The Toxicologist: Late-Breaking Supplement

These abstracts also are available via the SOT Event App and the Online Planner.

All late-breaking abstracts are presented on Thursday, March 14, 8:30 am–11:30 am.
Preface

This issue is devoted to the abstracts of the Late-Breaking Poster Session of the 58th Annual Meeting of the Society of Toxicology, held at the Baltimore Convention Center, Baltimore, Maryland, March 10–14, 2019.

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**THURSDAY POSTER SESSION MAP**

March 14, 2019—8:30 AM to 11:30 AM—Hall A

Poster Setup—8:00 AM to 8:30 AM

Late-Breaking Poster #s: P101–P388

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Entrance

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ABSTRACT NUMBER: 3314    Poster Board Number: P101
TITLE: In Vivo Cytogenetic Effect of Certain Pesticides on Egyptian Toads *Bufo regularis*


KEYWORDS: Pesticides; Ecotoxicology; Genotoxicity

ABSTRACT: Since Amphibians have highly permeable skin, they can be used as bio-indicator for pesticides that may reach the aquatic environment. One of the batteries of mutagenicity tests for pesticide registration in EPA is *in vivo* cytogenetic, which required to assess the potential effects of test chemical on genetic material in bone marrow and/or peripheral blood cells of animals, which usually rodents (mice or rats) other than toads. In the present study, evaluation of cytogenetic effect using micronucleus test of erythrocytes in Egyptian Toads *Bufo regularis* exposed to four pesticides was carried out. Copper sulfate, temephos, glyphosate and bifenthrin were examined at one tenth of the recommended field application rates as 0.001 g/L, 0.5 ml/L, 2 ml/L and 0.48 mL/L, respectively, in water for 96 hours under laboratory conditions. Erythrocytes micronuclei and nuclear abnormalities of treated toads were compared with positive control (2 mg/L cyclophosphamide) and negative control (water). It was found that all the tested pesticides significantly induced the frequencies of micronuclei and nuclear abnormalities such as double nucleus; nuclear pycnosis; multi nucleus; nuclear concavity; and nuclear protrusion in erythrocytes of treated toads. This test can identify which pesticide can cause cytogenetic damage that results in the formation of micronuclei containing lagging chromosome fragments or whole chromosomes. Bifenthrin and temephos caused frequency of nuclear abnormalities and micronuclei more than either positive or negative control. However, glyphosate and copper sulfate caused less frequencies of nuclear abnormalities and micronuclei than positive control but still higher than negative control. This study showed that micronucleus assay data in toad’s erythrocytes can identify the cytogenetic damage and can be used as a biomarker. In conclusion, pesticides might cause global decline of non-target organisms such as amphibian, and might resulted to mutagenicity. Therefore, toads can be postulated as bio-indicator.

ABSTRACT NUMBER: 3315    Poster Board Number: P102
TITLE: Detection and Occurrence of Microplastics in the Gut of Food Fish Species from a Municipal Water Supply Lake in Southwestern Nigeria

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: A. O. Adeogun1, O. R. Ibor2, A. V. Chukwuka3, D. E. Omogbemi3, and A. Arukwe4. 1University of Ibadan, Ibadan, Nigeria; 2University of Calabar, Calabar, Nigeria; 3National Environmental Standards and Regulation Enforcement Agency, Abuja, Nigeria; and 4Norwegian University of Science and Technology, Trondheim, Norway.

KEYWORDS: Ecotoxicology; Food Safety/Nutrition; Exposure, Environmental

ABSTRACT: Microplastics (MPs) are physical anthropogenic pollutants and their ability to act as contaminant vectors in biological matrices is of serious ecosystem and human health concern. Herein, we screened and detected MPs for the first time in the guts of some commonly consumed fish species.
from a municipal water supply (Eleyele) lake in Nigeria. 109 fish samples consisting eight (8) species: 38 Coptodon zillii (CZ), 43 Oreochromis niloticus (ON), 19 Sarotheron melanotheron (SM), 3 Chrysichthys nigrodigitatus (CN) and one (1) each of Lates niloticus (LN), Paranchanna obscura (PO), Hemichromis fasiatus (HF) and Hepsetus odoe (HO) were collected between February-April, 2018. Fish gut content was removed, screened for the presence of MPs using the density gradient separation technique (NaCl hypersaline solution) and viewed with a fluorescence microscope. MPs were present in all the species screened except H. fasciatus with a frequency of 69.7% positive individuals in the examined population. MPs prevalence was highest in ON (34%) > CZ (32%) > SM (13%) > CN (6%) and 5% each, for PO, HO and LN. Generally, 1-6 MPs (12 µm-1 mm) were detected per individual with 34 MPs in the gut of S. melanotheron. Given that microplastics may contain several synthetic chemical additives/compounds that can be easily absorbed in the gut of ingested organisms; our findings raise concerns on the potential human/wildlife health effects of MPs in these economically/ ecologically important food fishes since MPs and their leachates may enhance bioaccumulation and biomagnification of contaminants along the food web.

ABSTRACT NUMBER: 3316   Poster Board Number: P103
TITLE: Evaluation of Oxidative Stress in Alevin Salmonid Species Exposed to Fluridone Based Herbicide Formulation

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: M. Bolotaolo, M. Stillway, and S. Teh. 
University of California Davis, Davis, CA. Sponsor: B. Puschner

KEYWORDS: Aquatic Toxicology; Pesticides; Ecotoxicology

ABSTRACT: Fluridone is a systemic herbicide which interferes with the synthesis of RNA, proteins, and carotenoid pigments inhibiting photosynthesis of targeted plants. Fluridone formulations, such as slow release pellets sold under the name Sonar Q, are widely used for invasive aquatic plant mediation. Herbicide application occurs directly to the water body, increasing potential for off target exposure. Fluridone toxicity is thoroughly studied, however studies are lacking on the toxicity of the formulation, especially on non-lethal endpoints such as oxidative stress. Alevin rainbow trout, representing a sensitive life stage of the salmonid species, were exposed to Sonar Q via immersion for 14 days. Behavior as well as multiple oxidative stress endpoints were evaluated including total glutathione, glutathione S-transferase, lipid peroxidation, superoxide dismutase, and catalase. The EC50 of ecologically dead individuals increased from early exposure to termination. The EC50 (1 day) was 5260 µg/L; (7 day), 7066 µg/L; and (14 day), 7623 µg/L. Individuals appeared to recover throughout exposure; however, in the wild this initial incapacitation could be detrimental to the individual’s survival. Non-lethal effects from exposure could also be of concern. Trends of oxidative stress induction with concentration of Sonar Q exposure will be discussed.
ABSTRACT NUMBER: 3317    Poster Board Number: P104

TITLE: Cumulative Effects of Subchronic Exposures to WTC Ambient Particles at Different Environmental Temperature in Mice: Heart Rate, Heart Rate Fluctuation, and Body Temperature

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: J. Li1, W. Yu1, M. Zhong1, J. HWANG2, L. Tian3, and L. Chen1. 1New York University, New York, NY; 2Academia Sinica, Taipei, Taiwan; and 3University of Hong Kong, Hong Kong, Hong Kong.

KEYWORDS: Environmental Toxicology; Exposure, Environmental; Cardiopulmonary

ABSTRACT: For the last fifteen years, those who worked and resided near the World Trade Center (WTC) site at the time of the September 11, 2001 attack and thereafter, have been plagued by cardiorespiratory ailments. The increasing environmental temperature showed some ambiguous health effects for the people who had particle exposure. Normal mice (FVB/N) were divided into two groups randomly. All the mice were implanted with electrocardiograph (ECG), core body temperature, and motion transmitters and exposure to the WTC particles suspended in water by nasal aspiration method once a week for three months. Control mice were exposed to water. The environmental temperature was transiently increased using an electric heater outside of the exposure chamber to explore how the climate temperature changes affect the particulate matters exposure. Time series of ECG monitoring for all the parameters' measurements were obtained for each animal in the WTC particle aspiration group and control groups. The daily crude effects were tested by the time-varying model and the associations between temperature and measurement indicators were quantified using a Poisson time-series model. With the passage of time, the body temperatures and activities of mice did not show statistical differences between the WTC-exposed and water-exposed groups while heart rate had significant fluctuations. After the chamber temperature was gradually increased over a period of 3-4 hours to a maximum 38 °C and lasted 30 minutes before the chamber temperature gradually fell to room temperature, there were significant increasing patterns of heart rate for the mice which have been exposed to the WTC particles subchronically over the three months (P<0.05). Moreover, the differences for these two indicators between the two groups reached the maximal gap in the temperature-change periods to the 2.10-fold and 1.73-fold separately. Using ECG telemetry of unrestrained conscious mice shows that environmental climate temperature changes perturb cardiac autonomic functions to a greater degree in mice that were exposed to WTC particles. Our data implied that extreme weathers episodes can potentially augment the health impact of air pollution.

ABSTRACT NUMBER: 3318    Poster Board Number: P105

TITLE: The Effects of 8-Prenylnaringenin on eNOS Phosphorylation and Nitric Oxide Synthesis via AMPK/CaMKII-dependent Pathway


ABSTRACT: Humulus lupulus L. (Hops) used in beer production is a source of polyphenols such as xanthohumol (XN), and its metabolites isoxanthohumol (IXN) and 8-prenylnaringenin (8-PN). Hops present potential health-protective properties, which are likely attributed to their effect on oxidative stress and inflammation. Nitric oxide (NO), produced by endothelial nitric oxide synthase (eNOS), plays critical roles in the regulation of vascular function and maintenance. This study investigated the intracellular pathway underlying eNOS activation by 8-PN. XN, IXN, and 8-PN increased eNOS
phosphorylation and NO production in endothelial cells. However, 8-PN is strongly increase eNOS phosphorylation and NO production in these Hops sources. 8-PN induced phosphorylation of AMP-activated protein kinase (AMPK) and calcium/calmodulin-dependent kinase II (CaMKII) in a concentration-dependent manner. Each inhibitor of AMPK and CaMKII reduced 8-PN-induced eNOS phosphorylation. These results indicate that 8-PN stimulates eNOS phosphorylation and NO production via activation of AMPK/CaMKII-dependent pathway. Therefore, 8-PN may be useful for the treatment or prevention of endothelial dysfunction associated with vascular disease.

ABSTRACT NUMBER: 3319    Poster Board Number: P106
TITLE: Ultrafine Particulate Matter Combined with Ozone Promotes Arrhythmogenesis in Mature Adult Rats with Cardiovascular Disease


KEYWORDS: Environmental Toxicology; Exposure, Environmental; Cardiovascular System

ABSTRACT: Background: Individuals with cardiovascular disease (CVD) are uniquely susceptible to air pollution-related cardiopulmonary morbidities and mortalities. In this study, we examined the arrhythmic effects of an acute exposure to either ultrafine particulate matter (UFPM), ozone (O₃) or UFPM and O₃ combined (UFPM+O₃), in mature adult Wistar-Kyoto (WKY) and Spontaneously Hypertensive (SH) rats. The aim of this investigation was to evaluate the overall hypothesis that the combination of ultrafine particulate matter (UFPM) and ozone (O₃) enhances the arrhythmogenic effects of exposure, increasing arrhythmia incidence and severity. Furthermore, CVD promotes the development of a proarrhythmic state, enhancing susceptibility to exposure-induced arrhythmogenesis.

Methods: Conscious, mature adult Wistar-Kyoto and Spontaneously Hypertensive rats were exposed to one of the following experimental atmospheres: filtered air (FA), UFPM (~250 μg/m³), O₃ (1.0 ppm) or UFPM+O₃ (~250 μg/m³ + 1.0 ppm) for 6 h, followed by an 8 h filtered air recovery period. ECG tracings recorded during experimental protocol were evaluated by a board certified veterinary cardiologist without knowledge of strain or exposure group assignment. Numeric (continuous; total number of events) and categorical scoring schematics were used to assess arrhythmias, based on origin: (1) ventricular ectopy (VE), (2) supraventricular ectopy (SV) or (3) atrioventricular block (AVB). Overall arrhythmia severity score was calculated as sum of categorical severity scores. Results: UFPM+O₃ significantly increased arrhythmic event total number and severity compared to FA. For WKY rats, UFPM+O₃ increased total number of VPC and AVB events as well as VE and overall severity scores. For SH rats, UFPM+O₃ increased total number of VPC and AVB events as well as VE, AVB and overall severity scores. Conclusion: The results of the present study demonstrate that O₃ augments the arrhythmogenic effect of UFPM in both WKY and SH rats. Additionally, we found that mature adults with CVD are particularly vulnerable to UFPM and O₃-combined, and thus, at greater risk for increasingly severe exposure-induced arrhythmic events.
ABSTRACT NUMBER: 3320   Poster Board Number: P107
 TITLE: A Systematic Review of the Health Effects Associated with the Gaseous and Particulate Fractions of Diesel Exhaust

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ABSTRACT: Diesel exhaust, comprised of gases and particulate matter, is a contributor to ambient air pollution, and its negative impacts on human health are well-documented. Recent changes in diesel engine technology have significantly altered the composition of exhaust, primarily by lowering levels of particulate matter. Health effects following exposure to whole diesel exhaust continue to be reported in controlled human exposure and animal toxicological studies. However, it remains unclear whether these effects are associated with the gas or particle fraction of the exhaust. To gain an understanding of the role of both the gaseous and particulate fractions of diesel exhaust on specific health outcomes, we conducted a systematic review in which we examined animal inhalation and controlled human exposure studies that included a comparison between whole and particle filtered diesel exhaust from more recent diesel engine technologies on any health endpoint. We identified 23 studies from a literature search in PubMed, Web of Science, and ToxLine that met both the inclusion and study evaluation criteria. For studies that focused on respiratory outcomes, changes in biomarkers were primarily associated with exposure to the gaseous phase of exhaust. For cardiovascular, neurological, and reproductive outcomes, effects were observed from exposure to both fractions, with particulate matter primarily affecting cardiovascular health and the gaseous phase primarily affecting the reproductive system. Results from this systematic review demonstrate that exposure to the gaseous and particulate fractions from newer diesel engines can have distinct and independent health effects. Thus, research to further elucidate effects of diesel exhaust should include filtration to examine both fractions rather than whole diesel alone, which could inform future strategies and technological advances to potentially mitigate risk.

ABSTRACT NUMBER: 3321   Poster Board Number: P108
 TITLE: Diesel Exhaust Particles Polarize Macrophages from Multiple Strains of Mice to a Mox-like Subtype

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KEYWORDS: Macrophage; Genomics

ABSTRACT: Diesel exhaust particles (DEP) are a major source of air pollution and a prominent contributor to cardiopulmonary effects, but the mechanisms mediating those effects are unknown. Macrophages are highly responsive to inhaled particles. Therefore, we hypothesized that macrophage activation and polarization are essential in mediating DEP-induced cardiopulmonary effects. We conducted a genome-wide analysis of macrophages treated with a methanol extract of DEP (DEPe) to help elucidate potential mechanisms that mediate acute effects induced by air pollution exposures. Peritoneal macrophages were harvested from 24 strains of mice from the Hybrid Mouse Diversity Panel (HMDP). Cells were treated for 4 hours with either DEPe or media only. Gene expression was analyzed using Affymetrix HT MG-430A arrays. Analysis of the microarray data showed that 1,284 genes were...
upregulated and 1,428 genes were downregulated with DEPe treatment (out of 13,278 detected genes). Pathway analysis using PANTHER tools identified cellular responses to external stimuli (stress and heat stress), transcription, and IL-1 signaling being among the most prominent upregulated pathways. Many of the upregulated genes included antioxidants such as Hmox1, Txnrd1, Srxn1, and Gclm as well as inflammatory genes like Irak1 and Ikbkg. Using X2K software to investigate key transcription factors involved revealed that Nrf2 was the most significant driver (p<1e-41). Importantly, evaluation of macrophage polarization gene profiles determined that DEPe led these cells to polarize to a Mox-like macrophage subtype, typically induced by oxidized phospholipids and dependent on Nrf2 expression, as opposed to M1 or M2 subtypes. In fact, 53.1% of the Mox marker genes were significantly upregulated at least 1.5-fold, compared to less than 5% each of M1 and M2 marker genes. DEPe dysregulated a large number of genes in peritoneal macrophages from multiple strains of mice. Macrophage responses were similar across the various strains, dominated by antioxidant responses driven by the transcription factor Nrf2, as well as pro-inflammatory responses. Brief treatment with DEPe induced macrophage polarization to a Mox-like subtype, likely to depend on Nrf2 expression as well, which may have significant implications in the cardiopulmonary toxicity induced by air pollution.

**ABSTRACT NUMBER:** 3322  
**Poster Board Number:** P109  
**TITLE:** Early Life Ozone Exposure Changes Lung Growth  
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**KEYWORDS:** Inhalation Toxicology; Oxidative Injury; Developmental Toxicity; Post-Natal  
**ABSTRACT:** Inhaled ozone is a widespread concern; over 50% of the US population lives in areas that exceed the national ambient air quality standards set to protect human health. Early life exposure to ozone air pollution can alter lung functional growth. However, what is not well understood are the structural and cellular changes that occur in the lung when exposed to ozone early in life, which may change how the lung grows. Our prior work established that early life ozone-exposure resulted in abnormal lung growth when measured in the conducting airways of male adult rats. This abnormal growth was evident as distal conducting airways that were smaller in both diameter and length. The purpose of this study is to define how these changes occur by examining the lungs immediately after the early life ozone exposure in rats of both sexes and after a recovery period in adults. Male and female rats inhaled 0.5 ppm ozone from postnatal day 7 to 28 or filtered air (FA). Samples were taken 3-4 days after the last ozone exposure (juveniles) and after 4 weeks of recovery in FA (adults) and included tissues for targeted and nontargeted gene expression analysis, histopathology and bronchoalveolar lavage (BALF) cell differentials. We assessed conducting airway (proximal, distal airways) and alveolar gene expression in the lung using microdissection of RNA-later inflated tissue. Genes involved in airway epithelial differentiation (Club Cell Secretory Protein, CCSP), proliferation (Proliferating Cell Nuclear Antigen, PCNA) and antioxidant enzyme responses (Glutathione S-transferase Pi, GST-pi) were measured. Ozone exposure significantly decreased the percentage of macrophages in BALF. This change was persistent and significant even after the FA recovery period. Cell proliferation (PCNA gene expression) and airway epithelial differentiation (CCSP gene expression) was significantly decreased in the distal airways of females. While male rats had similar trends in reduced CCSP and PCNA expression in distal airways, these did not reach statistical significance. GSTpi was increased in the proximal airways and reduced in the distal airways of females exposed to ozone but not in males. We conclude that
females are more susceptible to ozone induced changes to conducting airways than males and that the
distal airway reduction in growth may be due to decreased Club cell differentiation and proliferation at
this site in both sexes. Support: P30 ES023513

ABSTRACT NUMBER: 3323  Poster Board Number: P110
TITLE: Pro-inflammatory Potential of Indoor Dust Collected from Households in San Juan, Puerto Rico in
the Aftermath of Hurricane Maria

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PR.

KEYWORDS: Exposure, Environmental; Inhalants; Particulates

ABSTRACT: In September 2017, Hurricane Maria caused extensive devastation in Puerto Rico, including
substantial wind- and water-damage to homes. The extremely slow pace of recovery and repair of the
water-damaged homes increases the risk of inhabitants’ long-term exposure to pro-inflammatory indoor
air pollutants. To assess the pro-inflammatoryatory potential (PIP) of indoor particulate matter in the
aftermath of Hurricane Maria, dust samples were collected from 50 homes from an affected community
in San Juan, PR. Based on self-reported survey of the household occupants, 26 of these homes were
located in non-flooded areas (13 of them experienced some internal water damage) and 24 in flooded
areas (13 were subjected to flooding inside and 11 had some water damage). Dust samples’ extracts
were evaluated for their pro-inflammatoryatory potential (PIP). The PIP was based on the extracts’ potency to
induce the release of cytokine interleukin (IL)-1 beta from peripheral human blood leukocytes.
Household occupants were also surveyed about their home environment and household damage
resulting from the hurricane. Preliminary results show that some home environment factors such as pet
ownership, self-reported allergen exposure, and smoking were not associated with increase in PIP. In
homes whose interiors were flooded during the hurricane, damage to the kitchen area and poor
ventilation were associated with a 1.2 (p = 0.009) and 2.35 fold (p = 0.03) increase in PIP of the dust
samples. Flooding damage that required removal of floors, roof, or wooden materials in the homes were
associated an increase in the PIP of the dust samples (p = 0.02 to 0.004). These results suggest that
indoor conditions that result from long term, unrepaired flood damage in homes with less ventilation,
may increase the risk of occupant exposure to particulate matter with higher pro-inflammatoryatory
potential. Based on these preliminary results, timely repairs in the aftermath of water damage to
households is recommended to reduce the risk of exposure to indoor pollution that could negatively
affect the health of occupants following hurricanes.
TITLE: The Effect of Varying Doses, Sources, and Durations of Particulate Matter (PM2.5) Exposure on Myofibroblast Differentiation

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: N. Craig, and S. Huang. University of Michigan, Ann Arbor, MI. Sponsor: R. Loch-Caruso

ABSTRACT: Rational: Exposure to air pollution, specifically particulate matter (PM2.5) has been associated with a number of different lung disorders, including lung cancer and asthma. Recent epidemiologic studies suggest that exposure to PM2.5 may be linked to the development of Idiopathic Pulmonary Fibrosis (IPF), a deadly lung disease of unknown etiology. How PM2.5 contributes to the development or progression of IPF is unknown. Here, we seek to examine the direct effect of PM2.5 on the functions of lung fibroblasts, the main effector cells in IPF. Methods: A cell line of primary human fibroblasts (CCL-210), as well as primary fibroblasts from normal and IPF lungs, were cultured on 6-well plates and exposed to varying concentrations of PM2.5 (.001 to 30 µg/cm²) every day or every three days for 7 days. Cells were treated with PM2.5 obtained either from Washington, DC (DC-PM2.5) and the National Institute of Standards and Technology, or from manual samplers that collected PM2.5 from the rooftop of Peking University School of Public Health in Beijing, China during January 2015 (B-PM2.5). Cell lysates were analyzed for smooth muscle actin (SMA) expression and expression of bone morphogenetic protein (BMP) 2. Results: Fibroblasts exposed to low doses of DC-PM2.5 repeatedly over time, but not to higher doses or for shorter intervals, exhibited increased expression of α-SMA, a marker of myofibroblast differentiation. This contrasts with the response of fibroblasts to B-PM2.5, which exhibited a dose dependent increase in α-SMA protein expression in response to exposure. Interestingly the increase in α-SMA protein was not reflected by changes in mRNA. Both DC-PM2.5 and B-PM2.5 significantly unregulated expression of BMP2, which was further sensitized by longer durations of repeated exposure. Conclusion: Direct exposure of fibroblasts to PM2.5 in vitro resulted in differentiation of fibroblasts to myofibroblasts, which is critical to the development of pulmonary fibrosis. These changes occur in response to long, but not short, durations of exposure, and indicate that low levels of PM2.5 exposure can cause changes in fibroblast response that are distinct from higher doses. These data suggest that longer exposure times may be necessary to accurately model chronic disease. Funding: This work was supported by HL127203 from NHLBI.

