro* toxicology assays is limited by OECD guidelines by the maximum allowable solvent levels that can be used, as well as the concentration of the generated condensate. Here we describe two HTP aerosol collection methods using commercially available IQOS devices (v2.4) and Marlboro Amber heatsticks. The first method involved collection of HTP aerosol using a Borgwaldt RM20E rotary smoke machine. A total of 80 sticks were smoked under Health Canada Intense conditions in 60-90 minutes. Custom sized 30 mm CFPs were placed immediately after the IQOS devices for collection of TPM. Gas phase components were collected using two in-line impingers containing 30 mL of PBS each. The use of additional impingers affected the puff profile. The second method involved collection of HTP aerosol using a Burghardt dual syringe puff engine connected to a custom-made 10-port automated adapter. As with the first method, a total of 80 sticks were smoked and custom sized 30 mm CFPs were used. Gas phase components were collected using three in-line impingers containing 30 mL of PBS each. One
impinger was placed before the dual syringe and two were placed behind it to avoid changes in the puff profile. In both methods, CFPs were extracted with DMSO to yield a final concentration of 200 mg/mL. Following extraction, the DMSO was filtered using centrifugal filters. DMSO extracts were analyzed for glycerol, nicotine, water and carboxyls. PBS extracts were analyzed for glycerol, nicotine, and carboxyls. DMSO extraction efficiency was measured by re-extracting the CFP. In both methods, >85% of the glycerol and nicotine were captured in the first DMSO extraction. No nicotine or glycerol was detected in the first impinger contents. Approximately 37% of the total formaldehyde was detected in the CFP (first extraction) and 53% in the first impinger in both methods. Acetaldehyde and acrolein were primarily detected in the PBS. With both methods, ~40-65% and ~30-42% of the analytes were detected in the first and second impingers respectively. Both collections were completed within a reasonable amount of time (60-90 min) and resulted in concentrated extracts for in vitro toxicology assessment of Heated Tobacco Products.

ABSTRACT NUMBER: 5006  Poster Board Number: P107
TITLE: Male Reproductive Toxicity of PFNA: New Additions to California’s Proposition 65 List
AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: P. Iyer1, Y. Niknam2, L. Li1, M. Campbell2, F. Moran1, A. Kim1, F. Kaufman1, and M. Sandy2. 1California Environmental Protection Agency, Sacramento, CA; and 2California Environmental Protection Agency, Oakland, CA.
KEYWORDS: Perfluoronated Agents; Reproductive Tract; Male; Regulatory/Policy; Proposition 65; PFNA
ABSTRACT: California’s Developmental and Reproductive Toxicant Identification Committee (DARTIC) for Proposition 65 (Safe Drinking Water and Toxics Enforcement Act) determined that Perfluorononanoic acid (PFNA) and its salts has been clearly shown through scientifically valid testing according to generally accepted principles to cause male reproductive toxicity. PFNA is a persistent environmental pollutant and is detected in virtually all Californians. To support the DARTIC’s deliberations, OEHHA reviewed available epidemiologic and animal toxicology studies, as well as mechanistic evidence relevant to the biological plausibility of observed apical outcomes resulting from PFNA exposure. Dose-dependent reductions in epididymal and testis weight and histopathological changes in the testis of rats and mice (germ cell degeneration, cytoplasmic vacuolization in Sertoli cells, and interstitial cell atrophy) were observed. Dose-related reductions in sperm count, sperm motility and viability were reported in studies of rats or mice treated with PFNA. At dosing levels that caused histopathological lesions, PFNA also reduced serum testosterone levels in rats and mice. Prenatal treatment with PFNA reduced intratesticular levels of testosterone with an apparent reduction in the Sertoli cell population in the testis in neonatal mice. While potential mechanism(s) underlying the testicular effects of PFNA remain to be determined, changes in gene and protein expression of several steroidogenic regulatory factors (e.g., steroidogenic acute regulatory protein (StAR)) and steroidogenic enzymes in mice and zebrafish were reported. Also changes in receptor function (e.g., androgen, estrogen) were reported. PFNA has been shown to interfere with thyroid hormone binding, serum levels, and function using in vivo, in vitro, and in silico test systems. The use of key characteristics to efficiently identify, organize, and summarize data from mechanistic studies, was also explored. Epidemiologic evidence in humans for effects on developmental landmarks of the male reproductive system, serum testosterone levels, and sperm quality was mixed, although some associations with decreases in serum testosterone in young boys and adolescents have been reported. PFNA and its salts are now listed under Proposition 65.

The Toxicologist: Late-Breaking Supplement 2022
ABSTRACT NUMBER: 5007  Poster Board Number: P108

TITLE: Relative Characterization of Biological Responses to Perfluoroalkyl Substances (PFAS) and Aqueous Film-Forming Foams with In Vitro Hepatocyte Transcriptomics


KEYWORDS: Persistent Organic Chemicals; Perfluorinated Agents

ABSTRACT: Aqueous film-forming foams (AFFF) are utilized to extinguish flammable liquid fires. Per- and poly-fluorinated chemicals substances (PFAS) grant these formulations beneficial properties to protect firefighters and resources. However, these chemicals are a cause for concern due to their environmental persistence and risk to human health (e.g., liver toxicity). We have conducted high-throughput transcriptomic screening of 5 PFAS-containing AFFFs, alongside 25 additional reference chemicals (e.g., liver injury, nuclear receptor activators), in human hepatocytes (HepaRG). Initial dose-range finding guided selection of exposure concentrations (ATP Depletion). Subsequent hepatocyte transcriptomic assays (S1500++, TempO-Seq), previously qualified to estimate the potencies for human liver injury using clinical drugs, were performed and analyzed using BMDExpress 2.3. Test substances were classified as liver-injury or non-injury for the exposure range examined. Twenty of the thirty tested compounds produced benchmark concentrations (BMCs) greater than 105, which serve as screening-level estimates for biological activity in human liver. This enabled relative characterization of biological activities. Longer carbon chain PFAS were more potent than shorter chain PFAS, AFFFs, and non-fluorinated surfactants, which is consistent with emerging understanding of PFAS toxicity. PFAS have been linked with hepatic receptor pathways PPARα and CAR with established associations to liver cancer. Transcriptomic data were analyzed for modulation of PPARα and CAR pathways, and BMC-normalized efficacies were tabulated for comparisons. AFFFs displayed moderate-to-low proficiency to alter PPARα and CAR pathways when compared with reference chemicals such as PFOA and PFOS, respectively. Biological response similarity analysis was performed using hierarchical clustering for the identified transcriptomic pathways across the 30 test substances evaluated. These data revealed important insights for chemical similarity efforts, and enhance our understanding of the toxicity potential of AFFFs.

ABSTRACT NUMBER: 5008  Poster Board Number: P109

TITLE: Occupational Jet Fuel Exposure Is Related to Compensation Claims for Neurological Outcomes in Air Force Veterans

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: I. B. H. Samuel1,2, G. Wolff3, A. J. Werner2, J. Escobar1, A. Schneiderman2, K. Hancock1, and T. D. Vincent2. 1Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD; 2Department of Veterans Affairs, Washington, DC; and 3US Air Force School of Aerospace Medicine, Wright-Patterson AFB, OH.

KEYWORDS: Nervous System; Inhalation Toxicology; Volatile Organic Compound(s)

ABSTRACT: Exposure to jet fuels is a commonly experienced occupational hazard in military service. Although the acute effects of jet fuel exposure are well-documented, less is known about the long-term effects. The Department of Veterans Affairs and the US Air Force School of Aerospace Medicine
investigated associations between occupational exposure to jet fuels during service in the Air Force and claims submitted for compensation due to adverse health outcomes. The study cohort (N=312,903) included individuals who served from 1995-2019 that had occupation codes for jobs with or without expected exposure to jet fuels. Exposure duration was calculated by summing time-in-job for all jobs with potential exposure. Logistic regression was used to determine claims pursuit for the entire cohort. Subsequent analyses used logistic regression and general linear models to determine relationships between jet fuel exposure and claims for neurological conditions. In general, the exposed group pursued claims more than the unexposed group (odds ratio: 1.22). For individuals that filed at least one claim, the rate of claim pursuit for a neurological condition was similar for the exposed and unexposed groups. The high exposure group had a significantly higher claim grant rate compared to the unexposed group. Further, there was a positive, linear relationship between the relative exposure level and combined percent of disability. These findings suggest that occupational jet fuel exposure may be associated with adverse health outcomes in a population of Air Force Veterans that filed for compensation claims. It is important to note that various factors can influence both a veteran's decision to submit a claim, as well as claim outcomes. Thus, future studies are needed to further investigate these relationships using healthcare data to better inform policy on care and compensation for health outcomes related to jet fuel exposure, as well as improvement of preventive measures.

ABSTRACT NUMBER: 5009    Poster Board Number: P110
TITLE: Prolonged Neurologic Injury in Rats after Chlorine Gas Inhalation


KEYWORDS: Inhalation Toxicology; Neurotoxicology; Behavioral

ABSTRACT: Chlorine gas is a toxic inhaled chemical that is usually thought to cause respiratory defects after exposure. Recent reports have suggested injury may also occur to the nervous system, with neuromuscular and neurocognitive defects, and possible seizures after acute inhalation. In this study, we aimed to study the acute and long-term neurological impacts in a rodent model of high dose chlorine exposure. We exposed awake Sprague Dawley rats (300-350 g, n=19) to chlorine gas (LD$_{50-6h}$) in a whole-body inhalation chamber, and followed rats for 28 days. Video electroencephalography (EEG) was used to evaluate brain electrical activity (power) immediately after exposure and every 7 days. Adhesive Removal Test and Accelerated Rotor-Rod Tests were performed to assess sensorimotor coordination and motor learning every 7 days. Oxygen saturation and respiratory rate were evaluated over 28 days. We found that brain activity in rats by EEG power analysis was decreased to 45% of baseline at 30 min after chlorine exposure, and that recovery of brain activity over 28 days was slow and incomplete (at 88% of baseline EEG power at 28 days; p<0.001). Similarly, we found significant sensorimotor coordination and motor learning impairments in chlorine-exposed rats, both acutely and during the 28-day recovery period. Latency to fall was significantly decreased immediately after chlorine exposure (31 vs. 221 seconds, chlorine vs. naïve; p<0.0001), with great improvement to day 7 (208 vs. 291 seconds, chlorine vs. naïve; p=0.0015), but without additional improvements from day 7 to 28. Adhesive Removal was similarly impacted, with time to removal of right paw adhesive greatly increased immediately after chlorine (115 vs. 35 seconds, chlorine vs. naïve; p<0.0001), but with complete recovery by day 7 (15 vs. 9
ABSTRACT NUMBER: 5010    Poster Board Number: P111
TITLE: Bisphenol A Modulates Leptin Signaling Pathways in Human Neuroblastoma Cells

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: I. F. Ngoka. University of Maryland Eastern Shore, Princess Anne, MD.

ABSTRACT: Bisphenol A (BPA) is an endocrine disrupting chemical and is one of the most utilized industrial chemicals worldwide. Early life exposure to BPA is known to result in various adverse health effects. Several studies have shown that BPA increased the risk of metabolic disorders and obesity by changing the endocrine-metabolic pathways in adipose tissue. Obesity occurs as a result of genetic, environmental, behavioral, social, and economic factors. Increased intake due to excessive consumption of palatable food has contributed to the rise of obesity. It is well established that the hypothalamus of the central nervous system plays a central role in regulation of energy balance and food intake to maintain the body’s physiological requirements. An extensive body of evidence has demonstrated that endocrine regulator such as leptin mainly acts on the hypothalamus to regulate food intake and body weight. In addition, expression of leptin receptors in other regions of the brain such as the dopaminergic (DA) neurons suggests that the leptin signaling can act on other brain regions to mediate the reward value of nutrients. Recent studies have indicated the midbrain dopaminergic neurons as a potential site for leptin’s action on mediating the feeding behaviors and therefore affecting the energy balance. Emerging evidence support the hypothesis that environmental contaminant exposures, particularly those occurring in early-life, may interfere with homeostatic control and induce or exacerbate obesity. The aim of this study was to investigate the effects of BPA on leptin signaling pathway in human neuroblastoma SH-SY5Y cells. Cells were treated with leptin or BPA at various concentrations alone or in combination. Cell viability, Leptin levels and level of signal transducer and activator of transcription (STAT3) were assessed. Low concentration (0.01-50 µM) of BPA showed no significant effect in cell viability. Exposure to 0.1-100 nM of leptin led to concentration-dependent increase in coupling of leptin receptor with activation of STAT3. NSC74859, a selective STAT3 inhibitor, blocked this activation by leptin. BPA inhibited the leptin-induced STAT3 phosphorylation in a concentration-dependent manner. SB202190, a p38 mitogen-activated protein kinase (MAPK) inhibitor, partially blocked the BPA inhibition of leptin-induced STAT3 activation. These results suggest that BPA concentration-dependently inhibit leptin action, in part via p38 MAPK. Supported by Title III.
**ABSTRACT:** Phenotypic analysis of small model organisms such as *Caenorhabditis elegans*, *Drosophila*, and zebrafish are gaining attention due to their use in rapid screens and translatable screening outcomes to humans. Among these small models, *C. elegans* is amenable to high-throughput and high-content screening that recapitulates sub-lethal toxicology phenotypes found in higher animal models. To facilitate *C. elegans* based screening, we have developed a microfluidic technology to immobilize multiple populations of *C. elegans* and enable high-resolution imaging in various formats including 96-well, 64-well, and 384-well formats. Here, we demonstrate the use of the 64-well chip, with 4.5 mm well-to-well spacing in an ultra-high-density format, to immobilize up to 1,920 animals in 3 min. The high trapping efficiency and high-resolution imaging enable high-content phenotyping of cellular and subcellular features. We demonstrate the use of the microfluidic device for fast screening using multiple neuronal strains labeled with fluorescent proteins. To demonstrate the use of our robust immobilization, we carried out high-content screening of a pan-neuronal protein aggregation model, which expresses 67 repeats of poly-glutamine proteins (PolyQ67) in all the neurons under the control of the promoter for F25B3.3. The animal shows age-dependent localization of fluorescence signal and forms discrete foci, which are evident in the head ganglion and ventral cord regions of the *C. elegans*.

We treated the animals with dronedarone hydrochloride, which was shown to have protein homeostasis activity in the body wall muscles, to characterize its role in maintaining fluorescence signals from the retrovesicular ganglion. We found a 10% increase in the size with the detectable fluorescence intensity when the animals are treated with 50 µM dronedarone as compared to the vehicle control. The repeatable orientation of the animals enabled us accurately to measure the length of the animals to be 785.5 ± 11.14 µm and 426.2 ± 12.36 µm for the animals treated with 50 µM dronedarone and vehicle, respectively. Using the microfluidic chip we were able to identify all 26 GABAergic motor neurons that are labeled with unc-25::gfp at single-neuron resolutions for toxicology studies. We characterized the motor neuron degeneration in the animals when treated with methyl mercury and rotenone in a dose-dependent manner for toxicology outcomes.

**ABSTRACT NUMBER:** 5012  
**Poster Board Number:** P113  
**TITLE:** Developmental Neurotoxicity of Brominated and Organophosphorus Flame Retardants Using 3D hiPSC-Derived Brain Spheres  
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**KEYWORDS:** Neurotoxicity; Developmental; In Vitro and Alternatives; Induced Pluripotent Stem Cells; Flame Retardants  
**ABSTRACT:** Flame retardants (FRs) are suggested to be developmental neurotoxicants and might contribute to the increase in the prevalence of neurodevelopmental disorders. FRs are extensively used chemicals in consumer products, including children’s products such as toys, and are therefore a potential health concern. This study aimed to evaluate FRs (brominated and organophosphorus) potential to induce developmental neurotoxicity in a human 3D in vitro model (brain spheres). The model consists of different cell types of the central nervous system (CNS) such as neurons, astrocytes,
and oligodendrocytes and has shown to be relevant for key cellular processes involved in neurodevelopment. 3D induced pluripotent stem cell (NIBSC8) derived brain spheres were exposed for 7 days to 2,2',4,4'-tetrabromo diphenyl ether (BDE-47: 1 µM, 10 µM, 20 µM), Triphenyl phosphate (TPHP: 1 µM, 10 µM, 20 µM), Isopropylated phenyl phosphate (IPP: 1 µM, 10 µM, 20 µM) and Tetrabromobisphenol A (TBBPA: 0.01 µM, 0.1 µM, 1 µM, 10 µM) at 8 weeks of differentiation. Non-cytotoxic concentrations were, determined by no significant difference in the percentage cell viability of the brain spheres exposed to these FRs vs. solvent controls [dimethyl sulfoxide 0.1%(v/v)] using resazurin assay. qRT-PCR was performed for selected non-cytotoxic concentrations to evaluate gene expression of markers for neurons (Btub3), synapse formation (Syn1), proliferation (Ki67), and oligodendrocytes (PLP1, CNP, MBP). Several of the genes evaluated had altered expression after exposure to these flame retardants and indicate developmental neurotoxic effects in the 3D brain sphere model. The oligodendrocyte markers (CNP and PLP1) and marker for synapse formation (Syn1) were especially affected with upregulated (CNP and PLP1) and downregulated (Syn1) expression in a dose-dependent manner. These results indicate that synapse formation and oligodendrocytes differentiation and maturation are affected by exposure to these flame retardants in concordance with previous studies using an in vitro testing battery for the assessment of developmental neurotoxicity. Immunohistochemistry and z-stack confocal imaging are currently being performed to confirm these effects.

ABSTRACT NUMBER: 5013  Poster Board Number: P114
TITLE: Glyphosate Crosses the Blood-Brain Barrier: A Driver of Neuroinflammation and a Potential Risk Factor for Alzheimer’s Disease

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KEYWORDS: Neurotoxicity; Pesticides; In Vivo Models; Pesticides; Alzheimer’s Disease; Glyphosate

ABSTRACT: Herbicides are environmental contaminants that have gained much attention due to the potential hazards they pose to human health. Glyphosate, the active ingredient in the broad-spectrum herbicide Roundup, is the most heavily applied herbicide worldwide. The recent rise in glyphosate application to corn and soy crops has a strong, positive correlation with increased death rates due to neurodegenerative disorders such as Alzheimer’s disease and other forms of dementia. Glyphosate has been shown to cross the blood-brain barrier (BBB) in vitro but has yet to be verified in vivo. Additionally, reports have shown that glyphosate exposure increases pro-inflammatory cytokines in the periphery, particularly Tumor Necrosis Factor alpha (TNFα), which can induce detrimental cellular outcomes. Here, we examine whether glyphosate infiltrates the brain and elevates TNFα levels in two cohorts of male and female 4-month-old C57BL/6J laboratory mice. Mice received either 125, 250, 500 mg/kg/day of glyphosate or a vehicle control via oral gavage for 14 days. Brain tissue analysis revealed that glyphosate infiltrated the brain in a dose dependent manner. Brain glyphosate exposure correlated positively with urine glyphosate levels. We also found upregulation of TNFα in both blood plasma and brain tissue post glyphosate exposure. Notably, brain and urine glyphosate measures correlate with brain and blood TNFα. In vitro application of glyphosate to primary cortical neurons derived from the APP/PS1 mouse
A model of Alzheimer's disease showed elevated soluble Aβ40 and Aβ42 production at 40μg/mL as well as increased cytotoxicity. In addition, RNA sequencing revealed a number of genes which were dysregulated in a dose-dependent manner, and significantly expressed in oligodendrocytes and GABAergic neurons. Collectively, these results show that glyphosate can reach the brain and increase the expression of TNFα, suggesting that exposure to this herbicide may have detrimental outcomes regarding the health of the general population.

**ABSTRACT NUMBER:** 5014  **Poster Board Number:** P115

**TITLE:** Structure-Based Refinement of Uncharged, Bis-Oxime Antidotes against Organophosphate Intoxication: Acetylcholinesterase-Templated Design, Synthesis, and In Vitro Characterization

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**KEYWORDS:** Organophosphates; Neurotoxicity; Pesticides; Protein Structure; Bis-Oxime Reactivator

**ABSTRACT:** Hydrolytic degradation of acetylcholine, catalyzed by human acetylcholinesterase (hAChE; EC 3.1.1.7) is blocked by covalent binding of organophosphates (OPs) to the active Ser203. Exposure to OP pesticides and nerve agent OPs can lead to states of serious cholinergic intoxication and death. Life-saving therapeutic recovery of hAChE activity is currently achieved by nucleophilic aldoxime antidotes based on pyridinium structural scaffold. Unlike the offending OPs that distribute in exposed tissue and diffuse across biological membranes, including the blood-brain-barrier (BBB), the pyridinium-based oxime antidotes remain in peripheral circulation, unable to cross BBB and reactivate OP-inhibited hAChE in the CNS. We have previously developed a series of non-pyridinium, uncharged bis-oxime reactivators with promising 

The Toxicologist: Late-Breaking Supplement 2022
TITLE: Electronic Cigarette Exposure Increases Antioxidant Response Genes in Mouse Lungs


KEYWORDS: Metals; Lung; Pulmonary or Respiratory System

ABSTRACT: Recently, there has been a rapid rise in electronic cigarette (e-cig) use, specifically among youth. Several studies have shown that e-cig can induce cell death, oxidative stress, oxidative damage, and altered metabolic activity. However, what specific components and mixtures in e-cig aerosols cause these health effects are still unknown. Studies have pinpointed flavorings and metals as cytotoxic components. Metals have been found at concentrations that would expose e-cig users to levels that exceed regulatory standards. Ethyl maltol, a particular flavoring of interest, is found in approximately 50%-80% of e-liquids and has been demonstrated to facilitate metal uptake into cells. We investigated the effect of ethyl maltol and metals on electronic cigarette toxicity in a mouse model. Mice (8-10 weeks old) were exposed to one of the following 5 conditions: room air, Propylene Glycol/Vegetable Glycerin (PG/VG), PG/VG+nicotine, PG/VG+ethyl maltol, and PG/VG+ethyl maltol and nicotine (n=6 per exposure). Mice were exposed 5 hours a day, 5 times a week for 2 weeks in a whole-body exposure chamber using the popular Suorin air pod device. One day after the last exposure organs were harvested, airway function was assessed with a FlexiVent, and the diffusion capacity of carbon monoxide was evaluated. Aerosol into each chamber was collected and analyzed for metals using ICP-MS. Mice in the ethyl maltol exposure group had increased compliance at baseline and increased total bronchial alveolar lavage cells. mRNA was extracted from harvested lungs, and real-time PCR was performed for genes in the nuclear factor-erythroid factor 2-related factor 2 (Nrf2) antioxidant defense pathway. An increase in Nrf2 and glutathione-s-transferase mRNA was observed in ethyl maltol and nicotine exposed mice. Increases in nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 1 mRNA levels were observed in ethyl maltol exposed mice. Lastly, increases in glutathione peroxidase 2 and catalase were observed in mice exposed to nicotine only. These results indicate that e-cig aerosols with ethyl maltol or nicotine can increase oxidative stress.

ABSTRACT: Intestinal Toxicity of a Chronic Exposure to Inorganic Arsenic


KEYWORDS: Inflammation; Microbiome; Barrier Impairment; Inorganic Arsenic

ABSTRACT: Chronic exposure to inorganic arsenic [As(III) and As(V)] affects about 200 million people, and is linked to a greater incidence of certain types of cancer and other pathologies such as diabetes type II and cardiovascular diseases. Drinking water is the main route of exposure, so, in endemic areas, the intestinal mucosa is constantly exposed to the metalloid. In vitro studies have shown an adverse effect of inorganic As on intestinal epithelial cells. Studies in rodents have shown effects of As(III) on the
The objective of this study was to evaluate the toxicity of a chronic exposure to As(III) and As(V) on the intestinal mucosa and its associated microbiota. For this purpose, BALB/c mice were exposed during 6 months through drinking water to As(III) (15 and 30 mg/L) and As(V) (15, 30 and 60 mg/L). Both As species increased reactive species (ROS/RNS), being greater the pro-oxidant response caused by As(III) (43-64%). A pro-inflammatory response was also observed, evidenced by an increase of fecal lactoferrin [As(III): 23-29%, As(V): 13-34%] and a higher neutrophil infiltration of intestinal mucosa and submucosa. As(III) treated mice also showed an increase of the colonic levels of pro-inflammatory cytokines (IL-1β: 101-201%, IL-2: 97-166%, TNFα: 24-94%). Moreover, exposure to As, especially to As(III), produced an hyperplasia of goblet cells, affecting the mucus production. Both treatments also affected intestinal microbiota metabolism with reductions of fecal concentration of short chain fatty acids. Moreover, As(III) exposure resulted in changes in gut microbial alpha diversity but no differences in beta diversity. This suggested that the abundance of some taxa was significantly affected by As(III), although the composition of the population did not show alterations. Analysis of differential taxa agreed with this, 21 ASVs were affected in abundance or variability, specially ASVs from family Muribaculaceae. The effects observed on different components of the intestinal barrier may be responsible of the impaired permeability, evidenced by an increase of fecal albumin in As(III) treated mice (48-66%). The disruption of intestinal barrier could be one of the causes of the systemic diseases observed in population chronically exposed to inorganic As.
intestine was also evidenced at all concentrations of Hg(II) and MeHg, with increased levels of reactive species (ROS/RNS: 19-37%) and lipid peroxidation (14-37%). Intestinal permeability was also affected by Hg exposure, as evidenced by a higher content of albumin in feces at all concentrations of Hg(II) (27-59%) and at the highest dose of MeHg (43%), which is an indicative of intestinal barrier disruption. Structural studies of large intestine showed a shortening in the crypts of the mice exposed to both Hg species as well as mucus hypersecretion when animals were exposed to MeHg. Moreover, changes in fecal short fatty acids profile suggested alterations in intestinal microbiota metabolism.

ABSTRACT NUMBER: 5018       Poster Board Number: P119
TITLE: Functional Similarities and Differences in Vertebrate Drug Transporter Interactions with Transporter-Interfering Chemicals (TICs)

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KEYWORDS: Environmental Toxicology; Xenobiotic Transporters; Persistent Organic Chemicals

ABSTRACT: Marine pollutants bioaccumulate at high trophic levels of marine food webs and are transferred to humans through consumption of apex species. Yellowfin tuna (Thunnus albacares) are marine predators, and one of largest commercial fisheries in the world. Previous studies have shown that yellowfin tuna can accumulate high levels of persistent organic pollutants, including Transporter Interfering Chemicals (TICs), which are chemicals shown to bind to mammalian xenobiotic transporters and interfere with their function. We examined the extent to which these same compounds might interfere with the activity of the yellowfin tuna (Thunnus albacares) ortholog of this transporter. To accomplish this goal we identified, expressed, and functionally assayed tuna ABCB1. The results demonstrated a common mode of vertebrate ABCB1 interaction with TICs that predicts effects across these species, based on high conservation of specific interacting residues. Importantly several TICs showed potent inhibition of Ta-ABCB1, such as the organochlorine pesticides Endrin (EC₅₀ = 1.2 ± 0.2 μM) and Mirex (EC₅₀ = 2.3 ± 0.9 μM). However, unlike the effects observed on mouse Abcb1a, low concentrations of the organochlorine pesticide TICs p,p’-DDT and its metabolite p,p’-DDD co-stimulated verapamil-induced Ta-ABCB1 ATPase activity possibly suggesting a low transport activity for these ligands in tuna. These results provide a mechanistic basis for understanding the potential vulnerability of tuna to these ubiquitous pollutants.

ABSTRACT NUMBER: 5019       Poster Board Number: P120
TITLE: Identifying Photolocomotor Behavioral Response Profiles to a Protein Synthesis Inhibitor in Zebrafish across the Freshwater-to-Marine Continuum

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KEYWORDS: Aquatic Toxicology; Behavior; Ecotoxicology
ABSTRACT: For decades, behavior has been examined in fish models during ecology and ecotoxicology research, and then applied for diverse environmental diagnostic applications. Photolocomotor behavioral response profiles in the zebrafish model have emerged as useful endpoints during pharmacology and toxicology studies, as efforts to shift to alternative vertebrate models have increased. Here we examined how cycloheximide, commonly used in biomedical research to inhibit protein synthesis in eukaryotic cells in vitro, influences swimming activity of zebrafish and fathead minnows during interchanging photoperiods following previously reported methods. We followed common standardized regulatory guidelines for toxicity studies with zebrafish (OECD) and fathead minnow (US EPA) to facilitate the comparability of our findings to other studies. Zebrafish (4-6 hpf) and fathead minnows (<48 hr post hatch) were exposed for 96 hours to cycloheximide before photolocomotor responses were observed using behavioral analysis software. Behavioral observations occurred for 50 minutes, including 10 minutes of acclimation, two 10-minute dark periods, and two 10-minute light periods. Total locomotor behavior significantly reduced after exposure to cycloheximide during the dark phases (p < 0.05); however, no distinctive pattern emerged. Photomotor responses of zebrafish were significantly decreased during the dark phases (p< 0.10), with more pronounced responses observed during the second dark phase (p < 0.05). Because contaminants in surface waters may occur across the freshwater to marine continuum, we performed additional experiments to understand behavioral modifications across a salinity gradient. Locomotion significantly decreased after co-exposure at the highest combination of the two experimental factors during the dark phases (p <0.05). Further, for zebrafish, there were significant changes in photomotor behavior for all treatment levels (p <0.01) during the second dark phase, and at the highest concentration for the first dark (p <0.01) and second light phases (p <0.05). Ongoing work is examining transcriptomic responses to cycloheximide across the salinity gradient, and aims to inform future research of environmental contaminants, including cyanotoxins, exerting toxicity through protein synthesis inhibition.

ABSTRACT NUMBER: 5020   Poster Board Number: P123

TITLE: Deciphering the Gut Microbiota and Associated Metabolism in Antibiotics-Treated Wistar Rats

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KEYWORDS: Gastrointestinal; In Vivo Models; Metabolomics; Gut Microbiome; Oral Toxicity Study

ABSTRACT: Gut microbiome is known to play an important role in the health and development of the host. We investigated the changes in gut microbiota and consequently the fecal and plasma metabolomes following a 28-day oral treatment with two antibiotics - Tobramycin and Colistin sulfate in male and female Wistar rats. Tobramycin was administered at doses of 150 and 1000 mg/kg body weight/day, and Colistin at 10 and 100 mg/kg body weight/day, respectively. Blood plasma samples were collected on days 7, 14 and 28 and feces on day 28 of the study. Plasma and feces samples were used to analyze the metabolomes using targeted MS-based profiling and gut bacteria were analyzed from 16S marker gene-based sequencing data of the feces. The results showed that the plasma metabolome of Tobramycin treated rats had a strong response which includes strong alterations in amino acids, lipids, bile acids (BAs), carbohydrates and related classes. Previously established key-
metabolites, known to be associated with the gut microbiome, including indole-3-acetic (IAA), indole-3-propionic acid (IPA), 3-indoxylsulfate (IS) and hippuric acid (HA), were all significantly downregulated in Tobramycin treated rats compared to controls in both sexes, indicating potential effects on gut microbiota. The plasma metabolome of Colistin treated rats, showed significant changes but to a lesser extent than Tobramycin group, indicating a weak effect of Colistin on the gut microbiome. In line with this, the diversity and relative abundance analyses of 16S bacterial families from fecal samples, showed a strong shift in the Tobramycin group compared to controls. Compared to plasma, feces showed an even higher number of significant metabolome changes upon Tobramycin treatment. Particularly, a strong accumulation of primary BAs and significant downregulation of secondary BAs in the feces was observed, suggesting an impairment of gut bacterial deconjugation reaction. Colistin, on the other hand showed a much weaker effect on the gut bacterial composition and consequently also on the metabolome profiles. These results, help to gain an understanding of the contribution of gut microbiota and its associated metabolic capacity on host metabolites and indicate an important role in BA metabolism.

**ABSTRACT NUMBER:** 5021  **Poster Board Number:** P124

**TITLE:** Development of Physiologically Based Pharmacokinetic Modeling to Predict Pharmacokinetics of Pesticide Molecules in Rats

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** Y. Wu. Syngenta, Greensboro, NC. Sponsor: D. Wolf

**ABSTRACT:** Physiologically based pharmacokinetics (PBPK) modeling is a robust tool that can be used to predict the *in vivo* pharmacokinetic behavior of pesticide molecules. The modeling approach progressively integrates different information into a mechanistic framework as soon as they become available in the early stages of pesticide development. For this reason, it has become increasingly popular in pesticide discovery and development in agricultural industry and is now widely accepted by regulatory authorities. In this work, we developed PBPK models of different structures and complexities for rat, which were parameterized with experimentally measured *in vitro* data including hepatic metabolic clearance, plasma protein binding, Caco-2 permeability, octanol-water partitioning, solubility, and molecular weight. A total of 4 models were developed, including a simple 1 compartment pharmacokinetic (PK) model, 2 compartment PK model with consideration of chemical dissolution, a more complicated 5 compartment PBPK model, and the 5-compartment, diffusion-based PBPK model. Model evaluation involved 64 pesticide molecules for which *in vivo* time course concentration in blood were available for orally administrated rats. The comparison shows that the simple 1 compartment PK model exhibits the best performance in predicting maximum blood concentration ($C_{\text{max}}$) and the area-under-the-curve (AUC) of the blood concentration. 7.2% of $C_{\text{max}}$ predictions were not within the same order of magnitude of *in vivo* observations, and 58.8% of the $C_{\text{max}}$ predictions yield more than two-fold differences from the experimental observations. For AUC, 18.3% of the predictions were not within the same order of magnitude of *in vivo* observations, and 66.3% of the AUC predictions had the fold error greater than 2 when compared with experimental observations. Sensitivity analysis based on Morris test for the 1 compartment PK model indicated that volume of distribution was generally the most influential parameter throughout the modeling period, while the importance of elimination parameter increased with time. In summary, the study demonstrated that predicting *in vivo* pharmacokinetic behavior of pesticide molecules based on *in vitro* assays was possible by development of in-house PBPK tools. Future
Improvement could integrate the multi-compartment gastrointestinal (GI) tract model into a compartment PBPK model to simulate the detailed absorption and dissolution of pesticides throughout different parts of GI tract.

**ABSTRACT NUMBER:** 5022  **Poster Board Number:** P125  
**TITLE:** Investigating the Gut Microbiome and Metabolome following Treatment with Two Artificial Sweeteners in Wistar Rats

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**KEYWORDS:** Food Safety; Gastrointestinal; In Vivo Models; Metabolomics; Oral Toxicity Study

**ABSTRACT:** Gut bacteria are important for the host and therefore it is vital to understand their metabolic functionality and contribution to the host’s metabolism. Here, we examine the effects of two doses of artificial sweeteners, Acesulfame potassium (Ace-K) and Saccharin on the metabolomes (plasma and feces) and fecal 16S bacterial composition to elucidate their influence on the gut microbiome. Ace-K was administered at doses of 40 and 120 mg/kg body weight/day, and Saccharin at 20 and 100 mg/kg body weight/day, respectively in a 28-day oral toxicity study with Wistar rats. Blood plasma samples were collected on days 7, 14 and 28, and feces on day 28. Targeted MS-based metabolome profiling of plasma and feces were performed and community analysis via 16S marker gene sequencing of feces was conducted. The results indicated that overall, the sweeteners induce very minor changes in the gut microbiota and in line with this, fecal metabolomes of the sweeteners-treated animals were also similar to controls. In contrast, the plasma metabolomes of the sweeteners-treated animals showed more profound alterations; Saccharin inducing about three-fold higher number of statistically significant changes than Ace-K in both male and female rats. Saccharin significantly altered metabolites belonging to amino acids, lipids, energy metabolism, bile acids (BAs) and related classes. Interestingly, plasma metabolome profiles of Ace-K treated animals showed sex-specific changes. Large alterations in glycine conjugated primary and secondary BAs in males. Previously established plasma biomarkers associated with the gut microbiome (indole derivatives, hippuric acid) did not show any change, which is in line with the observation that Ace-K and Saccharin have very low/no influence on fecal microbiota and metabolite profiles. MetaMapTox, an in-house database, which consists of metabolic profiles from more than 1000 compounds, was used to compare and predict the modes of action of the two sweeteners, which indicated the absence of any kind of toxicity. Overall, there were no indications that the plasma metabolome changes should be considered as adverse, but rather as an adaptation to the administered doses of the two sweeteners.

**ABSTRACT NUMBER:** 5023  **Poster Board Number:** P126  
**TITLE:** Establishing OELs for Sensory Irritants with Limited Data Using Predictive and In Silico Models

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**KEYWORDS:** Computational Toxicology; QSAR
ABSTRACT: Sensory irritation is one of the most common health endpoints that serve as the basis for OELs. Numerous research efforts have been conducted to derive OELs based on predictive methods, various data types, and mathematical models for sensory irritation. One study by Schaper (1993) described a linear relationship with high correlation between measured RD$_{50}$s and ACGIH TLVs for airborne chemical irritants and demonstrated the use of the RD$_{50}$ as a benchmark for occupational exposures. There is a need to develop a predictive model for deriving short-term exposure limits (STELs) for sensory irritants. The aim of our study was to establish a model capable of correlating the relationship between RD$_{50}$ and STELs in order to derive OELs for sensory irritants. A focused review of scientific literature was conducted. The results of this review provided evidence that the potency and onset of sensory irritation is not governed by Haber’s Law, but instead is governed primarily by concentration. The review also resulted in the identification of a NTP database based on Schaper (1993) that included chemicals with both RD$_{50}$ and established STEL. Some of the chemicals included in the NTP database had multiple RD$_{50}$S and STEL. In such cases, each RD$_{50}$ and STEL was analyzed separately. Based on these criteria, a total of 136 observations associated with 47 unique chemicals were eligible for inclusion in the analysis. Further, the data underwent log transformation because it was determined that RD$_{50}$S were log normally distributed. The correlation between LnSTELs and LnRD$_{50}$ values were determined via a linear model using R software. A strong correlation between RD$_{50}$S and STELs was identified, with a predictive equation of Ln (STEL) = 0.77 * Ln (RD50) - 1.49 and an R$^2$ value of 0.80. Sensitivity analyses, including evaluation of outliers, showed that the model was robust and independent from extreme values. This model supports the use of RD$_{50}$S to derive STELs for chemicals without existing exposure recommendations. Further, for chemicals that are sensory irritants with no data, predicted RD$_{50}$S from in silico QSAR models could be used to derive STELs. For an example, a case study using acetone demonstrated the application of the model (predicted STEL: 758 ppm; actual STEL: 750 ppm). Hence, in silico methods and statistical modeling can present a path forward for establishing reliable OELs and improving worker safety and health.

ABSTRACT NUMBER: 5024   Poster Board Number: P127

TITLE: Hepatotoxicity Potential of 3,5,8-Trihidroxy-6-Metoxy-2-(5-Oxohexa-1,3-Dienyl)-1,4-Naphthoquinone (TMON) and Erythrostominone (ERY) Assessed by In Vitro and In Silico Approaches

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KEYWORDS: Computational Toxicology; In Vitro and Alternatives

ABSTRACT: Synthetic dyes that are widely used in industrial manufactures. However, several of these synthetic dyes have intrinsic toxicity. Thus, dyes obtained from natural sources gain space as an alternative to the use of synthetic dyes, intensifying the search for new compounds that are safe for human and environmental health. ERY (Erythrostominone) and TMON (3,5,8-trihidroxy-6-metoxy-2-(5-oxohexa-1,3-dienyl)-1,4-naphthoquinone) primary and secondary metabolites, respectively, isolated from an endophytic fungus, both with red color, emerges as great candidates for commercial application. On the other hand, data on the toxicity of these dyes are scare or non-existent. In this context, we used hepatocarcinoma cell line (HepG2) as in vitro model, and in silico models to assess the hepatotoxicity of TMON and ERY. MTT assay and SRB (Sulforhodamine B) assay was performed to assess the cell viability and cytostatic effect of TMON and ERY in HepG2 cells. AOPLiver™, from an in silico
Toxicology Platform (iS-Tox®) from Altox Ltda. was used for prediction of hepatotoxicity in silico. In silico prediction for TMON showed that the compound present structural alert. Prediction of Molecular Initiating Event (MIE) showed reactivity, activation of biochemical pathways such as chemicals that stimulate oxidative stress (antioxidant response element, ARE) and aryl hydrocarbon receptor (AhR). However, cellular response for HepG2 cells was predicted as non-toxic. The model predicted that the dye is hepatotoxic for non-rodent, rodent, and human. The in vitro tests using HepG2 cells showed that TMON was able to reduce cell viability in dose-dependent manner after 24 or 48 hours of exposure and concentration above 10 µM, with higher toxicity after 48h of exposure. For ERY, like TMON, in silico models predicted a structural alert, ARE agonism activity, week-moderate toxicity for HepG2 cells and hepatotoxicity for non-human models. Finally, in vitro tests showed that cytotoxic effect of ERY in MTT assay only occurs in 50 and 100 µM, and cytostatic effect observed with SRB assay in concentrations above 10 µM. In this way, both TMON and ERY presents potential hepatotoxicity evidenced by in vitro and in silico methods.

ABSTRACT NUMBER: 5025        Poster Board Number: P128
TITLE: Positioning the “Worm-on-Chip” Technology as Part of the Toolbox for NAMs

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: L. Mouchiroud1, E. Katsyuba1, D. Liaskos1, D. Rouquié2, A. Letamendia3, and M. Cornaglia1. 1Nagi Bioscience SA, Ecublens, Switzerland; and 2Bayer CropScience, Sophia Antipolis, France.

KEYWORDS: Alternatives to Animal Testing; Reproductive and Developmental Toxicology; Predictive Toxicology

ABSTRACT: Currently existing toxicology testing still implies an extensive experimentation in mammalian models, which is expensive and associated with important ethical concerns. The alternative methods to animal testing are typically based on cellular models. The main limitation of these in vitro approaches is that they often cannot predict complex responses at the level of an organism, usually involving a multi-organ crosstalk and metabolic processing of the test molecules. Nowadays nematode Caenorhabditis elegans (C. elegans) starts to get recognition as a valuable alternative model in predictive toxicology studies, that can complement in vitro models to better predict the outcomes in mammals. However, experimentation in C. elegans is still mainly based on manual handling techniques and direct observation by the operator, hence largely limiting the potential of the worms for high-throughput and high-content screenings required for toxicology studies. We developed a microfluidic-based robotic platform that can perform automated high-content phenotypic analysis of C. elegans and execute different types of toxicology assays, including the assessments of fertility, embryotoxicity and acute toxicity. As an illustration, we present here the results of a study characterizing the effects on reproduction of seven benchmark chemicals: bisphenol A, thalidomide, hydroxyurea, lithium chloride, methoxycetacid, methotrexate and busulfan. Synchronized populations of worms were chronically exposed to different doses of these compounds starting from the last larval stage (L4) until day 3 of adulthood. The images of each worm were recorded every hour and time-resolved phenotypic readouts were then extracted from the collected images, including growth dynamics, sexual maturity, fertility, embryonic viability, progeny accumulation rate and survival rate. The phenotypic outcomes were compared to those of positive (5-fluorouracil) and negative (1% DMSO) controls. Out of the tested compounds lithium chloride showed the most pronounced negative effects on embryonic viability, while
methoxyacetic acid strongly affected growth dynamics of the mothers. In conclusion, we propose an innovative solution for rapid identification of toxic compounds and their potential mechanism of toxicity, using a biological model that perfectly bridges the gap between \textit{in vitro} and \textit{in vivo} assays.

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<th>ABSTRACT NUMBER: 5026</th>
<th>Poster Board Number: P129</th>
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<td><strong>TITLE:</strong> Improving Quality and Reliability of \textit{In Vitro} Data through the Implementation of Good \textit{In Vitro} Method Practices (GIVIMP)</td>
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<td><strong>AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:</strong> A. Ulrey, and E. Hill. \textit{Institute for In Vitro Sciences Inc., Gaithersburg, MD.}</td>
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<td><strong>KEYWORDS:</strong> \textit{In Vitro} and Alternatives; Regulatory/Policy; Quality</td>
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<td><strong>ABSTRACT:</strong> Over the past several years the scientific community has put renewed focus on improving the reproducibility of experimental data. In the field of \textit{in vitro} toxicology there are several resources that can help with this goal including systematic reviews of published work, the publication and updates to Good Cell Culture Practices, improvements the method validation process, and the increased application of Good Laboratory Practices (GLP) to \textit{in vitro} studies. Another tool for improving the reproducibility of \textit{in vitro} work is the implementation of the practices described in the OECD’s guidance document Good \textit{In Vitro} Method Practices, or GIVIMP. The GIVIMP guideline is applicable to academic laboratories developing new alternative methodologies, established laboratories participating in validation programs and performing routine \textit{in vitro} studies, and industry laboratories intending to submit \textit{in vitro} data to regulatory agencies. These institutions have differing capabilities for designing and implementing quality systems that both adhere to published recommendations and their unique operational needs. This poster shares a practical strategy for using GIVIMP principles to assess a laboratory’s current processes and implement improvements designed to increase the reproducibility of work performed. It is anticipated that through these quality improvements, data, reports, and manuscripts will be more scientifically robust and lead to increased reproducibility of \textit{in vitro} work.</td>
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<th>ABSTRACT NUMBER: 5027</th>
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<td><strong>TITLE:</strong> A Weight-of-Evidence Approach for Androgen Receptor Conservation across Vertebrate Species</td>
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<td><strong>KEYWORDS:</strong> Endocrine; Androgens; \textit{In Vitro} and Alternatives; Other: Data Integration</td>
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| **ABSTRACT:** The US Environmental Protection Agency’s Endocrine Disruptor Screening Program (EDSP) is tasked with assessing chemicals for their potential to perturb endocrine pathways such as the androgen receptor (AR). EDSP employs a tiered toxicity testing strategy that includes whole-animal studies; however, this has proven challenging due to the extensive time, resources, and animals needed to evaluate a single chemical. To address this challenge, EDSP is transitioning towards the use of \textit{in vitro} high-throughput (HT) assays to screen chemicals efficiently. The ability of these mammalian-based assays to accurately reflect chemical interactions in non-mammalian targets, however, remains uncertain. Therefore, a goal of the EDSP is to evaluate biological conservation across taxa to understand how broadly HT results can be extrapolated. To assess cross-species conservation of the AR pathway, we
used a combination of computational analyses and systematic literature review (SLR) methodology to conduct a comprehensive analysis of existing data at in silico, in vitro, and in vivo levels. First, molecular target conservation was assessed across 585 diverse species based on the structural similarity of androgen receptors. These results indicate vertebrate AR shares a high degree of similarity relative to invertebrates and are predicted to be similarly susceptible. Novel, semi-automated SLR efforts targeted published toxicity data relevant to in vitro and in vivo AR assays across non-mammalian species. Database searches resulted in 3,216 in vitro articles and 1,775 in vivo articles. Following human- and machine learning-driven article screening, 33 in vitro and 245 in vivo articles were judged relevant for data extraction. Across relevant in vitro articles, most data (25 articles) were for fish species with less data obtained for other non-mammalian taxa (5 avian, 2 amphibians, and 1 reptile). Similarly, relevant in vivo articles also contained primarily fish data (196 articles) with less representation across other taxa (20 avian, 29 amphibians, and 1 reptile). Although data analysis is ongoing, preliminary analyses of extracted data suggests androgenic responses are conserved across vertebrate species. The views expressed in this abstract do not necessarily reflect the policies of the US EPA.