TITLE: Offspring Growth Inhibition in a Multiplex Model of Paternal Alcohol and Maternal Air Pollution Exposure


KEYWORDS: Developmental Toxicity; Prenatal; Particulates; Respiratory Sensitization

ABSTRACT: Epidemiological studies suggest a combination of parental histories and environmental exposures intersect in the etiology of disease. To date, no studies have addressed the possibility that preconception male exposures have the capacity to potentiate dysgenesis induced by an environmental pollutant encountered by the mother. To identify potential interactions, we combined two separately established models to generate a novel multiplex model. Herein, a mouse model of paternal
preconception alcohol exposure preceded maternal gestational exposure to particulate air pollution. Male C57Bl/6 mice were provided 10% (w/v) EtOH and 0.066% (w/v) saccharin or 0.066% (w/v) saccharin alone (Control) for 70 days. Subsequently, males were mated with females that were exposed to filtered air (FA) or 99.52 µg/m³ ultrafine particulate matter (PM) for 6-h daily from gestational day 0-18. To evaluate airway inflammatory responses, offspring underwent suboptimal immunization and challenge with ovalbumin (OVA) or PBS. Offspring allergen exposure had no effect on body weight. Thus, we compared differences between paternal (EtOH or Control) and maternal (PM or FA) exposure groups. At 28 days, offspring from the EtOH-PM group had significantly reduced body weight (8.9 ± 0.3) in comparison to the Control-PM (12.3 ± 0.4), Control-FA (11.7 ± 0.3), and EtOH-FA (12.5 ± 0.5) groups, \( P < 0.001 \). Growth inhibition was significant in both sexes and persistent from day 17-28. Significant differences in BALF white blood cell counts were not seen, although analysis on selected growth factor expression is ongoing. In conclusion, we created a novel multiplex model that uncovered a synergetic interaction between two environmentally-relevant parental exposures.

**ABSTRACT NUMBER:** 3326   **Poster Board Number:** P113

**TITLE:** A Pilot Study to Evaluate the Immunotoxicity of Perfluoro-2-methoxyacetic Acid (pfmoaa) in Orally Exposed Male and Female C57Bl/6 Mice

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**KEYWORDS:** Perfluoronated Agents; Immunotoxicity; Immunotoxicity

**ABSTRACT:** Per- and polyfluoroalkyl substances (PFAS) comprise over 5,000 synthetic fluorinated chemicals used extensively in numerous products and processes. Sub-groups of “long-chain” PFAS that contain more than six or eight carbons and carboxylic or sulfonic acid functional groups, have been phased out of production and use in U.S. and several European countries due to persistence, bioaccumulation, and toxicity (PBT). Replacements for phased out PFAS are being detected in surface and drinking water; however, many replacements are understudied with respect to PBT. Two PFAS presumed to be immune hazards to humans by the US National Toxicology Program raises concerns about immunotoxicity of other PFAS. Perfluoro-2-methoxyacetic acid (PFMOAA) was identified as the dominant PFAS in raw and finished drinking water from the Cape Fear River of North Carolina. No data exist in the published literature on PBT of this PFAS. Therefore, the study objective was to begin to evaluate its immunotoxicity in mice. Adult male and female C57BL/6 mice were given PFMOAA (0, 0.00025, 0.025, or 2.5 mg/kg in 0.5% Tween) or a 7.5 mg/kg perfluorooctanoic acid (PFOA) positive control via gavage for 30 d. On day 26, animals were injected with a T cell-dependent antigen. At study terminus, blood and organs were collected. Spleen and thymus were immediately prepared to determine cellularity and lymphoid cell immunophenotype. Body weights did not differ across dose groups over the study period or at terminus. Liver weight, a typical marker of PFAS exposure, increased 140-200% in both sexes by PFOA, but was not increased by PFMOAA. Absolute and relative lymphoid organ weights and cellularity were not changed by PFMOAA or PFOA. This pilot study suggests that at these administered doses, PFMOAA, a PFAS comprised of three carbons, does not induce changes in body or liver weight or lymphoid organ weight and cellularity often observed after exposure to other PFAS. Additional analyses of immune-specific endpoints, notably the T cell-dependent antibody
response, will determine if PFMOAA also poses immunotoxicological concerns similar to phased-out
PFAS.

ABSTRACT NUMBER: 3327    Poster Board Number: P114
TITLE: Persistent Organic Pollutants Disrupt Gluconeogenesis in Differentiated HepaRG Cells

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KEYWORDS: Metabolism; Persistent Organic Chemicals; Gene Expression/Regulation

ABSTRACT: Many epidemiological studies suggest a role for some environmental persistent organic pollutants (POPs) in the increased incidence of obesity and type 2 diabetes (T2D) during the last decades. Only few \textit{in vitro} studies using human-derived cell lines support such a role. Further, the mechanisms of action for many of these POPs are unknown. The aim of the DiaPOPs study is to better understand the relationship between POPs and T2D by combining epidemiological and mechanistic approaches using a human hepatocyte cell line (HepaRG) to analyze disruptions of carbohydrate and lipid metabolism. In a first step, we identified a set of four “diabetogenic” POPs (p,p’-DDE, β-HCH, PCB 156 and PCB 180) by studying associations between the measured concentrations of circulating POPs and the incidence of T2D in the French D.E.S.I.R. cohort with a 9-year follow-up. Then, HepaRG cells were exposed for 24 or 72 hours to each POP alone or in a mixture at 10 and 100 fold higher concentrations than those found in the cohort. RNA-sequencing showed that two gluconeogenic enzymes (glucose-6-phosphatase and phosphoenolpyruvate carboxykinase) were down-regulated by β-HCH, alone and in the mixture. Down regulation of the enzymes was also found by RT-qPCR for p,p’-DDE, β-HCH and PCB 156, alone and in the mixture, after 24h and 72h of POP exposition. Secretion of glucose (as measured by bioluminescent methods) was decreased in the culture supernatants of HepaRG cells exposed to each POP, alone or in the mixture for 72 but not 24 hours. These results suggest that the gluconeogenesis might be a targeted metabolic pathway of “diabetogenic” POPs. The effects of POPs on gluconeogenesis should be taken into consideration by stakeholders when determining potential links between environmental stressors and metabolic disruption in humans.

ABSTRACT NUMBER: 3328    Poster Board Number: P115
TITLE: Defining the Neurotoxic Potential of Polychlorinated Biphenyl Concentrations in Environmental and Human Samples

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KEYWORDS: Persistent Organic Chemicals; Polychlorinated Biphenyls; Neurotoxicity; Developmental

ABSTRACT: Coplanar polychlorinated biphenyls (PCBs) are termed dioxin-like due to their aryl-hydrocarbon receptor (AhR) mediated toxicity similar to the dioxin TCDD. For risk assessment, individual PCBs are assigned a TCDD equivalency factor (TEF) based on their AhR activity relative to TCDD. Once corrected by congener concentrations present in a mixture TEF values are summed to develop a TCDD
equivalent (TEQ) to estimate exposure risks for human or wildlife populations. Non-coplanar PCBs, termed non-dioxin-like (NDL) due to little to no activity at the AhR, constitute greater than 50% of PCB burdens in environmental and organismal samples but are considered non-toxic contributors to PCB mixtures under the current TEQ scheme. Several neurotoxic relative potency schemes have been developed for PCBs due the presence of altered neurodevelopmental and cognitive and behavior deficits seen in exposed organisms. Here, we assess the applicability of both schemes by applying neurotoxic equivalencies to PCB burdens found in previously published research. We then summarize the neurotoxic potential found in environmental exposure scenarios and in human serum samples. We find that greater than 8% of lakes in the United States have fish with PCB burdens that present a potential consumption risk for neurotoxicity. Application to air also demonstrates potential risks to children in urban schools due to the presence of PCBs in indoor air. Our finding suggest that a neurotoxic scheme combined with the established TEQ would likely provide a more inclusive measure of risk presented by environmental PCB mixtures.

ABSTRACT NUMBER: 3329  
Poster Board Number: P116

TITLE: Impact of Polychlorinated Biphenyl Congeners on Cell Proliferation and Estrogenic Activity in Human Placental Trophoblast Cells


KEYWORDS: Polychlorinated Biphenyls

ABSTRACT: Polychlorinated biphenyls (PCBs) are common environmental organic pollutants that are widely considered to have various toxic effects, including reproductive toxicity. Despite a worldwide ban on their production, PCBs remain to be an environmental problem due to their high persistency and ongoing leaking to the environment from existing applications and waste. Numerous reports have described PCB-dependent adverse effects on human fetal growth, including increased risk for intrauterine growth restriction, changes in endocrine function and hormone metabolism, and neurological deficits. PCBs are recognized as estrogenic endocrine disruptors (EEDs) because they induce or block estrogen (E2) actions to interfere with reproductive and developmental pathways in animals and humans. Estrogen is mediated by the two receptors isoforms: estrogen receptor alpha (ERα) and beta (ERβ). Both receptors are expressed in human tissues and have different action profiles. Estrogen receptor alpha (ERα) is the major regulator of placenta. 17β-estradiol (E2) promotes cell proliferation in both normal and transformed epithelial cells by modifying the expression of hormone responsive genes involved in the cell cycle and/or programmed cell death. The aim of this study was to examine (i) the toxic potency of PCB-118 and PCB-153 and (ii) the effect of PCB-118 or PCB-153 on estrogen-induced cell proliferation in the human placental trophoblast (BeWo) cells. Cells were treated for different exposure time and various concentrations of PCB-118, PCB153, or estrogen alone or in combination. Cell proliferation, viability and apoptotic cell death were assessed. Results indicated that exposure to PCB-118 or PCB-153 significantly decreased cell proliferation in a concentration-dependent manner in BeWo cells after 24 h of exposure. Low concentrations (0.01-10 nM) of PCB-118 promotes estrogen-induced cell proliferation. The ER-α antagonists, ICI 182,780, and tamoxifen did block these effects indicating that this effect is mediated through ER receptors involved in the control of proliferation in the placenta. PCB-153 showed a weak estrogenic activity in BeWo cells. These results demonstrate that the human
placenta is a target of PCB toxicity, and that current environmental PCB exposure levels are a risk to reproductive health. Supported by Title III.

ABSTRACT NUMBER: 3330    Poster Board Number: P117
TITLE: Cigarette Smoke Exposure Exacerbates Pulmonary Fibrosis Induced by Polyhexamethylene Guanidine in Mice

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KEYWORDS: Inhalation Toxicology

ABSTRACT: Cigarette smoke (CS) is associated with chronic diseases, particularly lung diseases such as chronic obstructive pulmonary disease (COPD), lung cancer, and desquamative interstitial pneumonia. The present study aimed to assess the effects of repeated exposure to CS in polyhexamethylene guanidine (PHMG)-induced pulmonary fibrosis. Mice were exposed nose-only inhalation to CS (300 mg/m³) for 4 hours/day, 7 days/week for 2 weeks. The following four experimental groups were evaluated: vehicle control (VC), PHMG, CS, and PHMG + CS. Animals in the PHMG group exhibited increased the numbers of total cells and inflammatory cells in the bronchoalveolar lavage fluid (BALF), lung hydroxyproline (HP) content, and histopathological changes, including macrophage infiltration and granulomatous inflammation/fibrosis in the lungs. These parameters were exacerbated in lungs of mice in the PHMG + CS group. In contrast, mice in the CS group alone displayed only minimal macrophage infiltration in pulmonary tissue. The expression of inflammatory cytokines was markedly increased in lungs of mice in the PHMG or CS groups compared to VC group. However, the expression of these cytokines in lungs of mice in the PHMG + CS group was not increased more than that of the PHMG or CS group. On the other hand, the expression of fibrogenic mediators was significantly elevated in lungs of mice in the PHMG group compared with that VC group, and the expression was further increased in lungs of mice in the PHMG + CS group. Although, an enhanced expression of inflammatory cytokines following CS exposure in lungs of PHMG-treated mice was not observed, and these results demonstrate that repeated exposure to CS may enhance the development of PHMG-induced pulmonary fibrosis.

ABSTRACT NUMBER: 3331    Poster Board Number: P118
TITLE: Inhalation Risk Assessment and Protective Airborne Paraquat Inhalation by Different Respirators Use during Simulate Spray Operation

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: S. Chaiklieng, Khon Kaen University, Khon Kaen, Thailand.

KEYWORDS: Risk Assessment; Inhalation Toxicology; Pesticides

ABSTRACT: The aims of this study were to evaluate the protective effect of the respirators while simulated PQ spraying operations in experimental chamber. Inhalation risk assessment was estimated following US.EPA-IRIS. Airborne PQ concentration was collected from an experimental spray chamber with controlled level lower than 50% standard of NIOSH. Air monitoring was performed under the conditions setting of in- and out-respirators. The polytetrafluoroethylene filter membrane was used to
collect PQ and analyzed with HPLC-UV detector following NIOSH standard method 5003 for sampling and analysis. The protective respirators were the convenient sponge mask, one protective mask mostly used by sprayers, and others respirators were cup shape mask and cartridge respirator. The concentration of out-respirator was 12.34 µg/m³, the concentration in-respirator was significantly decreased to be 3.27 µg/m³. The cup shape mask is most effective of reducing the concentration of PQ. The inhalation risk assessment for long term, short term and chronic pneumonitis effects of without-respirator were 13.49%, 13.49%, and 3.45% of sprayers, respectively. The health risk was decreased to be 6.90%, 3.98% and none of sprayers with -respirator condition, respectively. As a result, the respirators decrease both concentrations and risk on PQ exposure, especially for chronic pneumonitis.

ABSTRACT NUMBER: 3332 Poster Board Number: P119
TITLE: Low-Dose Polyhexamethylene Guanidine Phosphate Exposure Exacerbates Ovalbumin-induced Asthma in Mice

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KEYWORDS: Respiratory Toxicology

ABSTRACT: Polyhexamethylene guanidine phosphate (PHMG-P), one of the main components of humidifier disinfectants, induces pulmonary inflammation and fibrosis. Although there is increasing evidence about lung toxicity of PHMG-P, there are no reports on asthma exacerbation following PHMG-P exposure. We assessed whether repeated exposure to low-dose PHMG-P induces steroid-resistant asthma exacerbation in a mouse model of ovalbumin (OVA)-induced asthma. Mice with OVA-induced asthma were administered five different doses of PHMG-P five times through intratracheal injection. To concept of steroid-resistant asthma exacerbation, the mice received dexamethasone (1 mg/kg) by gavage. At 24 and 48 h after the last OVA challenge, lung function measurement and sampling were performed, respectively. After sampling, cellular changes and inflammatory cytokine levels in the bronchoalveolar lavage fluid (BALF), total immunoglobulin E (IgE) production in serum, and histological changes in lung tissues were investigated. OVA-induced mice exhibited increased number of total cells and inflammatory cells in the BALF, increased production of total IgE in serum, and enhanced airway hyperresponsiveness (AhR) compared with the naïve control. These asthma parameters were exacerbated by low-dose exposure to PHMG-P in OVA-induced mice. In addition, histological evaluation revealed that OVA-induced inflammatory cell infiltration, mucus production, and goblet cell hyperplasia were markedly increased by PHMG-P exposure in a dose-dependent manner. Furthermore, PHMG-P exposure induced alveolar/interstitial fibrosis, bronchiololaveolar hyperplasia, and alveolar emphysema in OVA-induced mice. The asthma parameters exacerbated by low-dose PHMG-P were not suppressed by dexamethasone in OVA-induced mice. These results showed that low-dose exposure to PHMG-P can exacerbate OVA-induced asthma in mice. Our results provide the first experimental evidence that PHMG-P aggravates allergic asthma. This study was funded by the Korea Ministry of Environment (MOE) as “the Environmental Health Action Program (2017001360002) and Korea Institute of Technology (KK-1905-02).”
The Role of MicroRNA in Polyhexamethylene Guanidine Phosphate Induced Pulmonary Fibrosis

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: Y. Park, and K. Chung. Sungkyunkwan University, Suwon, Korea, Republic of. Sponsor: T. Krebs

ABSTRACT: Polyhexamethylene guanidine-phosphate (PHMG-p) which is a major ingredient in the humidifier disinfectants, has been proven in many studies to cause pulmonary fibrosis. Many studies revealed that dysregulation of microRNAs (miRNAs) is one of the mechanisms of lung fibrogenesis. Previously, up-regulated miR-6126 was observed in PHMG-p treated human lung alveolar epithelial A549 cells with epithelial-mesenchymal transition (EMT) which is key mechanism in lung fibrosis. Therefore, we explored the functional role of miR-6126 in PHMG-p induced EMT.

In this study, we found out that miR-6126 specifically expressed by PHMG-p exposure, not by paraquat, transforming growth factor-β and bleomycin which are EMT-inducing chemicals. Overexpression of miR-6126 elevated the expression of α-smooth muscle actin (α-SMA), a characterized marker of myofibroblast, whereas inhibition of miR-6126 repressed PHMG-p-induced α-SMA activation during EMT. Moreover, the MAPK and Akt/GSK-3β pathways which have been known to be involved in the pathogenesis of fibrotic response, were regulated by miR-6126 modulation. As a target of miR-6126, we predicted that protocadherin9 (PCDH9), a mediator of cell adhesion by multiple bioinformatics analyses and identified through target validation assays. In conclusion, miR-6126 plays a pivotal role in PHMG-p-induced EMT regulating multiple signaling pathways including MAPK and Akt/GSK-3β pathway via targeting PCDH9. Based on our results, we suggest that miR-6126 can be utilized as a novel diagnostic marker for PHMG-p-induced pulmonary fibrosis.

Guanidine-based Polymers Induced EMT through Membrane Toxicity in A549 Cells

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: D. Kim. Sungkuynkwan University, Suwon, Korea, Republic of. Sponsor: T. Krebs

ABSTRACT: In 2011 Korea, a number of people who were exposed to humidifier disinfectants exhibited signs of pulmonary fibrosis. Epidemiological and toxicological studies demonstrated that polyhexamethylene guanidine-phosphate (PHMG-p) was an active component to induce pulmonary fibrosis. However, chemicals based on guanidine structures are widely used in various fields because of their outstanding antibacterial, antifungal, and antiviral activities. Therefore, in this study, pulmonary fibrotic response of guanidine-based disinfectant, PHMG-p, polyhexamethylene biguanidine (PHMB) and oligo(2-(2-ethoxy)ethoxyethyl guanidinium chloride (PGH) in type II alveolar epithelial A549 cells were identified to figure out their risk increasing pulmonary fibrosis. The WST-1 assay and plasma membrane toxicity tests were conducted to measure cellular toxicity. The epithelial mesenchymal transition (EMT), which plays a important role in the mechanism of pulmonary fibrosis, was investigated by measuring a-SMA in protein level. Although PHMG-p, PHMB, and PGH showed different toxic potency, all three substances exhibited cellular toxicity and fibrotic responses. PHMG-p and PGH caused the highest and lowest damage, respectively. Similar to PHMG-p, EMT was induced in PHMB and PGH. Moreover, the p-AKT, which was signaling protein related to EMT pathway, was increased by treating each of 3 substances and AKT inhibitor attenuated chemial induced EMT. Taken together, three substances were
demonstrated to induce EMT through cell damage and had common mechanism involved in AKT pathway. Therefore, the use of guanidine-based disinfectants should be based on tight regulation by assessing their potential toxicity on the lungs.

**ABSTRACT NUMBER:** 3335  
**Poster Board Number:** P122  
**TITLE:** Prevalidation of an Alternative Method for Acute Inhalation Toxicity Assessment Using Calu-3 Ali Culture Model

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** D. Kim, M. Jeong, and Y. Han.  
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*Sponsor: T. Krebs*

**ABSTRACT:** As many chemicals have been developed in recent decades, the incidence of caused by chemical exposure have also increased. Inhalation toxicity is responsible for a large proportion and the demand for acute inhalation assessment test is increasing rapidly. Although *in vivo* inhalation toxicity test was developed in many ways, it has limits to assess the toxicity of many chemicals for economic and ethical reasons. Therefore, we have developed an *in vitro* acute inhalation toxicity test system and the goal of this study is to validate it. Transferability and reproducibility were evaluated to validate *in vitro* acute inhalation assessment with 3 independent institutions including one lead laboratory Sungkyunkwan university (SKKU) and two good laboratory practice compliance certificated institutions, BiotoxTech (BT), Korea institute of toxicology (KIT). To confirm the transferability of the test method, 2 positive and 2 negative controls were tested in each laboratory after being transferred from lead laboratory. Analysis of agreement of 3 repeated assays for 10 chemicals in each laboratory was attempted to establish intra-reproducibility. Moreover, each laboratory conducted the assay for 20 chemicals to assess inter-reproducibility. Results using four chemicals showed complete concordance, which meant that the assay was transferred to other laboratories well. Toxicity tests for 10 and 20 chemicals showed excellent intra-laboratory agreement (SKKU:100%, BT:95%, KIT:100%) and inter-laboratory agreement (95%), respectively. It suggests that *in vitro* acute inhalation toxicity test has enough high intra- and inter-laboratory reproducibility. Finally, the accuracy as a predictive performance (85%) was obtained by taking the above data together. In this study, we validated *in vitro* acute inhalation toxicity test system by evaluating transferability, intra- and inter-laboratory reproducibility. Although there was a limit to the small number of the chemicals, the predictive performance of the assay was also considerable. Therefore, it is necessary to test much more chemicals so that our results imply that the assay can be accepted widely to predict and screen inhalable chemicals. *This research was supported by a grant (18182MFDS466) from the Ministry of Food and Drug Safety in 2019.*

**ABSTRACT NUMBER:** 3336  
**Poster Board Number:** P123  
**TITLE:** Experimental Validation of AOP 206 on PPARγ Antagonism Leading to Pulmonary Fibrosis through TGF-β Signaling Pathway

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** D. Lim, J. Jeong, S. Bae, and J. Choi.  
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**KEYWORDS:** Lung; Pulmonary Or Respiratory System

**ABSTRACT:** Pulmonary fibrosis is a respiratory disease in which scars are formed in the lung tissues, leading to serious breathing problems. It is an immunological process that is known to be regulated by
the immune modulator Peroxisome proliferator-activated receptors γ (PPARγ) and transforming growth factor β (TGF-β). Previously, we developed an AOP on PPARγ inactivation leading to lung fibrosis based on literature search and compilation of relevant information, PPARγ inactivation as MIE and TGF-β activation as KE. In this study, we conducted validation of MIE, KE and AO of this AOP using inhibitors of MIE, SR16832 (PPARγ inhibitor) and KE, SB525334 (TGF-β inhibitor). Bleomycin was used as positive control of fibrosis (AO). KE relationships (KERs) were investigated by examining effect of downstream event after inhibition of upstream event. The validation was conducted in a human bronchial epithelial cell line (BEAS2-B), as well as primary cells (Normal Human Bronchial Epithelial cell, NHBE and Fibrosis-Disease Human bronchial epithelial cell, DHBE). Further in vivo validation was conducted on mouse model via intra-tracheal instillation exposure. It was found that PPARγ inhibition leads to TGF-β activation and increase in collagen expression. To maximize applicability of this AOP for inhalation toxicity screening, additional validation was conducted using chemicals, of which their exposure route was made through inhalation. Potential inhalation exposure chemicals were selected from global chemical regulation lists. Common chemicals from these lists were sub-selected as potential stressors. Among these chemicals, disulfiram, as PPARγ agonists, whereas, rotenone as PPARγ antagonists were selected for validation of MIE of this AOP. Overall results confirm AOP of PPARγ antagonism leading to pulmonary fibrosis through TGF-beta signaling pathway. Acknowledgement: This work was supported by a grant from the Korean Ministry of Environment through 'Environmental Health R&D Program' (2017001370001).