ABSTRACT NUMBER: 5028        Poster Board Number: P131
 TITLE: Alternative Testing Strategies for Elucidating the Effects of Exposure to Antibiotics on the Interplay between Gut Microbiota and the Bile Acid Pool

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: N. Zhang¹, J. Wang², W. Bakker¹, W. Zheng¹, M. Baccaro¹, B. Ravenzwaay², and I. M. C. M. Rietjens¹. ¹Wageningen University and Research, Wageningen, Netherlands; and ²BASF SE, Ludwigshafen, Germany.

KEYWORDS: In Vitro and Alternatives

ABSTRACT: Bile acid composition and concentrations in blood and intestinal content are directly related to the composition of the gut microbiota and the microbial conversion of bile acids. The aim of the present study was to develop novel in vitro testing strategies able to study the effects of xenobiotics on the interplay between gut microbiota and the bile acid pool. To this end the effects of a selected series on non-absorbable antibiotics (tobramycin, colistin sulfate, meropenem trihydrate, doripenem hydrate) which have low or no systemic toxicity and are able to alter the intestinal microbiome composition, on the profiles of the microbiome and related bile acids was studied in anaerobic rat fecal incubations as an in vitro model. 16S rRNA analysis was used to profile the changes in the gut microbiota composition. Tobramycin treatment had the largest impact on abundant microbial family levels among all the treatment. Tobramycin also appeared to affect the bile acid pool to a larger extent than other antibiotics resulting in time dependent reduction of the available bile aids upon prolonged incubation, while also inhibiting the deconjugation of conjugated bile acids. Comparison of the results obtained from in vitro fecal incubation study to data acquired from a 28 day rat in vivo study revealed that the substantial increase in taurocholic acid (TCA) levels observed in the in vivo study could only in part be explained by the reduced deconjugation. Additional in vitro studies using Caco-2 cell layers as a model for intestinal reabsorption of bile acids revealed an inhibiting effect of tobramycin on the transport of TCA across the intestinal cell layer. This may further explain the observed increase in the intestinal levels of TCA upon tobramycin treatment in vivo, thus providing an explanation for the differences of the in vivo and in vitro study.
ABSTRACT NUMBER: 5029  
POSTER BOARD NUMBER: P132

TITLE: Addition of Mammalian Cell Culture Medium Impacts Nanoparticle Toxicity in Zebrafish


KEYWORDS: Nanoparticles; Embryo; Developmental/Teratology; Nanomaterial, Zebrafish; Graphene Oxide

ABSTRACT: Engineered nanomaterials (ENM) are widely used in modern society and their impact on the environment and human health remains largely unknown. There have been many studies that investigate nanomaterial effects, but the materials used, the experimental assay, the model, and the platform (in vivo or in vitro) are highly diverse. Without toxicological data collected systematically (with similar materials, relevant platforms, and assays), it will be challenging to identify potential risks associated with ENM exposures. Most in vitro screening approaches utilize media-rich ionic species Roswell Park Memorial Institute (RPMI) and proteins fetal bovine serum (FBS). These ions and proteins are critical for cellular viability function, but studies have shown that the toxicity of certain ENM differs between cell culture and zebrafish experiments. Some of the differences in toxicological outcomes could be caused by differences in the media used in the various assays. Previously, zebrafish have been found to develop normally in low ionic water (Ultra-Pure, UP). First, we compared the use of UP along with RPMI and FBS to mimic cell culture media conditions using our standard zebrafish assay and found that zebrafish tolerate a maximum of 50% RPMI, 4% FBS, and an antibiotic cocktail (Amphotericin B 0.25 µg/mL, Penicillin 100 units/mL, and Streptomycin 100 µg/mL) to prevent bacterial growth. Embryonic zebrafish were dechorionated and exposed to 0 to 50 µg/mL of 2 different size ENM in a solution containing RPMI, FBS, and antibiotics in UP from 6 to 120 hours post-fertilization (hpf). A total of 13 mortality and morphological defects were examined at 24 and 120 hpf to measure potential changes in developmental toxicity due to interactions between cell culture media and the ENM. Two graphene oxides (GOs) (250 x 250 nm [sGO]; 400 x 400 nm [mGO]) caused statistically significant morbidity and/or malformations in zebrafish by 120 hpf. The adverse effects observed in zebrafish exposed to 3 and 25 µg/mL sGO was reduced from 100% effect to 46.67% and 75%, respectively in conditions similar to cell culture medium (p<0.05). The same trend was observed for the large graphene oxide (mGO) where the survival rate increased from 31.3% to 93.7% at 30 hpf with only the addition of 4% FBS. The apparent toxicity of ENM is influenced by the presence of components found in cell culture media.

ABSTRACT NUMBER: 5030  
POSTER BOARD NUMBER: P133

TITLE: Histopathological and Inflammatory Indices Comparison of Mouse Lung following Repeat Exposure to Nanoclay and Machined Nanoclay Composite Dusts

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: L. Rojanasakul1, T. Stueckle1, J. Coyle1, H. Shane1, M. Orandle1, J. Jensen2, K. Roach1, C. Johnson1, R. Derk1, L. Battelli1, D. Richardson1, S. Friend1, R. Gupta3, C. Dinu1, and E. Lee1. 1NIOSH, Morgantown, WV; 2Harvard University, Boston, MA; and 3West Virginia University, Morgantown, WV.

KEYWORDS: Nanotechnology; Inhalation Toxicology; Nanoparticles; Nanoclay
**ABSTRACT:** Nanocomposite (NC) development continues to grow through use of nanomaterials for a wide diversity of applications. Incorporation of organomodified nanoclays (ONCs) to improve barrier function and strength of plastic components suggests occupational exposure to ONC and ONC-enabled composite dust is likely in the future. Quaternary ammonium compounds (QACs) are one class of coatings on ONCs that possess inflammatory and cytotoxic properties, however, little data exists on how repeat ONC exposure impacts lung health. Female Balb/C mice were repeat exposed via six aspirations over 3 weeks to Cloisite 93A (16.7 or 41.7 µg), virgin polypropylene (VPP) machined dust, or 1% Cloisite 93A PP NC machined dust (50 or 150 µg) to compare lung pathological responses along the ONC lifecycle. Lungs were collected, fixed, and stained to evaluate particle deposition, inflammatory, and pathological response. Flow cytometry analyses of bronchioalveolar lavage and lung cells were performed to differentiate cell populations. Microscopic evaluation of H&E and PAS-stained sections of lung exposed to 250 µg Cloisite 93A showed cytological evidence of allergic airway disease, including perivascular and peribronchiolar infiltrates of mixed inflammatory cells, nodular accumulations of lymphocytes, and PAS-positive goblet cells in the terminal bronchiole epithelium. These abnormalities often localized to areas of particle deposition and persisted through the end of the 28-day study period. VPP and 1% Cloisite 93A PP NC dusts showed minimal multifocal granulomas with occasional alveolar histiocytosis. Cloisite 93A exposed animals showed elevation in neutrophil and CD4+ T cells, CD11b+, and CD86+ CD103+ dendritic cells while all particle exposures caused transient elevations in dendritic cells, interstitial macrophages, and activated monocytes. Lung inflammatory cytokine signal profiles identified acute inflammatory response signature with high dose Cloisite 93A displaying persistent elevated Th2-mediated signaling profile correlating with pathological observations. These results indicate that repeat occupational exposure to QAC-coated ONCs early in their lifecycle possess potential chronic inflammatory disease risk.

**ABSTRACT NUMBER:** 5031      **Poster Board Number:** P134

**TITLE:** Toxicological Assessment of Carbon Nano-Onions in Female BALB/c Mice

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** Y. Tan, L. Thomsen, and R. J. Rosengren. 
*University of Otago, Dunedin, New Zealand.*

**KEYWORDS:** Nanotoxicity, CYP450, CYP3A, Malondialdehyde; Carbon Nano-Onions

**ABSTRACT:** Conventional chemotherapy in cancer treatment non-selectively targets rapidly proliferating cells which ultimately leads to various side effects. Nanomedicine is a promising approach for targeted drug delivery for cancer treatment. The development of nanoscale drug carriers offers precise tumor targeting with reduced side effects alongside with an increase in the efficacy of chemotherapy. Carbon nano-onions (CNOs), a potential carbon-based drug carrier, have shown excellent biocompatibility in vitro. However, the whole animal safety of CNOs is unknown. Hence, this study aimed to investigate the toxicity of oxidized carbon nano-onions (oxi-CNOs) following intravenous administration in female BALB/c mice. Oxi-CNOs were dispersed in 50% w/v polyethylene glycol and had an average hydrodynamic diameter of ~50 nm analyzed by dynamic light scattering. Mice were administered either a single dose (125, 250 or 500 µg) or three doses of oxi-CNOs (125, 250 or 500 µg, every four days). Histological examination of organ slices revealed that oxi-CNOs predominantly accumulated in the liver, spleen and lungs. However, this accumulation did not cause any significant changes to organ weight, plasma alanine aminotransferase activity and creatinine levels. To determine if the accumulation of oxi-
CNOs in the liver would impact drug metabolism, total CYP450 and CYP3A protein and catalytic activity were determined. The results showed that total hepatic CYP450 as well as CYP3A catalytic activity and protein levels were unchanged in all treatment groups compared to control. Furthermore, no oxidative stress was induced by oxi-CNOs as indicated by the unchanged malondialdehyde level in liver microsomes and spleen homogenates in the treatment groups compared to control. Our data provide the first evidence that IV administration of oxi-CNOs is non-toxic in female mice, indicating a potential safe new drug carrier.

ABSTRACT NUMBER: 5032  Poster Board Number: P135

TITLE: Automation and Validation of the OrganoPlate LiverTox for Hepatotoxicity Detection

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: K. Bircsak¹, A. Alsebahi¹, R. Reddinger¹, D. Goubert¹, R. DeBiasio², M. Miedel², A. Saleh¹, T. Shun², L. Vernetti², and A. Gough². ¹MIMETAS, Gaithersburg, MD; and ²University of Pittsburgh Drug Discovery Institute, Pittsburgh, PA.

KEYWORDS: In Vitro and Alternatives; Predictive Toxicology; Hepatic; Organ-on-a-Chip

ABSTRACT: Drug-induced liver injury (DILI) is one of the leading causes of market withdrawal in the pharmaceutical industry and poses a serious health risk to affected patients. Identification of hepatotoxic compounds in the preclinical phase of drug development is key to preventing DILI, however currently employed animal and two-dimensional (2D) in vitro models do not adequately predict human hepatotoxicity. Existing models suffer from many challenges including species differences, throughput limitations, and lot-to-lot variability of primary human hepatocytes. Here, we developed a functional 3D in vitro model of the human liver, the OrganoPlate LiverTox compatible with automated liquid handling and validated for hepatotoxicity screening. To build the model, up to 96 independent 3D perfused cultures were established on MIMETAS’ OrganoPlate 2-lane using automated liquid handling to seed cells, dose, collect media and add assay reagents. For cell seeding, induced pluripotent stem cell-derived hepatocytes (iHep) in extracellular matrix were added to a microfluidic channel, following which endothelial and Kupffer cells were added to an adjacent channel to mimic the liver sinusoid. Characterization of the model revealed long-term hepatocyte function including CYP3A4 activity, as well as albumin and urea production for up to 14 days of culture. Fetal hepatocyte marker alpha-fetoprotein (AFP) dramatically declined over the 14 day culture, supporting iHep maturation in the OrganoPlate LiverTox. Assay validation studies using troglitazone as a positive hepatotoxic control compound revealed robust Z-factors ≥ 0.2 for albumin, urea, iHep viability (propidium iodide staining), and iHep nuclear size (Hoechst 33342 staining) assay readouts. Using these assays, 159 compounds of known hepatotoxicity were screened in the OrganoPlate LiverTox (50 µM, 72 h) and ranked by a composite score by combining the assay readouts. A follow-up dose response evaluation of select hits suggested the albumin assay to be the most sensitive readout in calculating TC₅₀ values. Taken together, the OrganoPlate LiverTox is a promising platform for hepatotoxicity detection and has the potential to be used in a high throughput screening capacity.
ABSTRACT NUMBER: 5033    Poster Board Number: P136
TITLE: Disentangling Tenofovir Toxicity by Combined High-Throughput Imaging and Multi-‘Omics in Human Kidney Cells

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: A. Pearson¹, D. Haenni², J. Bouitbir³, M. Hunt⁴, B. Payne⁴, S. Nlandu-Khodo⁵, and A. Hall². ¹NIEHS, Research Triangle Park, NC; ²University of Zurich, Zurich, Switzerland; ³University of Basel, Basel, Switzerland; and ⁴Newcastle University, Newcastle, United Kingdom. Sponsor: S. Ferguson

KEYWORDS: Clinical Toxicology; In Vitro and Alternatives; Kidney; Tenofovir

ABSTRACT: Nephrotoxicity from xenobiotics is a major cause of kidney disease and a frequent reason for failure in drug development. The proximal tubule (PT) is the most common site of nephrotoxicity. Innate PT cell functions expose them to high concentrations of drugs and may result in impaired solute reabsorption, leading to systemic disruption of endogenous processes with clinical ramifications. Moreover, the cellular mechanisms driving the toxic effects of many drugs are unknown. Realistic in vitro models and suitably sophisticated analysis techniques are required to emulate these toxicological dynamics and identify molecular perturbations that drive functional phenotypes. The anti-viral drug tenofovir disoproxil fumarate (TDF) provokes changes in mitochondrial morphology and solute transport defects in the PT, for reasons that have remained elusive for over twenty years. The aim of our study was to establish representative models of TDF toxicity, using differentiated human-derived PT cells, to investigate the underlying cellular events. We developed a high-throughput image analysis pipeline, using machine learning, that enabled rapid and quantitative measurement of solute transport and mitochondrial morphology. We then leveraged this to establish TDF treatment regimens that reliably reproduce phenotypes reported in patients. We subjected these newly established in vitro models to detailed multiparametric analysis - including oxygen consumption measurements, metabolomics, and transcriptomics - and validated key findings in biopsy specimens from patients with TDF-associated proximal tubulopathy. By integrating data from different orthogonal experimental approaches, we elucidated a highly robust metabolic fingerprint of TDF toxicity. Crucially, we identified that inhibition and suppression of complex V (ATP synthase) of the respiratory chain is a pivotal event in the pathogenesis, triggering responses that explain the human phenotype, including disruption of cristae formation, ATP depletion, and oxidative stress. Thus, we demonstrate an efficient imaging approach to screen for disease relevant functional defects in kidney cells in vitro and reveal a new molecular paradigm for understanding the pathogenesis of a frequent cause of nephrotoxicity.

ABSTRACT NUMBER: 5034    Poster Board Number: P137
TITLE: Translational Safety Overview of 2021 New Drug Approvals


KEYWORDS: Predictive Toxicology; Clinical Toxicology; Bioinformatics

ABSTRACT: 50 new drugs were approved by the FDA’s Center for Drug Evaluation and Research (CDER), plus 10 new drug approvals by the Center for Biologics Evaluation and Research (CBER) - a total of 60 new drug approvals (NDAs). The goal of this overview is to identify drug discovery trends and evaluate
the translation of safety findings in preclinical models to clinic. Drug discovery has greatly benefited from new technologies and that is reflected in 2021’s NDA list. In 2021, 52% of the approved therapies were classified as biotechnologies/peptides vs. 48% small molecules, suggesting that traditional approaches and new technologies share the drug discovery space now. Of these NDAs and BLAs, new cancer drugs accounted for 31% of approvals, followed by drugs intended to treat infections/infestation, immune mediated disorders, congenital and genetic disorders, psychiatric disorders, and metabolic disorders (18%, 11%, 8% and 5% of approvals, respectively). To analyze the utility of preclinical safety packages in hazard identification and risk assessment, preclinical and clinical safety data was compiled from FDA’s submission packages for these recently approved drugs. To assess hazard identification performance of preclinical species vs. clinical data were compared and the most common adverse events (AE) were identified and classified by severity. Overall, the most common AE in human were nausea, headache, diarrhea, abdominal pain, vomiting and fatigue. When classified by severity, 30% of NDA reported serious AE (grade >= 3) vomiting, diarrhea, increased liver enzymes in humans. Preclinical species demonstrated 67-38% and 100-84% sensitivity and specificity predicting these SAE. This analysis suggests that preclinical species are important for understanding drug safety, but these models alone still may not strongly translate common SAE. These data provide an updated evaluation on the strengths and limitations of preclinical safety packages in 2021 and which toxicities and modalities are in greatest need of scientific investment and improvement in 2022.

ABSTRACT NUMBER: 5035    Poster Board Number: P138

TITLE: Adipose Inflammation Modulation by Both Methylparaben-Enriched and High-Fat Diets

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: E. M. Hill¹, A. Tapaswi¹, K. Polemi¹, I. L. Bergin², D. E. Brenner³, D. Bridges¹, D. C. Dolinoy¹, and J. A. Colacino¹. ¹University of Michigan School of Public Health, Ann Arbor, MI; ²University of Michigan, Ann Arbor, MI; and ³University of Michigan Medical School, Ann Arbor, MI.

KEYWORDS: Inflammation; Carcinogenesis; Methylparaben

ABSTRACT: An estimated 10% of all breast cancers are linked to obesity. Increased adipose tissue inflammation and inflammatory cytokine secretion associated with the obese state is being investigated as a major factor. Recently, obesogens have been shown to disrupt lipid metabolism and promote adipogenesis and obesity. However, it is unclear obesogens affect adipose inflammation and cytokine secretion. Forty-two female MMTV-erbB2 mice were exposed to a methylparaben diet, a high fat diet, or control diet for either a short term (12 weeks, n = 7 for each diet) or long term (30 weeks, n = 7 for each diet). Mice were weighed and palpated weekly for breast tumors. To determine differences in macrophage infiltration, adipose from the early timepoint was fixed, sectioned, and stained for Mac2 and crown-like structures were numerated. Finally, adipose was also collected and cultured ex vivo and the adipose-conditioned media was then collected. Secreted cytokines were then measured using Luminex. After 30 weeks, mice fed the high fat diet were heavier on average than those fed either the methylparaben or control diets. Surprisingly, mice fed the methylparaben were no heavier than the control mice by week 13, however, were significantly smaller than mice given the control diet by week 30. Mice fed the high fat diet were more likely (6/7) to develop any tumors compared to control mice (4/7) and methylparaben-fed mice (1/4). After 13 weeks, mice fed the high fat diet had adipose tissue with more crown-like structures, followed by mice fed the methylparaben, followed then by the control
mice. Finally, leptin secretion at the early timepoint decreased in the high fat diet and methylparaben groups compared to control. However, leptin secretion in the mice sacrificed at 30 weeks showed no differences between groups. Instead, levels of C-reactive protein and IL-6 both showed increased levels in the high fat diet mice compared to controls.

**ABSTRACT NUMBER:** 5036  **Poster Board Number:** P139

**TITLE:** Cellular and Metabolic Toxicity of Oral Nicotine Pouch Products in Oral Epithelial Cells

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** S. Jabba, S. Howell, P. Silinski, and S. Jordt. Duke University, Durham, NC.

**KEYWORDS:** Tobacco Products; In Vitro and Alternatives

**ABSTRACT:** Nicotine pouches are a new smokeless tobacco product category, whose sales rapidly increased (>300%) in the last 2 years. Nicotine pouches are tobacco- or tobacco-leaf free, consisting of filler, nicotine or nicotine salts, sweeteners and a variety of characteristic flavors, including mint, menthol, wintergreen and sweet-associated flavors (fruity and candy flavors such as berry, citrus, cinnamon and coffee). Though nicotine pouches are marketed with claims of reduced risk, it is currently unknown whether the use of flavored nicotine pouch products has adverse effects on oral tissues, and, if so, what the product characteristics are that contribute to the toxicological effects. The potential cytotoxic effects from exposure to flavored nicotine pouch extracts were assessed using LDH and fluorescence Live/Dead cell assays in UM-SCC-1 cells, an oral squamous cell carcinoma cell line. Live-cell metabolic assays were carried out in UM-SCC-1 cells to determine the effects of these flavored pouch extracts on key bioenergetic parameters of mitochondrial function. Exposure to several flavored nicotine pouch extracts (on! mint, wintergreen; Zyn coffee, spearmint, cinnamon) for 18-24 hours increased LDH release in UM-SCC-1 cells by ~1.5-2.0 fold. Cells exposed to either nicotine (3 mM) or the active flavor chemical present in these products (menthol, carvone or methyl salicylate at 3 mM), produced no significant LDH activity over untreated control. Extracts of VELO products (Citrus and Mint) did not increase LDH activity. In the Live/Dead assay, significant dose-dependent cytotoxicity was observed upon exposure to various dilutions of Zyn and VELO Cinnamon extracts. In metabolic assays, compared with their individual constituents (nicotine, active flavor chemical), several extracts of flavored Zyn, VELO and on! products diminished key parameters of cellular energy metabolic functions, including basal respiration, ATP production, and spare respiratory capacity. Nicotine pouch extracts have differential toxicological and metabolic effects, with several extracts increasing LDH release, cell death and reducing mitochondrial function.

**ABSTRACT NUMBER:** 5037  **Poster Board Number:** P141

**TITLE:** Modeling Changes in Exposure to Persistent Organic Pollutants from Transitioning to Plant-Based Diets: A Global Perspective

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** J. D. Hader, and M. MacLeod. Stockholms Universitet, Stockholm, Sweden. Sponsor: J. Hader, Society of Environmental Toxicology and Chemistry

**KEYWORDS:** Exposure Assessment; Food Toxicity; Persistent Organic Chemicals
ABSTRACT: A transition away from animal-based proteins is seen as an important aspect of sustainable development in the 21st Century. The market share of plant-based protein dietary sources is currently dwarfed by that of animal-based proteins, however the production and consumption of these alternative protein sources is rapidly increasing. Animal-based foods are a key exposure route for some persistent organic pollutants (POPs), particularly beef and dairy products. To explore the possible impact that a dietary shift towards plant-based proteins may have on dietary POP exposure, we paired spatially-explicit global chemical fate and transport modelling of an archetypal POP with bioaccumulation and human exposure modelling of a range of possible future diets. Projected annual emissions of PCB-153 through 2049 were taken from the literature and used to drive the BETR-Global chemical fate and transport model, with a grid spacing of 15° latitude/longitude. Modelled chemical fugacities in air, water, and soil were then passed to a steady-state model based on the ACC-HUMAN model, which simulates the uptake of POPs through the aquatic and terrestrial food chains, ultimately calculating human dietary exposure. As a preliminary analysis, we considered three future dietary scenarios for humans - a ‘business as usual’ diet, a vegetarian diet (no animal flesh as food and a 1.25x increase in plant food consumption), and a vegan diet (no animal products in the diet and a 1.5x increase in plant food consumption) - and calculated the geospatially-varying resultant ingestion exposures to PCB-153. In the bounding case in which all food was produced and consumed locally, the transition to a vegetarian and vegan diet resulted in a ~1 and ~2.5 orders of magnitude decrease in exposure, respectively, in the eastern United States, and a factor of ~2.5 and ~2 orders of magnitude decrease, respectively, in central Europe. Results for other regions and dietary patterns, the effect of regional and international food trade, and modelling with additional chemicals will provide a more complete picture of exposure routes and levels under alternative scenarios. This study highlights the potential co-benefit of reduced human exposure to POPs that may occur with a transition away from animal-derived protein sources in response to the environmental concerns of animal agriculture.

ABSTRACT NUMBER: 5038    Poster Board Number: P142
TITLE: Estimate Consumer Exposure to Intimate Hygiene Products and Their Effect on Vulvar pH
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Sponsor: J. Naciff
KEYWORDS: Exposure Assessment; Clinical Trials/Human Studies; Intimate Hygiene
ABSTRACT: Intimate wash formulated with mild cleaning agents provides an attractive intimate hygiene option for consumers. Different forms of intimate washes (gels and foams) and intimate cleaning wipes are available on the market today. As published consumer habits and practices (H&Ps) and exposure parameters for intimate washes are limited, we initiated the investigation to understand this area. In diary studies, participants were asked to use the given intimate gel or foam for 21 days (n=394 pre-menopausal China women and more frequent use than their normal habits) and 28 days (intimate gel and wipe (a regimen design), n=60 for H&Ps and 119 for skin pH in pre- and post-menopausal women in the US study). Surveys, post-use questionnaires, and interviews were also conducted to collect H&Ps. Additionally, pH was measured at introitus, L. minora, L. majora, and perineum at baseline after washout and at the end of the 28-day study. Results: Intimate wash was used on the vulva (98%), introitus (92%), perineum (72%), perianal area (45%), and intertriginous area (11%) shown in the China study. Their frequency of use was 4.33 ± 1.96 and 5.63 ± 1.98 times a week during menstruation and
non-menstruation respectively, while one, two, and three times/day (50th, 90th, and 95th percentile) were observed in the US study. Regarding average washes used, 2.45 g per use or per day were shown in the China study, and 3.95 g/use and 5.63 g/day in the US study. In addition to the shower use, a washbowl was used by 25.8% of China users. For intimate washes, the retention factor (1%) we derived to estimate product remaining on the skin after rinsing is proportional to body surface area (intended and unintended contact with washes) and inversely proportional to water volumes for rinsing. The 28-day study showed that intimate skin pH values are comparable between post- and pre-menopausal subjects though the former has a trend of higher pH than the latter. Also, mean pH values are comparable before and after use of the intimate hygiene products as well as between pre- and post-menopausal groups. Main pH ranges are similar in pre- and post-menopausal subjects i.e., 5.5-7.0, 4.5-7.0, and 4.5-5.5 for introitus and perineum, L. minora, and L. majora respectively. Taken together, the intimate hygiene products tested do not seem to alter normal vulvar pH in women, suggesting helping to limit the potential for changes in vulvar microflora. Importantly, the deposition and other exposure parameters would help improve safety assessment for intimate washes.

ABSTRACT NUMBER: 5039    Poster Board Number: P143

TITLE: EAS-E Suite: A Data Integration and Modeling Framework for Chemical Safety and Sustainability

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ABSTRACT: Thousands of new and existing chemicals are subject to ecological and human health risk assessment; however, extensive data gaps for exposure estimation present obstacles to addressing regulatory objectives. The Exposure And Safety Estimation (EAS-E) Suite platform is a community resource that can aid regulatory objectives and the safe and sustainable production and use of chemicals in society. EAS-E Suite is freely available on-line (www.eas-e-suite.com) and contains databases of chemical information and various models and tools to support chemical evaluations including (i) retrospectively for existing chemicals and use, and (ii) prospectively (forecasting) for new chemicals and for proposed changes of use of existing chemicals. We summarize key concepts of the EAS-E Suite platform and include a case example of its application for chemical assessment and management. The PROduction-To-EXposure High Throughput (PROTEX-HT) model in EAS-E Suite consolidates the principal elements of the production-to-outcome continuum for tens of thousands of chemicals. PROTEX-HT quantifies relationships between production volumes, chemical emissions throughout the lifecycle, fate and transport in natural and manufactured environments (e.g., homes), persistence, bioaccumulation, toxicokinetics in ecological receptors and humans and aggregate human and ecological exposures to estimate the potential for effects. We present the case example results of PROTEX-HT using 95 chemicals for which human biomonitoring data are available. Seventy-nine percent and 97% of the PROTEX-HT human exposure predictions were within one and two orders of magnitude, respectively, of independent human exposure estimates inferred from biomonitoring data from the US population. The results in this presentation show how PROTEX-HT in EAS-E Suite can support: (i) the screening and ranking of chemicals based on various exposure and risk metrics, (ii) setting chemical-specific maximum allowable tonnage based on user-defined toxicological thresholds, and (iii) identifying the most relevant emission sources, environmental media, and exposure routes of concern. The case
example application and results also show that high chemical tonnage does not necessarily result in high exposure or health risks.

ABSTRACT NUMBER: 5040 Poster Board Number: P144
TITLE: Application of Bayesian and Probabilistic Approaches for Cancer Dose-Response Assessment Incorporating Model Uncertainty and Human Variability

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ABSTRACT: The traditional cancer slope factors (CSF) are derived with limited consideration of uncertainties in dose-response model choice, interspecies extrapolation, and human variability. These limitations, noted previously by the National Academies, can be addressed applying probabilistic methods, but such approaches have only been demonstrated in a small number of case studies. We hypothesized that these limitations can be addressed through Bayesian cancer dose-response modeling and probabilistic methods, enabling broad application across chemicals in a standardized workflow. We collected 342 sets of animal bioassay cancer dose-response data previously used by governmental agencies, of which 273 had sufficient data for applying our approach. In contrast with the current method for model selection (lowest Akaike Information Criterion), we incorporate model uncertainty through use of a Bayesian weighted model averaging approach that considers the relative likelihood of different models fitting the data. We then applied inter-species extrapolation and incorporated human variability probabilistically using distributions from World Health Organization guidance, and derived predictions for population cancer incidence in terms of the risk-specific dose (RSD) at \(10^{-6}\) to \(10^{-4}\) incidence. We found that traditional CSFs, which are defined as “approximating a 95% confidence limit,” typically correspond to 55-69th percentiles after more fully incorporating uncertainty and variability. Moreover, probabilistic 95th percentile RSDs were typically 7- to 40-fold more stringent than traditionally-derived ones. However, in a small (<5%) number of cases of highly non-linear dose-response, probabilistically-derived RSDs at \(10^{-6}\) incidence were 3- to 300-fold less stringent, though probabilistically-derived RSDs at \(10^{-4}\) incidence where no more than 7-fold less stringent. We conclude that implementing Bayesian and probabilistic methods in dose-response modeling and extrapolation processes will provide a more scientifically rigorous basis for cancer dose-response assessment and thereby improve overall cancer risk characterization.

ABSTRACT NUMBER: 5041 Poster Board Number: P145
TITLE: A Risk Assessment of Perfluorooctane Sulfonic Acid (PFOS) and Perfluorooctanoic Acid (PFOA): Considerations for Obesity


KEYWORDS: Perfluorinated Agents; Risk Assessment; Biomonitoring; Perfluorooalkyl Substances (PFAS)

ABSTRACT: Obesity has been linked to exposure to a variety of environmental pollutants in the United States. Perfluorooalkyl substances (PFAS) are a class of highly stable chemicals that are ubiquitous in the environment and cause widespread human exposure. A multi-database literature review was conducted
to assess the risk of increased obesity associated with exposure to perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), two common PFAS. The relationship between human serum PFOS and PFOA concentrations and body mass index (BMI) data from the 2017 to 2018 cycle of the National Health and Nutritional Examination Survey (NHANES) was examined. The literature review revealed mixed conclusions with regards to associations of either PFOS or PFOA with obesity, with exposure thresholds and demographics possibly influencing the results. Analysis of NHANES data revealed statistically significant correlations that varied within demographic groups. In the overall population, linear regression showed a statistically significant inverse relationship for both [PFOA] and [PFOA] with BMI. For PFOS, the correlation coefficient was -0.002. For PFOA, the correlation coefficient was -0.037. Linear regression of [PFOS] and BMI in females showed a coefficient of -0.038. Logistical regression of overweight status (defined as a BMI ≥ 25) and [PFOA] and [PFOS] for various demographics also had statistically significant results. Adults aged 65 years and up exposed to PFOA or PFOS had higher odds of being overweight than similarly exposed adults 20 to 45 years old, with odds OR=1.87 for PFOA and OR=1.79 for PFOS. Mexican Americans had higher odds of obesity than White people with similar PFAS exposures, with OR = 2.49 for PFOA and OR=2.60 for PFOS. Other Hispanic persons had higher odds of obesity than White people with similar PFAS exposures as well, with OR = 2.20 for PFOA and OR = 2.23 for PFOS. The juxtaposition of inverse relationships in the overall population with higher odds ratios for specific demographics found in the NHANES analysis may help to explain the mixed literature results and highlights the need for additional demographic-level epidemiological studies of the effects of PFAS exposure on weight. More research is needed to determine the effects of exposures in utero and at young ages.

ABSTRACT NUMBER: 5042          Poster Board Number: P146
TITLE: Application of In Vitro to In Vivo Extrapolation (IVIVE) to Informing In Vivo Point of Departure
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KEYWORDS: Risk Assessment; Alternatives to Animal Testing; In Vitro to In Vivo Extrapolation
ABSTRACT: Traditional chemical risk assessment is often based on published mammalian in vivo toxicity data that is used to derive a point of departure (POD) for a potential adverse effect by a specific route of exposure. Certain uncertainty factors are then applied to the in vivo POD for the most sensitive effect to derive the ATSDR minimum risk levels (MRLs). However, time and resource requirements prohibit efficient multi-target organ toxicity assessments for the large number of environmental chemical pollutants. In vitro high-throughput screening (HTS) assays and other new approach methodologies (NAMs) could address this problem using reverse dosimetry to contextualize HTS concentration responses to an in vivo system. In this study, we selected 36 priority chemicals (e.g., permethrin, hexachloroethane, 2,4-D, pentachlorophenol) for which oral MRL values are available for l neurotoxicity, hepatotoxicity, or developmental toxicity. We obtained in vitro activity concentrations from curated HTS assays for these chemicals through the NTP Integrated Chemical Environment database (https://ice.ntp.niehs.nih.gov/) and conducted in vitro to in vivo extrapolation (IVIVE) to estimate the daily equivalent administered dose (EAD) that would result in plasma concentrations equivalent to the in vitro activity concentrations. For chemicals that were inactive in all HTS assays, the maximum testing
concentration was used for IVIVE. The ranges of EADs were then compared to MRLs and in vivo PODs used to derive the MRLs for specific endpoints. Although there are differences depending on the endpoints evaluated, our results showed that for most chemicals, the ranges of EAD estimates are within 10-fold of in vivo PODs. However, the ranges of EAD estimates are higher than most MRLs, suggesting an in vitro uncertainty factor may be needed for predicting MRLs. Specific assays providing the most accurate predictions for in vivo PODs need further evaluation. These findings demonstrate the usefulness and limitations of HTS data and IVIVE approaches in risk assessment. This project was funded in whole or in part with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C. The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry.

ABSTRACT NUMBER: 5043  Poster Board Number: P147
TITLE: US Army Public Health Center Toxicity Assessment and Fit-for-Purpose Testing of 1-Methyl-2,4,5-Trinitroimidazole (MTNI)

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KEYWORDS: Chemical Hazard Assessment; Hazard Identification/Reduction; In Vitro and Alternatives; Imidazole

ABSTRACT: The US Army is evaluating the novel compound 1-methyl-2,4,5-trinitroimidazole (MTNI) as a melt-pour explosive under a Strategic Environmental Research and Development Program (SERDP) effort. Toxicity and physicochemical estimates were generated using computational modeling based on chemical structure using the software tools: BIOVIA (generates oral, inhalation, dermal, reproductive and developmental, genotoxicity, carcinogenesis, and ecological endpoints), ECOSAR (generates acute and chronic aquatic toxicity endpoints), and EPISuite™ (estimates physicochemical properties and environmental fate). MTNI was identified within the azole antibiotic chemical class. MTNI was then subjected to a four-part in vitro toxicity screen that included bacterial genotoxicity, marine bacterial luminescence (MarBL) test, skin sensitization potential, and an rat oral LD₅₀ estimate derived from in vitro cytotoxicity data. MTNI was found to be mutagenic, a skin sensitizer, and had a predicted rat LD₅₀=260.7 mg/kg. In the MarBL assay, MTNI was highly toxic - EC₅₀=0.035 mg/L. During testing, MTNI was observed to rapidly degrade in aqueous solutions. In order to address both the concern around the antibiotic properties and determine if the rapid degradation alters MTNI toxicity profiles, additional acute aquatic testing with Daphnia magna was conducted. D. magna toxicity was approximately one order of magnitude lower with fresh MTNI (0.558 mg/L) as compared to the MarBL EC₅₀. Aging MTNI for 1 week in water prior to testing yielded an EC₅₀ of 18 mg/L - an additional 2-orders of magnitude reduction in toxicity. The overall reduction in toxicity from the bacterial to invertebrate assessments was 500-fold. MTNI was used as a proof of principle for the development of a rapid-screening approach for MarBL where the number of test conditions were expanded using a 96-well plate format. The MarBL permitted the simultaneous assessment of MTNI under various aged conditions. These data will be presented and the ramifications for occupational and environmental exposures, in light of MTNI aqueous degradation will be discussed. Disclaimer: The mention of any non-federal entity and/or its
products is not to be construed or interpreted, in any manner, as federal endorsement of that non-federal entity or its products.

ABSTRACT NUMBER: 5044  Poster Board Number: P148
TITLE: Establishing Safe Harbor Levels for Trace Proposition 65 Contaminants: Dichloroacetic Acid, Methyl Chloride, and Propylene Oxide

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KEYWORDS: Safety Evaluation; Risk Assessment; Regulatory/Policy

ABSTRACT: Complying with California's Proposition 65 regulations can be challenging when chemicals do not have established Safe Harbor Limits (SHLs). Specifically, trace contaminant chemicals listed by Proposition 65 without established SHLs represent a knowledge gap. Using appropriate Proposition 65 guidance as well as knowledge of how the Office of Environmental Health Hazard Assessment (OEHHA) has historically calculated SHLs, we propose surrogate SHLs for evaluating exposure to three trace contaminants sometimes found in consumer products: dichloroacetic acid, methyl chloride, and propylene oxide. The purpose of this work was to establish whether product warning statements would be necessary. While in some cases it can be relatively simple to calculate a surrogate SHL using Proposition 65 guidance, other cases require more complex modeling. Although consumer product companies strive to avoid trace contaminants in their products, the product chemistry sometimes makes this unavoidable. For dichloroacetic acid and propylene oxide, SHLs were based on existing guideline values published by regulatory agencies, and for methyl chloride, toxicity data from animal studies were used. Based on these approaches, we calculated an SHL of 14 \( \mu g/d \) based on carcinogenic potency and a SHL of 812 \( \mu g/d \) based on developmental and male reproductive toxicity for dichloroacetic acid, a SHL of 1.0 mg/day based on testicular toxicity for methyl chloride, and an oral SHL of 2.9 \( \mu g/d \) and an inhalation SHL of 54 \( \mu g/d \) for propylene oxide. While these SHLs were calculated based on oral data (with one exception), based on review of available toxicokinetics studies, they should be protective when other exposure routes are applicable. Utilization of these conservative SHLs in exposure assessments resulted in exposure margins several orders of magnitude above that which would prompt any discussion of need to label. Based on our evaluation, these methods provide an acceptable and relatively efficient means to address potential safety concerns related to Proposition 65 chemicals in consumer products. Further, this collaboration reinforces the importance of peer review amongst experts when making such determinations. Subsequent to the calculation of our surrogate SHL for dichloroacetic acid, OEHHA published a NSRL of 17 \( \mu g/d \), illustrating that the methods used provide a reasonable approximation aligned with the OEHHA process.

ABSTRACT NUMBER: 5045  Poster Board Number: P149
TITLE: Establishing Safe Harbor Levels for Trace Proposition 65 Fragrance Ingredients: Estragole, Methylugenol, and Pulegone

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Complying with California’s Proposition 65 can be challenging when chemicals do not have established Safe Harbor Limits (SHLs). SHLs provide a benchmark that can be used to compare to exposures from daily use of consumer products. Specifically, fragrance chemicals listed by Proposition 65 without established SHLs represent a significant knowledge gap. Using appropriate California Proposition 65 guidance as well as knowledge of how California's Office of Environmental Health Hazard Assessment (OEHHA) has historically calculated SHLs, we propose surrogate SHLs that can be used for evaluating consumer product exposures to three fragrance compounds sometimes found at trace levels in consumer product goods: estragole, methyleugenol, and pulegone. The purpose of this work was to establish SHLs based on the state of the science and regulatory guidance from OEHHA in order to supplement product safety assessments and establish whether or not product warning statements would be necessary. While in some cases it can be relatively simple to calculate a surrogate SHL using Proposition 65 guidance, other cases may require more complex modeling. Many considerations must be factored in: exposure routes based how products will be used, consideration of the exposed population, and other chemical specific factors. For estragole and pulegone, benchmark dose modeling was conducted in order to establish an appropriate dose level for SHL derivation, while for methyleugenol, an existing toxicity guideline value from OEHHA interpretive guidance was used. Based on these approaches, we calculated SHLs of 0.004 μg/day for estragole, 1.3 μg/d for methyleugenol, and 0.12 μg/d for pulegone. While these SHLs were calculated based on oral data, a review of available toxicokinetics studies indicate they should be protective when other exposure routes are applicable, such as by the dermal route coinciding with the appropriate use of consumer products. Utilization of these conservative SHLs in exposure assessments resulted in exposure margins several orders of magnitude above that which would prompt any discussion of need to label. Further, this collaboration reinforces the importance of peer review amongst experts when making such determinations. Based on our evaluation, these methods provide an acceptable and relatively efficient means to address potential safety concerns related to Proposition 65 chemicals in consumer products.
clinical and microscopic histopathological evaluation one day following the final exposure and after a 14-day recovery period. In this study, a No-Observable-Adverse-Effect-Concentration (NOAEC) of 250 ppm was established based upon adverse findings which included losses in body weights, a dehydration/volume contraction, and adverse histopathological changes in the nasal cavity at ≥ 500 ppm. In the reproductive and developmental study, male and female rats were exposed to 0, 250, 750, or 1,500 ppm 6-hr/day, during premating, mating, gestation, and lactation. A parental NOAEC of 250 ppm was established based upon reduced body weights and adverse nasal pathology findings at ≥ 750 ppm C4 isonitrile. A fertility NOAEC of 750 ppm was established based upon acyclic estrous cycles that were associated with adverse body weight reductions and a lower mean number of pups per litter observed at 1,500 ppm, and a developmental NOAEC of ≥ 1,500 ppm was established. Therefore, based upon an overall weight of evidence assessment, a NOAEC of 250 ppm is established as a point of departure (PoD) for C4 isonitrile human health risk assessment purposes demonstrating that this compound has a satisfactory hazard profile for use within gas-insulated equipment in the electric power industry.

**ABSTRACT NUMBER:** 5047  
**Poster Board Number:** P151  
**TITLE:** Initial Testing of an Ion Channel Panel for Early In Vitro Detection of Seizure Liability  
**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** K. Rockley, H. Jennings, E. Johnson, K. Jones, R. Roberts, and M. Morton. ApconiX, Macclesfield, United Kingdom.  
**KEYWORDS:** In Vitro and Alternatives; Ion Channels, Seizure  
**ABSTRACT:** Seizure liability remains a significant cause of attrition throughout drug development. Seizures were the most common CNS issue encountered in preclinical drug development in an SPS industry survey published in 2016 (encountered by 67% of respondents). Between 2005-2010 the CNS was the organ system most frequently associated with safety-related attrition in clinical development. A loss of competitiveness, delays, increased costs, and a considerable safety risk all emphasise the need for improved methodologies to accurately detect seizure liability earlier in drug development. Current methods rely on nonclinical rodent and non-rodent studies. Ideally, novel, high-throughput in vitro methods would provide an earlier prediction of seizurogenic risk that could eliminate liabilities early in discovery while there are still options in chemistry and reduce the reliance on costly animal studies. The involvement of numerous ion channels in seizure provides an opportunity for a new paradigm in screening. Akin to the success of screening against a panel of ion channels to reduce cardiovascular safety liability (CiPA), the involvement of ion channels in seizure suggests that a similar approach is valid. An initial panel of 15 seizure-related ion channels have been selected (Nav1.1, Nav1.2, Nav1.6, Kv7.2/7.3, Kv7.3/7.5, Kv1.1, Kv4.2, KCa4.1, Kv2.1, Kv3.1, KCa1.1, Cav2.1, GABA α2β2γ2, nicotinic α4β2, NMDA 1/2A) based on weight of evidence from genetic/pharmacological studies and recombinant cell lines created. The activity of the HESI NeuTox compound set (4-AP, acetaminophen, amoxapine, amoxicillin, chlorpromazine, enoxacin, linopirdine, pentyleneetetrazole, picrotoxin, pilocarpine, phenytoin, strychnine) and other seizure causing drugs (bupropion, clozapine, diphenhydramine, paroxetine, quetiapine) on this ion channel panel was determined using automated electrophysiology. Of the ion channels tested, clozapine, diphenhydramine, chlorpromazine, amoxapine, paroxetine, bupropion and linopirdine inhibited two or more ion channels. The potassium channels were sensitive to more compounds than the sodium channels. All seizure causing compounds demonstrated at least one
“hit” against our seizure panel. These initial studies highlight the potential utility of a seizure ion channel screening panel to provide mechanistic information and support optimal drug design in early development before animals, resources and time have been wasted.

ABSTRACT NUMBER: 5048 Poster Board Number: P152
TITLE: Preclinical Detection of Drug-Induced Liver Injury as a Predictive Measure for Future Drug Labeling


KEYWORDS: Safety Evaluation; Hematotoxicity; Methods/Mechanism

ABSTRACT: Drug induced liver injury (DILI) remains a major adverse event leading to both the withdrawal of drugs from the market and the cessation of clinical trials. Although, preclinical strategies have been developed to predict preclinical DILI, there is a lack of information connecting the preclinical detection of DILI to the labeling of approved drugs. The hypothesis for this study was that preclinical detection of DILI increases the potential for correct labeling of approved drugs (i.e., withdrawn, black box warning, precautions). Public databases were used to determine to the frequency of DILI in patients, the preclinical detection rates of DILI, and whether the preclinical detection of DILI could be an indicator of future labeling of an approved drug. Using the FDA’s DILI Rank Dataset, compounds with high clinical DILI concern (N = 23) and severe clinical DILI (N = 23) were identified. For this analysis, the occurrence of liver function test (LFT) elevations was used as an indicator of DILI. Next, using the NIH’s LiverTox database, the compounds with clinical DILI were put into two categories, occurrence of LFT elevations (N = 31) and occurrence of severe LFT elevations (N = 23). To determine the frequency of DILI of these drugs and whether there was preclinical detection of DILI, previously published reports were used. It was found that the preclinical detection rates for LFT elevation and severe LFT elevation were 19/31 (61.2%) and 11/23 (47.8%) respectively. Furthermore, to determine the validity of preclinical detection as a predictive indicator of future FDA drug labeling, a confusion matrix was used to compared the preclinical detection and the FDA drug labeling. For the occurrence of LFT elevation, it was found that the sensitivity and the specificity were 78.9% and 8.3%, with a precision of 93.7%. This suggests that preclinical detection of DILI has a higher predictive power for labeled drugs than non-labeled. The sensitivity and specificity for severe LFT elevation were 55% and 100%, with a precision of 76.9%, which suggests a higher predictive capability for non-labeled drugs than labeled. Overall, it was found that preclinical detection of DILI can adequately identify about half of clinical occurrences of LFT elevations.
Additionally, preclinical detection can predict the future labeling of drugs with LFT elevations, but not severe LFT elevations.