ABSTRACT NUMBER: 3337 Poster Board Number: P124

TITLE: Exploring WTS ROS Generation as Screen for Waterpipe Tobacco Flavors Toxicity

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: O. Pelaez, J. Reed, E. Mackintosh, S. Chen, K. Bernd, and C. DeForest Hauser. Davidson College, Davidson, NC.

KEYWORDS: Inhalation Toxicology; Oxidative Injury

ABSTRACT: Recently, waterpipe tobacco smoking has enjoyed increasing popularity worldwide due, in part, to the availability of a wide array of shisha tobacco flavors. While commercial producers are likely to develop flavors from chemicals that have been shown to be safe for oral consumption, little data is available regarding the effect of flavoring on waterpipe tobacco smoke's (WTS) inhaled toxicity. The aim of this study was to characterize the feasibility of using the oxidative potential of WTS particulate phase to screen the relative toxicity of different flavors of WTS. The waterpipe setup and puff protocol were held constant and the oxidative potential and toxicity of Starbuzz™ exotic Apple Americano (AA) and Blue Mist (BM) shisha were determined. The acellular dithiothreitol (DTT) assay has been used to quantify ROS (oxidative potential) of a variety of particles; however, no studies to date have used this method to characterize and quantify the ROS generation potential in WTS. DTT consumption per smoking session and per gram of shisha consumed were 1.96 µM/min and 2.253 µM/min, respectively for AA and 0.481 µM/min and 1.85 µM/min, respectively for BM. When rat type II alveolar cells were exposed to a single session of WTS at the air-liquid interface both WTS flavors decreased membrane integrity when compared to non-smoking control conditions. AA WTS was cytotoxic, decreasing lysosomal function (NRU) and membrane integrity (FDA) by ≥ 30% while BM WTS did not meet this cytotoxicity threshold. These data show that different flavors of shisha demonstrate different levels of oxidative potential and that there is a correlation between higher oxidative potential and cellular
toxicity. Thus, these data suggest that the oxidative potential of WTS particulate may be used as tool to categorize the relative toxicity of different flavors of shisha.

ABSTRACT NUMBER: 3338    Poster Board Number: P125
TITLE: Waterpipe Tobacco Smoke: The Impact of Puff Protocol on Particulates and Toxicity

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: M. Hodges, D. Carmack, E. Uffman, A. Gilbert, A. Shill, C. DeForest Hauser, and K. Bernd. Davidson College, Davidson, NC.

KEYWORDS: Inhalation Toxicology; Particulates

ABSTRACT: Waterpipe tobacco smoke (WTS) is formed by volatilizing shisha components with charcoal-heated air drawn through the pipe head and body. Many biological and chemical approaches to WTS research focus on chemical analysis of smoke constituents or how cells respond to WTS components. The Beirut smoking protocol is based on observational studies of smokers and has emerged as a standard for waterpipe research. The aim of this study was to investigate how modifications to the Beirut protocol, including smoke particulate physical characteristic analysis and model physiological exposure of cells at the air-liquid interface affect the dose and relative toxicity of the WTS produced. The waterpipe setup and the total volume smoked were held constant while puff topography was varied between the Beirut protocol: 3sec puff+17sec interpuff interval (IPI) and protocol with a longer IPI: 3sec puff+25 sec IPI. Rat Alveolar Type II cells were exposed to whole smoke at the air-liquid interface and two metrics of cell health analyzed. Compared with non-smoking controls, both puffing protocols generated smoke that decreased metrics of cellular health measured 24 hours after a single 57-minute exposure. Smoke produced using the Beirut protocol was cytotoxic, decreasing lysosomal function (NRU) and membrane integrity (FDA) by ≥ 30%. Particle concentrations and size distributions were measured using a TSI Engine Exhaust Particle Sizer. In a 30 minute session, as IPI increased we observed no change in particle size distribution with a decrease in total particle concentration (1.67x10^{14} to 7.02x10^{13}). These data show that different puff topographies generate particle dose changes with IPI length that are both harmful but do not demonstrate identical toxicity. This suggests that data regarding WTS physical characteristics can be used to inform cellular toxicity and it is important to insure puff topography parameters in referent studies generate smoke within the same dose range.

ABSTRACT NUMBER: 3339    Poster Board Number: P126
TITLE: Multi-walled Carbon Nanotubes Elicit Sustained Pulmonary Remodelling Effects Dependent on Gender Rather than a Functional Nrf2 Status

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KEYWORDS: Nanotechnology; Antioxidants; Inflammation

ABSTRACT: Multi-walled carbon nanotubes (MWCNT) are nanomaterials with various applications including food packages that can cause pulmonary inflammation, tissue remodelling and profibrotic lesions. Oxidative stress and redox-sensitive nuclear factor erythroid 2-related factor 2 (Nrf2) are implicated in MWCNT-induced pulmonary damage whereas the impact of gender and sustained
exposure are still largely unknown. Therefore, our aim was to evaluate the pulmonary damaging effects of MWCNT in Nrf2-/- and Nrf2+/+ male and female mice after 60 days exposure. Female (n=34) and male (n=25) Nrf2-/- and +/- mice aged 9-10 weeks were exposed to MWCNT (Mitsui & Co, average length of 13 µm and a diameter range from 40-100 nm) by a single pharyngeal aspiration of 40μl of 1mg/ml suspension per 20g body weight. Immediately before exposure, MWCNT were suspended in 2% mouse serum in double distilled water followed by sonification. After 60 days, animals were sacrificed to collect blood and lungs to analyse markers of pulmonary remodelling, oxidative stress and inflammation. MWCNT exposure significantly increased interstitial granuloma lesions, interstitial fibrosis and bronchoalveolar hyperplasia in all groups. Gender significantly affected the interstitial lesions and fibrosis whereas Nrf2-status had no significant role. MWCNT exposure significantly reduced systemic antioxidant capacity as well as pulmonary antioxidant levels in Nrf2 -/- males. Gene expression of Nrf2-responsive antioxidants catalase, heme oxygenase 1, superoxide dismutase 1 and gamma-glutamylcysteine synthetase was not significantly affected by the MWCNT exposure in all groups. Tissue inflammation was significantly enhanced in the lungs of all mice treated with MWCNT irrespective of their gender or Nrf2 genotype. Systemic inflammation was not significantly affected by MWCNT treatment although a trend was observed for elevated IL-10 plasma levels in Nrf-/- males (p=0.0053). In conclusion, MWCNT induce sustained pulmonary remodelling and inflammation in mice dependent of gender but independent of a functional Nrf2 suggesting that other not redox-regulated pathways may have a stronger impact on the pro-fibrotic effects of MWCNT.

ABSTRACT NUMBER: 3340    Poster Board Number: P127
TITLE: Charcoal and Shisha Constituents as Contributing Factors to the Dithiothreitol-measured Oxidative Potential of Waterpipe Tobacco Smoke

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: P. Conquest, O. Pelaez, C. Peng, E. Tayloe, K. Bernd, and C. Hauser. Davidson College, Davidson, NC.

KEYWORDS: Inhalation Toxicology; Exposure, Environmental; Particulates

ABSTRACT: Waterpipe tobacco smoke (WTS) can cause long term and acute damages on the human cardiovascular system and respiratory system. Most characterization of WTS to date has been restricted to targeted analyses of toxicants also present in cigarette smoke. These targeted studies fail to account for toxicants unique to WTS. Waterpipes employ a different smoke-producing mechanism and contain different additives (such as flavorings and syrups) than cigarettes, thus producing different chemical profiles. Here we present the results of non target analyses of semivolatile organic compounds using LCMS methods as well as a compartmental analysis of metals in the waterpipe system using FAAS to evaluate the potential chemical toxicity of WTS. This chemical analysis is coupled with the dithiothreitol (DTT)-measured oxidative potential of the particulate component of WTS. WTS was collected with charcoal and electronic heating sources for shisha and charcoal with syrup and a non reactive matrix in the head. The smoke was collected, extracted and analyzed using LCMS, FAAS and DTT. DTT analysis indicates that on a per mass basis WTS generated by shisha increases reactive oxygen species (ROS) by a factor of 1.6 in comparison to smoking syrup alone. Particulate matter generated by charcoal alone increases the ROS by a factor of 1.14 relative to the WTS generated by shisha smoked with charcoal. WTS generated using shisha and charcoal has a chromium concentrations of 85 ng/g with a 300% increase in concentration from the unsmoked shisha to the combination of smoked shisha, bowl water and particulate matter, indicating a significant contribution from charcoal during the smoking process.
We have found 18 compounds in WTS that are unique to shisha in comparison to syrup smoked with charcoal. Charcoal heating contributes 190 compounds to WTS that are not found with electronic heating and are not generated by charcoal alone. The inorganic and organic constituents additionally present in WTS smoked using charcoal and shisha may contribute to the increased ROS generation, as measured by DTT analysis, which serves as a proxy measurement for toxicity.
ABSTRACT NUMBER: 3342   Poster Board Number: P129

TITLE: Research of Inflammatory Cytokines Effect on PHMG-p-induced Lung Fibrosis Using Epithelium-fibroblast Tetra-culture Model

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: J. Kim, and M. Jeong. SKKU, Suwon, Korea, Republic of. Sponsor: T. Krebs

ABSTRACT: It is known that Polyhexamethylene guanidine phosphate (PHMG-p), one of humidifier disinfectants, induces human lung fibrosis. In lung fibrosis, activation of fibroblast is a key event. To determine mechanism of fibroblast activation caused by during lung fibrogenesis, communication between epithelia and fibroblast must be considered. However conventional mono cell culture model has a limitation to understand these sophisticated cross-talks. Therefore, we evaluated the effects of PHMG-p on fibroblast activation using tetra tetra-culture model that can mimics human lung epithelium environment. Tetra-culture model composed with two parts, tri-culture at apical chambers and fibroblast at basolateral chambers. One type of epithelia cells (Calu-3) and two types of immune cells (THP-1, HMC-5) were incubated in insert apical side and fibroblast cells (MRC-5) were incubated in basolateral side. To analyze the integrity of tri-culture barrier, trans-epithelial electrical resistance (TEER) value was measured using EVOM^2 until TEER reaches the maximum value. At day 11, when TEER value reaches peak, PHMG-p was treated to apical for one day. Inserts were transferred to basolateral fibroblast plates and incubated it for two days. At day 14, apical barrier was collected and conducted cytokine analysis and fibroblasts at basolateral were conducted western blot to confirm fibroblast activation marker. TEER increased steadily and highest at day 11 as evidence of a well-developed tight junction of the tri-culture barrier. IL-6, IL-8, IL-1β levels were increased in PHMG-p treatment group. Extracellular matrix protein Collagen and fibroblast activation marker CTGF and PAI-1 level were increased at fibroblast with PHMG-p treated group. In conclusion, PHMG-p induce lung epithelium barrier breakdown and inflammation cytokines that caused by epithelium breakdown actives fibroblast. Furthermore our research suggests this tetra-culture system can be a screening system for inhalant toxicants that might induce lung fibrosis.

ABSTRACT NUMBER: 3343   Poster Board Number: P130

TITLE: Released β-catenin from β-catenin/E-cadherin Complex by PGH Induces Epithelial-mesenchymal Transition

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: J. Kim, and M. Jeong. SKKU, Suwon, Korea, Republic of. Sponsor: T. Krebs

ABSTRACT: In 2011 Korea, outbreak of lung fibrosis disease occurred by humidifier disinfectant. Researches elucidated PHMG-p, which is most frequently used humidifier disinfectant, induces Epithelial-mesenchymal transition (EMT) and causes lung fibrosis. Besides, PGH a humidifier disinfectant which has similar structural feature with PHMG-p but its toxicity mechanism is still obscure. In this study we elucidated the PGH induced EMT and its mechanism. A549, human lung epithelial cells were treated with PGH for 48, 72 hours. We measured EMT-associated proteins using Immunofluorescence (IF) and Western blot to identify the EMT. To certify nuclear signals that trigger EMT, nuclei were extracted separately from A549. EMT markers and related signals were measured from nuclear fraction. TOPFLASH/FOPFLASH reporter gene assay was conducted to confirm TCF/LEF signal intensity. The results of IF assay showed that, fluorescence of E-cadherin was decreased and alpha-Smooth Muscle

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Actin (α-SMA) was increased. Furthermore, decrease of E-cadherin and increase of α-SMA was confirmed by Western blot. In A549 nuclei fraction, activated β-catenin and TCF/LEF family were increased. Catenin transcriptional activation was higher in PGH treated A549 cells than non-treated A549 cells when TOPFLASH/FOPFLASH reporter gene assay was conducted. In conclusion we found out PGH induces EMT though β-catenin pathway. Our research will help to understand the pathology of PGH induced pulmonary fibrosis.

**ABSTRACT NUMBER:** 3344  **Poster Board Number:** P131

**TITLE:** Flavor Solvent Adducts in Electronic Cigarette Liquids Are Modulators of Respiratory Irritant Receptors with Distinct Toxicological Effects on Lung Epithelial Cells

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** S. V. Jabba, A. I. Caceres, and S. E. Jordt. Duke University, Durham, NC.

**KEYWORDS:** Respiratory Toxicology; Cytotoxicity; Oxidative Injury

**ABSTRACT:** US Food and Drug Administration and US Surgeon General have categorized the current rapid increase in youth e-cigarette use to be of “epidemic” proportions. This increase in youth e-cigarettes prevalence is attributed to the availability of e-liquids in a wide range of appealing flavors (>7,700 flavors). Some popular E-liquid flavors include cherry, vanilla and cinnamon flavors, which are reactive aldehydes and upon inhalation have been demonstrated to have respiratory irritant responses and cardiovascular toxicological effects. Using chemical analysis of these popular e-liquids and their aerosols, we recently demonstrated that flavor aldehydes in e-liquids react with solvent chemicals like propylene glycol (PG) and glycerol, to form stable conversion products called “flavor-solvent adducts” or aldehyde-PG acetals. While Flavor Extract Manufacturers Association (FEMA) has categorized some flavor aldehyde PG acetals as GRAS (Generally Recognized As Safe) for perfume and food applications, their cellular and systemic toxicities, especially in the respiratory system, remain to be examined. Using BEAS-2B cells, a normal human bronchial epithelial cell line, as a cellular model, we conducted cellular toxicity tests (live/dead assay, LDH assay), mitochondrial function assays to measure metabolic function, and transcriptional approaches to examine gene expression changes. In live/dead and LDH assay, some aldehyde-PG acetals demonstrated higher cytotoxicity than parent aldehydes at several concentrations tested. In mitochondrial functional assays, exposure to certain aldehyde-PG acetals impaired several key parameters of cellular metabolism more profoundly than the parent aldehydes. Gene expression analysis demonstrates that PG acetals induce differential expression of several genes involved in anti-oxidant mechanisms and immune responses. Using calcium imaging of 293T cells expressing respiratory irritant receptors (TRP ion-channels; TRPA1 & TRPV1), we demonstrated that, compared to their parent aldehydes, several PG acetals activated TRPA1 & TRPV1 with increased efficacy, indicating their potential to produce respiratory irritation and pain. In conclusion, our data demonstrates that chemical reaction products formed in E-liquids like aldehyde-PG acetals have potent pharmacological, metabolic, transcriptional and toxicological effects that are different from parent constituents.
ABSTRACT NUMBER: 3345    Poster Board Number: P132
TITLE: Impact of Phthalates and Phthalate Mixtures on Pregnant and Lactating Mice

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: M. Zima, K. Neier, C. Harris, L. Svoboda, and D. Dolinoy. University of Michigan, Ann Arbor, MI.

KEYWORDS: Glutathione; Endocrine Disruptors; Developmental Toxicity; Prenatal

ABSTRACT: Gestational exposure to phthalates, chemicals found in plastics and personal care products, has been implicated as a risk for adverse metabolic effects in offspring, but little attention has been given to the effects of endocrine disrupting chemicals on maternal outcomes. The etiology of metabolic syndrome is marked by systemic oxidative stress. Mono(2-ethylhexyl) phthalate has been indicated in cell culture studies to induce the release of reactive oxygen species and/or impair antioxidant defenses leading to a state of oxidative stress, and epidemiological findings have linked maternal phthalate exposure and increase oxidative stress biomarkers in urine and plasma. Maternal target tissue responses to phthalates, however, have not been previously studied. An established mouse model of perinatal exposure was used to evaluate target tissue effects of phthalate exposed pregnant mice. Dams (N=14-17/group) were fed 1 of 6 diets two weeks before mating through gestation and lactation: 1) 7% corn oil control, 2) 25 mg di(2-ethylhexyl) phthalate (DEHP)/kg chow, 3) 25 mg dibutyl phthalate (DBP)/kg chow, 4) 75 mg diisononyl phthalate (DINP)/kg chow, 5) 25 mg DEHP + 75 mg DINP/kg chow, or 6) 25 mg DEHP + 25 mg DBP + 75 mg DINP/kg chow. Body weights, liver weights, and mesenteric adipose tissue weights were collected four days post-weaning. Cysteine and glutathione redox potentials in maternal liver and adipose were measured by HPLC. One-way analysis of variance was used to compare outcomes across exposure groups. DBP-exposed dams had a statistically significant decrease (5.67% vs. 4.75%) in the relative weight of liver tissue to body weight as compared to controls (p=0.05). There were no statistically significant changes observed in body weights or adipose tissue weights. Maternal exposure to phthalates during pregnancy did not have direct impacts on cysteine and glutathione redox potentials at the measured time point. Since maternal exposure to DBP was associated with decreased relative liver weight, future work will evaluate how this influences metabolism in the offspring. Additional future directions include measuring liver redox potentials at different time points in gestation and investigating redox potentials in maternal blood.

ABSTRACT NUMBER: 3346    Poster Board Number: P133
TITLE: Investigating the Ability of Synergistic Activators of the Nrf2/Antioxidant Response Element Pathway to Protect Cells from Hydrogen Peroxide Exposure


KEYWORDS: Undergraduate Student; Natural Products; Antioxidants

ABSTRACT: A cell can better withstand the cytotoxic effects of oxidative and electrophilic stress through pre-exposure to activators of the nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response element (ARE) pathway, which controls the expression of a battery of cytoprotective genes. Numerous small molecules activate this system, including relatively non-toxic ones such as the electrophile sulforaphane, the subject of clinical trials for a variety of chronic diseases. An area of emerging interest is the effect of combining two or more small molecules on activation of ARE-regulated gene expression.
We find that sulforaphane-induced expression of ARE-regulated genes is significantly and synergistically enhanced in HaCaT human keratinocytes by 18 μM 2,5-di-tert-butyl-1,4-hydroquinone (dtBHQ), a generator of reactive oxygen species (ROS). In addition, the maximum extent of activation obtainable with sulforaphane treatment alone is doubled with the inclusion of dtBHQ. Interestingly, at low doses of dtBHQ and sulforaphane, the interaction is unexpectedly antagonistic. We hypothesized that inclusion of dtBHQ with sulforaphane would increase sulforaphane’s ability to protect cells from subsequent oxidative stress, using concentrations of each that are high enough to give additive or synergistic activation of the ARE pathway. HaCaT cells were pretreated with varying combinations of sulforaphane and dtBHQ, followed by exposure to toxic concentrations of hydrogen peroxide. Sulforaphane pretreatment resulted in a protective effect as expected. However, combinations of various concentrations of dtBHQ with sulforaphane revealed that dtBHQ co-treatment is unable to further protect cells from hydrogen peroxide toxicity.

ABSTRACT NUMBER: 3347    Poster Board Number: P134
TITLE: In Vitro High-Throughput Screening of Chemical-Induced Oxidative Stress Using HepaRG Cells


ABSTRACT: Oxidative stress is thought to be critical in the pathogenesis of many diseases including inflammation and cancer. In vitro high-throughput screening approaches are needed to characterize the potential of chemicals to induce oxidative stress. In this study, chemical-induced cytotoxicity and oxidative stress were evaluated in vitro using a high-throughput human hepatocyte (HepaRG) culture model. This evaluation focused on a set of chemicals which were previously tested by the NTP, mostly in 2-yr bioassays, and thus have established in vivo toxicity data in mice and rats. This set included chemicals that were carcinogenic and non-carcinogenic to the liver. Human HepaRG cells were exposed to 9 doses of chemical (or vehicle control) in a 384-well plate format for 48 or 96 hr at 37°C. Culture media containing chemical was replaced with fresh media/chemical after 48 hr of exposure. Cell viability and oxidative stress were measured after 48 or 96 hr of exposure using the CellTiter-Glo and reactive oxygen species (ROS)-Glo assays, respectively. Menadione and diclofenac were used as positive controls, and ginseng was used as a negative control. Initial data has shown that some chemicals which are carcinogenic to the liver in vivo, such as perfluorooctanoic acid (PFOA), fenofibrate, pentabromodiphenyl ether mixture (DE-71), and triclosan, induce significantly increased production of ROS by HepaRG cells in vitro after 96 hr of exposure; whereas some liver carcinogens, such as di(2-ethylhexyl) phthalate (DEHP), tetrachloroazobenzene (TCAB), pulegone, and bromodichloroacetic acid (BDCA), do not. Some chemicals, such as milk thistle extract and ethinyl estradiol, induce production of ROS by HepaRG cells after 96 hr of exposure but are not hepatotoxic in vivo. These discrepancies with regards to in vivo vs. in vitro chemical-induced hepatotoxic effects may be attributable to species-specific differences and/or the metabolic capacity of hepatocytes. This in vitro human hepatocyte model has the potential to be used for high-throughput screening of chemical-induced oxidative stress.
ABSTRACT NUMBER: 3348  Poster Board Number: P137

TITLE: Triclocarban Exposure Exaggerates Colonic Inflammation and Colon Tumorigenesis in Mice

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: H. Yang. Harvard Medical School, Brookline, MA.