ABSTRACT NUMBER: 5049 Poster Board Number: P153
TITLE: Preclinical Organoids as an In Vitro Tool to Study the Intestinal Response to Cytotoxicants

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ABSTRACT: Cross-species comparison of drug responses at the organoid level could help to determine the human relevance of animal findings. To this end, we must evaluate the in vitro-in vivo translatability of preclinical organoids. Here, we took 5-FU as an exemplar drug to test if the in vivo gut response to this cytotoxicant is preserved in murine enteroids and colonoids. We developed a mouse model of 5-FU-induced diarrhea with an exposure similar to the clinic. 5-FU at 50 mg/kg BID (high dose) induced an early peak in crypt apoptosis followed by crypt atrophy in small intestine (SI) and colon and villus shortening in SI. There was a rapid increase in Caspase-3-positive crypt cells after 5-FU, whilst Ki67 and Olfm4 staining declined gradually over time. To enable comparison between the response in organoids and their corresponding in vivo region, top nominal in vitro doses causing significant cytotoxicity were chosen. The inferred intracellular concentration in organoids at 1000 µM was within the exposure range related to gut toxicity in vivo. 5-FU at ≥ 100 µM decreased ATP levels and increased Caspase-3 activity in enteroids and a similar trend was observed in colonoids. Image-based morphometric analysis revealed phenotypic changes indicative of cell death at ≥ 100 µM in enteroids. In keeping with the in vivo findings, 5-FU raised Caspase-3 cell counts and reduced Ki67 staining. At the transcriptome level, there was an overlap in the activity of pathways related to 5-FU’s mode of action, lipid and cholesterol metabolism and integrin signaling between setups. The best in vivo-in vitro correlation was observed between organoids at ≥ 100 µM and high dose treated mice at 24h. However, several pathways such as those related to drug metabolism showed opposite activities across systems. The predicted activity state of upstream regulators was overall well preserved between setups, with p53, CDKN2A and other cell cycle genes among the top. Collectively, results suggest that organoids represent an adequate tool to explore the gut response to cytotoxicants.
convulsions and neurodegeneration. In contrast, nonclinical species showed a higher sensitivity (75%) and much lower specificity (30.4%). The neural spheroids demonstrated higher likelihood ratio positive and invert likelihood ratio negative compared with nonclinical safety studies. This assay has the potential to be used as a predictive assay to detect neurotoxicity in early drug discovery, aiding in the early identification of compounds that eventually may fail due to neurotoxicity.

**ABSTRACT NUMBER:** 5051  **Poster Board Number:** P155

**TITLE:** Do CD-1 Mice Used in Behavioral Studies Metabolize Illicit Synthetic Cannabinoids Like Humans?

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**KEYWORDS:** Metabolism; Cytochrome P450; Neurotoxicology

**ABSTRACT:** In 2019 synthetic cannabinoid receptor agonists accounted for more than one-third of new drugs of abuse worldwide. Human studies are not ethically feasible to assess health risks and establish regulatory guidelines. Thus, exposure studies with CD-1 mice have become the gold standard. Observed responses ultimately depend on metabolism that alters drug structure and potency. However, there are no reports on synthetic cannabinoid metabolic pathways for CD-1 mice. Such evidence is critical for ascribing their suitability as surrogates for human responses to exposures. We then compared steady-state metabolism of N-(1-adamantyl)-1-(5-pentyl)-1H-indazole-3-carboxamide (5F-APINACA/5F-AKB48) by human and CD-1 mouse liver microsomes avoiding confounding effects of typical extensive metabolism of synthetic cannabinoids. For both species, results showed two metabolic pathways for 5F-APINACA: oxidative defluorination of the pentyl tail and two sequential adamantyl hydroxylations. The kinetic profiles indicated equal competition between pathways except oxidative defluorination dominated at higher concentrations. Adamantyl dihydroxylation kinetics had a lag and was less efficient than initial hydroxylation. When fit to traditional models, all reactions had very low micromolar Kms and at high substate levels, substrate inhibition with high Kis. When normalized to total CYP (not protein) levels, human and mouse metabolic rates were comparable. Analysis of kinetic plots treated data sets individually and so, we are globally analyzing all data with differential equations for possible mechanisms to identify the most probable one. This strategy more specifically determines how pathways interact and compete with one another for comparing metabolism between species. Based on chemical inhibitor studies, CYP2C and CYP3A enzymes dominated metabolism, yet human CYPs were more selective for reactions than mouse Cyps. In sum, 5F-APINACA metabolic pathways and clearance were similar between humans and CD-1 mice suggesting suitability for assessing the impact of 5F-APINACA metabolism on behavioral and toxicological responses. Whether this trend applies to other synthetic cannabinoids requires further study given known variations in CYPs responsible for metabolism.
ABSTRACT NUMBER: 5052    Poster Board Number: P156

TITLE: Development of a Pharmacokinetic Model for Aerosolized Carfentanil Inhalation


KEYWORDS: Inhalation Toxicology; Pharmacokinetics; Pharmaceuticals; Carfentanil

ABSTRACT: Opioid misuse is an unwavering public health crisis highlighted by a consistent rise in opioid-related overdoses and deaths in the past twenty years. The threat posed by opioid abuse is further amplified by the recent emergence of more potent synthetic opioids, such as carfentanil (CRF) and remifentanil (REM), and their potential use as incapacitating agents. This potential was demonstrated during the 2003 Moscow Theater Incident, where an aerosolized combination of CRF and REM was used to incapacitate a theater full of people. In the wake of this incident, the need to understand the inhalation threat of opioids is undeniable. In this study, a physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) model of aerosolized CRF in ferrets was developed to assess cardiopulmonary effects of CRF inhalation. A physiological model of a ferret was developed from tissue weights reported in literature, blood flow rates scaled allometrically to tissue volume, and experimentally determined hepatocyte clearance and lipophilicity of CRF. This PBPK model was validated using pharmacokinetic data reported by McCranor et al. (2020) for subcutaneous dosing of 25 µg/kg of CRF in ferrets. This validated model was then used to determine the PK of aerosolized CRF inhalation at a concentration-time product of 14.4 mg × min/m$^3$ (0.72 mg/m$^3$ for 20 min) with a mass median aerodynamic diameter of 3 - 4 µm. The delivered dose was calculated from individual subject minute volumes and the average concentration of CRF. The developed inhalation PBPK model was then compared to PD effect-time profiles of observed cardiopulmonary effects, represented as a fraction of baseline values, during and after exposure. For PD model development, subjects were randomly separated into training and test cohorts. A direct linear model with a negative slope was found to best fit the relationship between plasma concentrations of CRF and breathing frequency (f) with an RMSE of 0.584 in the training cohort and 0.570 in the test cohort. This relationship indicates that an increase in plasma CRF leads to a decrease in f. A sigmoidal model was found to best fit mean arterial pressure (MAP) with RMSE of 0.327 and 0.248 for the training and test cohorts, respectively, indicating higher CRF concentration leads to an increase in MAP up to a maximum value. Given the similarity of human and ferret lungs, these relationships may be used to develop real-world countermeasure protocols and aid crisis response.

ABSTRACT NUMBER: 5053    Poster Board Number: P157

TITLE: Metabolic Clearance and Lipophilicity of Select Opioids and Opioid Antagonists


KEYWORDS: Pharmacokinetics; Opioids

ABSTRACT: Opioid abuse is a global public health issue, exacerbated by the emergence of more potent synthetic opioids, particularly fentanyl and its analogs. While competitive antagonists exist, the
therapeutic potential of current antagonists against synthetic opioids is largely unknown. Furthermore, renarcotization remains an ongoing concern due to the relatively short durations of action of current antagonists. In this study, physicochemical properties, specifically metabolic activity and lipophilicity, were characterized for fentanyl-class opioids and common opioid antagonists in an effort to aid computational model development and drug discovery. Select fentanyl-class opioids (fentanyl, remifentanil, carfentanil) and opioid antagonists (naloxone, naltrexone, nalmefene) were subjected to metabolism via individual cytochrome P450 (CYP) enzymes and hepatic spheroids. Substrate decay was quantified using liquid chromatography with tandem mass spectrometry (LC-MS/MS), and Michaelis-Menten parameters were derived from initial reaction velocities. Lipophilicity, as measured by the octanol-water partition coefficient (logD), was characterized across multiple pH using a miniaturized shake-flask method followed by LC-MS/MS quantification. Predicted pKa were optimized using experimental determined logD to better inform tissue-plasma partition coefficient calculations. For all substrates, intrinsic hepatic metabolism was higher than the composite of CYP activities. Of the CYP isozymes investigated, 3A4 yielded the highest absolute and relative metabolism across all substrates, with largely negligible contributions from 2D6 and 2C19. Comparative analysis of systemic clearance for opioids and opioid antagonists highlighted elevated lipophilicity and diminished CYP3A4 activity as potential considerations for the development of more efficacious opioid antagonists. In terms of pKa optimization, a larger discrepancy was observed for opioid antagonists compared to agonists. Finally, computational models were refined using experimental inputs, with significant improvements observed in both absorption and clearance. Together, these results provide multiple screening criteria for early stage drug discovery, as well as computational models with which to evaluate potential countermeasure candidates.

ABSTRACT NUMBER: 5054    Poster Board Number: P158
TITLE: Elucidation of Metabolic Interactions, A Focus on AMB-FUBINACA and Other Xenobiotic Compounds
KEYWORDS: Biotransformation and Toxicokinetics; Metabolism; Other: Carboxylesterase-1; AMB-FUBINACA
ABSTRACT: Within New Zealand alone, the synthetic cannabinoid AMB-FUBINACA has been associated with at least 64 deaths between May 2017 and February 2019. Little is known about the mechanism of synthetic cannabinoid toxicity and while it is hypothesized to be mediated through CB1 (cannabinoid receptor type 1), it may not be as simple as high potency or affinity at the receptor. It is currently unknown what enzyme is responsible for the rapid hepatic conversion of AMB-FUBINACA to the hydrolyzed metabolite, AMB-FUBINACA acid. As AMB-FUBINACA acid is inactive at CB1, inhibition of this metabolic pathway may result in additional CB1 activation by AMB-FUBINACA. Initial experiments with human liver microsomes indicated that this hydrolysis reaction is extremely rapid (t1/2 = 0.21 min) and not mediated by CYP450 enzymes. It was hypothesised that CES1 (carboxylesterase-1) is responsible for the metabolism of AMB-FUBINACA, xenobiotics which inhibit CES1 would result in sustained concentrations of AMB-FUBINACA, likely resulting in prolonged CB1 activation in vivo. Metabolism was assessed in vitro with recombinant CES1 and concentrations of AMB-FUBINACA were determined using
high-performance liquid chromatography. AMB-FUBINACA metabolism by CES1 was significantly inhibited by the steroidal saponin digitonin and the angiotensin II receptor blocker telmisartan. Previous literature indicates cannabinoid delta-9-tetrahydrocannabinol and ethanol also inhibit CES1; however, AMB-FUBINACA metabolism was not significantly inhibited in the presence of these compounds. Further studies should assess AMB-FUBINACA metabolism in the presence of xenobiotics also detected within the blood of these fatal New Zealand AMB-FUBINACA poisonings to establish a link between epidemiological data and in vitro metabolism research. As AMB-FUBINACA metabolism occurs so rapidly, minor inhibition of this pathway could prove toxic, understanding these interactions may give insight into why some users experience high levels of harm.

ABSTRACT NUMBER: 5055    Poster Board Number: P161
TITLE: Identifying Candidate Reference Chemicals for In Vitro Testing of the Retinoid Pathway from Public Data and Literature Mining

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KEYWORDS: Developmental/Teratology; In Vitro and Alternatives; Developmental Toxicity; Prenatal; Retinoic Acid

ABSTRACT: Using in vitro bioactivity of chemicals to predict in vivo toxicity is an important component of animal-free toxicity testing. Sets of reference chemicals and data measuring their activity against targets of interest are essential both to validate and establish confidence in in vitro assays. The retinoic acid (RA) signaling pathway is especially important in developmental processes and toxic exposure. Employing data extraction methods and advanced literature mining tools, we assembled a set of candidate reference chemicals for activity on ten protein family targets in the retinoid system (serum retinol binding protein, stimulated by retinoic acid 6, cellular retinol binding protein, cellular retinoic acid binding protein, retinoic acid 4-hydroxylase, retinol dehydrogenase, retinal dehydrogenase, retinoic acid receptors alpha, beta and gamma (RARA, RARB, RARG)). The collection was assembled from Protein Data Bank, ChEMBL, ToxCast/Tox21, and the biomedical literature in PubMed. The process produced approximately 220 putative reference chemicals. When this set was inserted into the Abstract Sifter literature tool and PubMed searches were performed, around 137 (approximately 75%) of the chemicals showed literature connections to developmental toxicity. When the queries are structured to focus on one developmental adverse outcome associated with retinoid disruption - limb defects - we find approximately 55 chemicals have some supporting literature. These methods produced a strong pool of candidate reference chemicals. The integration of the chemical set into an Abstract Sifter literature tool facilitates the investigation of the chemicals’ known downstream effects and provides connections via hyperlinks to the EPA Chemicals Dashboard with its rich chemical information encompassing in vitro profiling, in vivo data and computational predictions. This abstract does not necessarily represent US EPA policy.
ABSTRACT NUMBER: 5056    Poster Board Number: P162
TITLE: Exploring Sensitivity Methods to Increase the Model Confidence of and Improve Pregnancy and Non-Pregnancy PBPK Models

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KEYWORDS: Biological Modeling; Computational Toxicology

ABSTRACT: Labetalol is a beta blocker that is commonly prescribed to treat hypertensive crises in many patients including pregnant women. The objective of this work is to use various sensitivity analysis methods to determine which is best suited to analyze a predictive model of labetalol. Furthermore, we determine which parameters are the most sensitive to help increase model confidence. Physiologically based pharmacokinetic (PBPK) models use mathematical equations to predict the absorption, distribution, metabolism, and excretion (ADME) of chemical substances, such as a drug of interest, in humans and other species. These models have multiple compartments with each representing an organ or system in the body. Compartment defining parameters that represent filtration rate, organ volumes, blood flows, etc. can be altered to see how the ADME is affected. In this project, a PBPK model of labetalol was translated between two dissimilar coding languages, Berkeley Madonna code to R using mrgrsolve package notation. The R coding language was chosen due to the amount of available sensitivity packages. The initial global sensitivity analysis yielded sensitivity coefficients of 0.7, 0.6, and 0.6 for cardiac output, body weight, and fractional flow rate to the liver, respectively. Additionally, a parameter sensitivity package was used to perform a local sensitivity analysis on the modified labetalol PBPK model. From these results, we are aware of sensitive parameters for labetalol such as body weight, albumin, and clearance. In the future we will further explore global sensitivity analysis which can more beneficial than a local sensitivity analysis because the prior allows for the quantification of parameter interactions which will highlight parameter contributions to model confidence. Furthermore, other packages may be explored to compare multiple results and methods of parameter sensitivity analysis. Finally, sensitive parameters between pregnant women and non-pregnant women will be compared to elucidate pregnancy specific parameter sensitivities.

ABSTRACT NUMBER: 5057    Poster Board Number: P163
TITLE: Population Pregnancy Physiologically Based Pharmacokinetic/Pharmacodynamic Modeling of Labetalol

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KEYWORDS: Physiologically Based Pharmacokinetics; Biological Modeling; Pharmacokinetics; Labetalol

ABSTRACT: Gestation hypertension, a common ailment in pregnancy, can be a precursor to conditions like pre-eclampsia. Since many physiological changes occur during pregnancy, pharmacokinetics (PK) may need to be reevaluated in pregnancy to ensure efficacy and safety. Yet, pregnancy is often an exclusion factor in clinical trials due to ethical concerns, which leads to data scarcity and an increased awareness of the need for change in research conducted for this population. Physiologically based
pharmacokinetic (PBPK) modeling, which is a series of mathematical equations, can serve as a tool to predict drug PK in various life-stages. Additionally, PBPK modeling can help to identify and explore data gaps in PK parameters. The goal of this project is to generate a population model that well predicts PK and pharmacodynamic (PD) variations in blood pressure (BP) for pregnancy using labetalol which is primarily metabolized by UGT1A1 and to a greater extend by UGT2B7. In our previous research, we coded a PBPK model calibrated with observed literature data from healthy male plasma concentrations. Then, we created a UGT2B7 ontogeny equation for pregnant women de novo to predict the clearance of labetalol over the duration of pregnancy. This was applied as an activity factor in UGT2B7 metabolism in the pregnancy population model to predict variations in the PK profile. Furthermore, the PD component was coded and calibrated with healthy male BPs and implemented within the pregnancy model. Population parameters were varied between 10-30%. The mean PK simulation at the 2nd and 3rd trimester captured >50% of the observed data points for pregnant women within 2 average fold-error (AFE). BP simulation contained 100% of points for pregnant women at the 3rd trimester within 1.5 absolute average fold error (AAFE). 100% of points for non-pregnant normotensive women were within the AAFE. Thus, the variation in the population may still account for wide range of differences in pregnant and non-pregnant individuals alike. This work shows that the population PBPK model can estimate the range of PK changes during pregnancy and the variabilities in population. It highlights BP mild changes over pregnancy versus non-pregnancy. Hence, the model serves as a predictive tool to estimate dose and response range in pregnant women which could be used for starting or target dosing strategy. This and similar analyses could be used as tools in regulatory or clinical evaluations.

**ABSTRACT NUMBER:** 5058  **Poster Board Number:** P164

**TITLE:** Development of a Device to Perform Automated High-Throughput Processing of Comet Assay

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**ABSTRACT:** Single cell gel electrophoresis (aka. the comet assay) is a highly sensitive method to measure DNA strand breaks in both in vivo and in vitro models. The comet assay is widely used to study the causes and mechanisms of genotoxicity in various fields, such as public health, carcinogenesis, and the environmental sciences. Recently, several comet assay methods have been described, which increase sample throughput, reduce reagent usage, together with improving the sample processing and running procedures. However, despite this, comet assay sample workup remains time-consuming, and has the potential to introduce human errors, contributing to the widely reported intra- and inter-laboratory variability. We have developed a device which can perform high throughput, automated processing of comet assay slides. This removes human involvement and hence error, decreases run time, and allows samples to be processed 24/7, with an overall decrease in time and cost. This device automates all slide handling steps, including loading, and removing buffers, washing, drying, and staining; the duration of these steps, together with temperature and voltage are fully controlled, and can be adjusted to provide optimal conditions. Furthermore, our results showed improved assay precision over a manual high throughput comet assay system.
ABSTRACT NUMBER: 5059    Poster Board Number: P165
TITLE: The Toxicity of the Pesticides Diclofop-Methyl and Its Active Form Diclofop Determined by In Silico Models Showed High Potential of Toxicity to Humans

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KEYWORDS: Computational Toxicology; Pesticides

ABSTRACT: In silico tools and models developed for toxicity assessments can be implemented in tiered testing approaches, such as integrated testing strategies (ITS), integrated approaches to testing and assessment (IATA), and adverse outcome pathways (AOP) frameworks, balancing transparency, mechanistic interpretability, and predictivity. In this regard, here we used the iS-Tox® Platform, which was developed under the sponsor of FAPESP (São Paulo Research Foundation - process number 2016/08322-5) to evaluate and predict the toxicity of 2 pesticides, diclofop and diclofop-methyl. These pesticides are used in wheat, soybean, beans, onions and oats in order to inhibit the growth of harmful grasses and, therefore, increase agricultural production. The herbicide is used to suppress the photosynthesis and chlorophyll synthesis damaging the membrane of protoplasts. It is one of the most commonly detected herbicides in environment, both in aquatic environments and in products from agriculture; and the assessment of its toxicity to non-target organisms is extremely important. In this way, the in silico prediction showed that both diclofop-methyl and diclofop were categorized as class 4 for acute toxicity, but, unlike of diclofop-methyl (which has a non-hepatotoxic potential of 61%), the active form (diclofop) showed a higher potential of about 60% of hepatotoxicity. Both substances have toxicity probability in relation to development and reproduction, as well as a genotoxic potential - of the three machine learning methods used on the platform, both diclofop-methyl and diclofop proved to be mutagenic in at least one of them. As for the analyses of metabolic parameters evaluated, diclofop acted as an inhibitor of at least one of the cytochrome P450 (CYP) enzymes, while diclofop-methyl acted as an inhibitor in 5 out of 9 pathways evaluated, indicating that further tests needs to be done in order to better evaluate their toxic potential to non-target organisms.

ABSTRACT NUMBER: 5060    Poster Board Number: P166
TITLE: A Chemical Landscape Based on In Silico Data Availability Profile across Diverse In Vitro/In Vivo Assays to Support Read-Across Evaluations

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KEYWORDS: Computational Toxicology; QSAR

ABSTRACT: The U.S. EPA CompTox Chemicals Dashboard has curated over 830K environmentally relevant compounds. Out of 830,280 chemicals, less than 12% have experimental testing data (either in vitro or in vivo). We hypothesize that the uncertainty in prediction (i.e., binary value of in- or out-of-applicability domain) from a large collection of quantitative structure-activity relationship (QSAR) models can be used to identify regions of chemical space where there is limited or no information, and which therefore should be targeted for read-across evaluations. To perform this analysis, we first clustered 830,280 chemicals based on their chemical structures defined using molecular substructural
fingerprints (Saggar, Sedykh A et al. 2021). The clustering was done using a modified recursive k-means algorithm, resulting in 15,334 clusters. The median cluster size is 31. We then mapped out-of-domain prediction data generated from 129 Leadscope QSAR models on the 830,280 chemicals to the 15,334 clusters. For each cluster, we characterize its in silico data availability profile using the fraction of out-of-domain predictions of chemicals against each of the 129 QSAR models. The 15,334 x 129 matrix served as the input for a dimension reduction algorithm (Uniform Manifold Approximation and Projection, UMAP) to render a 2-dimension (2D) landscape for visualization. The endpoints of these 129 QSAR models cover 22 toxicity categories, including ‘carcinogenicity in vivo’ (4 models), ‘cardiotoxicity in vivo’ (13 models), and ‘neurotoxicity in vivo’ (4 models), which are the three health effects emphasized in the strategic plan of DNTP. Out of 15,334 clusters, only ~4% (557 clusters), where all the chemicals in the cluster, were out-of-domain in all the carcinogenicity models, ~10% (1455 clusters) were out-of-domain in all the cardiotoxicity models, and about 14% (2102 clusters) were not covered by any of the neurotoxicity models. Some chemical substructures found in these ‘pan-out-of-domain’ clusters include benzothiophene, azulene, alkyl-cyclohexane, etc. Complementary data such as production volume, estimated human exposure, and experimental testing data can be further integrated to identify data-poor regions of chemical space that exhibit high predicted exposure, and therefore require further evaluations.

ABSTRACT NUMBER: 5061   Poster Board Number: P167
TITLE: Development of a Climate Vulnerability Index: A Case Study in Will County, Illinois

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ABSTRACT: The goal of the Climate Vulnerability Index (CVI) Project is to create a national GIS Environmental Mapping Tool to identify areas that both have baseline vulnerabilities as well as will be adversely impacted by climate change. Baseline vulnerabilities include those related to socio-economic status, health, infrastructure, and environment, while climate change vulnerabilities include adverse impacts on the economy, on health, and on the incidence and severity of extreme events. Toxicological Prioritization Index (ToxPi) and ArcGIS were utilized to integrate and create visualizations of each of the baseline vulnerability categories as well as of the overall baseline vulnerability. This pilot study uses Will County, Illinois as a starting point to demonstrate this approach. We first curate and merge the datasets across indicators, then use the ToxPi methodology to integrate those data into an overall score, and finally apply hierarchical clustering to examine whether vulnerabilities cluster geographically. In addition to those vulnerabilities addressed by the nation-wide CVI indicators, Will County, Illinois has seen a large increase in the number of warehouses, and the community is concerned with potential risks associated with warehouse expansion. We manually identified warehouse locations using Google Maps satellite images and worked with local ground-truthing by citizen scientists. Our preliminary results indicate that the census tracts with the highest ToxPi scores for the baseline vulnerability categories and the overall baseline vulnerability are also concentrated near where many of the warehouses are also located. However, annual averages of air pollutants NO2, O3, and PM2.5 did not appear to correlate with warehouse density. To address spatial and temporal variability, we are also partnering with local...
community groups to assist in citizen air pollution monitoring using mobile and stationary sensors to gather additional data on the relationship between warehouses and air pollution.

ABSTRACT NUMBER: 5062       Poster Board Number: P168
TITLE: Preparation of a Harmonized Resource of Publicly Available Chemical Safety Data, ToxValDB

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KEYWORDS: Bioinformatics; Computational Toxicology; Risk Assessment

ABSTRACT: Chemical safety assessment requires thorough and systematic evaluation of a broad range of applicable evidence. However, not all decisions require that level of scrutiny. Also, already-assembled repositories of more general knowledge potentially available for tens of thousands of chemicals can serve as starting points for thorough systematic reviews. To support these and other efforts, ToxValDB contains over 1.2M records of quantitative in vivo toxicology and risk data on over 30K chemicals harmonized across over 40 aggregate sources and the open literature. The current project describes the steps taken to assure the fidelity of the transformed records to the original source material, including the normalization of the structure of the information and terminology used across many sources to a common format. This is a unique challenge due to the heterogeneity of experimental or regulatory values collected in this resource. We also present the resulting chemical coverage for experimental study types, showing this resource’s possibly utility for answering research questions or identifying gaps in chemical space and experimental coverage. Summaries of the assembled information are available through the U.S. EPA CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard/). This abstract does not necessarily reflect US EPA policy.

ABSTRACT NUMBER: 5063       Poster Board Number: P169
TITLE: Collaborative Analysis of Complex Nitrosamines


KEYWORDS: Computational Toxicology; Genetic Toxicology; Genotoxicity; Nitrosamine

ABSTRACT: The recent discovery of small-molecule nitrosamine impurities in marketed drugs, starting with nitrosodimethylamine (NDMA) in batches of Valsartan in 2018, has led to significant regulatory response, including drug recalls and regulatory guidance that requires the evaluation of all synthetic and formulation routes for the potential presence of nitrosamine impurities. Due to the wide range of potential routes of formation for nitrosamines [Lopez-Rodriguez et al (2020), Org. Process Res. Dev. 24, 1558-1585], many active pharmaceutical ingredient (API) structures are themselves liable to be nitrosated, either during the later stages of the synthetic process or as the formulated drug product. This poster describes the formation of a data-sharing initiative, coordinated by Lhasa Limited, which has the following aims: Firstly, since many APIs are generic drugs and are manufactured by multiple companies, the reduction of duplicate testing and the availability of an Ames test result conducted to
the highest standards and in appropriate conditions should reduce potential uncertainties for regulatory submissions. Secondly, the consortium aims to investigate differences between these compounds and the small molecules which comprise the majority of publicly available nitrosamine data [Thresher et al (2020), Regul. Toxicol. Pharmacol., 116, 104749] and elucidate any structure activity trends and significant differences. Key among these is that based on initial reports and the small amount of data for complex structures that is currently available, the proportion of these compounds which are Ames negative may be higher than the proportion of small molecule nitrosamines, for reasons such as differences in metabolic potential or the proposed adducts formed. Full testing of this hypothesis requires the curation of a large high-quality dataset, which can only be achieved via cross-industry collaborations such as this.

ABSTRACT NUMBER: 5064
Poster Board Number: P170
TITLE: Developing and Validating Machine Learning Models for Acetylcholinesterase Inhibitors across Multiple Species


KEYWORDS: Computational Toxicology; Hazard Identification/Reduction; Organophosphates; Acetylcholinesterase Inhibitors

ABSTRACT: Acetylcholinesterase (AChE) is a target for both drugs and poisons in different contexts. Overdose of reversible AChE inhibitors, such as those used to treat Alzheimer’s disease and other dementias, or exposure to irreversible AChE inhibitors, such as those used as commercial pesticides or chemical weapons, can lead to accumulation of acetylcholine and overstimulation of nicotinic and muscarinic receptors in the central nervous system. This can also lead to both acute and chronic symptoms, ranging from headache and breathing problems, to coma and death. Because of this, AChE is a target of toxicological interest and identifying compounds that inhibit AChE is crucial step for identifying potential environmental toxins. Using our Assay Central software, we previously described Bayesian models for human and eel AChE using inhibitor data from ChEMBL and performed 5-fold cross validation with ROC values of 0.92 and 0.91, respectively. We developed and used an auto-curation tool to standardize and curate datasets pulled from ChEMBL, Tox21, ToxCast and PubMed. As there is a wealth of such data available for several species (rat, mouse, eel, human etc.) in these databases, we have used these resources to expand our model comparisons using 15 different machine learning models (regression and classification models) using ECFP6 fingerprints and 3D pharmacophore fingerprints. For example, we have used our updated Assay Central software to create classification models for eel AChE based on eight different algorithms, using a threshold of activity at IC50 20 µM. While all the algorithms performed well against a test set of 150 compounds, the k-nearest neighbor model performed the best overall, with AUC, precision, recall, accuracy, and specificity scores of 0.78, 0.86, 0.77 0.76, and 0.74, respectively. By consensus, these different classification models accurately predicted activity in 115 of 150 (77%) compounds in this external set. We will use this machine learning approach to generate and test models for other species similarly. The best validated models will then be used to profile compounds for potential to inhibit AChE across species. We will gain an understanding of our model applicability domains using different visualization tools. Our AChE machine learning models
will also enable rapid profiling of massive virtual and DNA encoded libraries to identify compounds with potential clinical or environmental implications.

**ABSTRACT NUMBER:** 5065  **Poster Board Number:** P171  
**TITLE:** Higher-Throughput, Allometrically-Scaled, Two-Chamber Liver-Organ Co-culture System for Toxicity Testing  
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**KEYWORDS:** Alternatives to Animal Testing; Biotransformation; In Vitro and Alternatives  
**ABSTRACT:** Ingested chemicals and drugs often undergo hepatic xenobiotic metabolism and generate a spectrum of metabolites. These metabolites can exhibit greater or lesser toxicity than the parent compounds for a given biological target through hepatic bioactivation and bioinactivation, respectively. Here, we describe our two-chamber liver-organ co-culture model in a higher-throughput 96-well format for the determination of toxicity on target tissues in the presence of physiologically relevant human liver metabolism. Importantly, this platform is designed to be allometrically scalable to produce sufficient quantities of metabolites to simulate the in vivo exposure setting. Our two-chamber system is a hydrogel formed within each well consisting of an outer ring-shaped trough (human liver tissue chamber) with a central well (target tissue chamber). The target tissue can either form a three-dimensional (3D) spheroid-shaped microtissue, or a two-dimensional (2D) cell layer. The hydrogel is equilibrated with cell culture media that freely diffuses between the two chambers. We selected the human liver NoSpin differentiated HepaRG cells (Lonza) to form our 3D human liver tissue, as the utility of primary human hepatocytes for chemical safety evaluation is limited by donor-specific variability, and a rapid loss of hepatocyte functionality in vitro. Histological analyses showed that HepaRG cells formed viable 3D organoids that exhibited robust tissue protein expression of albumin, Phase I cytochrome P450 (CYP3A4) enzyme, MRP2 transporter for biliary excretion, and asialoglycoprotein receptor for glycoprotein uptake. Our 3D human liver tissues actively metabolized phenacetin, bupropion and verapamil into their metabolites, acetaminophen, hydroxybupropion, and norverapamil, respectively. Importantly, we demonstrated that both phenacetin and its metabolite rapidly diffused and equilibrated between the 2 chambers, thus demonstrating the efficacy of our co-culture model in testing drug/chemical toxicity on target tissues with physiologically relevant human liver metabolism. These results support the use of our liver-organ co-culture platform to provide critical and higher-throughput testing for metabolism-dependent bioactivity drugs/chemicals, to better recapitulate the biological effects and predict potential human health hazards of compounds.

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**ABSTRACT NUMBER:** 5066  **Poster Board Number:** P172  
**TITLE:** Organ-on-a-Chip Meets High-Throughput Screening: Measuring Trans Endothelial Electrical Resistance  
**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** A. Nicolas, F. Schavemaker, G. Rabussier, S. Trietsch, H. Lanz, P. Vulto, and D. Goubert. MIMETAS, Oegstgeest, Netherlands. Sponsor: K. Queiroz, American Association for Cancer Research

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The Toxicologist: Late-Breaking Supplement 2022
KEYWORDS: In Vitro and Alternatives; Safety Pharmacology; Predictive Toxicology; Organ-on-a-Chip

ABSTRACT: We demonstrate a method to study low Trans Epithelial Electrical Resistance values in endothelial models. We exposed 36 Huvec tubules to a varying concentration range of the toxicant staurosporine and acquired real-time data over 24 hours using OrganoTEER, showing a useful dynamic range down to 1 Ohm.cm² and the capability of label-free real time barrier integrity measurements in a state of the art blood vessel on a chip model. In vitro endothelial barrier models, such as the blood-brain barrier, are a crucial element of studying the safety and delivery mechanism of therapeutic drugs and toxicants. Endothelial models have much lower TEER values than their in vivo counterparts, which makes them challenging to measure. Here we demonstrate the suitability of the OrganoTEER platform for TEER measurements on low TEER endothelial tubules grown in the OrganoPlate. The developed system makes use of an electrode interface compatible with the OrganoPlate. TEER values can be determined for up to 80 perfused tubules in 40 independent chips within 60 seconds. The instrument can be placed within an incubator to enable automated long-term monitoring of TEER over the entire duration of an epithelial/endothelial study. Collagen I at 4mg/ml was deposited in the gel compartment using Phaseguide technology. Huvec tubules were cultured against collagen I for 5 days and exposed to Staurosporine for 24 hours. Prior to toxicant addition, TEER values were recorded in the incubator to be used as t0 values. Medium containing a range of Staurosporine concentrations (0 mM, 11 nM, 33 nM, 100 nM, and 300 nM) was added to the perfusion and gel channels of the chips before starting a time-lapse measurement. We have shown the effect of staurosporine-induced toxicity for a wide range of concentrations. TEER values are being affected in both time and dose-dependent manners. Values were recorded down to 1 Ohm.cm², showing a tenfold reduction from peak values. The sensitivity of this system is combined with the possibility to perform label-free measurement throughout incubation without interruption of the experiment. We demonstrated the implementation of TEER measurements on an Organ-on-a-Chip platform in combination with a HUVEC based endothelial model, with TEER values reaching down to 1 Ohm.cm². We propose this system for the study of healthy and damaged models of endothelial barrier tissues in a non-invasive way to provide a valuable tool for drug toxicity and transport studies.
study was to adapt and modify a microphysiological human placental barrier model comprised of human placental epithelial and endothelial cells in order to use it as a model system for placental drug transfer studies. The engineered placental barrier model was characterized by assessing confluent monolayer formation of epithelial and endothelial cells, intercellular junctions, barrier permeability, and structural integrity. To test the feasibility of the system as a drug screening tool, we examined the barrier transfer of three small molecule drugs (glyburide, rifaximin, and caffeine) that have different molecular weights. Non-compartmental pharmacokinetic analysis (NCA) was also performed using drug concentration data from the placenta barrier models. From our preliminary tests, we observed that the three drugs used in this study crossed the engineered placenta barrier at different transfer rates: glyburide (MW = 494 Da) < rifaximin (MW = 786 Da) < caffeine (MW = 194 Da). The results will be further compared to reported human drug transfer rates. This preliminary finding suggests that the placental drug transport may not depend solely on drugs’ molecular weights but may depend on other factors such as lipid solubility, drug ionization, and transporters expressed by placental cells. In conclusion, our findings suggest that, although further optimization and characterization are still needed, the microphysiological human placental barrier model adapted in our study may have a potential to serve as a viable in vitro testing tool to study placental drug transport mechanisms. The NCA analyses of drugs used in the microphysiological system will further help inform computational models for the in vivo pregnant women.

ABSTRACT NUMBER: 5068    Poster Board Number: P174
TITLE: Single Crystal Structure of 4-Methoxy-2-Nitroacetanilide: A Comparative Study with Implications to the Mechanism(s) of Toxicity of Phenacetin

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KEYWORDS: Biotransformation and Toxicokinetics; Methods/Mechanism; Metabolic Activation; Single Crystal Structure; 119-81-3 N-(4-Methoxy-2-nitro-phenyl)acetamide

ABSTRACT: The analgesic/antipyretic use of 4-alkoxyacetanilides, in particular, 4-ethoxyacetanilide (phenacetin or 4-EA) predates the First World War. 4-hydroxyacetanilide (popularly known as Tylenol or acetaminophen) and 4-EA were introduced into markets at around the same time; however, 4-EA due to its carcinogenic and kidney-damaging properties was withdrawn from the markets some three decades ago. Although there has been extensive research on Phase I and II biotransformation of 4-alkoxyacetanilides, little or no information is available on nitrated and other oxidation products that could be formed in reactions with the peroxynitrite anion-NO₂⁻. We have previously shown that the putative free radical products of •NO₂ and CO₂ formed in peroxynitrite-CO₂ reactions constitute an important source of non-enzymatic biotransformation of 4-hydroxyacetanilide, apocynin, and clozapine. Towards understanding this and to shed light on molecular targets leading to the organ-specific toxicity of 4-EA, we have synthesized 4-methoxy-2-nitroacetanilide through acetylation of 4-methoxy-2-nitroaniline using excess acetic anhydride under reflux conditions. Following removal of the unreacted acetic anhydride through reaction with ethanol, 4-methoxy-2-nitroacetanilide was recrystallized twice from water. The single crystals of 4-methoxy-2-nitroacetanilide were analyzed using the technique of X-ray diffraction. We find that, in 4-methoxy-2-nitroacetanilide, the three substituents vary in the degree...
of lack of planarity with the central phenyl ring. The methoxy group is nearest to coplanar, with a C-C-O-C torsion angle of 6.1(5)°. The nitro group is less coplanar, with a 12.8(5)° twist about the C-N bond. The acetamido group is considerably less coplanar with the central ring, having a 25.4(5)° twist about the C-N bond to the ring. These deviations are much larger than those observed in the analogous 4-hydroxy-2-nitroacetanilide. The NH group forms an intramolecular hydrogen bond to nitro O having N⋯O distance 2.632(4) Å and N-H⋯O angle 137(4)°. Together with the recent understanding of the mechanisms of action of acetaminophen which proceeds through hydrolysis and subsequent formation of arachidonic acid conjugates and their binding cannabinoid receptors, the results of this study may be useful in providing insights into molecular targets for 4-EA and its metabolites.

ABSTRACT NUMBER: 5069  Poster Board Number: P175
TITLE: In Vitro Metabolite Identification of Toxin Payload, DM51, in Human and Cynomolgus Monkey Hepatocytes

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KEYWORDS: Other (open text box); Metabolism; Hepatocytes; Antibody-Drug Conjugate

ABSTRACT: A number of antibody drug conjugates (ADCs) are in clinical development or have recently been approved for the treatment of cancer. A unique feature of ADCs is the small molecule toxin component (payload) coupled, using various linker technologies, to an antibody that targets delivery of the toxin. In vivo, the payload is released from the antibody and then acts to inhibit growth of cancer cells by interfering with cellular processes such as by blocking microtubule production and/or function. DM51 is a small molecule maytansine derivative that has been developed as an ADC payload. The current study was undertaken to determine the metabolism of DM51 in a species typically used for toxicology studies and to establish the potential relevance of DM51 in human cancer therapy. DM51 was incubated in the test system of primary cynomolgus monkey (n=3) and human hepatocytes (n=5) with known profiles of CYP activities and >70% post-thaw viability. After a 48 h adaptation period, hepatocytes cultured in a collagen-sandwich configuration were treated once and cultured. Triplicate wells for each of the cultures (2.2 million cells/mL for monkey, 1.3 million cells/mL for human) were treated in 48-well plates with supplemented Williams’ E medium (37 ± 2 °C) containing DM51 (10 µM) or the positive control 7-ethoxycoumarin (500 µM). DM51 and six related components (C1 through C6) were detected and the presence of DM50 was confirmed during all incubations. After 0, 2, 6 and 24 h, the reactions were terminated by the addition of an equal volume of methanol containing 5 mM N-ethylmaleimide (NEM) for the DM51 samples and placed on ice for 1 hour. DM51 and all related components were present at levels high enough for UV detection in each species although DM51 was only present at the 0 h time point and only after NEM derivatization. C1, C5, C6 appear to be products of chemical conversion rather than the result of enzymatic biotransformation of DM51. C2 (S-methylation plus oxygenation to yield a sulfoxide) was the most abundant metabolite in both species. No human-specific metabolites were detected, but DM50 was more abundant in human, compared with monkey, hepatocytes. The predominant metabolic pathways in these primate species included: S-methylation, oxygenation, ester hydrolysis and cysteine conjugation or combinations thereof.
**ABSTRACT NUMBER:** 5070  
**Poster Board Number:** P176  
**TITLE:** Acute Electronic Cigarette Exposure Alters Polyunsaturated Fatty Acids in Mice  

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** D. H. Gonzalez, J. Castellanos, R. Gupta, S. Dutchover, A. Louie, and J. A. Araujo. David Geffen School of Medicine at UCLA, Los Angeles, CA.

**KEYWORDS:** Inhalation Toxicology; Lung; Pulmonary or Respiratory System; Cardiovascular System; Electronic Cigarettes

**ABSTRACT:** The United States has seen a surge in electronic cigarettes (EC) use, particularly in adolescents. Adverse pulmonary effects associated with oxidative stress and severe inflammation have been well documented in EC users. Yet the molecular mechanisms responsible for the proinflammatory effects of EC use are poorly understood. Here, we assessed the changes in polyunsaturated fatty acid (PUFA) levels in bronchoalveolar lavage fluid (BALF) and plasma from acute EC exposure in mice. We employ mice deficient in myeloid heme oxygenase 1 (mHO1-KO) and floxed at the HO1 genomic cassette (mHO1^fl/fl^) as controls (C57BL/6J background). mHO1-KO (N=5) and mHO1^fl/fl^ (N=5) mice in were exposed EC aerosol or filtered air (FA) for two hours sessions over three consecutive days. Arachidonic acid (AA), Linoleic Acid (LA) and oxidized metabolites (5-HETE, 13-HODE) were assessed by liquid chromatography-mass spectrometry. mHO1^fl/fl^ mice exposed to EC exhibited decreased AA in BALF (p<0.05) and plasma (p=0.15) compared to controls. This was accompanied by increased plasma 5-HETE/AA (p<0.05) and 13-HODE/LA (p=0.07). These effects were not observed mHO1-KO mice, but EC exposure tended to increase BALF LA levels (p=.09) in these animals. We observed no significant differences in plasma LA and oxidized LA metabolites in both strains of mice exposed to EC. In conclusion, merely three days EC exposure led to reduced levels of AA in BALF/plasma accompanied by increased plasma 5-HETE/AA in mHO1^fl/fl^ mice. Paradoxically, these effect are not observed in EC exposed mHO1-KO mice, suggesting a protective role of myeloid HO1 deletion in this context.

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**ABSTRACT NUMBER:** 5071  
**Poster Board Number:** P177  
**TITLE:** Urinary Phthalate Metabolites and Risk of Dyslipidemia among Adult Population in the US: NHANES 2007-2016  

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** N. Hasan, N. Trisha, and T. Roh. Texas A&M School of Public Health, College Station, TX.

**KEYWORDS:** Phthalates; Lipids; Cardiovascular System; Dyslipidemia, Plasticizers, Metabolic Syndromes; Phthalates

**ABSTRACT:** Dyslipidemia or abnormalities in plasma lipids is a major component of metabolic syndromes and a well-known risk factor to be associated with adverse cardiovascular outcomes. In the present study, a cross-sectional study was performed to investigate whether exposure to phthalates plays a role in abnormal plasma lipids among US adults. Socio-demographic, health, behavioral factors, and plasma lipids data were collected from five cycles (2007-2016) of the National Health and Nutrition Examination Survey (NHANES). Dyslipidemia was analyzed comprehensively along with its individual contributing factors such as total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), non-HDL, and atherogenic index (AI). Other covariates including age, gender, race, body mass index, poverty index ratio, urinary creatinine, serum cotinine level, hypertension, diabetes,
physical inactivity, and alcohol intake were categorized and adjusted to the model. Both linear and multivariate logistic regression models were fitted to estimate crude and adjusted odds ratios. Among the phthalate metabolites, di(2-ethylhexyl) phthalate has a significant positive association as its adjusted odds ratios of high TG, high LDL, and high Al, and overall dyslipidemia are 1.40 (95% CI 1.07, 1.83), 1.32 (95% CI 1.06, 1.63) and 1.37 (95% CI 1.11, 1.69), and 1.39 (95% CI 1.13, 1.71), respectively. Other phthalate metabolites such as mono-ethyl phthalate and mono-n-butyl phthalate are also significantly associated as the odds ratios of dyslipidemia are 1.25 (95% CI 1.08, 1.44) and 1.29 (95% CI 1.02, 1.63) after adjusting for covariates. From this study, we concluded that increased exposure to phthalate may induce a higher risk for dyslipidemia among the adult population.

ABSTRACT NUMBER: 5072    Poster Board Number: P178
TITLE: Toxic Effects of Photodegraded Anthracene on Epidermal Inflammatory Response

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KEYWORDS: Chemical of Concern; Polycyclic Aromatic Hydrocarbons; Toxicity; Acute; Skin; Anthracene

ABSTRACT: Like many other Polycyclic Aromatic Hydrocarbons (PAHs), anthracene is a major pyrogenic and petrogenic chemical that can be identified in wild-fires, automobile exhaust, and oil refining processes. Anthracene is ubiquitous in areas of heavy industry and has been reported as a toxic compound to humans by the US Environmental Protection Agency. The structural changes of anthracene under UV radiation have been recognized, but the modification of toxicity to humans has not been studied. The objective of the present study is to assess the phototoxic effects anthracene and provide a potential molecular pathway for the initiation of an inflammatory response by keratinocytes using in vitro and in vivo models. Anthracene was degraded under simulated sunlight for 1, 4, 8, and 24 hours. Treatments with degraded or non-degraded anthracene were applied daily to 3D cultured keratinocytes to measure the effect on epidermal stratification with histological analysis. Monolayer human keratinocytes were treated with photodegradation products for 4 and 16 hours and harvested for gene expression analysis. Cell viability and migration was estimated using monolayer keratinocyte cultures. Hairless, immunocompetent mice were treated daily with photodegraded product for two consecutive weeks and sacrificed for histological and gene expression analysis. Histological analysis demonstrated that 4 hour photodegraded products increase epidermal thickness above control values for both mouse and 3D cultured keratinocytes. Photodegraded anthracene also altered the viability and migration of keratinocytes with cell viability decreasing as degradation time increased. Keratinocyte migration was negatively impacted at 0, 1, and 4 hour photodegraded treatments. The gene expression analyses in mouse epidermis demonstrated that proinflammatory factors, il1b and s100A9 were upregulated in 4, 8, and 24 hour photodegraded treatments. A cell-cell junction protein, Connexin43, was found to be downregulated for the same treatments. Gene expression in human keratinocytes showed downregulation of s100A8, and two matrix metalloproteinases, mmp1, and mmp9 at 0 and 1 hour photodegraded treatments. The results of this study indicate that photodegraded anthracene initiates unique inflammatory responses in keratinocytes. These results can inform risk assessment for public exposure to other photodegraded PAH compounds and support the development of targeted therapeutics.
ABSTRACT NUMBER: 5073  
POSTER BOARD NUMBER: P179  
TITLE: Tobacco-Disrupted Transcriptional Regulation Skews Osteoblastogenesis  

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: N. R. L. Sparks, D. Williams, and N. I. zur Nieden, University of California Riverside, Riverside, CA.  