ABSTRACT: Triclocarban (TCC) is a widely used antimicrobial ingredient in consumer products and is a ubiquitous contaminant in the environment. A better understanding of its impact on human health could be important to establish further regulatory policies. However, the actions of TCC on inflammation and cancer are unknown. Here, we examined the effects and mechanisms of TCC exposure on colonic inflammation and colitis-associated colon tumorigenesis in mice. To study the biological effects of TCC, we treated mice with TCC via diet and studied its effect on dextran sodium sulfate (DSS)-induced colitis in C57BL/6 wild-type (WT) mice, spontaneous colitis in Il-10-/- mice, and azoxymethane (AOM)/DSS-induced colon tumorigenesis in C57BL/6 WT mice. To investigate the mechanisms of TCC, we studied its impact on gut microbiota using 16S ribosomal RNA (rRNA) sequencing, and tested the roles of gut microbiota in the biological actions of TCC using antibiotic cocktail-induced suppression of gut microbiota. We found exposure to low-dose TCC increased DSS-induced colitis in C57BL/6 WT mice, exaggerated spontaneous colitis in Il-10-/- mice, and enhanced AOM/DSS-induced colon tumorigenesis in C57BL/6 WT mice, illustrating its pro-inflammatory and pro-neoplastic actions. Regarding the mechanisms, TCC reduced diversity and altered composition of the gut microbiota, and failed to promote colitis in antibiotic cocktail-treated mice, supporting that TCC increased colitis through gut microbiota-dependent mechanisms. It is indicated that exposure to TCC exaggerates colonic inflammation and colitis-associated colon tumorigenesis, through modulation of gut microbiota.

ABSTRACT NUMBER: 3349  Poster Board Number: P138

TITLE: Relationships between Formation of DNA Double-Strand Breaks (DSBs) and t(9:22) Translocations in Human HL60 Promyelocytic Cells Exposed In Vitro to Butadiene Diepoxide

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KEYWORDS: Alkylating Agents; Carcinogenesis; Mode-Of-Action

ABSTRACT: 1,3-Butadiene (BD), a major industrial chemical, is a rodent carcinogen that IARC classified as a known human carcinogen based mainly upon associations between BD exposure and cancer of hematolymphatic tissues, including a possible link to chronic myelogenous leukemia (CML). CML is defined by the presence of the Philadelphia chromosome, a translocation between chromosomes 9 and 22 [(t9:22)] that produces a BCR-ABL fusion gene as a constitutively expressed oncogene sufficient to initiate this leukemia. The carcinogenicity of BD operates by a genotoxic mechanism that involves formation of three DNA-reactive epoxides. Butadiene diepoxide (DEB) is the most genotoxic BD metabolite, forming DNA-DNA crosslink adducts that can lead to DNA strand breaks. Thus, a study was designed to determine if (±)-DEB exposure of HL60 cells, a promyelocytic leukemia cell line lacking the BCR-ABL fusion gene, can produce t(9:22) translocations. Experiments performed to define the relationships between cytotoxicity and the induction of micronuclei (MN), as a dosimeter of DNA DSBs, showed that single acute (24 h) exposures of HL60 cells to 0 to 5.0 μM DEB (i) caused a significant linear dose-response for diminution of cell survival with a correlation between exposure level and the degree...
of cytotoxicity ($R = 0.9977$, $p = 0.002$) and (ii), at 48 h post exposure, there was a significant positive correlation between dose level and the frequency of MN ($R = 0.9839$, $p = 0.016$). To determine the relative induction of MN and t(9;22) translocations following exposures to DEB, or x-rays as a positive control for formation of t(9;22) translocations, HL60 cells were exposed for 24 h to 0, 1, 2.5, or 5 μM DEB or to 0, 2.0, 3.5, or 5.0 Gy x-rays, or doses that yielded 0, 20%, 50%, or 90% cytotoxicity based upon preliminary data. Treatments between 0 to 3.5 Gy x-rays caused significant dose-related increases in both micronuclei ($p < 0.001$) and t(9;22) translocations ($p = 0.01$), whereas DEB exposures causing similar levels of cytotoxicity did not induce an increase in translocations over background. These data indicate that, while DEB induces DNA DSBs required for formation of MN and translocations, this chemical does not appear to produce these lesions at sites required for induction of t(9;22) translocations. Research funded by the ACC’s Olefins Panel.

**ABSTRACT NUMBER:** 3350  
**Poster Board Number:** P139  
**TITLE:** The Loss of the Aryl Hydrocarbon Receptor Progresses the Pathogenesis of Pancreatic Ductal Adenocarcinoma  
**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** M. Walcheck. University of Wisconsin - Madison, Madison, WI. Sponsor: C. Bradfield  
**ABSTRACT:** In 2018, an estimated 55,440 people were diagnosed with pancreatic ductal adenocarcinoma (PDAC) in the United States with ultimately about 44,330 (80%) dying of their disease. The pathogenesis of PDAC remains poorly understood, hindering efforts to develop more effective PDAC therapies. Therefore, there is an urgent need to identify new therapeutic targets and improve the treatment options for PDAC. Recent discoveries show the Aryl Hydrocarbon Receptor (AhR) plays a crucial role in the pathogenesis of several cancers, but its role in pancreas cancer has not been well studied. In this investigation, we evaluated the role of AhR in the development of PDAC in vivo by using a mouse model of PDAC, where Kras mutation (the most common mutation in PDAC) is isolated to pancreas lineage cells (KC mouse). The KC mice were crossed to AhR null (Ah+/--) mice to understand how decreased AhR (Ah+/--KC) and loss of AhR (Ah+/-KC) affects PDAC formation and progression. Using this model, at 10 months of age we compare the incidence and grade of lesions and cancers between KC and AhKC mice, and also evaluated littermate controls. We found the control mice had absence of spontaneous development of pancreatic cancer precursor lesions (PAN-IN) or PDAC (n= 74). To date, the KC mice (n=3) have developed a range of pathology, including PAN-IN 1 (n = 1), PAN-IN 2 (n = 1) and PDAC (n = 1). Interestingly, each of the Ah+/-KC mice (n=2) have developed PDAC. There have not been any Ah+/--KC mice that have come to 10 months age, but we have 6 that will be evaluated over the next several months. Furthermore, there are currently 30 KC mice and 27 Ah+/-KC mice that will reach 10 months in the same timeframe. Although early, it appears there is some evidence that AhR may play a protective role in PDAC development, and warrants further exploration including ultimately as a possible therapeutic target.
ABSTRACT NUMBER: 3351    Poster Board Number: P140
TITLE: 4SP65, a Novel Therapeutic for Drug-Resistant Lung Cancer

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: D. M. Walker¹, and V. E. Wallker². ¹The Burlington HC Research Group, Inc., Jericho, VT; and ²University of Vermont, Burlington, VT. Sponsor: M. Poirier

KEYWORDS: Pharmaceuticals; Signal Transduction; Toxicity; Acute

ABSTRACT: Non-small cell lung cancer (NSCLC) is one of the most common human malignancies with estimated 5-year survival rates at <15%. 4SP65 is a novel drug composed of WR1065, amifostine’s major active moiety, conjugated to the arms of FDA-approved thiol-terminated 4-star polyethylene glycol (PEG). In the cytosol of all cells tested to date, WR1065 is released from the PEG scaffold by thiol-disulfide exchange reactions. WR1065 is reported to increase tumor cell apoptosis, and to reduce invasion, metastasis, angiogenesis, and to alter redox-regulated pathways including PI3K/Akt, MAPK/ERK, NF-kB, and p53. Cell uptake of 4SP65 is by transporters resembling those of the amino acid/polyamine system. This work tested the hypothesis that 4SP65/WR1065 can alter the intracellular environment to reactivate programmed cell death pathways and to reduce significant cytotoxicity in NSCLCs by (1) reducing ROS levels, (2) restoring approximate redox poise, and (3) establishing a cytosolic reducing environment. CyQuant, Presto Blue, and trypan blue staining were used to determine changes in cell viability of NCI-H460, NCI-H1437, and A549 cells following a single 48 hour exposure to 4SP65, cisplatin, or 4SP65+cisplatin. Linear regression lines fit to CyQuant-based standard curves for each cell line had \( R^2 \) values of > 0.995. Experimental designs and data evaluations used published methods for drug combination studies. Regression lines fit to curves of the log of \([(fa)/(1 - fa)]\), (where \( fa = \) percent survival inhibition/100), plotted against the log of 4SP65 concentrations, had \( R^2 \) values of 0.95 or greater, demonstrating high quality data. In A549 cells, 4SP65+cisplatin at 8 + 0.36 microM (the EC50s), respectively, had a combination index (CI) of 0.64 (3+ synergy) with a Dose Reduction factor of 3 for both drugs. In NCI-H460 cells, 4SP65+cisplatin at 0.3 + 0.6 microM (the EC50s), respectively, had a CI of 0.45 (3+ synergy), and dose reduction factors of 1.2 for 4SP65 and 2.7 for cisplatin. In primary epithelium, the highest level of 4SP65 toxicity was 22%, resulting in selectivity indexes of >100 for all NSCLCs tested. The Presto Blue staining results showed a novel mode of action for 4SP65/WR1065. Dye reduction in cisplatin-exposed cells was linear with cell number, but the reducing capacity of 4SP65-exposed cells remained at \(~100\%\) until the fraction inhibited exceeded 96\% of controls. These pilot study results reinforce the above hypothesis and support further investigation of 4SP65 and its analogs.

ABSTRACT NUMBER: 3352    Poster Board Number: P141
TITLE: The Kinetics of GST-P Positive Foci after Cessation of Treatment with Genotoxic Hepatocarcinogens or Furan Derivatives in the Liver of Rat

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KEYWORDS: Liver; Carcinogenesis; Histopathology

ABSTRACT: Glutathione-S-transferase placental form (GST-P) positive foci are well-known as preneoplastic lesions in rat hepatocarcinogenesis. We previously reported the existence of two types of
GST-P positive foci in terms of their kinetics after cessation of carcinogen treatment. Namely, GST-P positive foci induced by diethylnitrosamine, a genotoxic hepatocarcinogen, were increased after cessation of the treatment, but those by furan, an unclassified hepatocarcinogen, were decreased. In the present study, to investigate whether other genotoxic hepatocarcinogens or furan derivatives induce the same phenomena, we examined their kinetics using 2-amino-3-methylimidazo[4,5-f]quinolone (IQ) and estragole (ES) as a genotoxic hepatocarcinogen, or 2-methylfuran (2-MF) and 2-pentylfuran (2-PF) as furan derivatives. 6-week old male F344 rats were given IQ (100 ppm) in their diet or ES (150 mg/kg/day), 2-MF (30 mg/kg/day) or 2-PF (100 mg/kg/day) by gavage for 13 weeks. Quantitative analysis for GST-P positive foci was performed just after the cessation of the treatment and 7 weeks later. In the IQ group, the number and area of GST-P positive foci were not significantly changed after cessation. Those in the 2-MF, 2-PF or ES groups were decreased. In the 2-MF and ES groups, GST-P positive foci with mosaic appearance were scattered. Difference in the kinetics between the IQ and ES groups indicates that there were no common features in GST-P positive foci induced by genotoxic carcinogens. The same phenomenon as in the case of furan was observed in 2-MF and 2-PF groups, indicating that GST-P positive foci induced by furan derivatives commonly possess the nature of regression of GST-P expression. Mosaic appearance might result from the fact that GST-P positive hepatocyte with the property of being regressed exist in normal-appearing GST-P positive foci.
effects were compared against historical data in dogs, and overall profile was comparable across both species; confirming that the rat model is a suitable alternative to large animal models for assessing potential inotropic effect of novel compounds.

**ABSTRACT NUMBER:** 3354  **Poster Board Number:** P143  
**TITLE:** Exposure to BDE-99 Impedes Cerebrovascular Growth and Increases Vascular Permeability during Early Development in Zebrafish  
**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** Y. Wei, X. Zhong, J. Kang, and J. Qiu. Sun Yat-sen University, Guangzhou, China.  
**KEYWORDS:** Persistent Organic Chemicals  
**ABSTRACT:** Polybrominated diphenyl ethers (PBDEs), the ubiquitous environmental pollutants, are known to exert toxic effects on development, such as neurodevelopment. Vascular system which supplies oxygen and nutrients, maintains homeostasis and protects from toxic agents, is crucial for tissue development, such as brain development. However, the adverse effects of PBDEs on vasculature and the underlying mechanisms are largely unknown. In this study, we investigated the impacts of 2,2',4,4',5-pentabrominated diphenyl ether (BDE-99), a predominant PBDEs congener, on vascular growth and vascular barrier formation, with an emphasis on cerebral blood vessels, in the early life stage using a zebrafish model. No general toxicity was observed in zebrafish larvae exposed to 0-0.5 μM BDE-99 at 72 hpf. BDE-99 exposure did not result in pronounced developmental impairment in somatic blood vessels, including intersegmental vessels (ISV) and common cardinal vein (CCV), whereas both 0.05 μM and 0.5 μM of BDE-99 led to a reduction in cerebrovascular density as well as down-regulation of VEGFA and VEGFR2 in head. In addition, BDE-99 exposure increased vascular leakage both in cerebral and truncal vasculatures at 72 hpf, and the accentuated vascular permeability was observed in brain. Consistently, mRNA levels of tight junction molecules decreased in BDE-99-exposed larvae, and more robust reductions of Cldn5, ZO1 and Jam were detected in head as compared with trunk. Moreover, the proinflammatory factors including Tnfα, Il-1β and Icam-1 were induced and the expression of neurodevelopment-related genes was suppressed in head following BDE-99 exposure. Taken together, these results reveal that developmental exposure to BDE-99 impedes cerebrovascular growth and disturbs vascular barrier formation. The cerebral vasculature in developing zebrafish, a sensitive target for BDE-99, may serve as a promising tool for the assessment of early neurodevelopmental effect by PBDEs exposure.

**ABSTRACT NUMBER:** 3355  **Poster Board Number:** P144  
**TITLE:** Baseline Cardiac Phenotypes across a Diverse Population of Mice: Implications for Cardiotoxicity Risk Assessment  
**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** J. Popp, and D. Threadgill. Texas A&M, College Station, TX.  
**ABSTRACT:** Although cardiotoxicity is often the most sensitive parameter for determining threshold exposure levels of toxicants, relatively few environmental chemicals have been tested successfully for cardiotoxicity. This is due, in part, to incomplete, although ongoing, development of models of cardiotoxicity, both in vitro and in vivo. Simplistic in vitro models lack receptor diversity, while in vivo
models usually make use of a homogenous population. Of those chemicals tested, translating findings from current models to human risk assessment is challenging due to the lack of genetic diversity represented in these models, as well as limited epidemiological data to validate translation to humans. Animal models have been the gold standard for risk assessment, and with the use of individuals from a genetically diverse mouse reference population, the Collaborative Cross (CC), we can model the genetic diversity present in the human population. In 31 strains of mice, electrocardiogram measurements indicate a high strain-dependent variability ranging from 300 BPM to 800 BPM as well as significant differences in QT interval duration. Cardiac high frequency ultrasounds were also acquired in both conscious and unconscious mice; isoflurane had profound strain-dependent effects on cardiac function. Results demonstrate inter- and intrastrain variability in unexposed mice as well as in their response to chemicals. While the goal is to create a powerful diverse testing panel whose baseline phenotypes are characterized before and after exposure to chemicals, these baseline measurements are crucial for identifying the ideal model for testing toxicity, ultimately allowing more informative risk assessments.

ABSTRACT NUMBER: 3356    Poster Board Number: P145
TITLE: Reliable Method for Intravenous Dosing in Guinea Pigs

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ABSTRACT: The guinea pig is widely accepted as an appropriate small animal model for predictive preclinical testing due physiological similarities to humans in comparison to other small animal models. This is particularly true when exploring cardiovascular changes due to the similarities of myocardial function and ion channels. Historically, intravenous administration in the guinea pig has required surgical implantation of a vascular access button (VAB) which allows for simple accessibility of the femoral vein (or equivalent). Although this has been moderately successful, risk of infection and loss of patency increase the potential to exclude subjects during repeat dosing assessments. In order to mitigate these issues, intravenous pedal vein dosing route was investigated as an alternate method precluding the requirement for surgical implantation of a VAB. This method has proven to be a successful alternative (to VAB dosing) and is suitable for both single dose and repeat dose designs. Eight telemetered guinea pigs were all successfully dosed intravenously via the pedal vein during cardiovascular telemetry collection. A 30-minute baseline of CV data were collected in order to assess the stress attributable to the dosing of each animal. The resulting data showed an increase in HR of 38 BPM and mean BP of 1.9 mmHG through 10 minutes post dosing, which is consistent with the excitability state of the animals under normal handling procedures. Following dose administration, the animals returned to their baseline state within 30 minutes post dosing which is further indicative of limited procedural stress from this route of administration. Our results present intravenous pedal vein dosing as a novel alternative intravenous dosing route while maintaining a high rate of success rate with a limited amount of procedural stress affecting the data. This method was successfully used for daily IV administration in a 28-day reproductive toxicity study in female guinea pigs with minimal bruising at the injection site(s).
**ABSTRACT NUMBER:** 3357  
**Poster Board Number:** P146  

**TITLE:** An Evaluation of the Guinea Pig for Use on Cardiovascular Safety Studies  

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** M. Waines, K. Ford, S. Foust, B. Brna, B. Walling, and B. Roche. Charles River Laboratories, Ashland, OH.  

**KEYWORDS:** Safety Pharmacology  

**ABSTRACT:** As the guinea pig heart possesses cardiac ion channels similar to man, this species has long been considered an adequate surrogate for assessing cardiac physiology during preclinical drug development. However, the robustness of historical data for the guinea pig is scarce and/or incomplete for some measurable endpoints. The objective of this study was to evaluate cardiovascular endpoints typically assessed in cardiovascular safety studies as well as adding to the historical control data base within CRL using male Hartley guinea pigs. Cardiovascular endpoints were collected from several study types; whole animal including bioanalysis/clinical pathology, conscious telemetry including LVP, and isolated hearts to include HR, ECG interval, arterial blood pressure, LVP parameters (±dP/dt), end diastolic developed pressure (DevP), arrhythmia analysis, echocardiography and histopathology with cardiac biomarkers for inclusion in the poster. Results will be presented as mean values ±SE as well as “typical” ranges for a given parameter; briefly, cardiovascular end points of heart rate (200-300 bpm in conscious animals and 150-250 bpm in the Langendorff); blood pressure (diastolic 51.6-58.3 mmHg, systolic 73.3-80.8 mmHg); body temperature (37-39°C); dP/dt+ (3185.4-4517.5 mmHg/sec); clinical pathology end point of HCT (42.6-55.8%); echocardiography stroke volume (.29-.40 mL) with echocardiographic assessment revealing no appreciable difference in left ventricular or left atrial size, shape and systolic function; arrhythmias (PVCs were observed in control animals); and histopathology (Rhabdomyomatosis, or Nodular Glycogen Infiltration, was observed in some of the control guinea pig hearts during histopathological evaluation). Establishing historical control data is vital to provide reference ranges to compare when conducting studies with test articles that have the potential to alter these parameters.

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**ABSTRACT NUMBER:** 3358  
**Poster Board Number:** P147  

**TITLE:** Plasticizer Interaction with the Heart: Chemicals Used in Plastic Medical Devices Can Interfere with Cardiac Electrophysiology  

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** R. Jaimes, D. McCullough, B. Siegel, L. Swift, D. McInerney, J. Hiebert, and N. G. Posnack. Children's National Health System, Washington, DC.  

**KEYWORDS:** Phthalates  

**ABSTRACT:** Phthalate esters are frequently employed as ‘plasticizers’ in the manufacturing of flexible, plastic medical products. Since phthalates are non-covalently bound to plastic polymers, these chemical additives will leach from the parent product under normal conditions of use. As a result, patients can be subjected to high phthalate exposure through contact with plastic medical products. Studies suggest that phthalate exposure may be an underlying contributor to cardiovascular dysfunction. We investigated the safety and biocompatibility of mono-2-ethylhexyl phthalate (MEHP), a phthalate with documented exposure in intensive care patients, by evaluating cardiac electrophysiology in intact hearts. Cardiac electrophysiology was assessed and high-speed optical mapping of transmembrane voltage (Vm) was performed on isolated, Langendorff-perfused hearts. The dose of MEHP was chosen based on the...
reported blood concentration following an exchange transfusion procedure. Acute, 30-min exposure to MEHP slowed atrioventricular conduction and increased the atrioventricular node effective refractory period. Optical mapping revealed prolonged action potential duration (APD) at slower pacing cycle lengths, akin to reverse use-dependence, resulting in a steep monophasic APD restitution curve in MEHP-exposed hearts. Following MEHP-exposure, the action potential shape became triangulated (APD90: 54.7 ± 13.6 msec to 71.0 ± 8.9 msec, mean ± SD) and the ventricular effective refractory period was prolonged (77.5 msec to 115 msec). MEHP-exposure also slowed ventricular epicardial conduction velocity (57 cm/sec to 43 cm/sec). This is the first study to highlight the impact of acute MEHP exposure, using a clinically-relevant dose, on cardiac electrophysiology in the intact heart. The presented findings suggest that heightened clinical exposure to plasticized medical products may have cardiac safety implications - given that action potential triangulation is a risk factor for early after depolarizations and cardiac arrhythmias. Future translational studies are necessary to discern whether secondary cardiac complications may be a risk factor for patients undergoing invasive medical procedures that employ plasticized products.