KEYWORDS: Stem Cells; Developmental Toxicity; Prenatal; Tobacco Products; Osteoblasts  

ABSTRACT: Birth defects that affect skeletal tissues are frequent and impose life-long health concerns for the affected individual and their families. Tobacco exposure during pregnancy has been associated with adverse skeletal birth outcomes. Yet, the molecular mechanisms of tobacco exposure and skeletal birth defects are understudied. Since proper development of osteoblasts crucially depends on regulatory genes, we hypothesize that exposure to harm reduction tobacco products (HRTPs), that include ultra-filtered cigarettes and chewing tobacco, alter such genes that adversely affect osteoblast differentiation, resulting in a skeletal defect. In this study, we used human embryonic stem cells (hESCs) differentiating into osteoblasts to assess the developmental toxicity of HRTPs. Subtoxic doses of the HRTPs, Camel Blue (CB) sidestream smoke and Snus extracts (STE), inhibited osteoblast differentiation. HRTP exposure altered FOXO transcription factors correlating to attenuated osteogenesis. Decreased FOXO mRNA and nuclear protein levels analysis revealed a link between HRTP exposure and poor osteoblast differentiation. shFOXO cell cultures phenocopied the reduction of osteoblast production as seen with the HRTP treatment. RNA-sequencing analysis revealed additional mechanisms of osteogenic inhibition. The HRTP exposure resulted in differentially expressed genes (DEGs) playing key roles in osteogenic signaling pathways, including WNT, TGFβ, FGF, and Hh. CB downregulated genes including FOXO, GATA2, EGFR, and IGF1R and STE downregulated T, EOMES, CDX1/2, and CER1. Gene ontology (GO) of CB and STE DEGs impacted biological processes suggesting bone developmental toxicity. Combining the RNA-seq with a FOXO ChIP-seq analysis, suggested that the lack of FOXO correlates to defects in bone differentiation and development. Where 52% and 35% of the down-and upregulated Camel Blue DEGs and 52% and 51% of the down-and upregulated STE DEGs are FOXO targets. Overall, our data suggests that HRTP-related attenuation of FOXO correlates to low osteoblast differentiation. Further, HRTPs disrupt embryonic skeletal development by modulation of HRTP-sensitive transcription factors that are regulated early in osteoblast differentiation and the misregulation of which can result in a skeletal birth defect. Consequently, HRTPs may be more harmful to a developing fetus and infant by perturbing developmental processes by influencing factors that control osteogenesis.

ABSTRACT NUMBER: 5074  
POSTER BOARD NUMBER: P182  
TITLE: Environmentally Relevant Perfluorobutanoic Acid (PFBA) Levels Induced a Non-Monotonous Dose-Response on the Beet Armyworm (Spodoptera exigua) Transitional Cycle  

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: E. W. Omagamre, S. A. Zebelo, and J. S. Pitula. University of Maryland Eastern Shore, Princess Anne, MD.  

KEYWORDS: Perfluoronated Agents; Non-Mammalian Species; Environmental Toxicology; Perfluorobutanoic Acid  

ABSTRACT: It has been observed that perfluorobutanoic acid (PFBA) is more readily transported to the aerial parts of plants compared with other perfluoroalkyl substances (PFAS). A potential implication of
this accumulation is the exposure to insect herbivores feeding on the leaves and fruits of such plants. In this study, the impact of dietary exposure to environmentally relevant concentrations of PFBA on the development and metamorphosis of the beet armyworm, Spodoptera exigua was investigated. Artificial diet for the beet armyworm larvae was prepared and thereafter dosed with PFBA to concentrations of 100 pg/g, 1 ng/g, 100 ng/g, and 1 µg/g alongside a control diet with no added PFBA. The prepared diets were fed to 3rd instar larvae (n=18 per group) and critical developmental indices were studied through the larval-adult transition. The results showed that each of the PFBA exposed groups consumed about 3 times more diet than the controls at 1-day post-dietary exposure (dpe). The diet consumption subsequently showed a non-monotonous curve from 2-dpe to 6-dpe with significant hormesis at 5-dpe when modeled with the Brain-Cousens 4 parameter model (4.06<f<52.95; p<0.1). The 1 µg/g group showed the highest weight gain at 1-dpe which then changed to the 100 ng/g group at 2-dpe and then the 1 ng/g group at 3-dpe to 5-dpe. Finally, the 100 pg/g group had the highest weight gain at 6-dpe before the larvae began their larval-pupae transition. The pre-pupation and pupation trends were also non-monotonous, with the controls showing the least larval-pupal transition at 9-dpe (i.e., 43%) and the 1 ng/g group showing the highest transition (i.e.,100%). The average pupal weight of the 100 pg/g, 1 ng/g, 100 ng/g and 1 µg/g treatments were 89, 96, 100 and 96 mg respectively. The weights of the emerged adults showed a U-shaped curve, with the controls showing the most average weight (72.5 mg) and the 1 ng/g group showing the least (57.7 mg). The overall data suggest that PFBA may have modulated the juvenile and 20-hydroxyecdysone hormonal pathways responsible for the regulation of larval feeding and inter-instar/larval-pupal metamorphosis.

ABSTRACT NUMBER: 5075        Poster Board Number: P183
TITLE: Development of a Primate Model to Evaluate the Effects of Ketamine and Surgical Stress on the Neonatal Brain

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: C. Wang1, S. Liu1, F. Liu1, A. Bhutta2, T. Patterson1, and W. Slikker1. 1US FDA/NCTR, Jefferson, AR; and 2University of Arkansas for Medical Sciences, Little Rock, AR.

KEYWORDS: Neurotoxicity; Developmental; Ketamine

ABSTRACT: The effects of anesthetic exposure on the developing brain may be confounded by several factors including pre-existing disorders and surgery-induced stress. Ketamine, a noncompetitive N-Methyl-D-Aspartate (NMDA) receptor antagonist, has routinely been used as a pediatric general anesthetic. However, controversy remains about whether ketamine exposure may be neuroprotective or induce neuronal degeneration in the developing brain. Here, we report the effects of ketamine exposure on the neonatal nonhuman primate brain under surgical stress. Eight neonatal rhesus monkeys (PND 5-7) were randomly assigned to each of two groups: Group 1 animals (n=4) received ketamine at 2 mg/kg via intravenous bolus prior to surgery and a ketamine infusion of 0.5 mg/kg/hr during surgery in the presence of a standardized pediatric anesthetic regimen; Group 2 animals (n=4) received volumes of normal saline equivalent to those of ketamine given to Group 1 animals prior to and during surgery, also in the presence of a standardized pediatric anesthetic regimen. Under anesthesia, the surgery consisted of a thoracotomy followed by closing the pleural space and tissue in layers using standard surgical techniques. Vital signs were monitored to be within normal range throughout the anesthesia. Elevated levels of cytokines IL-8, and MCP-1 at 6 hrs after surgery were detected from
animals in Group 1. Fluoro-Jade C staining revealed significantly higher neuronal degeneration in frontal cortical areas in ketamine-exposed animals, compared with their control animals (Group 2). Intravenous ketamine administration prior to and throughout surgery in a clinically relevant neonatal primate model appears to elevate cytokine levels (blood samples) and increase neuronal degeneration. Consistent with previous data on the effects of ketamine on the developing brain, the results from the current randomized controlled study in neonatal monkeys undergoing simulated surgery (thoracotomy) show that ketamine does not provide neuroprotective or anti-inflammatory effects. The dose of anesthetic, duration of anesthesia, route of administration, presence or absence of surgical stress, and the maturity of the developing brain are all critical elements to consider in the design of developmental anesthesia exposure studies.

ABSTRACT NUMBER: 5076  Poster Board Number: P184

TITLE: Pharmacokinetic Analyses of Placental Transfer of Single Doses of Glyburide, Rifaximin, and Fentanyl in Pregnant Sprague Dawley Rats

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: D. Mehta¹, M. Li¹, N. Nakamura¹, M. Chidambaram¹, X. He¹, M. S. Bryant¹, R. Patton², K. Davis², and J. Fisher¹. ¹US FDA/NCTR, Jefferson, AR; and ²Toxicologic Pathology Associates, Jefferson, AR.

KEYWORDS: Pharmacokinetics; In Vivo Models; Reproductive and Developmental Toxicology; Glyburide, Rifaximin, Fentanyl

ABSTRACT: Although medicine use during pregnancy is common, information on exposure of drugs to the developing fetus and their potential teratogenic effects is lacking for most medications. In this study, we identified key drug properties that can have a significant impact on exposure to the fetus by examining the placental transfer of drugs using a rat model. Time-mated Sprague Dawley (SD:Hsd) rats aged 11-13 weeks were administered either glyburide, rifaximin, or fentanyl at gestational day 15 and maternal blood, placentae, and fetuses were collected at 5 min, 30 min and at 1, 4, 8, 24, 48, and 96 h post-dose. Our results showed that the fetal exposure was lowest for glyburide, accounting for only 2.2% of the maternal plasma exposure as measured by the ratio of their corresponding area under the concentration-time curves (AUC), followed by rifaximin (37.9%) and then fentanyl (172.4%). Although the placentae exposures were found to be 3-fold higher for rifaximin and 4-fold higher for fentanyl compared to the maternal plasma levels, the fetal AUC exposures compared to placentae were found to be 10.7% for glyburide, 11.8% for rifaximin, and 39.1% for fentanyl. These data suggest that the placenta acts as a protective shield for the fetus; however, the extent of this protection varies for different drugs and can depend on several factors, such as the physicochemical properties of drugs and transporter-driven mechanisms. Based on the reported physicochemical properties for glyburide, rifaximin, and fentanyl, we can infer that higher levels of plasma protein binding and lower levels of systemic absorption, lipophilicity, and acidic strength are likely to be key drug properties that could make it more difficult for drugs to cross the placenta and reach the fetus.
ABSTRACT NUMBER: 5077       Poster Board Number: P185
TITLE: Using Data Associated with a Developmental and Reproductive Toxicity Adverse Outcome Network to Aid Safety Assessments


KEYWORDS: Reproductive and Developmental Toxicology; Alternatives Assessment; Mechanisms

ABSTRACT: With the drive towards animal-free toxicity testing, increasing volumes of alternative assays, models and data are being developed. To conclusively undertake a risk assessment, all available relevant data should be utilized. However, as alternative assays often measure discrete biological steps in a mechanism of toxicity (and not traditional toxicity endpoints), it can be difficult to understand the context and significance of their results in isolation. Adverse outcome pathways (AOPs), a formalized approach to documenting mechanisms of toxicity, provide this contextualization. AOPs comprise of key events (KEs) linked to each other through key event relationships (KERs). Each KE must be measurable - as a result it should be possible to link an assay to each KE. This feature allows for the grounding of relevant assays in a mechanistic rationale and for the review of multiple assay types within the framing of a mechanism of toxicity. However, for AOPs to become a useful tool in risk assessment, it is likely that comprehensive networks of pathways leading to AOs of regulatory significance, will be needed. To this end, in this work, an AOP network relevant to developmental and reproductive toxicity (DART) was developed through expert review of the public literature. This resulted in a network of over 280 KEs, including 54 MIEs, supported by 507 references. 50 assays (e.g. AhR binding) with over 400,000 study observations were then associated to the network. At most, only a small number of these pathways would be relevant to an individual compound of interest and therefore unguided access to the entire network may not be useful. Therefore, an approach was explored where assay data and similarity searching were used to identify potentially relevant AOPs for individual compound to help guide safety assessments. This approach was validated by screening 199 compounds deemed to be developmental toxicants in a publicly available Zebrafish assay dataset against the network, using a proprietary Tanimoto similarity method and a similarity threshold of 80%. Potentially relevant AOPs were identified for 136/199 compounds through data associated to 28/50 assays within the AOP network (e.g. 11 compounds were indicated as potential AhR ligands) using this method. By identifying potentially relevant mechanisms, this network may prove to be a useful reference tool and guide when undertaking a safety assessment.

ABSTRACT NUMBER: 5078       Poster Board Number: P186
TITLE: Assessing the Toxicity of Engineered Nanomaterials in the Male Reproductive System: Developing Improved Methods and Strategies for Hazard Assessment

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KEYWORDS: Nanoparticles; Reproductive and Developmental Toxicology; Endocrine Disruptors
ABSTRACT: Reproduction is the only certain constant within animals and maintaining the integrity of this process is key to ensuring survival of the species. As male fertility rates in the Western world decline, the need to understand and manage any novel threats to reproductive health is paramount. However, the animal burden associated with such research is great. Consideration of how to reduce, refine or replace animal use in developmental and reproductive toxicity (DART) testing is therefore critical. This research aimed to assess the toxicity of engineered nanomaterials (ENM) in the male reproductive system and develop an improved testing strategy for hazard assessment of DART. Using a panel of highly representative ENM, assays to screen for toxicity in male testicular cell lines were established and optimised in vitro. Findings were validated for reliability and reproducibility against those generated using similar test systems with cells from alternative organs of the body. Assessment of cell and endocrine function also provided a deeper understanding of cellular responses following acute sub-lethal ENM exposure. Comparison of outcomes in vitro to in vivo was enabled by appraisal of tissues from animals exposed orally to the same ENM. Through this, a new method by which to stage tubules for histopathological analysis was developed, and for the first time a truly thorough morphological and stereological examination of tissues for markers of effect was provided. Assimilation of key findings made it possible to identify all silver ENM assessed as potential reproductive toxicants and endocrine disruptors in vitro and in vivo (sub-acutely). Development of the staging method contributes to simplifying the highly complex task of testicular histopathology analysis. In turn this will assist in accelerated generation of robust academic research which meets the stringent requirements for such analyses within the regulatory arena. Finally, the accumulated results were deemed useful to guide development of a novel Integrated Approach to Testing and Assessment (IATA) for male reproductive toxicity from ENM. This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 263215 (MARINA).

ABSTRACT NUMBER: 5079        Poster Board Number: P187
TITLE: Hydraulic Fracking Contaminants Exposures Induce Differential RNA Expression in Relation to Sex in Mice

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: R. Berube¹, Y. Chiang¹, K. Gurdziel¹, S. C. Nagel², and C. D. Kassotis¹. ¹Wayne State University, Detroit, MI; and ²University of Missouri, Columbia, MO.

KEYWORDS: Endocrine Disruptors; Endocrine Toxicology; Genomics

ABSTRACT: Unconventional oil and natural gas (UOG) production has increased in the United States over the last three decades. More than 1,500 different chemicals have been reported to be used for hydraulic fracturing. Previous research from our laboratory reported that mice gestationally exposed to a mixture of 23 UOG contaminants, suspected or known endocrine-disrupting chemicals, increased body weights, altered energy expenditure, decreased activity, and decreased ability to respond to diet challenge in offspring. While these studies demonstrated a robust metabolic phenotype in developmentally exposed mice, we have lacked information on causal mechanisms underlying these effects. To address this knowledge gap, we utilized livers collected from mice at postnatal day 85 following gestational exposure to four concentrations of the UOG chemical mixture. RNA was sequenced on an Illumina NovaSeq 6000, data were de-multiplexed using Illumina’s CASAVA 1.8.2 software. Differential gene expression analysis was used to compare transcriptome changes between groups and sexes, using significantly altered
genes (p-value ≤ 0.05; FDR ≤ 0.05). Principal components analysis revealed sex-specific differences in global gene expression, with male mice exhibiting less significant differences in gene expression than females when compared to non-exposed mice. This may be due to the males’ ability to compensate for anti-androgenic exposures by increasing testosterone production. We also observed a trend in female gene expression, with increasing mixture concentrations clustering closer to the positive control, supporting an anti-androgenic mechanism. In females, the high concentration mixture induced significant changes in expression of \( \text{cyp1a1} \) and \( \text{cyp1a2} \) (detoxification phase II), and of developmental genes, such as \( \text{greb1l} \), (kidney and genital development), \( \text{Fat1} \) (epithelial morphogenesis) and \( \text{Tmem119} \) (osteoblast differentiation). While the lower concentration mixture induced changes in expression of detoxification phase II (\( \text{ugt1a1} \)), and lipid and insulin metabolism (\( \text{lcat} \) and \( \text{irs2} \)). Gene ontology analysis demonstrated that the immune system, inflammation processes, and cellular differentiation were the most affected in females. Overall, this project will support a better understanding of the mechanisms underlying sex-specific UOG chemical-mediated impacts on metabolic health dysfunction.

ABSTRACT NUMBER: 5080  
Poster Board Number: P188

TITLE: Characterization of Reference RNAs and Lysates Made from Compound-Treated Cell Lines for Use in Transcriptomic Assays


KEYWORDS: Gene Expression/Regulation; Methods/Mechanism; Bioinformatics; Reference RNA

ABSTRACT: Reference RNAs are an important positive control for gene expression analysis, providing a standard sample for quality control and normalization between assay runs. The first widely adopted reference RNAs (Shi et al 1151-1161, doi:10.1038/ntb/1239) were the MAQC reference A and B. MAQC A (Universal Human Reference RNA, UHRR, Agilent) is a pool of total RNA extracted from 10 cell lines. MAQC B (FirstChoice® Human Brain Reference RNA, Ambion), no longer available, was a pool of total RNA from brain tissue of 23 donors. No commercial reference RNAs include induced expression of genes relevant to toxicology or modulated cell function. With EPA contract funding, we pursued the reproducible manufacture of reference RNAs and lysates. We found that a single cell line expressed 50 to 75% of the genes in a mixture of 10 highly diverse cell lines, and that each successively added cell line to that initial one resulted in diminishing additive contributions approaching an asymptote of 12,000 to 15,000 expressed genes depending on the cell lines used. In contrast, by treating cells with select compounds and formulating a mixture of untreated and treated cells, we could produce a reference that contained >13,000 expressed genes including genes relevant to compound exposures using only 3 cell lines and 2 compounds. These cells were pooled in different proportions to make two reference samples. This smaller number of cell sources improved batch-to-batch repeatable. The resulting BioSpyder Universal Reference RNAs (BURR1 and BURR2) were prepared as blends of total RNAs that exhibited an overall RIN of 9.8. Matched BioSpyder Universal Reference Lysates (BURL1 and BURL2) were prepared for use with the extraction-free TempO-Seq® assay in a compatible buffer. Performance metrics for both the RNAs and lysates were >4 logs dynamic range of expression, 12,000 to 13,000 genes expressed by each, with >11,000 expressed genes in common, and providing a range of 29 log2 fold change in differential expression between [BURR1 and 2 or BURL1 and 2 of >5,000 genes (p\text{adj} <0.05)] at a sequencing depth of 8M counts. Cross-platform correlation of log2 fold changes with RNA-seq analysis.
of the same RNAs was excellent, $R^2 = 0.94$, and consistency of sample mixtures was comparable or better than the original MAQC A and B samples. These references perform well as positive controls, with particular utility in toxicology and drug discovery due to inclusion of genes related to cellular responses.

**ABSTRACT NUMBER:** 5081  **Poster Board Number:** P189  
**TITLE:** Efficacy Assessment of Vincristine and Zoledronic Acid in Two Breast Cancer Orthotopic Models  
**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** K. Kabirov$^1$, M. Lindeblad$^1$, A. Lyubimov$^1$, A. M. Abukhdeir$^2$, and M. Cobleigh$^2$. $^1$University of Illinois at Chicago, Chicago, IL; and $^2$Rush University Medical Center, Chicago, IL.  
**KEYWORDS:** Carcinogenesis; In Vivo Models; Safety Pharmacology

**ABSTRACT:** Vincristine (VIN) and zoledronic acid (ZA) have been shown to inhibit RhoA's activity in cell cultures. NF1 mutant (Hs578T cells) and null cells (MCF-10ANF1-/- cells) were more sensitive to these drugs as single agents compared to wild type control cells (e.g., MCF-7). In combination, the effect was even more dramatic. In this study, inhibition of the RhoA activity was tested *in vivo*. Hs578T and MCF-7 cells were inoculated in NSG mice to form tumors. After tumor xenograft model development, VIN at 0.5 mg/kg, ZA at 2.5 mg/kg, or a combination of VIN and ZA at the same doses was given to animals intraperitoneally. Mice were treated with test articles once weekly for four weeks. MCF-7 xenograft: The tumor volumes (relative to Day -1) were statistically significantly decreased on Day 25 ($p<0.05$) in the dose groups administered VIN and VIN/ZA combination when compared to the control group. Growth of transplanted MCF-7 tumors was statistically significantly inhibited ($p<0.01$) following treatment with VIN, ZA, or VIN/ZA combination. Hs578T xenograft: Relative to Day -1 tumor volumes were statistically significantly decreased on Day 5 and Day 19 ($p<0.05$) in the dose group administered VIN/ZA combination when compared to the control group. Growth of transplanted Hs578T tumors was statistically significantly inhibited ($p<0.01$) following treatment with VIN, ZA, or VIN/ZA combination. In summary, treatment of NSG mice with 0.5 mg/kg vincristine, 2.5 mg/kg zoledronic acid or 0.5 mg/kg vincristine + 2.5 mg/kg zoledronic acid led to enhanced MCF-7 and Hs578T tumor xenograft suppression. The RhoA inhibition was more evident in the mutant cell line xenograft. Administration of vincristine alone and in combination with zoledronic acid resulted in more pronounced RhoA inhibition than administration of zoledronic acid alone and it will be applied to future studies with quantitative biomarker assessment. RhoA pathway inhibition seems to be an attractive pathway for future personalized therapy. A.M. Abukhdeir is currently an employee of the US FDA. The findings and views expressed in this publication do not reflect the views and opinions of the US Government or the US FDA. The study was sponsored by Rush University Medical Center.

**ABSTRACT NUMBER:** 5082  **Poster Board Number:** P190  
**TITLE:** Integrating Endocrine and Genotoxicity Datasets to Prioritize Potential Breast Carcinogens for Risk Reduction and Further Study  
**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** J. E. Kay$^1$, M. Schwarzman$^2$, and R. A. Rudel$^1$. $^1$Silent Spring Institute, Newton, MA; and $^2$University of California Berkeley, Berkeley, CA.  
**KEYWORDS:** Carcinogenesis; Endocrine Disruptors; Environmental Toxicology
**ABSTRACT**: Breast cancer is a major concern due to its prevalence and deadliness. While some breast carcinogens have been identified in studies in humans or experimental animals, many potentially hazardous exposures remain unrecognized despite their biologically relevant activity, impeding cancer research and prevention. Abundant evidence indicates that certain environmental exposures can raise breast cancer risk by increasing estrogen or progesterone signaling or inducing DNA damage. Since receptor-mediated effects and genotoxicity are both Key Characteristics of carcinogens, genotoxic endocrine disrupting chemicals (EDCs) are of particular concern. To identify these potential hazards, EPA data for steroidogenesis (HT-H295R) and estrogen receptor activation (*in vitro* and computational) and NTP, TOXNET, CCRIS, ECVAM, and eChemPortal genotoxicity databases were integrated. The enrichment of these Key Characteristics among rodent mammary carcinogens (MCs) was evaluated by comparing the fractions of MCs with these activities versus the fractions positive among all chemicals tested. In total, 720 chemicals were found to increase estrogen or progesterone steroidogenesis or ER activation out of 2,279 tested, and 241 were also genotoxic. Rodent MCs were enriched for these characteristics, with 46% of those being active for both genotoxicity and endocrine disruption, compared to 20% of all chemicals tested. Chemicals were further prioritized based on predicted exposures in the US. According to EPA and FDA databases, 184 genotoxic EDCs are found in consumer products, 143 in dietary sources, 48 in pharmaceuticals, and 137 in pesticide formulations. The highest priority candidates for exposure reduction include 19 genotoxic EDCs to which EPA estimates general population exposure at 100 ug/kg/day or more. By integrating publicly available datasets for two Key Characteristics, hundreds of potential breast carcinogens were identified, revealing numerous opportunities for risk reduction. Future experimental and epidemiological studies can help further characterize breast cancer-relevant chemicals and their risks to human health.