ABSTRACT NUMBER: 3359        Poster Board Number: P148
TITLE: Assessing Cardiac Gap Junction Intercellular Communication Toxicity in Response to Chemicals Used in Medical Devices
KEYWORDS: Phthalates
ABSTRACT: Background: Di-(2-ethylhexyl) phthalate (DEHP) is a main component of polyvinylchloride plastics used in many medical devices. DEHP can leach into blood or other lipophilic solutions where it is hydrolyzed to mono-(2-ethylhexyl) phthalate (MEHP). Pediatric ICU patients can have DEHP exposure 2 to 3 times higher than the average daily exposure for adults, making it important to quantify the toxicological effects of plasticizers on vulnerable pediatric populations. Objective: This study aimed to examine the toxicity of phthalate exposure on cardiomyocyte intercellular communication, using gap junction fluorescence recovery after photobleaching (GAP-FRAP) and microelectrode array (MEA) recordings. Methods & Design: Cardiomyocytes differentiated from human-induced pluripotent stem cells were treated with either 10 or 60 uM MEHP or 10 or 50 ug/mL DEHP for up to one week. For MEA experiments, field potential duration (FPD) measurements were recorded in response to external pacing (2 Hz) and during spontaneous beating. Local extracellular action potentials (LEAP) were recorded in response to external pacing (2 Hz). Parameters of interest included beating rate, FPD, LEAP 90% duration, spike amplitude, and conduction velocity. For GAP-FRAP experiments, cells were stained with Calcein-AM dye and imaged on a Leica TCS SP8 confocal microscope. Recovery after photobleaching was quantified and used as a measure of toxicity to cellular communication to explain potential MEA changes in beating rate and conduction velocity. Results & Discussion: Preliminary MEA results showed DEHP immediately decreased beat period (1 hr: 670+/−50 msec, 4 days: 460+/−10 msec) compared with controls (1 hr: 840+/−60 msec, 4 days: 880+/−40 msec). Sodium spike amplitude was decreased at 4 days (.33+/−.0.05 mV vs 2.41+/−.0.03 mV). No significant differences in MEA parameters were observed between control and MEHP-treated samples. In conclusion, the results of this study will be important for
helping to understand the effects of phthalate plasticizers on cardiac electrical conduction, specifically related to intercellular communication.

ABSTRACT NUMBER: 3360   Poster Board Number: P149
TITLE: Prenatal Exposure to Environmental Chemical Mono-2-ethylhexyl Phthalate Exposure Alters Neonatal Cardiomyocyte Development and Physiology
KEYWORDS: Phthalates
ABSTRACT: Di-(2-ethylhexyl)-phthalate (DEHP) is a main component of polyvinylchloride plastics used to soften otherwise rigid plastics. DEHP and its main metabolite, mono-2-ethylhexyl-phthalate (MEHP) have been detected in human amniotic fluid and umbilical cord blood, suggesting exposure to developing fetuses. Phthalates are known endocrine disruptors and prenatal exposure is likely to influence cardiac development and physiology. To investigate the direct effects of prenatal exposure on the developing cardiovascular system, pregnant rats were administered MEHP-treated water ad libitum. Pup body weight, heart weight, anogenital distance and heart rates were measured and PND1-2. Body weight and heart weight were slightly larger in prenatally exposed animals compared with controls (+7% and +12.8 %, respectively). No significant difference in anogenital distance was observed. Cardiomyocytes were isolated, cultured on glass coverslips and loaded with a calcium-sensitive dye to monitor calcium transients. The calcium transient reuptake phase was prolonged, mean time from t0 to 30% of cytosolic calcium reuptake (CaD30) for control cells was 239 ± 0.011 ms and 282 ± 0.022 ms for MEHP treated and from t0 to 80% of cytosolic calcium reuptake (CaD80) for control cells was 496 ± 0.026 ms and 659 ± 0.065 ms for MEHP treated. Cell viability and metabolic activity assay was performed, and we observed a decrease in metabolic activity (glycolysis-dependent) in MEHP exposed cells (-56.4% at 30min assay time point). qRT-PCR analysis revealed significant differences in key cardiac developmental and calcium handling genes, including: calsequestrin (CASQ2), phospholamban (PLB), Troponin I3 (TNNI3), ryanodine (RYR2) and GATA-binding protein 4 (GATA4). Phthalates are known endocrine disruptors and as such, in utero exposure may impact the developing heart.

ABSTRACT NUMBER: 3361   Poster Board Number: P150
TITLE: Exposure to Tris(1,3-dichloro-2-propyl)phosphate(tdcpp)inducesvascular Toxicity through Nrf2-vegf Pathwayin Zebrafishand Human Umbilical Vascular Endothelial Cells
AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: X. Zhong1, J. Qiu1, J. Kang1, X. Xing1, X. Shi2, and Y. Wei1. 1Sun Yat-sen University, Guang Zhou, China; and 2Wuhan University, Wu han, China.
KEYWORDS: Cardiovascular System; Mode-of-Action
ABSTRACT: The growing production and extensive use of organophosphate flame retardants (OPFRs) have led to an increase in their environmental distribution and human exposure. Developmental toxicity is a major concern of OPFRs’ adverse health effects. However, the impact of OPFRs exposure on vascular development and the toxicity pathway for developmental defects are poorly understood. In this study, we investigated the effects of exposure to tris(1,3-dichloro-2-propyl) phosphate (TDCPP), a frequently detected OPFR, on early vascular development, and the possible role of nuclear factor erythroid 2-
related factor (Nrf2)-dependent angiogenic pathway in TDCPP’s vascular toxicity. TDCPP exposure at 300 and 500 μg/L impeded the growth of intersegmental vessels (ISV), a type of microvessels, as early as 30 hpf. Consistently, a similar pattern of decreased extension and remodeling of common cardinal vein (CCV), a typical macrovessel, was observed in zebrafish at 48 hpf and 72 hpf. Developing vasculature in zebrafish was more sensitive than general developmental parameters to TDCPP exposure. The expression of genes related to VEGF signaling pathway dose-dependently decreased in TDCPP-treated larvae. In *in vitro* experiments using human umbilical vascular endothelial cells (HUVECs), the increased cell proliferation induced by VEGF was suppressed by TDCPP exposure in a dose-dependent fashion. In addition, we found a repression of Nrf2 expression and activity in TDCPP-treated larvae and HUVECs. Strikingly, the application of CDDO-Im, a potent Nrf2 activator, enhanced VEGF and protected against defective vascular development in zebrafish. Our results reveal that vascular impairment is a sensitive index for early exposure to TDCPP, which could be considered in the environmental risk assessment of OPFRs. The identification of Nrf2-mediating VEGF pathway provides new insight into the adverse outcome pathway (AOP) of OPFRs.

**ABSTRACT NUMBER:** 3362  
**Poster Board Number:** P151  
**TITLE:** Immunophenotyping Pig Immune Cells by Flow Cytometry: A Powerful Tool for Pharmacology and Toxicology Studies  
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**KEYWORDS:** Immunotoxicology; Biomarkers; Safety Evaluation  
**ABSTRACT:** Pigs are increasingly used in pharmacology and toxicology research because they share many physiological and anatomical commonalities to humans. Because the pig has been a focus of vaccine research for many years, pig-specific immunology reagents exist as research tools. Pigs have some unique aspects to their immunology: in general pigs have higher circulating leukocytes, a lower ratio of CD4:CD8 T cells, and express more double positive (DP) CD4+/CD8+ cells than other species. These DP cells increase with age and after vaccine and/or infection, and are thought to have memory phenotype. Pigs also have a higher percentage of γδ T cells than other species. γδ T cells are highest in younger animals, express SLA-DR (Swine Leukocyte Antigen, MHC II), and CD8 and thought to have cytolytic activity and memory. Unlike other species, SLA-DR is preferentially expressed on CD8+ T cells and is up-regulated on activated cells. We describe Immunophenotyping panels that were designed to identify pig immune cells using flow cytometry. Using EDTA-treated whole blood samples from naïve pigs, we identified pig T-cell populations including T-helper cells (CD3+CD4+CD8-), Cytotoxic T cells (CD3+CD4-CD8+), Double positive (CD3+CD4+CD8+) T cells, and γδ T cells (CD3+TCR γδ+). Consistent with the literature, we confirmed SLA-DR expression on CD8+ T cells, but not on CD4+ T cells. In addition we identified Pig Natural Killer Cells (NK, CD3-CD335+), Pig B cells (CD3-CD21+) and Pig Monocytes (CD3-CD14+CD163+). We conclude that we successfully identified pig adaptive (T and B cells), and innate (NK cells and Monocytes), immune cells using multi-parameter flow cytometry. We are currently working to optimize our method to identify Regulatory T cells (CD4+CD25+ FoxP3+) in the pig. Immunophenotyping in the pig has many potential applications, including monitoring immune cells in pharmacology models and vaccine development. Also, in recent years the use of the pig has markedly increased as an alternative to the use of canines and non-human primates in Toxicology studies and research. With the
current focus on immune-modulating reagents in drug development, monitoring pig immune cells in Toxicology studies is especially relevant.

ABSTRACT NUMBER: 3363  Poster Board Number: P152
TITLE: Assessment of Immunotoxicity by Xenobiotic Exposure Using Larval Zebrafish
AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: D. W. Phelps¹, A. Fletcher¹, I. Rodriguez-Nunez¹, M. Balik-Meisner¹, D. Tokarz¹, D. Reif¹, D. Germolec², and J. A. Yoder¹. ¹North Carolina State University, Raleigh, NC; and ²NIEHS, Research Triangle Park, NC.

KEYWORDS: Immunotoxicity

ABSTRACT: Current assessment practices for immunotoxicity involves a tiered approach for hazard identification and mechanistic studies, including observational studies, evaluation of immune function, and measurement of susceptibility to infectious and neoplastic disease. These studies generally use costly, low-throughput mammalian models. Zebrafish, however, offer an excellent alternative due to their rapid development, ease of maintenance, and homology to mammalian immune system function and development. Larval zebrafish also provide a convenient model to study the innate immune system with no interference from the adaptive immune system. In this study, we utilized a respiratory burst assay (RBA) to measure reactive oxygen species (ROS) production after xenobiotic exposure. ROS are produced in macrophages and neutrophils in response to pathogens in order to eliminate them from the host. Embryos were exposed to subteratogenic doses of chemicals from 6 hpf to 96 hpf with daily media changes, and at 96 hpf the ability to produce ROS was measured. Through the RBA, we identified five compounds that suppressed global ROS production: 17-β estradiol, benzo(a)pyrene, lead acetate, methoxychlor, and phenanthrene; these compounds have also previously been identified as immunosuppressive in mammalian innate immunity assays. In order to evaluate whether the suppression of ROS by these compounds was due to a decreased number of neutrophils or macrophages, we combined flow cytometry with transgenic zebrafish larvae to count the numbers of these cell types after chemical exposure. With this assay, we found benzo(a)pyrene altered macrophage number, but not neutrophil number. Taken together, this work demonstrates the utility of zebrafish larvae as a tool for identifying compounds that impact innate immune function at subteratogenic levels and identifies two mechanistic routes by which xenobiotic exposure may alter immune function.

ABSTRACT NUMBER: 3364  Poster Board Number: P153
TITLE: Hydroquinone Exposure Impairs Adaptive Immune Response to Influenza Vaccine In Mice
AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: S. H. P. Farsky¹, A. L. Fabris¹, G. H. O. da Rocha¹, M. Paula-Silva¹, P. L. Ho², and E. L. V. Silveira¹. ¹University of São Paulo, São Paulo, Brazil; and ²Butantan Institute, São Paulo, Brazil. Sponsor: S. Barros

KEYWORDS: Infectious Disease; Volatile Organic Compounds; Immunotoxicology

ABSTRACT: Exposure to environmental pollutants, including cigarette smoke, harms the efficacy of active vaccination. Cigarette smoking leads to exposure to more than 8,000 compounds, and here we investigated if a chronic in vivo exposure of hydroquinone (HQ), the most abundant oxidative compound of particulate phase in tobacco, could impair the adaptive immune response induced by the trivalent influenza vaccine. Male C57BL/6 mice were daily exposed to HQ (2500 ppm) or PBS for 8 weeks
At weeks 6 and 8, mice were immunized (i.m.) with 100µL of the aforementioned vaccine, and toxic and immunological parameters were analyzed. HQ exposure slightly reduced some of the erythrocytic parameters and did not alter body weight and biochemical markers of toxicity, such as enzymatic activities of glutamic oxalacetic transaminase and glutamic pyruvic transaminase, levels of creatinine, and morphology of lungs, liver, kidneys. However, HQ-exposed animals presented increased oxidative stress in splenocytes and altered morphology of spleen follicles; increased size of lymph nodes associated with decreased number of specific IgG-secreting cells; and reduced IgG2c subclass titers. Altogether, these data point out HQ as a harmful pollutant that impairs humoral response induced by influenza vaccination.
ABSTRACT NUMBER: 3366        Poster Board Number: P155
TITLE: PPAR-γ Regulates T Cell Responses in Polycyclic Aromatic Hydrocarbons-Induced Inflammation

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:  D. Kim, M. Sharma, and S. Huang.  University of Cincinnati College of Medicine, Cincinnati, OH.

KEYWORDS: Polycyclic Aromatic Hydrocarbons; Immunotoxicity; Inflammation

ABSTRACT: Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous lipophilic air pollutants released from incomplete combustion of organic materials, such as automobile fuel-burning, industrial gas emission, cigarette smoking, and food grilling. Exposure to PAHs increases the risk of various inflammatory diseases and conditions. However, whether and how PAHs interfere with immune cell responses and exacerbate inflammatory responses remain elusive. As known, dendritic cells (DCs) activate effector T cells through antigen presentation and cytokine stimulation, determining the balance of pro- and anti-inflammatory responses. Thus, we hypothesize that PAH-exposed DCs alter the cytokine production of T cells, leading to unbalanced inflammatory homeostasis. To test this hypothesis, we treated human DCs with benzo[a]pyrene (BaP), a common component of PAHs, and showed the increased production of inflammatory cytokine interferon-γ (IFN-γ) in DC-mediated T cell activation. To further determine which genes and pathways regulate the immune responses altered by BaP, we performed transcriptomic analysis on BaP-treated DCs and showed that BaP exposure inhibited the expression of the gene cluster mediated by the nuclear transcription factor peroxisome proliferator-activated receptor γ (PPAR-γ). To further test whether PPAR-γ regulates the effect of PAH exposure on T cell activation and inflammatory response, we treated DCs with PPAR-γ agonist (pioglitazone) and antagonist (GW9662) and measured the surface markers and intracellular cytokine production of T cells. Our results showed that DCs treated with PPAR-γ antagonist increased IFN-γ production of T cells and DCs treated with PPAR-γ agonist indicated a decreased IFN-γ production of T cells in response to BaP exposure. These accumulative results support that BaP exposure enhances pro-inflammatory responses, which can be partially alleviated by the activation of PPAR-γ pathway. Therefore, manipulation of PPAR-γ pathway provides a potential mean to regulate inflammatory diseases associated with PAH exposure.

ABSTRACT NUMBER: 3367        Poster Board Number: P156
TITLE: Pro-inflammatory Actions of Hydroquinone on Arthritic Synoviocytes


KEYWORDS: Environmental Toxicology; Receptor; Aryl Hydrocarbon; Immunotoxicity

ABSTRACT: The genesis of rheumatoid arthritis (RA) involves genetic and environmental factors. Cigarette smoke aggravates pre-existent RA, but the mechanisms involved are unknown. Hydroquinone (HQ) is one of the most abundant pro-oxidative compound featured in cigarette smoke and is a benzene metabolite. Our previous experimental data have demonstrated that HQ exposure in the initial or later phases of collagen induced arthritis (CIA) aggravates the symptomatology of the disease, suggesting HQ as a cigarette compound involved on aggravation of RA in smokers. Here we aimed to investigate the mechanisms of HQ exposure on synoviocytes from arthritic cavities. Male Wistar rats were daily exposed to saline, vehicle (ethanol:saline 5 %) or HQ (25 ppm) for 1 hour, during 35 days. Bovine collagen type II emulsified on complete Freund adjuvant was injected s.c. into the tail on the 7th (0.4
mg/200 µL), and a booster (0.2 mg/100 µL) was carried out seven days later. Synovial membranes were collected on the 35th day. Primary human fibroblast like synoviocytes from rheumatoid arthritis patients (RAHFLS) were cultured in Dulbecco’s modified Eagle’s medium (DMEM F12) supplemented with 10 % fetal bovine serum (FBS) and incubated with HQ (1 or 10 µM) and/or TNF-alpha (2 ng/mL) for 1 to 24 hours. Samples were analyzed by ELISA, FACS or histological techniques. Simultaneous in vivo exposure of HQ to rats during CIA development induced higher collagen deposition on the synovia, synoviocytes hyperplasia and proliferation and higher levels of IL-6 in synovial fluid. HQ increased the proliferation of RAHFLS. In addition, in vitro HQ treatment augmented the secretion of cytokines IL-6 and IL-8 evoked by TNF-alpha stimulation. Moreover, HQ directly evoked reactive oxygen species (ROS) production by RAHFLS. Furthermore, HQ increased the Aryl hydrocarbon receptor (Ahr) expression by synoviocytes but did not alter the NFkB translocation evoked by TNF-alpha stimulation. In conclusion, data herein obtained point out HQ exposure as a harmful compound of cigarette smoke in aggravation of RA, showing a toxic mechanism of HQ involving the activation of Ahr pathway by synovial cells.

ABSTRACT NUMBER: 3368 Poster Board Number: P157

TITLE: The Impact of Di(2-ethylhexyl) Phthalate Exposure on Colonic Immune Microenvironment in Mice


KEYWORDS: Immunotoxicity

ABSTRACT: Di(2-ethylhexyl) phthalate (DEHP) is a ubiquitous plasticizer contained in, but not limited to, personal care products, plastics, and contaminated foods and water sources. DEHP is noncovalently bound to plastics and can leach into foods and water sources and enter our system by ingestion. Since the digestive system is directly exposed to orally introduced DEHP, we hypothesized that acute exposure to DEHP alters the colonic immune microenvironment. In particular, the colon was investigated since it plays critical roles in immune defense and harbors the largest microbiome, which modulates the innate and adaptive immune responses. Therefore, this study aims to assess the effects of DEHP exposure on colonic inflammation using 3-month-old male CD-1 mice orally dosed with corn oil vehicle, 20 g, or 200 g DEHP/kg of body weight (BW) (n=6 mice/group) for ten days. Histological analysis unveiled decreased colon length (p=0.04), increased edema, and increased red blood cells (RBCs) in the distal colon in mice exposed to 20 g DEHP compared to control. The distal colons of mice treated with 200 g DEHP only showed increased RBCs compared to control. Flow cytometric analysis revealed a significant increase in lymphocyte population (CD45+, CD11b-) in the proximal (p<0.05) and distal (p=0.01) colons of mice dosed with 20 g DEHP/kg BW compared to control. In particular, the increase in lymphocytes was attributed to the increase in CD3+ T-cells in the proximal (p=0.03) and distal colons (p=0.01). Unlike the low dose group, mice dosed with 200 g DEHP showed no significant differences in the colonic immune population compared to control. Finally, a comparison between 20 and 200 g DEHP groups showed that the lower dose increased CD11b+ lymphocyte population in the proximal (p=0.02) and distal (p=0.02) colons. Altogether, these data suggest that DEHP exposure increased the T-cell population in the colon, which supports the hypothesis that exposure to DEHP alters the colonic immune microenvironment in a non-monotonic dose-dependent manner. Histological and flow cytometric analyses suggest that DEHP exposure results in colonic inflammatory responses. Future directions include an assessment of the gut...
microbiome and how it modulates immune responses and an evaluation of the immunological reaction in DEHP-exposed females.

**ABSTRACT NUMBER:** 3369  **Poster Board Number:** P158  
**TITLE:** Inflammasomes Are Important Mediators of Arsenical-induced Skin Lesions

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** M. P. Kashyap, J. Khan, M. Waseem, C. Li, R. K. Srivastava, and M. Athar, University of Alabama at Birmingham, Birmingham, AL.

**KEYWORDS:** Cutaneous Or Skin Toxicity; Immunotoxicity; Inflammation

**ABSTRACT:** Inflammasomes are cytosolic multiprotein signaling complexes consisting of caspase-1, PYCARD, and nucleotide-binding oligomerization domain-like receptor with pyrin domain protein (NLRP). Inflammasomes in immune cells as well as in epithelial cells act as sensors for pathogen-associated molecular patterns, stress, or danger stimuli. Here we show their role in chemical injury. Warfare arsenicals induce severe inflammatory and tissue disrupting cutaneous lesions. Recently, AIM2 inflammasome was shown to be activated by arsenic which regulates caspase-1 and enhances the secretion of IL-1β and IL-18. We therefore tested whether mechanism of arsenicals-mediated severe inflammation in the skin is regulated by inflammasome activation. Ptch+/−/SKH-1 mice exposed to PAO, a surrogate arsenical develop inflammatory response in the skin associated with NALP1 induction in F4/80 macrophage. The induction of NALP1 is accompanied by enhancement in the levels of NALP3, IL-1β and IL18. Topical application of OLT1177, an NLRP3 inhibitor was able to mitigate cutaneous inflammatory response significantly. In this regard not only caspase-1, IL-1β and IL-18 levels are attenuated but skin bi-fold thickness, erythema, edema and Draize score were also decreased. Then we show that PAO suppresses autophagy as evident by the decrease in its biomarkers, Beclin1, Atg-12, Atg-5, Atg-3, LC3A/B, LC3A and LC3B in the skin which could provide a danger signal to resident macrophage. Dysregulated autophagy also induce inflammation. We therefore treated PAO-exposed animals with rapamycin, an inducer of autophagy. This treatment reduced cutaneous inflammation. Similar effects were noted by the treatment with other arsenicals namely, Lewsite (LW), Diphenylchloroarsine (DPCL) and Diphenycyanoarsine (DPCY). In summury, these data suggest an important role of inflammasome activation in arsenical-mediated cutaneous inflammation and blistering.

**ABSTRACT NUMBER:** 3370  **Poster Board Number:** P159  
**TITLE:** Localized *Staphylococcus epidermidis* Infection Elicits Spatially Dependent Inflammatory Response

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** N. Prince1, J. A. Penatzer1, M. J. Dietz2, and J. W. Boyd2. 1West Virginia University, Morgantown, WV; and 2West Virginia University School of Medicine, Morgantown, WV.

**KEYWORDS:** Inflammation; Infection; Cytokines

**ABSTRACT:** Infection is a common surgical complication that remains the major cause of failure in revision total knee arthroplasty (TKA) procedures, accounting for approximately 44% of failures. *Staphylococcus epidermidis* is often implicated in the medical device-related infections that lead to these failures. However, inflammatory responses associated with localized *S. epidermidis* infection are not well understood, and this lack of information could be limiting clinical and pharmacological approaches to treatment. In order to evaluate tissue-level inflammatory response to *S. epidermidis,*
human inflammatory cytokines were profiled in clinical samples from patients undergoing revision TKA procedures. Samples were collected from male (N=5) and female (N=5) patients, varying in age (46-76 years) and comorbidities. Tissues were collected at seven locations, varying in proximity to prosthetic knee, to compare infected samples with controls, with respect to spatial orientation. Of the twenty cytokines profiled, seven were identified as significant for distinguishing immune differences based on presence of \textit{S. epidermidis} infection: IL-6, MCP-1, and MIP-1β were significant at p<0.05, MIP-1α was significant at p<0.01, and IL-1α, IL-1β, and IL-8 were significant at p<0.001; four cytokines produced spatially disparate inflammatory responses, with respect to proximity to infection, at statistically significant levels: IL-4 and IP-10 were significant at p<0.05, and IL-1α and IL-1β were significant at p<0.01. This demonstrates the role of \textit{S. epidermidis} in producing a unique localized cytokine profile in tissues surrounding a prosthetic device. Through the analysis, it was possible to identify regional variability and offer novel information regarding the differences localized inflammatory response in human tissues.

\textbf{ABSTRACT NUMBER:} 3371 \hspace{1cm} \textbf{Poster Board Number:} P160

\textbf{TITLE:} \textit{Anacardium occidentale} L. Leaf Extracts Resolve Inflammation in Histamine-Induced Bronchoconstriction in Guinea Pigs

\textbf{AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:} O. J. Awakan\textsuperscript{1}, S. O. Malomo\textsuperscript{2}, O. S. Adeyemi\textsuperscript{3}, A. E. Omonisi\textsuperscript{3}, A. Iggunu\textsuperscript{2}, V. A. Awakan\textsuperscript{4}, and S. Adegbite\textsuperscript{5}. \textsuperscript{1}Landmark University, Omu-Aran, Nigeria; \textsuperscript{2}University of Ilorin, Ilorin, Nigeria; \textsuperscript{3}Ekiti State University Teaching Hospital, Ado-Ekiti, Nigeria; \textsuperscript{4}Federal Teaching Hospital Ido-Ekiti, Ido-Ekiti, Nigeria; and \textsuperscript{5}University of Nottingham, Ningbo, China.

\textbf{KEYWORDS:} Inflammation; Lung; Pulmonary Or Respiratory System; Natural Products

\textbf{ABSTRACT:} Ethanolic extracts prepared from the leaves of \textit{Anacardium occidentale} L. and a bioactive compound (oleamide) isolated from the same source has been shown to possess anti-inflammatory and bronchodilatory properties. Hence, these were evaluated for their ability to reverse the histopathologic alterations caused by an inflammogen, histamine (0.2 %; nebulized) in the lungs of guinea pigs. A standard bronchodilator (Salbutamol) was used as the reference drug, and the lung sections were viewed under the microscope. The cytotoxic effect of the extracts and oleamide was also investigated on Human foreskin fibroblast (HFF) and Vero cells. Cell morphological changes were observed by using light microscope. \textit{In vitro} cytotoxicity was evaluated using [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] MTT assay. The results show that oleamide and the extracts possess significant effects in ameliorating inflammation and dilating the tracheobronchial tree as seen in the activities (e.g. respiratory epithelium devoid of infiltration by eosinophils, marked bronchoarteriolar dilatation) on the muscle and blood cells. The cell viability and IC\textsubscript{50} values also reveal safety in their use (99-76% and 100-80% cell viability for HFF and Vero cells respectively as the concentration of extracts and oleamide increased (1-100 μg/ml). Data do not only warrant further biochemical profiling of plant extracts and oleamide but also implicate the prospects of these plant extracts as potential source of safer bronchodilatory therapy.
ABSTRACT NUMBER: 3372    Poster Board Number: P161
TITLE: Identification of Functional Farnesoid X Receptor in Glioblastoma NCI-60 Cell Lines

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: C. A. Nadolny¹, J. Chambers¹, G. Guo², and J. R. Richardson¹. ¹Florida International University, Miami, FL; and ²Rutgers, The State University of New Jersey, Piscataway, NJ.

ABSTRACT: Glioblastoma is the most common primary brain tumor in adults. It is extremely aggressive and one of the least treatable types of cancers, with a median survival of only 15 months. Unfortunately, little progress has been made in development of new therapies within the past decade, demonstrating the need to identify new potential targets. Farnesoid X Receptor (FXR) is a transcription factor predominantly expressed in the liver and intestine, where it is has been reported to suppress hepatocellular carcinoma through anti-inflammatory activity and repair of liver injury. Our laboratory has found FXR to be expressed in mouse and human astrocytes, where it regulates the inflammatory response to pro-inflammatory cytokines. However, the presence and function of FXR in human glioblastoma cells has not been explored. Here, we evaluated the expression of FXR in six NCI-60 glioblastoma cell lines and one immortalized glioblastoma cell line (ATCC U118). We were able to measure basal levels of FXR by qPCR, indicating the presence of this gene product in glioblastoma cells. When cells were treated with an FXR agonist (10µM WAY 362450), a significant induction (~1.4-2.5 fold) of the validated downstream target gene, small heterodimer partner (SHP) was observed. To our knowledge, these are the first data to identify the expression of functional FXR in NCI-60 glioblastoma cells.

ABSTRACT NUMBER: 3373    Poster Board Number: P163
TITLE: The National Toxicology Program’s Approach for the Systematic Evaluation of the Application of Zebrafish in Toxicology (SEAZIT)


KEYWORDS: Alternatives to Animal Testing; Systems Biology

ABSTRACT: Zebrafish provide a powerful alternative animal model that has the potential to complement more traditional rodent animal models in toxicology studies. The US National Toxicology Program (NTP) has been engaged in collaborations with zebrafish experts to better understand the utility of this model in screening, prioritizing, or predicting toxicity as compared to human in vitro systems or traditional rodent models. However, challenges exist which hinder the broader adoption of the zebrafish model in toxicology. These obstacles include the lack of harmonized experimental approaches in embryonic and adult fish, incomplete knowledge regarding chemical absorption and inconsistent informatics approaches used to classify adverse outcomes. SEAZIT was established in 2014 to tackle these challenges by serving as a resource for information sharing and to conduct focused experimental studies. In 2016, an Information Gathering Group was established to identify sources of variability in toxic responses when conducting embryonic zebrafish assays. Investigations revealed substantial variability across design parameters, data collected, and analysis procedures. The presence of the chorion and renewal of exposure media were identified as design parameters that could potentially influence study outcomes and should be investigated further. Based on these results, SEAZIT is now facilitating an Interlaboratory Study to address the impact of protocol design with the prediction that no
chorion and renewal of exposure media will increase toxicity. Participating groups evaluate a 40-chemical library designed to provide overlap with other NTP studies and includes chemicals with a range of physicochemical properties and developmental effects. Results from the Interlaboratory study will confirm if the hypothesized experimental parameters (chorion off and renewal of exposure media) do contribute to excess toxicity in zebrafish embryos within and across different research labs. These studies are among the first in a series which will aid the harmonization of zebrafish testing and may support the broader adoption of zebrafish in toxicology.

ABSTRACT NUMBER: 3374   Poster Board Number: P164
TITLE: Body Weight Changes in a Partial Body Irradiation Model of Gastrointestinal Acute Radiation Syndrome in the Male Mouse


KEYWORDS: Carcinogenesis; Exposure, Environmental; Gastrointestinal

ABSTRACT: The objective of the study was to develop a lethality profile for Partial Body Irradiation (PBI) specific to this institution, an endeavor necessary to validate the model prior to conduction efficacy studies under the criteria of the FDA Animal Rule. In order to understand animal survival, changes in body weight post irradiation were monitored every 3 days and compared by dose level. As such, criteria for euthanasia were not based on body weight changes, but on posture, coat, and behavior. Sedated C57BL/6 male mice were placed in a small animal X-ray irradiator with lead tubes covering their hind limbs (estimated 5% of bone marrow shielding) and exposed to a single dose of 11.5, 12.5, 13.5, 14.5, and 15.5 Gy. Supportive care was limited to providing acidified water and administering subcutaneous fluids post irradiation from Days 1 to 8. Based on the observed mortality over 30 days post irradiation, the LD₁₀ (lethal dose, 10 %), LD₃₀, LD₅₀, LD₇₀, and LD₉₀ for partial body irradiation was 11.62, 12.5, 13.14, 13.82, and 14.87 Gy, respectively. Body weight decreased in a dose-dependent manner; weight loss among groups was more severe and occurred more rapidly as irradiation level increased. Animals irradiated at 15.5 Gy had an average body weight nadir on Day 7 with an average loss of 30.5% as opposed to animals irradiated at 12.5 Gy that had an average body weight nadir on Day 8 with an average loss of 25.2%. Body weight gains were also observed in a dose dependent manner. Although no animals returned to their initial (Day 0) body weight, animals surviving to Day 30 gained more weight after body weight nadir in the lower irradiated group when compared to the higher irradiated group. This study demonstrated that under the conditions of partial body irradiation, the mouse can overcome body weight losses of up to 25.2% at LD₃₀.

ABSTRACT NUMBER: 3375   Poster Board Number: P165
TITLE: Toxicity of Emerging Perfluoroether Carboxylic Acids in the Zebrafish Embryo Model

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: K. Gebreab, and J. Berry. Florida International University, Miami, FL. Sponsor: M. Reynolds

KEYWORDS: Exposure, Environmental; Toxicity; Acute; Embryo

ABSTRACT: Perfluoro alkyl substances (PFASs) are persistent organic contaminants that have been detected in wildlife, humans and in the environment. They have been associated with negative health
effects like different cancers, neurotoxicity, thyroid malfunction, reproductive problems, immunotoxicity, lung toxicity, and hepatotoxicity. Studies have shown that the toxicity of PFAS is determined by the carbon chain length as well as the attached functional group. Long chain perfluorinated compounds have been replaced with short chain perfluoro ether carboxylic acids (PFECAs) and fluorinated derivatives. Except a few studies, mostly from manufacturers, there is no detailed information regarding their toxicity, pharmacokinetic behavior, and release of these compounds. In the present study, the zebrafish embryo model was exposed to various concentrations of seven emerging PFECAs and PFOA through 7pfdd (post-fertilization date) and developmental toxicity, lethality and neurobehavioral effect were assessed. Rapid onset of toxicity was observed in 24 hrs pfdd and was related to the length of the chain of the PFECAs. Therefore, this study showed significant (p<0.0001) lethality (LC50), developmental and neurobehavioral toxicity of emerging PFECAs in the zebrafish embryo. To our knowledge, this is the first toxicological study of emerging PFECAs that included the LC50 values in a zebrafish (Danio rerio) model.

ABSTRACT NUMBER: 3376    Poster Board Number: P166
TITLE: Comparative Toxicity of Model Chemical Oxidative Stressors in nfe2l2a-Mutant and Wild Type Zebrafish Is Not Fully Predicted by Potency of nrf2a-Dependent Antioxidant Gene Expression

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KEYWORDS: Antioxidants; Aquatic Toxicology; Transcription Factors

ABSTRACT: The transcription factor nrf2a induces a cellular antioxidant response and can provide significant protection against chemical-induced oxidative stress. However, the extent of protection from acute toxicity as indicated by nrf2a induction is not well established. In order to address this existing data gap, we examined the comparative potency of a model set of seven industrial compounds to induce nrf2a-dependent antioxidant response and related oxidative stress gene expression in zebrafish larvae. Exposure to cumene hydroperoxide and R-(−)-carvone, as well as the model oxidative stressor tert-butyl hydroperoxide, elicited a strong nrf2a-dependent antioxidant gene response at 4dpf. Using zebrafish carrying several different null or constitutively-activating mutations in nfe2l2a (the gene encoding nrf2a), we confirmed the dependence on nrf2a for induction of the antioxidant genes (gclc, gstp, prdx1, and gpx1a) by our model activators, and identified a significant role for nrf2a in the baseline expression of these genes and sod1. Elimination of nrf2a strongly increased the sensitivity of the zebrafish to the acute toxicity of the two hydroperoxides tested, but the lack of nrf2a in the null mutants did not affect the acute toxicity of R-(−)-carvone. Therefore, our studies indicate that the potency of nrf2a-dependent gene induction upon chemical exposure may not predict the role of nrf2a in zebrafish toxicity.
ABSTRACT NUMBER: 3377  
Poster Board Number: P167

TITLE: An In Vitro Model of IL-2-Mediated Type 2 Innate Lymphoid Cell Proinflammatory Cytokine Secretion

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: R. Visconti, and K. Kolaja. Celgene, Summit, NJ.

KEYWORDS: Cytokines; Biological Modeling; Autoimmune

ABSTRACT: Aldesleukin, a recombinant form of human IL-2, is an effective melanoma and renal cell carcinoma treatment. High-dose IL-2 has been used for decades and is considered the first effective cancer immunotherapy. Unfortunately, high-dose IL-2 therapy is associated with vascular leak syndrome (VLS), gross eosinophilia, and pruritus. The inflammatory response responsible for eosinophilia is believed to be mediated by Type 2 innate lymphoid cells (ILC2). ILC2s express the IL-2 receptor (IL2R) α chain, CD25, and secrete Type 2 proinflammatory cytokines, like IL-4, IL-5, and IL-13. The immunosuppressive property of chronic low dose IL-2 therapy that selectively binds the IL-2 high affinity receptor (IL-2Rαβγ) on Tregs is being explored as a viable treatment for several autoimmune diseases. In a preclinical study where cynomolgus monkeys were treated with an IL-2 analog designed for IL-2Rαβγ selectivity (IL-2RαβγA), pathologists identified hepatic eosinophilia and fibroplasia with portal bridging. Human and cynomolgus monkey ILC2 in vitro models were created to study the direct impact of aldesleukin and IL-2RαβγA exposure on CD25 binding and subsequent proinflammatory cytokine release to recapitulate the in vivo findings and potentially predict human drug safety. Human and cynomolgus monkey ILC2s were isolated from blood and grown in the presence of costimulatory factors IL-25, IL-33, and thymic stromal lymphopoietin. Flow cytometric analysis of each respective ILC2 cell population yielded log-fold increases in expression of ILC2-related cluster of differentiation markers CD25, CD127, CD161, and CD294. These enriched ILC2 cell populations were treated with four different doses of aldesleukin and IL-2RαβγA for four days and the resulting supernatants were collected and monitored for Type 2 cytokine concentration via an electrochemiluminescent assay (MesoScale Diagnostics). A dose-dependent IL-5 increase was detected in samples derived from both species of ILC2 cells treated with aldesleukin or IL-2RαβγA. The change in IL-5 levels in supernatants collected from ILC2s exposed to aldesleukin or IL-2RαβγA were dose-dependent and marked a six to eight-fold increase over the negative control samples free of exogenous IL-2. This ILC2 in vitro model is effective at detecting IL-2RαβγA-mediated cytokine secretion.

ABSTRACT NUMBER: 3378  
Poster Board Number: P168

TITLE: Classification of Chemicals for Their Irritation Potential Using an In Vitro Method of Reconstructed Human Cornea-Like Epithelium (RhCE)

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KEYWORDS: Alternatives to Animal Testing; Ocular Toxicity

ABSTRACT: Consumer products such as household cleaners and cosmetics may induce serious damage upon contact with the eyes, so assessment of ocular toxicity is important to ensure the safety of these types of products. The Draize rabbit test has been criticized for its lack of reproducibility, overestimation, and the use of live animals. The reconstructed cornea-like tissue model is been widely
used by the cosmetic industry to evaluate the irritation potential of raw materials, as an alternative to Draize rabbit test and has been evaluated by ECVAM. In compliance with the Test Guideline for Reconstructed Human Cornea-like Epithelium (RhCE), OECD 492, test items was evaluated in duplicate for each trial while one set of two tissues was treated with DPBS and served as control and methyl acetate as positive control. Assessment of a chemical to produce serious eye damage/eye irritation in RhCE test methods is based on tissue viability that is determined by enzymatic reduction of MTT to formazan. For solid test items, 30 mg of test item was applied onto the tissue followed by an incubation period of 4 hours. At the end of the exposure period the test items was removed and the tissue kept for 30 min at room temperature followed by an incubation (at 32 °C) period of 18h. At the end of the incubation period the tissue was incubated with MTT for 180 minutes followed by extraction of formazan for minimum period of 2h. The optical density (OD) of the extracted formazan was determined in at 570 nm. At the end of the treatment the negative control OD values were found to be within the range of > 1.0 to ≤ 2.5. The mean tissue viability values for positive control for the solid test item was found to be < 20% and the variation within the replicates was below < 20% and therefore, the experiment are considered valid. Percent mean viability of <50 was observed in 2,5-dimethyl-2,5-hexanediol, sodium oxalate, sodium benzoate and camphene which were classified as “Irritant” as per the test guideline OECD 492. For classification of these test item further testing was required. Percent mean viability of >60 was observed in 3,4,4’-trichlorocarbanilide, 2,2’-Methylenebis[6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol] and potassium tetrafluoroborate and were classified as “Non-Irritant” and no further testing was required.

ABSTRACT NUMBER: 3379  
Poster Board Number: P169  
TITLE: The Assessment of Alveolar Toxicity and Fibrotic Potential In Vitro Using the MatTek EpiAlveolar/Macrophage Co-Culture Model  
KEYWORDS: Respiratory Toxicology; Inhalation Toxicology; Safety Evaluation  
ABSTRACT: Pulmonary fibrosis is a debilitating, typically fatal condition, caused by a variety of factors, including environmental or occupational, drugs, radiation and genetic predisposition. No current in vitro, ex vivo, in silico, or in vivo models of pulmonary fibrosis fully recapitulate all salient features of the human disease. The most frequently used model uses Bleomycin to cause the disease in rats in vivo. In this study, a novel in vitro organotypic, 3D airway model from primary human cells (EpiAlveolar™) with macrophages was challenged to develop aspects of pulmonary fibrosis. Tissues were exposed to known pulmonary fibrosis causing agents; bleomycin (0.0012, 0.12 or 0.12 µg/mL) or TGF-β1 (1, 5 or 10 µg/mL) in the culture media, or repeated aerosol applications of silica (50 nm particle size, ca 1 or 10 µg/cm²) for up to a 14 day period. Tissue viability was assessed by transepithelial electrical resistance (TEER) and lactate dehydrogenase (LDH) release every second day. Tissue samples were collected throughout the time course for analysis by pathology and immunohistochemistry to further characterise the healthy model and to identify aspects of pulmonary fibrosis developing over time. Spent media samples were also retained for biomarker analysis. LDH release (cytotoxicity) was negligible for all tissues at all time points. TGF-β1 treated tissues showed a dose response reduction in TEER. Silica and bleomycin did not show any change in TEER with respect to controls. These data confirmed that repeat dosing of EpiAlveolar™ via both apical and basal routes were successful and that this would be a suitable model to

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identify alveolar toxicity. Pathology, immunohistochemistry and biomarker end-points are currently under evaluation and will be presented. In conclusion, the EpiAlveolar™ co-culture model is a valuable new tool for assessment of toxicological end-points. This model has a potential to identify new pulmonary fibrosis treatments or causative agents whilst reducing the need for in vivo testing and for improving translation to human outcomes.

ABSTRACT NUMBER: 3380    Poster Board Number: P170
TITLE: In Vitro Skin Sensitization: Keratinocyte-Based Are-Nrf2 Luciferase Reporter Gene Test with Different Classes of Chemicals

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KEYWORDS: Alternatives to Animal Testing; Predictive Toxicology

ABSTRACT: Skin sensitization potential of agrochemical products is an important part of safety determination process. A recent trend is development of KeratinoSens™ assay as an alternative for the in vivo tests. This assay has been shown to be predictive of sensitization potential of both pure substances as well as multi-component mixtures. The KeratinoSens™ assay uses an immortalised adherent human keratinocytes cell line that is transfected with a selectable plasmid. In this laboratory, assay based on Keratinocyte-Based ARE-Nrf2 Luciferase Reporter Gene method was conducted to evaluate skin sensitization potential of different classes of sensitizing and non-sensitizing chemicals to include extreme, strong, moderate, weak and non-sensitizers. In KeratinoSens™ assay, Nrf2-dependent luciferase induction and MTT-viability assay were performed in parallel plates. KeratinosensTM (HaCaT) cell line was seeded in 96-well plates and after 24 h incubation, cells were treated with various test chemicals at concentrations ranging from 2000 µM to 0.098 µM or with positive control substances with a test range of 4 to 64 µM for 48 h. Following 48 h, cells were washed with DPBS and placed in passive lysis buffer and incubated for 20 min at 37° C. The cell lysate plates were evaluated in microplate reader to measure luminescence. Cell viability of treated cells was also evaluated parallel using MTT test. Imax, EC1.5 and IC50 values were calculated based on luciferase activity i.e. luminescence reading, while IC50 was calculated based on MTT results. Based on these values, test chemicals were discriminated between sensitizers and non-sensitizers. Under the specified experimental conditions, 2, 4-Dinitrochlorobenzene, 4-Methylaminophenol sulphate, Methyldibromo glutaronitrile, 2-Mercaptobenzothiazole, Ethylene glycol dimethacrylate and Cinnamyl alcohol were considered sensitisers, while Salicylic acid, Isopropanol, Lactic acid and Glycerol were deemed as non-sensitisers in KeratinoSens™ assay. These results validate the capability of KeratinoSens™ assay to predict hazard and potency of skin sensitizers in both qualitatively (sensitizing potential) as well as quantitatively (sensitising concentration) and shows this assay as a viable replacement for the in vivo sensitization tests.
ASSOCIATION NUMBER: 3381  Poster Board Number: P171
TITLE: Assessment Of Skin Sensitisation Via In Vitro Human Cell Line Activation Test Method (h-clat)

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: M. V. Patel¹, P. Mishra¹, K. Vashi¹, I. Barad¹, R. Nagane¹, R. Date¹, and V. Piccirillo². ¹Jai Research Foundation, Valvada, India; and ²VP TOX, LLC, Ashburn, VA.