**ABSTRACT NUMBER**: 5083  
**Poster Board Number**: P191

**TITLE**: Cadmium Alters MicroRNAs Expression and Promotes Cancer Aggressiveness in Prostate Cancer Cells


**KEYWORDS**: Carcinogenesis; Epigenetics; Metals; Cell Culture; Heavy Metals

**ABSTRACT**: Cadmium (Cd) is a toxic non-essential transition metal that poses a health risk at low exposure levels and has acute and chronic effects on human health and environment. The International Agency for Research on Cancer (IARC) has established that cadmium is carcinogenic agent and Cd exposure may be related to various types of cancer including prostate cancers (PCa). Exposure to Cd can induce epigenetic changes. One of the principal mechanisms that mediate the epigenetic regulation of gene expression includes microRNA (miRNA). MiRNAs are a class of small noncoding RNAs (∼22-25-nucleotides) that regulate gene expression at post-transcriptional level. It has been reported that miRNAs are involved in regulating many important biological processes such as cell proliferation, apoptosis, differentiation, and metabolism. Growing evidence has shown that heavy metals such as Cd might exert their toxicity through miRNA alterations. This mechanism is implicated in various pathophysiological conditions and signaling pathways, consequently leading to the development of cancer and other diseases. Dysregulation of miRNAs may act as either oncogenes or tumor suppressors in cancer. In this study, we aimed to evaluate the functional impact of miRNAs (miR-34a, miR-125b and...
miR-96) expression on PCa aggressiveness, and to investigate whether Cd plays a functional role in promoting PCa progression and aggressiveness. Two in vitro cell models derived from prostate cancer patients were used: PC-3 cells and LNCaP cells. RT-qPCR assays were performed to examine the miRNA expression profile in response to Cd exposure. MTS and BrdU-based assays were used to assess the effect of Cd on cell viability and cell proliferation mediated through miRNAs. Results showed that miRNA expression profile was altered after exposure to Cd for 48 hours. Specifically, miR-34a and miR-125b were down-regulated while miR-96 was up-regulated upon exposure to Cd. Exposure to Cd also induced enhancement of cell proliferation. Transfection of Cd-treated cells with miR-34a mimic, miR-125b mimic or miR-96 inhibitor significantly suppressed cell proliferation in a dose-dependent manner. These results suggest that Cd may promote PCa proliferation by inhibiting expression of tumor suppressive miR-34a and miR-125b and enhancing the expression of oncogenic miR-96. Further studies are needed to address the role of Cd-induced epigenetic modifications in prostate carcinogenesis.

**ABSTRACT NUMBER:** 5084

**Poster Board Number:** P192

**TITLE:** ROS-Dependent Defective Autophagy Leads to Prostate Carcinogenesis

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** A. Tyagi, and C. Damodaran. Texas A&M University, College Station, TX.

**KEYWORDS:** Carcinogenesis; Inflammation

**ABSTRACT:** Recent epidemiological, preclinical, and clinical evidence demonstrates that cadmium (Cd) is a potent carcinogen for the prostate. Earlier, we demonstrated that defective autophagy plays a critical role in the Cd-induced transformation of prostate epithelial cells. This study dissects the mechanism by which Cd-induced defective autophagy causes prostate carcinogenesis. We stably overexpressed either antioxidants (SOD1, and/or SOD2) or inhibited the upstream regulators of Reactive Oxygen Species (ROS): p67phox, p47phox, and NADPH oxidase) and Endoplasmic Reticulum (ER) stress sensor (ATF4) in normal prostate epithelial cells and exposed to Cd-chronically (~12 months). In addition, we periodically performed the cell viability, immunoblotting, immunofluorescence, and in vivo analysis to analyze the effect of ROS-ER stress-defective autophagy signaling axis in Cd exposed healthy prostate epithelial cells. Our results established that stably silencing ROS activators (p67phox or p47phox or NADPH oxidase) prevented Cd-induced ER stress, as well as defective autophagy, resulting in abrogation of transformation in prostate epithelial cells. Interestingly, these transf ectant(s) failed to form colonies and tumors in xenotransplanted mice. Similarly, overexpression of antioxidants, either SOD1 or SOD2, in prostate epithelial cells and then exposure to chronic exposure of Cd inhibited ROS-ER stress, preventing defective autophagy signaling. As anticipated, xenotransplantation of these transfected cells showed impeded tumor growth. Similar results were also seen in ATF4 silenced prostate epithelial cells exposed to Cd (~12 months) inhibited the transformation and tumor growth in mice models. We periodically determined the ROS-ER-autophagy signaling axis by measuring ROS generation, the expression of antioxidants (SOD1, SOD2), ER stress responders (protein kinase R-like ER kinase (PERK), eukaryotic translation initiation factor 2-alpha (eIF2-α) and ATF-4) and autophagy markers (Plac8, LAMP1 and LC3B) in all transfectants during the transformation. NOX complex is an upstream activator of ROS and ER stress, responsible for defective autophagy in Cd-induced prostate carcinogenesis. Restoring SOD1 and SOD2 function or silencing NOX complex abrogate cd prostate carcinogenesis in preclinical models of the prostate.
ABSTRACT NUMBER: 5085  Poster Board Number: P193
TITLE: Ochratoxin A and Pesticides in Craft Beers: A Pilot Study


KEYWORDS: Food Safety; Pesticides; Risk Assessment; Ochratoxin

ABSTRACT: More than 98% of breweries operating in the United States (U.S.) are considered craft breweries, many of which rely on local grains and ingredients for beer production. Authors of published studies have suggested that international craft beers can contain pesticide residues and mycotoxins, such as ochratoxin A (OTA). However, little data exist that examine pesticide and mycotoxin residues in craft beers in the U.S. In this pilot survey, lagers from four regional craft breweries located in the Cincinnati, Ohio metropolitan area and one national domestic lager were purchased in fall of 2020. Samples (N=3 cans/beer, 1 sample/can) were evaluated for presence of OTA through high performance liquid chromatography. In a separate experiment, samples (N=1 can/beer) were assessed for 500 pesticides using gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry. A search was conducted of U.S., European, and Canadian governmental residue limits for cereals and grains (e.g., hops and barley) and/or exposure limits for OTA and detected pesticides. Measurements of OTA and pesticides were converted to µg/kg bw/day doses using a standard volume of beer intake (2839 mL) and standard human body weight (70 kg). Calculated daily doses of OTA and the two detected pesticides were compared to the identified residue limits and/or exposure limits to calculate margins of safety (MOS). In all five beers (N=3/beer), OTA was <LOD of 0.25 ppb, with resulting MOS values of <0.001. Three of the four craft lagers and the national brand lager did not contain pesticides above the reporting limits; however, one craft lager contained boscalid and dimethomorph at 0.018 and 0.011 ppm, respectively. For boscalid, the identified residue limits in hops and barley ranged from 0.2 to 80 ppm across the US, Canada, and European Union (EU), and for dimethomorph, the identified residue limits ranged from 4 to 80 ppm. The boscalid and dimethomorph MOS in this craft lager ranged from 18 to 3180 and 222 to 4440, respectively. Based on the lack of OTA detected and 498/500 pesticides below the reporting limit in the craft lagers and the national brand lager, as well as the large MOS values for boscalid and dimethomorph, exposure to OTA and these 500 pesticides in the tested beers presents minimal risk to the average beer consumer. Additional studies are needed to verify these findings on a larger scale, and additional considerations may include climate, season, source of grain, and brewing practices.

ABSTRACT NUMBER: 5086  Poster Board Number: P194
TITLE: MicroRNA Expression Profile Analysis in A549 Cancer Cells Treated with Methyl Sulfonylmethane

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: C. Ezenwanne, Z. Bonelli, and A. DiLorenzo. Montclair State University, Montclair, NJ.

KEYWORDS: Natural Products; In Vitro and Alternatives; Lung; Pulmonary or Respiratory System

ABSTRACT: Clinical and Epidemiological studies have shown a correlation between Inflammation and lung cancer. Methylsulfonymethane (MSM) has been used for years as a dietary supplement and clinical studies have shown MSM benefits for anti-inflammation and prevention of stress of immune cells. In...
particular, these studies have shown that MSM potentially induces apoptosis in cancer cell lines, and prevents the spread of cancer cells. (Kang et al. 2017) However, the specific molecular control role that MSM plays has yet to be investigated. Experiments are in progress to determine if specific MicroRNA(miRNA) can be identified and shown to be significantly altered in presence of MSM. Identification and observation of miRNA has been of interest to our lab because of its key role in the regulation of gene expression. These small non-coding bases are known to post-transcriptionally fine-tune up to 30% of genes and are currently implemented in cancer-based therapy. Specific miRNA segments have been found to be up-regulated and down-regulated when cells start metastasis. Our lab is exploring the possible role that MSM plays in viability, cell proliferation, and possible molecular pathways of A549 human lung cancer cells in post MSM treatment. Our preliminary studies show that MSM concentration from 100mM to 200mM are significantly effective for initiating apoptosis and inhibiting the proliferation of A549 cells without toxicity. The apoptotic pathway seems to be an intrinsic pathway based on the data that gathered so far. As miRNA has been a growing therapeutic target for scientists it is hoped that if there is a link between MSM and miRNA down-regulation, this might further solidify the value of nutraceutical studies in the treatment and control of cancer.

ABSTRACT NUMBER: 5087  Poster Board Number: P195

TITLE: Novel Mechanistic Prediction of Androgen Receptor-Linked Stress in Oncogenic Mycotoxicity Network

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: N. Ray, A. Nagappan, and Y. Moon. Pusan National University School of Medicine, Yangsan, Korea, Republic of.

KEYWORDS: Food Toxicity; Toxicogenomics; Carcinogenesis; Oncogenic Mycotoxins, Androgen Receptors, miRNA; Mycotoxin

ABSTRACT: Xenobiotic-responsive microRNAs (miRNAs) contribute to the regulation of cellular homeostasis or pathological processes during carcinogenesis, by reprogramming target gene expression in insulted human cells. Herein, we predicted the targets of carcinogetic mycotoxin-responsive miRNAs and analyzed their association with disease and functionality. miRNA target-derived prediction indicated potent associations of oncogenic mycotoxin exposure with metabolism- or hormone-related diseases, including sex hormone-linked cancers. Mechanistically, the signaling network evaluation suggested androgen receptor (AR)-linked signaling as a common pivotal cluster associated with metabolism- or hormone-related tumorigenesis in response to aflatoxin B1 and ochratoxin A co-exposure. Particularly, high levels of AR and AR-linked genes for the retinol and xenobiotic metabolic enzymes were positively associated with attenuated disease biomarkers and good prognosis in patients with liver or kidney cancers. Moreover, AR-linked signaling was protective against OTA-induced genetic insults in human hepatocytes whereas it was positively involved in AFB1-induced genotoxic actions. Collectively, miRNA target network-based predictions provide novel clinical insights into the progression or intervention against malignant adverse outcomes of human exposure to environmental oncogenic insults. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2021R1I1A1A01056963).
ABSTRACT NUMBER: 5088    Poster Board Number: P196
TITLE: Cytotoxic Effects of Several Marine Cyanobacteria

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: R. T. Akande\textsuperscript{1,2}, H. Kim\textsuperscript{2}, E. Glukhov\textsuperscript{2}, L. McGaw\textsuperscript{1}, and W. H. Gerwick\textsuperscript{2}. \textsuperscript{1}University of Pretoria, Gauteng, South Africa; and \textsuperscript{2}University of California San Diego, San Diego, CA.

KEYWORDS: Cytotoxicity; Natural Products; Chemical Characterization

ABSTRACT: Cyanobacteria have previously been found to synthesize diverse secondary metabolites with the proposed function of developing their own defence, attack or signalling pathways. They have the ability to produce diverse structurally and biologically active secondary metabolites, with activities such as enzyme inhibition, anticancer, antibiotic, or antiparasitic effects. In this study, a green tufted spongy \textit{Lyngbya} sp. and black \textit{Symploca} sp. were extracted and fractionated. The compounds present in these fractions were annotated using the Global Natural Products Social (GNPS) molecular networks. The cytotoxic effects of the fractions were evaluated against the NCI-H460 human lung cancer cell line. Two known natural products, wewakazole and dolastatin 12 were among the compounds annotated by GNPS in polar fractions. The active fraction showed 13.6 and 59.1% survival rate at 10 and 1 \(\mu\)g/mL respectively. Further studies are ongoing on the isolation and identification of the molecular structures of the bioactive compounds.

ABSTRACT NUMBER: 5089    Poster Board Number: P197
TITLE: Associations of Exposure to Uranium and Arsenic with Chronic Kidney Disease among US Adults: NHANES 2007-2016

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: T. Roh, N. T. Hasan, N. F. Trisha, N. M. Johnson, and D. Han. Texas A&M University, College Station, TX.

KEYWORDS: Epidemiology; Metals; Environmental Toxicology

ABSTRACT: Chronic kidney disease (CKD) is a growing health concern globally. Recent studies have suggested there may be links between exposure to uranium and arsenic and CKD. In the present study, a cross-sectional study was performed to investigate the association between co-exposure to uranium and arsenic and CKD in US adults, based on data from the National Health and Nutrition Examination Surveillance (NHANES) (2007-2016). CKD was defined as glomerular filtration rate <60 mL/minute/1.73m\textsuperscript{2} or urinary albumin-creatinine ratio >30 mg/g. The logistic regression was conducted to calculate the Odds Ratios (ORs) for the associations of urinary uranium and arsenic with chronic kidney disease, adjusted for age, gender, race/ethnicity, poverty income ratio, education, urinary creatinine, serum cotinine level, BMI, and hypertension. The subjects with diabetes were excluded from the analyses. The adjusted odds ratio for chronic kidney disease was 1.62 (95% CI 1.22-2.15) in the highest quartile of urinary uranium level, compared to the lowest quartile, and a significant dose-response linear relationship was observed across the quartiles (p-trend <0.01). Arsenic alone did not show a significant association with chronic kidney disease. However, in the highest quartiles of urinary uranium and arsenic, a stronger association with chronic kidney disease was observed (OR 2.66, 95% CI 1.31-5.42). Our study provided evidence that co-exposure to uranium and arsenic may post a greater
risk of chronic kidney disease in adults. Prospective investigations are needed in the future to corroborate our findings.

ABSTRACT NUMBER: 5090    Poster Board Number: P198
TITLE: The Associations between Urinary Iodine Concentrations and Dyslipidemia among US Adults: a Cross-Sectional Study
AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: N. Trisha, N. Hasan, and T. Roh. Texas A&M University, College Station, TX.

KEYWORDS: Cardiovascular System; Food Safety; Lipids; Dyslipidemia; Iodine

ABSTRACT: Recent studies show that abnormally low or excessively high dietary iodine intake disrupts lipid metabolism. In this study, a cross-sectional analysis was conducted to evaluate the association between abnormal urine iodine concentration and dyslipidemia in US adults. We analyzed five consecutive cycles of the National Health and Nutrition Examination Survey (NHANES) data (2007-2016) for a total of 9068 subjects who were above 19 years old. Dyslipidemia is characterized by increased high triglyceride level (>150 mg/dl), total cholesterol (>200 mg/dl), low density lipoprotein (>160 mg/dl) or decreased high density lipoprotein (<40 mg/dl). The iodine intake was categorized into severe deficient (<50 ng/mL), mild deficient (50-99 ng/mL), adequate (100-299 ng/mL), and excessive (≥ 300 ng/mL), based on the urinary iodine levels. The odds ratios (ORs) were estimated using the multivariate logistic regression model after adjusting for demographic, socioeconomic, serum cotinine level, alcohol consumption, physical activity, comorbidities such as hypertension, diabetes mellitus, and chronic kidney disease. Pregnant and lactating women were excluded from the analysis. Compared to the adequate intake group, the odds ratio for the dyslipidemia was significantly higher in the severe deficient group (OR 1.33, 95% CI 1.02, 1.73) and excessive intake group (OR 1.26, 95% CI 1.02, 1.55) after adjusting for sociodemographic and other risk factors. Our results suggest that both insufficient and excessive iodine intake may increase risks for dyslipidemia. Future prospective studies are necessary to confirm our findings.

ABSTRACT NUMBER: 5091    Poster Board Number: P199
TITLE: Biomarkers of Circadian Disruption of Central and Peripheral Circadian Clocks in Night Shift Nurses in Working Environment
AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: Z. Yang1, K. Black1, P. Ohman-Strickland1, J. M. Graber1, H. Kipen1, H. Zarbl1, and M. Fang1,2. 1Rutgers, The State University of New Jersey, Piscataway, NJ; and 2New Jersey Department of Environmental Protection, Trenton, NJ.

ABSTRACT: Persistent night shift that disrupts circadian rhythm is strongly associated with female breast cancer. To develop biomarkers of circadian disruption, we assessed central clock markers, melatonin and cortisol in plasma, and peripheral clock genes expression and clock regulatory factors (NAD+/NADH and SIRT1 deacetylase activity) in peripheral blood mononuclear cells (PBMCs) over 24 hours in day (12) and night (10) shift nurses. In day shift nurses, cortisol appeared to reach the lowest level at midnight and the highest level in the morning; melatonin reached the highest level right before dawn and decreased later in the morning to the lowest in the afternoon, both showing significant rhythmicity with opposite phases at the same morning time. Major clock genes (PER2 and PER3) mRNA expression also
showed 24-hour rhythms with peak values in the morning. In contrast, night shift nurses showed disrupted circadian rhythms with significantly lower cortisol and clock gene expression levels in the morning compared to the day shift workers. Melatonin levels and rhythms were similar, suggesting a misalignment of melatonin and cortisol. Molecular clock regulators, NAD$^+/NADH$ and SIRT1 activity, neither showed clear 24-hour circadian rhythm in day shift nurses, nor any significant changes in the night vs day shift nurses. The changes of these markers were repeated with similar findings at early and late night timepoints in additional night (20) and day (19) shift nurses. To our knowledge, this is the first study to assess the circadian disruption in cortisol and clock genes expression and their misalignment with melatonin in night shift workers in the working environment. Morning cortisol and clock gene mRNA expression levels in peripheral blood should be further investigated as biomarkers of circadian disruption in shift workers. Supported by NIEHS R21ES026802 for M.F. and P30ES005022 for H.Z.

ABSTRACT NUMBER: 5092  Poster Board Number: P200

TITLE: Novel Chemical Adducts in E-Cigarette Liquids and Their Toxicologic Effects on Human Lung Epithelial Cells

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: S. Howell$^1$, S. V. Jabba$^1$, A. I. Caceres$^1$, H. C. Erythropel$^2$, T. M. deWinter$^2$, J. B. Zimmerman$^2$, and S. E. Jordt$^1$. $^1$Duke University, Durham, NC; and $^2$Yale University, New Haven, CT.

KEYWORDS: Respiratory Toxicology; Cell Culture; Chemical of Concern

ABSTRACT: According to the 2021 National Youth Tobacco Survey, 44% of high school students and 17.2% of middle school students regularly abuse flavored e-cigarettes, vaping 20-30 days per month. E-cigarette manufacturers have systemically marketed numerous flavor varieties that contain unstable constituents and underreported compounds that lack toxicological data. Amongst the unspecified components, our lab has reported flavorant acetals produced exclusively by reactions between the parent flavorant aldehyde and propylene glycol (PG) or vegetable glycerin (VG) solvents. Additionally, we have shown multiple PG acetals activate TRPA1 and TRPV1 sensory irritant ion-channels more robustly than some parent aldehydes in transfected human embryonic kidney (HEK) cells. Also, immortal bronchial epithelial cells (BEAS-2B) treated with PG acetals for 24 hours show significant increases in cytotoxicity and cytostasis while exhibiting reduced cellular metabolism and proliferation. Here, we assessed the toxicological effects of vegetable glycerin (VG) acetals present in e-cigarette liquid compared to their parent flavor aldehydes. Calcium microfluorimetry demonstrated that VG acetals activate TRP irritant receptors in transfected HEK cells, some more robustly than the corresponding parent aldehydes. Using toxicological assays (LIVE/DEAD, LDH, MTT and mitochondrial functional assay), 24-hr VG acetal exposures of BEAS-2B cells caused significant increases in cytotoxicity compared to parent aldehyde, while reducing cellular proliferation and metabolism. Moreover, gene expression analysis revealed that VG acetals modulate proinflammatory cytokine and oxidative stress transcriptional profiles in treated BEAS-2B cells. Thus, our results indicate that e-cigarettes contain unreported components that are toxic and may harm the lung health of e-cigarette users. This study will address knowledge gaps regarding the safety of e-cigarettes and inform the FDA about toxicological effects of novel flavor adducts.
Addressing Chemicals Management Approaches through the Lens of Functional Substitution


ABSTRACT: Faced with the near-impossible task of assessing and regulating chemicals of concern (CoC) on an individual-basis, governments need rapid decision-making approaches for moving towards safer chemicals. The Essential Use Concept (EUC) is one such emerging approach, with the primary goal of expediting the restriction of all but “essential use” chemicals “necessary for the health, safety, or functioning of society.” One challenge of the EUC approach is its focus on determining societal needs, which is hard to define and could lead to extended debates and slow decision-making over what uses are considered essential. Another approach to expedite chemical assessment and decision-making is that of “functional substitution” (FS), which starts with the chemical function or “service” the CoC provides, evaluates if that function is necessary for the application, and then determines whether safer and effective alternatives exist to fulfill the function at the chemical, product, process redesign, or product service levels. FS can be applied in two ways: (1) by focusing on the performance needs of the application to first broadly identify solutions that fulfill the CoC’s function or maintain sufficient performance in other ways, then assessing the safety of those options; and (2) by focusing on comparative toxicity where chemicals are first grouped by function and ranked by hazard characteristics, then assessed for performance based on the needs of the application. There is increasing convergence of the EUC and FS approaches - with a functional lens being the first step of the EUC process. Here, we illustrate how the two approaches can be complimentary and expedite decision-making on CoC’s through three examples: per- and polyfluoroalkyl substances (PFAS) use in occupational protective clothing; copper-containing anti-fouling boat paint; and selection of safer solvents. These case examples explore the toxicological concern of the chemical, how FS is used in each of the approaches, and present clarifying points for each of these cases related to stakeholder involvement and tradeoff considerations. The EUC may be an optimal method for decision-making in clear cases of non-essentiality, but for unclear cases, emphasizing the FS approach may help broaden the range of alternative solutions earlier, potentially avoiding essentiality discussions. Each case will be context-specific and may use a combination of approaches to achieve the ultimate goal in movement towards safer chemicals and materials.

Exposure and Risk Characterizations of Ochratoxins A and Aflatoxins through Maize (Zea mays) Consumed in Different Agro-Ecological Zones of Ghana

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ABSTRACT: Mycotoxin contamination of foodstuffs is a serious food safety concern globally as the prolonged ingestion of these toxins has the tendency to worsen the risk of hepatocellular carcinoma. This study aimed at estimating ochratoxin A (OTA) and aflatoxin (AF) levels above international (European Food Safety Authority, EFSA) and local (Ghana Standards Authority, GSA) standards as well as the health risks associated with the consumption of maize (n=180) sampled from six (6) regions representing the agro-ecological zones of Ghana. OTA and AF were measured with High-Performance Liquid Chromatography with a Fluorescence detector. Out of the 180 samples analyzed for total aflatoxins (AFtotal), 131/180 tested positive and 127 (70.50%) exceeded the limits of EFSA and ranged 4.27-441.02 µg/kg. While for GSA, 116 (64.44%) of samples exceeded this limit and ranged between 10.18-441.02 µg/kg. For OTA, 103/180 tested positive and 94 (52.22%) of samples between the range 4.00-97.51 µg/kg exceeded the tolerable limit of EFSA, whereas 89 (49.44%) were in the range of 3.30-97.51 µg/kg exceeded the limits of GSA. Risk assessment values for total aflatoxins (AFtotal) ranged between 50-1150 ng/kg bw/day, 0.4-6.67, 0-0.0323 aflatoxins ng/kg bw/day and 1.62-37.15 cases/100,000 person/yr for Estimated Daily Intake (EDI), Margin of Exposure (MOE), Average Potency, and Cancer Risks respectively. Likewise, ochratoxin (OTA) values were in the ranges of 8.6x10-3-450 ng/kg bw/day, 0.05-2059.97, 0-0.0323 ochratoxins ng/kg bw/day and 2.78x10-4-14.54 cases/100,000 person/yr. Consumption of maize posed adverse health effects in all age categories of the locations studied since the calculated MOE values were less than 10,000.