KEYWORDS: Alternatives to Animal Testing; Aquatic Toxicology

ABSTRACT: Skin sensitisation resulting in allergic contact dermatitis (ACD) is a common occupational and environmental health issue. Many hundreds of chemicals have been implicated as skin sensitisers. Classically in vivo tests such as local lymph node assay and Buehler test were methods of choice to study sensitisation potential of chemicals, however quick throughput in vitro assays (eg. human cell line activation test (hCLAT)), are being developed as replacements for these in vivo tests. Unlike these in vivo tests which involves use of laboratory animals, hCLAT is performed using THP-1 cell line, a surrogate of dendritic cells. The present study was conducted to validate in vitro human cell line activation test by evaluating skin sensitisation potential of different classes of chemicals. The chemicals included an in vivo extreme sensitisier (2, 4-Dinitrochlorobenzene), an in vivo strong sensitiser (4-Phenylenediamine), in vivo moderate sensitisers (Nickel sulfate, 2-Mercaptobenzothiazole), in vivo weak sensitisers (R(+) Limonene, Imidazolidinyl urea) and in vivo non-sensitisers (Isopropanol, Glycerol, Lactic acid, 4-Aminobenzoic acid). All chemicals were tested in two independent dose range finding experiments to determine CV75 value (concentration resulting in 75% cell viability of test chemical as compared to vehicle control). The expression levels of CD86 and CD54 cell surface markers were assessed in two or three independent experiments, following treatment of THP-1 cells with eight concentrations of each test chemical followed by incubation of 24 ± 0.5 h at 37 ± 1°C under 5 ± 1% CO₂. Following incubation, cells were stained with IgG1, CD86 and CD54 antibodies and propidium iodide and analysed on flow cytometer to evaluate expression levels of CD86 and/or CD54 markers at sub-cytotoxic concentrations. The relative fluorescence intensity (RFI) values of CD86 and CD54 markers were calculated and used to predict test chemicals as “positive” or “negative”. Our study clearly predicted known in vivo sensitising chemicals as “positive” and known in vivo non-sensitizing chemicals as “negative”. The present study showed high rate of accuracy for prediction of skin sensitisation when compared with in vivo prediction. Thus, this in vitro model can be considered a valid alternative to evaluate skin sensitisation potential of test chemicals.

ABSTRACT NUMBER: 3382  Poster Board Number: P172
TITLE: Establishment of an In Vitro Hepatocarcinogenesis Model: Cell Invasion, Migration, and Proliferation of AML12 Cell Line Cultured in Presence of NNK


KEYWORDS: Carcinogenesis; In Vitro and Alternatives; Liver

ABSTRACT: Hepatic neoplasias are very prevalent and preventive or therapeutic approaches are necessary. Many in vivo models of hepatocarcinogenesis are available, however, they are expensive and request the use of many animals. Therefore, the development of in vitro models for these neoplasms becomes essential to understand the molecular alterations associated with the carcinogenesis process,
and their control. This study aims to establish an in vitro model of hepatocarcinogenesis with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Here we present the cell growth, invasion and migration rate during in vitro culture. Normal hepatocyte cell line derived from mice (AML 12, Mus musculus, CRL-2254) was cultured in the presence of carcinogenic agent NNK (100 pM). The experiment was divided into two groups: treatment group, which was submitted to 30 cycles of carcinogen exposure; control group. Methods: For proliferation assay three independent experiments were performed in triplicate. Cell proliferation capability was measured by analysis of cell cycle using propidium iodine staining and flow cytometry. Invasion and migration assay was performed in matrigel-coated transwell chambers and invasive cells were measured using the cell viability reagent Alamar blue (Resazurin dye). The absorbance was read at 570nm in a spectrophotometer. Statistical analysis was performed using Student t-test. P values<0.05 were considered significant. In the proliferation evaluation, treatment group (2.49% ± 0.8502) showed a higher percentage of cells in S phase when compared with control group (10.42% ± 7.709 ) (P<0.05). In the invasion and migration assay, the results show that the NNK30 group (0.08383 ± 0.01915) presented a statistically significant higher invasion and migration index (P=0.0352) than the control group (0.05433 ± 0.02268). These results indicate NNK carcinogen was capable of increasing the growth and invasion rate of normal hepatocytes after 30 cycles exposure. The in vitro model has been established; further characteristics are under study, and it may contribute to chemoprevention studies.

ABSTRACT NUMBER: 3383    Poster Board Number: P173
TITLE: Classification of EPA Ocular Irritants and Non-Irritants by the OptiSafe Test Method
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KEYWORDS: Alternatives to Animal Testing; Predictive Toxicology
ABSTRACT: The EPA eye irritation classification system is routinely used to categorize ocular toxicity. The EPA system classifies chemicals that damage the eye after 24 hours (Category III, II, or I) and those that do not cause damage (Category IV). This latter EPA classification is aligned with the standard definition of an “ocular non-irritant” and is appropriate for test substances routinely applied to the eye area. The OptiSafe™ (“Optimized for Safety”) test is a novel, shelf-stable, test-tube based method that can be used to discriminate ocular irritants/corrosives from non-irritants and does not use animal tissues or cells. The OptiSafe™ test determines whether a substance is an ocular non-irritant by measuring damage via a proxy for the corneal stroma (water-soluble molecules), damage to phospholipid bilayers (water-insoluble molecules), and the potential to induce pH extremes in a system (pH buffering system of the eye). Chemicals in this study were selected based on a wide range of EPA classifications, chemical and physical properties, high quality in vivo reference data, and chemical stability. Selected chemicals (38) including surfactants not previously tested were aliquoted into coded vials and tested blind in triplicate. The coded vials were tested, and results were reported as either EPA Category IV (nonirritant) or not (EPA Category III, II, or I). The OptiSafe™ test method applied to these 38 test chemicals achieved a sensitivity of 100% (27/27), specificity was 81.8% (9/11), and overall accuracy was 94.7% (36/38). The better accuracy of OptiSafe™ (~ 95%) versus the OECD Test Guideline 492 EIT method (~ 86%) is best attributed to its higher sensitivity. These results suggest that OptiSafe™ may be an important tool in the
complete classification of eye hazards, especially for surfactants, as well as cosmetics and other substances applied to or around the eye.

**ABSTRACT NUMBER:** 3384  **Poster Board Number:** P174  
**TITLE:** Increased Throughput and Cryopreservation of Precision-Cut Lung Slices Extend the Utility of Human-Relevant, 3-Dimensional Pulmonary Test Systems  

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**KEYWORDS:** In Vitro and Alternatives; Predictive Toxicology; Inhalation Toxicology  

**ABSTRACT:** Human-relevant, in vitro/ex vivo assays are considered an ethical and economically viable manner by which to screen the thousands of chemicals requiring hazard assessment. Of the 3-dimensional models, human precision-cut lung slices (PCLS) are often considered the most physiologically relevant pulmonary test system, but lower throughput and difficulties in cryopreservation have hampered PCLS use. We have modified a tissue slicer to accommodate 3 tissue cores for simultaneous slicing. Increased slice production was quantified using agarose and tissue cores in the slicer. To evaluate cryopreservation of PCLS, we have tested 5 cryopreservation formulations using PCLS (frozen on the day of slicing, or after overnight culture). Thawed slice viability in each of the groups was assessed with the WST-8 viability assay, prior to fixation and histological evaluation. The slicer modification resulted in 2.8-fold and 2.4-fold more slices from agarose cores, and lung cores, respectively. Cryopreservation efforts indicated freezing after slicing yields better average viability (48-73% of fresh, non-frozen control) than culturing overnight and freezing (13-54% of control) when assessing health over 4 days, post-thaw. Cryopreservation buffers containing University of Wisconsin preservation solution preserved viability the best (54%-90% of non-frozen control). Histological findings concurred with WST-8 viability results and indicated the retention of healthy lung tissue features (>75% of normal), post-thaw. The increased PCLS production indicates larger (or multiple) studies can be initiated from one donor lung. The promising cryopreservation results suggest slices can be banked and utilized at a later date, potentially even allowing the same donor’s tissue to be used repeatedly.

**ABSTRACT NUMBER:** 3385  **Poster Board Number:** P175  
**TITLE:** Ethanol as an Alternative Vehicle for Determining Skin Sensitization Potential Using the Human Cell Line Activation Test (h-CLAT)  

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**ABSTRACT:** Proper identification and classification of the skin sensitization potential for new consumer products, chemicals, and pharmaceuticals are important for hazard communication and subsequent risk management, upon which in vitro toxicology methodologies are heavily relied. The h-CLAT method, as defined by the OECD 442E test guideline, uses the dendritic cell proxy THP-1 monocyctic cell line, to addresses the third key event in the sensitization Adverse Outcome Pathway (AOP). The third key event
in the AOP is marked by dendritic cell activation, during which time the cell surface markers CD86 and CD54 are induced. In this assay, if a test article (a volatile liquid fragrance) induces CD54 and/or CD86 expression to a level of at least 200 and 150 RFI, respectively, it is predicted to be a skin sensitizer. A major limitation to the versatility of the h-CLAT is the number of recommended vehicles: Saline, media, or dimethyl sulfoxide (DMSO). However, the OECD 442E test guideline states that another vehicle may be used if sufficient scientific rationale is provided. In this study, the test article was not soluble in the recommended vehicles. Therefore, we sought to determine a suitable alternative vehicle and provide a sufficient scientific rationale for using such a vehicle. A previous in-house study suggested ethanol to be a potential alternative vehicle in this test (Abstr. No. 2264; SOT 2019), so the test article’s solubility was tested in ethanol. When it was determined the test article was indeed soluble in ethanol up to the recommended 100 mg/ml test concentration, the h-CLAT was performed as otherwise recommended by the guideline. Additionally, 2-mercaptobenthiazole and isopropanol; positive and negative controls, respectively, were concurrently tested using ethanol as their vehicle. The h-CLAT correctly predicted the isopropanol negative control not to be a skin sensitizer, while correctly predicting the positive control 2-mercaptobenthiazole to be a skin sensitizer. Using ethanol as the vehicle, the test article was predicted to be a skin sensitizer, with the RFI levels of CD54 and CD86 being induced above 200 and 150, respectively. Here we provide scientific evidence and rationale for ethanol to be used as a suitable vehicle with the h-CLAT method.

ABSTRACT NUMBER: 3386       Poster Board Number: P176
TITLE: Exosomes as a Systemic Mediator of Nanotube-Induced Dysfunction
AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: C. G. Canal1, E. Mostovenko1, T. Young2, A. Erdely3, M. J. Campen2, and A. K. Ottens1. 1Virginia Commonwealth University, Richmond, VA; 2University of New Mexico, Albuquerque, NM; and 3NIOSH, Morgantown, WV.
KEYWORDS: Nanoparticles; Proteomics; Neurotoxicology
ABSTRACT: Extra-pulmonary effects of nanoparticle inhalation have been well documented; however, mechanisms for conveying peripheral bioactivity remain elusive. Recent studies point to indirect molecular mediators shed into the circulation. Protein and lipid alternatives to cytokines or chemokines have been proposed, yet the diversity among responses suggests a broader assemblage of factors. Recently we have proposed proteolytic fragment peptides as an additional source of bioactivity, which interestingly were significantly associated with exosomal proteins after enrichment analysis. Here we tested whether modeled exposure to multi-walled carbon nanotubes (MWCNT) could alter the composition and bioactivity of circulating exosomes. Male C57BL/6 mice were exposed by oropharyngeal aspiration to 10 or 40 µg MWCNT-7 or vehicle dispersion media (0.6 mg/ml mouse albumin and 0.01mg/ml DPPC) with serum collected 4 h after. Serum-exosomes were isolated using size exclusion chromatography (SEC), with purity affirmed by Western blot, electron microscopy and particle size assays. A dose-dependent increase in the quantity and size of serum exosomes was observed with MWCNT exposure, reaching significance at the 40 µg dose. Subsequent proteomic mass spectrometry revealed that MWCNT treatment significantly altered serum exosome protein cargo, with particular increases in focal adhesion proteins and decreases in protease inhibitors, changes consistent with altered endocytosis and signaling. Following up on earlier work that showed MWCNT exposure impaired the blood-brain barrier and promoted neuroinflammatory glial responses, we treated primary astrocyte cultures with the exosomal fraction. GFAP immunofluorescence showed a dose-dependent reactivity of
astrocytes, with thicker processes and increased GFAP staining. Overall, results here demonstrated that MWCNT exposure has a significant effect on circulating exosomes, altering their trafficked cargo, cell-adhesion characteristics, and bioactivity. Consistent with barrier disruption and neuroinflammation in the brains of exposed animals, MWCNT-altered serum-exosomes promoted an astrogliosis phenotype that supports their involvement in driving neurological outcomes after nanoparticle exposure.

**ABSTRACT NUMBER:** 3387  
**Poster Board Number:** P177

**TITLE:** Impact of Engineered Nanomaterial Exposure on Lung Toxicity and Epithelium

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**KEYWORDS:** Metals; Macrophage; Cytotoxicity

**ABSTRACT:** Lung toxicity after exposure to engineered nanomaterials (ENMs) is related to alveolar macrophage removal of particles and local dose. To study how ENMs may alter the balance of epithelial injury and repair at non-cytotoxic concentrations, a combination of *in vivo* and *in vitro* approaches were used. *In vivo* studies of the interaction of ENM exposure and wound healing utilized a rat model exposed to ozone and ENM. *In vitro*, differentiated epithelial cells were used to determine the impact of various ENMs on acute cytotoxicity. Adult male mouse primary tracheal epithelial cells were differentiated at an air liquid interface and exposed to 8 ENMs: zinc sulfide (ZnS 100nm), vanadium pentoxide (V\(_2\)O\(_5\) 100nm), magnesium oxide (MgO 20nm), tungsten oxide (WO\(_3\) 15nm), zinc oxide (ZnO 50 nm), titanium dioxide of two sizes (TiO\(_2\) 30nm and 100nm), and silver nanoparticles (AgNP 20nm). Cell density and permeability was assessed after a 24-hour exposure. Our previous studies found 6 metal oxide nanomaterials do not significantly impact cell density or permeability at concentrations less than 250µg/mL. Indeed, 7 out of 8 of the current materials tested did not impact cell density even at 250µg/mL. However V\(_2\)O\(_5\) at 250µg/mL and 100µg/mL significantly decreased cell density. To investigate the impact of a not directly cytotoxic ENM exposure in an injured lung, adult male Sprague-Dawley rats were nose only exposed to 1% Ag(SiO\(_2\)) for 6 hours and a subset of rats were exposed to ozone at 1.0 ppm for 6 hours the day prior to filtered air or Ag(SiO\(_2\)) exposure. Both bronchoalveolar lavage (BALF) and lung tissue was collected. Autometallography (AMG) on Ag(SiO\(_2\)) exposed animals demonstrated silver staining in both macrophages and BALF solution only in the Ag(SiO\(_2\)) exposed groups. This was verified with hyperspectral darkfield images on AgSiO\(_2\) animals to show characteristic silver spectra in both macrophages and BALF solution, matching with the AMG stain. Cell debris indicated toxicity was present in animals exposed to both ozone and AgSiO\(_2\) and more nanoparticles positive for silver spectra were found in the dual exposed group than in those exposed to AgSiO\(_2\) alone. In conclusion, while lung epithelial cells are resilient to cytotoxicity, it is evident that in a wounded model, nanomaterials such as AgSiO\(_2\) are more persistent which may impact toxicity. *Funded by U01 ES027288.*
ABSTRACT NUMBER: 3388     Poster Board Number: P178

TITLE: Anticancer Efficacy of Phytosynthesized Silver Nanoparticles in Colorectal Cancer Cells (Caco-2) via Beclin-1 Mediated Autophagy

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ABSTRACT: Silver nanoparticles (Ag-NPs) is one of the promising nanoparticles and claimed to induce cytotoxicity in different cell line but its function on induction of autophagy mediated cell death is remaining to be delineated. Because autophagy is an exactingly controlled catabolic process and involves intracellular components degradation through lysosome and promising to prevent cancer. Therefore, the aim of this research was to elucidate the function and mechanism of green silver nanoparticles induced autophagic cell death on Colorectal cancer cell (Caco-2 cell) line in vitro. Ag-NPs were successfully synthesized from the reduction of Ag⁺ using AgNO₃ solution as a precursor and Brassica rapa var. nipposinica leaf extracts as a reducing and capping agent. The characterization of Ag-NPs was carried out using UV-vis spectrometry, energy dispersive X-ray (EDX) spectrometry, Fourier transform infrared (FT-IR) spectrometry, Field emission scanning electron microscopy (FESEM), X-ray diffraction (XRD), Atomic absorption spectrometry (AAS), and Transmission electron microscopy (TEM). Characterization confirmed that synthesized particles are spherical shaped face entered cubic structured and nanosized crystalline phased. With the 12 h exposure of three different concentration of Brassica Ag-NPs (1, 5, and 10 µg/mL) in to Caco-2 cell line, cell viability decreased significantly in a concentration dependent manner. Moreover, increase of lactate dehydrogenase (LDH) and a decrease of GSH is a mark of induction of intracellular reactive oxygen species. Finally, upregulation of Ikb, Beclin-1, P53, Lc3-II, cleaved caspase 3 and down regulation of NF-kB, Akt and mTOR is evident of autophagy regulated cell death in Caco-2 cell. Therefore, for the first time we showed Brassica Ag-NPs induced Beclin-1 mediated autophagic cell death in caco-2 cells. Thus, this finding supports a novel function of Brassica Ag-NPs in promoting death of colorectal cancer cell.

ABSTRACT NUMBER: 3389     Poster Board Number: P179

TITLE: Role of Substrate Stiffness in Human Fibroblast Responses to Multi-wall Carbon Nanotubes

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: A. E. Simmons, I. Wong, and A. B. Kane. Brown University, Providence, RI.

KEYWORDS: Nanoparticles; Predictive Toxicology

ABSTRACT: Mechanical properties of the substrate are likely to impact pulmonary fibroblast differentiation following exposure to rigid engineered nanomaterials (ENMs). 2D in vitro assays for fibrogenic nanomaterials are usually performed on stiff, plastic tissue culture substrates that are 1000 times stiffer (~1GPa) than human lung tissue (~1.6kPa). Stiff substrates may produce aberrant mechanosignaling that outcompetes rigid ENM mechanostimuli. Additionally, the relationship between nanomaterial interaction and autocrine and paracrine signaling at the single cell level is not well understood. To tackle this problem, I aim to evaluate a model of fibroblast to myofibroblast differentiation following exposure to ENMs using high-content image analysis on lung tissue substrate stiffness (~2kPa). I hypothesize that exposure to rigid ENMs drives fibroblast to myofibroblast differentiation through p-SMAD2/3 transcription factor nuclear translocation and both mechanical and
paracrine signaling and that this response is dampened in traditional cell culture assays. IMR90 cells (human normal, lung, embryonic fibroblasts) were exposed to ENMs with a variety of physical properties and geometries, including carbon black spherical particles (M120), crocidolite asbestos fibers, Mitsui multi-wall carbon nanotubes (Mitsui-7), and two-dimensional graphene to evaluate material properties that drive fibroblast to myofibroblast differentiation on soft and stiff substrates. Significant p-SMAD2/3 translocation to the nucleus and subsequent myofibroblast differentiation in response to exposure to rigid carbon nanotubes for 48 hours was detected on soft substrates as compared to stiff cell culture substrates. This response is significantly related to direct cell/nanomaterial interaction as evaluated by high-content quantitative image analysis of coordinate and percent area interaction. Future work will evaluate the relationship between nanomaterial interaction, TGF-β1 expression, α-smooth muscle actin (α-SMA) polymerization, and collagen expression in differentiated myofibroblasts. This research is supported by the NIEHS Training Grant T32 ES07272 and the NIEHS Superfund Research Program P42 ES013660.

ABSTRACT NUMBER: 3390    Poster Board Number: P183
TITLE: Porcine Model of Arsenicals Injury


KEYWORDS: Chemical & Biological Weapons; Inflammation; Mechanisms

ABSTRACT: Vesicants also referred to as “blister agents,” were developed as chemical weapons to debilitate the military and civilian populations during World War-I/II. These are highly reactive chemicals cause rapid and severe painful inflammatory and tissue damaging responses immediately after exposure. Arsenicals are also vesicants, which have also been weaponized. The molecular pathogenesis of inflammation and tissue injury following exposure to these chemicals is poorly understood primary due to lack of appropriate animal models. Here, we developed a porcine (Gottingen minipig) model to demonstrate dose- and time-dependent progression of the cutaneous injury. Cutaneous exposure to lewisite and other arsenicals diphenylchlorarsine (DPCA) and diphenylcynoarsine (DPCYA) caused marked redness, which quickly turned to fluid filled blisters. In this regard, lewisite was most potent as compared to DPCA and DPCYA at molar equivalent doses. Microscopically, a dose- and time-dependent increase in both size and number of micro-vesicants (mv) could be seen. MV are characterized by the separation of dermis from epidermis. In addition, a huge infiltration of inflammatory leukocytes occurred as early as 8 h after arsenicals administration, which progressed with time up to 72 h. These inflammatory responses were characterized by the release of pro-inflammatory cytokines IL-1β, IL-6, IL-8, IL-10 and IL-1RA along with the recruitment of T cells, macrophages & neutrophils in the dermal region of the injured skin. The underlying mechanism involved enhanced production of Reactive Oxygen Species (ROS) and associated upregulation of unfolded protein response (UPR). UPR transcription factor, ATF4 and its target CHOP were augmented. Furthermore, arsenical exposed skin showed disruption of tight (Yap/ZO-1) and adherens (Yap/α-Catenin) junctions. Alterations in these molecular signaling pathways following arsenical exposure were associated apoptotic with cell death of epidermal keratinocytes, which progressed to massive tissue damage by invoking RIP kinases-regulated necrosis. Our data demonstrate that porcine model, is a faithful model, which could recapitulate toxicity of vesicants as observed in humans.
ABSTRACT NUMBER: 3391  
Poster Board Number: P184

TITLE: Pulmonary and Hematological Effects of Phosphine Poisoning

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KEYWORDS: Inhalation Toxicology; Hematotoxicity; Mechanisms

ABSTRACT: Phosphine gas (PH3) is a widely used pesticide in the grain storage industry and a common reagent in the synthesis of complex compounds. Despite its commercial uses, PH3 also poses a substantial threat to human health due to its highly toxic nature and widespread availability. While PH3 affects all major organ systems and is thought to specifically target oxidative respiration, the exact mechanism of PH3 toxicity remains largely unknown. An improved understanding of underlying PH3 interactions is essential for focused research in the development of countermeasures. Female rats were exposed to PH3 at concentration-time products ranging from 15015 to 21250 ppm × min using a custom whole-body exposure system with integrated real-time physiological monitoring capabilities. Post-exposure, rats were euthanized at 1, 3, 6, or 24 hrs, and whole blood was collected for further biochemical analyses. PH3 exposure induced an increased respiratory drive shown by elevations in minute volume of 150 - 200% above control animals. Despite this marked increase, clinical observations, including hiccups, ataxia, postictal-like state, and convulsions, collected during and after PH3 exposure indicate a response to hypoxic conditions. An increase in blood plasma absorbance at 415 nm post-exposure also suggests possible hemolysis, which may occur under hypoxic conditions. Combined, these results imply an interaction between PH3 and blood which may decrease the bioavailability of oxygen (O2) in the absence of O2-deficient conditions. To explore possible hematological reactions, a bubble-through system was designed to examine the interactions of various gases with hemoglobin (Hb) solutions. Comparisons between the absorption spectra of air- and PH3-exposed Hb showed a decrease at approximately 410 nm, indicating a possible interaction between PH3 and heme. This suggests that PH3 and O2 may interact with Hb in an analogous manner. These results also indicate possible competitive binding that may interfere with O2 transport or reaction between PH3 and O2 that may lead to O2 sequestration. Both mechanisms result in decreased O2 bioavailability, which may be a driver of PH3 toxicity. These insights provide a foundation for the focus of further studies involving other heme-containing compounds and can lead to the development of mechanistic therapeutics.

ABSTRACT NUMBER: 3392  
Poster Board Number: P185

TITLE: Large-Scale Transcription and Pathway Analysis of Cyanide-Exposed Hearts


KEYWORDS: Inhalation Toxicology; Chemical & Biological Weapons; Gene Expression/Regulation

ABSTRACT: Severity of metabolic poisoning in smoke inhalation victims from structure fires is related to the combined toxicity of carbon monoxide and hydrogen cyanide (HCN). In addition to cyanide’s wide industrial use and availability, its fast action to induce incapacitation and death has made HCN a potential chemical weapon in a military or terrorist context. Symptoms of cyanide poisoning include...
cardiorespiratory suppression and arrest, and Parkinson’s-like disorders are observed later in life in some survivors. Current FDA-approved cyanide antidotes such as Nithiodote® are administered intravenously, slow in action, and consequently unsuitable for use in mass casualty incidents. In pursuit of a better countermeasure, dimethyl trisulfide (DMTS) is being developed as an intramuscular antidote for next generation of field treatment of cyanide poisoning. To contribute to the development of a safe, effective, and field-ready cyanide treatment, we determined the molecular pathways altered by HCN exposure in cardiac tissues with and without countermeasures. Unanesthetized adult male mice were exposed to HCN at 324 ppm or air in nose-only inhalation chambers and treated with vehicle, 100 mg/kg DMTS, or 200 mg/kg sodium thiosulfate (STS). Cardiac tissues of surviving animals were collected at 1, 2, 4, and 24 hours post-exposure, and total RNA was isolated and processed for microarray analysis. Identified genes (p-value ≤ 0.05, fold change ≤ -1.5 or ≥ 1.5) were imported into Ingenuity Pathway Analysis software to identify canonical and biological pathways and diseases and disorders altered in response to HCN exposure with or without treatment. The analysis indicated that HCN induced extensive disruption of transcriptional homeostasis particularly at 24 h without treatment, including gene networks involved in cardiac hypertrophy, cardiac necrosis, hypoxia-inducible factor signaling and neuronal cell death. Importantly, a large subset of genes was restored to normal level by DMTS treatment, and such genes are associated with cardiac fibrosis, oxidative stress response of heart and coronary artery disease. Our results support the development of DMTS as a therapeutic, and further data analysis may provide insight into therapeutic management of acute cyanide exposure and mechanisms for long-term pathophysiology of cyanide survivors.

ABSTRACT NUMBER: 3393    Poster Board Number: P186
TITLE: Transcriptomic Characterization of Phosgene-Induced Cardiotoxicity and Evaluation of Candidate Therapeutics


KEYWORDS: Chemical & Biological Weapons; Genomics; Inhalation Toxicology

ABSTRACT: Phosgene (CG) is a highly toxic industrial chemical that is used extensively as an intermediate in the production of plastics, synthetic chemicals, and pesticides. Approximately 1 million tons of phosgene are produced each year in the United States. Due to the large scale industrial use of CG and current lack of therapeutic options, the possibility of accidental exposure or deliberate release of CG is an area of concern. Inhalation of CG results in severe respiratory symptoms, with the most prominent being distal lung damage and pulmonary edema. Delayed toxic effects of CG inhalation include hemolysis and cardiovascular toxicity leading to cardiovascular collapse. The mechanism(s) causing the cardiotoxic effects of inhaled CG has not been elucidated. In order to investigate these pathways a transcriptomic analysis was performed using male CD-1 mice exposed to either CG (8-10 ppm) or filtered air using whole-body inhalation for 20 minutes. Animals were administered either Sildenafil (SD, p.o.) or Captopril (CP, i.p.)15-20 minutes post-exposure. The heart was collected from animals at 4, 8, 12, or 24 hours post-exposure. Total RNA was extracted and processed for microarray analysis. Genes presenting significant differences between exposure groups were identified using Partek Genomics Suite and mapped to toxicologically relevant pathways using Ingenuity Pathway Analysis (IPA) software. CG-exposed animals displayed changes in gene expression associated with cardiovascular disease pathways,
cardiac enlargement, and cellular function/maintenance beginning at 8 hours post-exposure. Treatment with SD or CP resulted in decreased activation of the acute phase response signaling pathway, compared to untreated animals, beginning at 8 hours post-exposure. Additionally, SD treatment resulted in decreased activity of the SAPK/JNK signaling pathway, relative to untreated animals. Furthermore, CP treatment activated pathways associated with cell viability, survival, and homeostasis. These data suggest that the heart responds promisingly to therapeutics intended to treat CG exposure and can be used to identify additional molecular pathways and druggable targets involved in phosgene intoxication.

**ABSTRACT NUMBER:** 3394  
**Poster Board Number:** P187  
**TITLE:** *In Vivo* Anti-inflammatory Efficacy of NDH-4338 Nanosuspensions in Thermosensitive Gels for Treating Nitrogen Mustard Skin Exposure

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**KEYWORDS:** Chemical & Biological Weapons

**ABSTRACT:** Sulfur mustard is a chemical warfare vesicant that damages exposed skin, eye, and lung tissues. Nitrogen mustard (NM) is used as a model for sulfur mustard, as it shares similar mechanisms of cytotoxicity and dermal injury. New therapies to treat mustard burns are needed to effectively reduce tissue inflammation and promote rapid healing in order to avoid infection. Nanosuspensions (NSs) are useful in the delivery of lipophilic drugs, as the reduced particle size can improve drug availability by providing an increased dissolution rate. In this study, NS technology was used to enhance the delivery of NDH-4338, a multi-inhibitor prodrug construct used to inhibit cyclooxygenase and acetylcholinesterase. These enzymes are upregulated by NM skin exposure and further exacerbate the injury. NSs were prepared via precipitation and ultrasonication in the presence of d-α-tocopheryl polyethylene glycol 1000 succinate (a stabilizer), in a Pluronic F127-based thermosensitive gel. The anti-inflammatory efficacy of two NS formulations, 1% and 2% w/v, was evaluated in CD-1 mice. Mice were exposed to 20 μl of 1M NM (20 μmol) in 20% deionized water/80% acetone (v/v) on day 1. The experiment consisted of six groups (n=6), including three treatment groups (1 and 2 % w/v NDH-4338 twice daily [BID], and 2% w/v once daily), and three control groups (the first exposed to NM without treatment, the second unexposed to NM and treated with vehicle once daily, and a third group unexposed to both the vesicant and NS treatments). After 3 days, punch biopsies of the treated area were weighed to assess the degree of skin inflammation in comparison to the control groups. Biopsies obtained from the NM exposed control group weighed an average of 130 mgs, while those from the unburned/untreated control group weighed an average of 55 mgs. All three NS treatment groups demonstrated a positive anti-inflammatory effect, however, inflammation was completely eliminated in the 2% NS/BID group that yielded an average punch biopsy of 57 mgs after treatment. In conclusion, the 2% NS dosed BID will be the lead immediate release formulation in studies investigating the controlled release properties of NDH-4338 loaded polymeric nanoparticles on healing efficacy.
ABSTRACT NUMBER: 3395    Poster Board Number: P188
TITLE: Differential Seizure Dynamics following Sarin- vs. Soman-Induced Status Epilepticus and Anticonvulsant Treatment.


KEYWORDS: Chemical & Biological Weapons; Neurotoxicity; Pesticides; Organophosphates

ABSTRACT: Pharmacological control of status epilepticus (SE) induced by organophosphate (OP) nerve agents (NA) is essential to preventing progressive brain damage and neuroinflammation. The response to anticonvulsant treatment, however, has been shown to vary after exposure to different NAs. Here we compared multiple aspects of soman- versus sarin-induced SE and the responses of this activity to anticonvulsant treatment in order to determine differences in the electrophysiological dynamics between these agents. Mice were surgically prepared two weeks prior to the experiment with electrodes to record brain activity. The oxime HI-6 (50 mg/kg; IP) was given prior to nerve agent exposure to increase survival without affecting the onset of seizure activity. Mice were exposed to the nerve agent soman (147 µg/kg; SC) or sarin (256 µg/kg; SC; control animals received an equivalent volume of saline) to elicit seizure activity followed by the anti-muscarinic atropine (1-10 mg/kg; SC). SE was terminated with midazolam (5 mg/kg; IP) and the centrally active oxime monoisonitrosoacetone (MINA) (50 mg/kg; SC) at 30 min following onset. Brain tissue was collected at 7 d to evaluate neurodegeneration. Both agents were found to have similar electrophysiological properties in relation to the onset and intensity of SE. Differences were observed in the decay of seizure power following max intensity and following anticonvulsant treatment, particularly in the delta band. The results suggest that underlying neurochemical or neurostructural mechanisms may play a role in the seizure dynamics and anticonvulsant responses following exposure to different NAs.

ABSTRACT NUMBER: 3396    Poster Board Number: P189
TITLE: Assessment of Nerve Agent Reactivators Using the Mouse Phrenic Nerve Hemidiaphragm from Wild Type or Human Ache Knock-In Mice


KEYWORDS: Chemical & Biological Weapons; Organophosphates

ABSTRACT: Nerve agents are highly potent acetylcholinesterase (AChE) inhibitors that lead to an inability of the AChE enzyme to hydrolyze the neurotransmitter acetylcholine (ACh). The resulting build-up of ACh leads to overstimulation of neuromuscular junctions, glands and central synapses, which results in salivation, bronchoconstriction, tremors, seizures and central respiratory failure. Treatment with atropine, a reactivator and an anticonvulsant can provide life-saving therapy. We have employed an ex vivo mouse phrenic nerve hemidiaphragm preparation to evaluate the efficacy of a reactivator in restoring physiologic function following inhibition by the nerve agents sarin (GB) or cyclosarin (GF) in wild type mice (C57BL/6J) or human AChE knock-in mice (C57BL/6-AChEtm1.1loc/J), 8- to 10-week-old males. Hemidiaphragm/ phrenic nerve bundles/ribs were dissected and maintained in tissue baths with oxygenated (95%O2/5%CO2) Tyrode’s buffer solution at 37°C. Tetanic stimulation parameters were 100
Hz, 2 sec train, 5 volts, resting tension ~3.5 g every 10 min. The experimental protocol consisted of five phases: Acclimation (buffer only), 1 hr; Baseline (buffer only), 30 min; Agent exposure, 30 min; Reactivation, 1 hr; and Recovery (buffer only), 30 min. The agent bath concentrations used were 5 x 10^{-6} M for GB and 1 x 10^{-6} for GF. Bath concentration for each reactivator was 200, 100 or 50 uM. The reactivators evaluated were MMB-4 and 2-PAM. MMB-4 gave significant reactivation of GF- and GB-inhibited activity in both wild types and knock-in mice, with slightly better reactivation observed in the knock-ins. This was especially notable at the 50 uM dose. The oxime reactivator currently fielded by the US Army is 2-PAM. In this study it was only evaluated against GF and was shown to be a poor reactivator in the diaphragms of both wild type and human AChE knock-in mice. This work supports the development of an animal model expressing human AChE that can be used in the evaluation of reactivators. These results indicate that MMB-4 provides an improvement over 2-PAM and has the benefit of also being centrally active, which could better protect the CNS in an in vivo model. This research complied with the Animal Welfare Act and implementing Animal Welfare Regulations and adhered to the principles noted in The Guide for the Care and Use of Laboratory Animals.

**ABSTRACT NUMBER:** 3397  
**Poster Board Number:** P190  
**TITLE:** Aberrant Changes in Metabolic Landscape Are Associated with Vesicant Action of Arsenicals  
**KEYWORDS:** Chemical & Biological Weapons; Metabolomics; Inflammation  
**ABSTRACT:** Cutaneous exposure to warfare arsenicals such as lewisite causes painful inflammation and blistering. The underlying mechanism associate with devastating action of these chemicals remains undefined. Here, we show that arsenicals-induced changes in cutaneous metabolic landscape occur soon after their exposure and progress with time. The alterations in the energy and lipid metabolism in the skin exposed to Lewisite (LW), Diphenylchloroarsine (DPCA) and Diphenylcyanoarsine (DPCY) were assessed using (RP)/UPLC-MS/MS platform. These metabolomics/lipidomics data suggest that arsenicals target glycolysis and Krebs cycle; and elevate levels of polyunsaturated fatty acids (PUFAs) and cAMP. To further explore the mechanism underlying these changes, we used phenylarsine oxide (PAO) as a surrogate arsenical. We used Ptch+/-/SKH-1 murine model as well as HaCaT and RAW 264.7 cells. PAO treatment caused identical changes in molecular profile of proteins involved in energy and lipid metabolism. Proteins involved in β-oxidation pathway, CPT1A, ACADM and ACADL were elevated. This augmented fatty acid (FA) metabolism is accompanied by the increase in free FAs and inhibited de novo FA synthesis which was observed in LW, DPCA and DPCY exposed skin. These data also suggest an enhanced FA hydrolysis from stored cytoplasmic lipids against high energy demand in arsenical exposed skin. FA oxidation/catabolism are regulated via a complex process involving perilipin-5/PGC1α nexus involving PKA and Sirt1, which also regulate mitochondrial biogenesis and oxidative function. Interestingly, these arsenicals reduce these proteins significantly. HADHA which catalyzes last three steps of mitochondrial β-oxidation was also downregulated. A transcription factor, SREBP1 which involved in de novo lipid synthesis is downregulated. This is further confirmed by the reduced expression of its transcriptional targets ACC1 and FASN. Further evidence for the involvement of SREBP1 in arsenicals-mediated tissue disruption is provided by our observations that its inhibitor Fatostatin exacerbated PAO-mediated cutaneous damage. AMPK diminishes lipid synthesis by dampening the activation of SREBP1. Dorsomorphin, an AMPK inhibitor not only restored SREBP1 activation but also
reduced PAO-induced inflammatory responses and cytokines/chemokines levels. These data identified a novel molecular pathogenesis of arsenicals-mediated cutaneous nociception.

**ABSTRACT NUMBER:** 3398  **Poster Board Number:** P191

**TITLE:** Novel Approaches to Immediate Personnel Decontamination

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Sponsor: H. Maibach

**KEYWORDS:** Chemical and Biological Weapons; Cutaneous or Skin Toxicity

**ABSTRACT:** A mass casualty chemical warfare agent (CWA) contamination event presents a percutaneous decontamination challenge. A mass casualty decontamination event aims to prevent the absorption of CWAs into skin to mitigate adverse health effects. The adverse effects associated with CWA contact are reduced the once CWA skin absorption is mitigated. Typical skin decontaminant studies use a protective ratio (defined as the LD50 of the treatment condition divided by the LD50 of the untreated group) as a metric of decontaminant performance. These ratios relate to death observed in the animal model as opposed to the quantitative measurement of CWA removal from the skin. A quantitative study of the reduction of CWAs sulphur mustard, soman, and [2-{Diisopropylamino}ethyl]-O-ethyl methylphosphonothioate (VX) from the skin model Strat-M and excised pig skin using extraction methods was completed. Extraction verification studies determined the percent recovery of CWAs from pig skin. 95% of the originally applied CWA was recovered from pig skin after a 5 minute duration, with at least 80% of the CWA recovered after 60 minutes. Multiple personnel decontamination products are investigated, including common off the shelf products, field relevant decontaminants such as reactive skin decontamination lotion (RSDL), bleach, soapy water, and novel technologies such as Zirconium Hydroxide. The products were applied to substrates contaminated with neat CWA and evaluated via solvent extraction to determine the amount of CWA removed by the decontaminant. Notable results show that the sorbent powder Zirconium Hydroxide is able to remove over 90% of initially applied CWAs (approximately 2 mg) from excised pig skin. In most CWA-pig skin combinations, the Zirconium Hydroxide powder removed more CWA from the pig skin than RSDL, a fielded decontaminant. The remaining agent results for a decontaminant on pig skin was typically lower than the same product on the Strat-M, but the overall performance trends were the same across the two substrates. The comparison of the Strat-M and pig skin indicate that Strat-M can be a useful tool in base lining decontamination technologies for skin. The study successfully illustrates differences in the amount of CWA retained in or on the Strat-M or pig skin after a decontamination treatment and provides a novel high throughput technique to establish an initial ranking of decontaminant product efficacy.
ABSTRACT NUMBER: 3399    Poster Board Number: P192
TITLE: Molecular Mechanism Underlying Nordihydroguaiaretic Acid-Mediated Protection against Arsenical-Induced Cutaneous Injuries


KEYWORDS: Metabolism; Inflammation; Cutaneous Or Skin Toxicity

ABSTRACT: Arsenicals are warfare chemicals, which cause severe cutaneous injury. The mechanism underlying these effects remains undefined. In metabolome data analysis, we observed a remarkable increase in metabolites generated by the activation of lipoxygenases. These include 5-HETE, 12-HHTrE and leukotriene B4 (LTB4). Nociceptive leukotriene and prostaglandins are generated by lipoxygenase and cyclooxygenase, respectively. We found an augmented production of prostaglandins also. Here, we investigated the effect of pan lipoxygenase inhibitor, nordihydroguaiaretic acid (NDGA) on arsenical-induced cutaneous injury. Topical application of NDGA significantly downregulated the protein expression of Lipoxygenase-15 in PAO treated Ptch+/−/SKH-1 mice. Furthermore, NDGA mitigated PAO-mediated cutaneous inflammatory response as evident by the decrease in skin bi-fold thickness, erythema and edema. NDGA also significantly ameliorated the PAO-induced levels of pro-inflammatory cytokines/chemokines such as IL6, MCP-1, IL-1β, IL18, GM-CSF, GROα, MIP-1α, MIP-1β, ENA78 and MIP2 in the skin. We show that PAO induced expression and activity of AMPK while reduced the expression of ACC1 and FASN which regulate lipid metabolism. These effects were accompanied by the induced expression of CPT1A, a protein involved in mitochondria transport of fatty acids and ACADM and ACADL, proteins involved in mitochondrial β-oxidation. NDGA treatment ameliorated PAO-induced alterations in lipid metabolism. We also found that inflammatory response induced by arsenicals is regulated at least in part by dysregulated autophagy. NDGA treatment restored the altered levels of autophagy markers, Atg12, Atg5, Atg3, LC3A/B, LC3A and LC3B. AMPK is an upstream regulator of fatty acid metabolism. Treatment with its inhibitor, Dorsomorphin restored fatty acid metabolism, autophagy and diminished inflammation. These data suggest that arsenical mediated inflammation is in part regulated by the lipoxygenase-derived lipid mediators. The underlying molecular mechanism involves AMPK-mediated disruption of lipid metabolism, inhibition of which could provide a novel antidote for blocking the inflammatory pathogenesis by these chemicals.

ABSTRACT NUMBER: 3400    Poster Board Number: P193
TITLE: Toxicity of Zinc Oxide Nanoparticles to Corneal Epithelial Wound Healing


KEYWORDS: Ocular Toxicity; Cytotoxicity; Nanotechnology

ABSTRACT: The cornea, the outermost layer of the eye which is covered by tear film, is a major route of exposure from aerosolized nanoparticles. Furthermore, many commercially available facial products including sunscreens contain zinc oxide nanoparticles (ZnO NP). However, there is a knowledge gap regarding the effects of ZnO NP on the cornea in health and disease. The purpose of this study was to determine the effects of ZnO NP on the cornea with or without wounding. Initially, the viability of immortalized human corneal epithelial (hTCEpi) cells was assessed using the calcein-AM assay following
treatment with ZnO NP (50 nm; 0.01 - 250 µg/mL) for 24 hours. A round wound healing assay with a monolayer of hTCEpi cells was performed using ZnO NP at concentration between 0.05 to 10 µg/mL. Subsequently, ZnO NP (50 µg/mL; n=6) and balanced salt solution (BSS; n=6) were topically applied 6 times daily in vivo rabbits to test their effects to the tear film and corneal epithelial wound healing. The Schirmer tear test, tear meniscus, tear film thickness and breakup time were measured at 1 and 7 days following treatment. Then, an 8-mm epithelial debridement was performed only in the right eye of all rabbits following treatment with ZnO NP or BSS in both eyes. Fluorescein stain and digital photography were performed twice daily to monitor the wound area until complete healing had occurred. Rabbits were subsequently euthanized, and hyperspectral microscopy was performed on histologic sections of the enucleated eyes to determine penetration of ZnO NP. One-way ANOVA followed by Holm-Sidak’s multiple-comparison test was used for statistical analysis. Cell viability and migration were significantly decreased with ZnO NP at ≥ 5 µg/mL (P < 0.001 and P < 0.05, respectively). Prior to wounding, topical treatment of ZnO NP showed no significant changes to the tear film. However, epithelial wound healing was significantly slower in rabbits treated with ZnO NP (P < 0.001) versus BSS. Hyperspectral images demonstrated that more ZnO NP penetrated through all layers of the wounded versus unwounded corneas; ZnO NP were found in the iris stroma and ciliary body of the wounded corneas. Exposure of ZnO NP to the wounded cornea can inhibit corneal epithelial cell migration and may result in accumulation to ZnO NPs within the eye. Further studies should be performed to ascertain the ocular toxicity of ZnO NP in vivo. Supported by NIEHS U01 ES027288 and NEI.
silico models can benefit from additional high quality in vivo data sources (e.g., European Chemicals Agency dossiers) and by including additional variable inputs such as in vitro eye irritation test method results. This work was funded with US federal funds from the NIEHS/NIH/HHS under Contract HHSN273201500010C.

ABSTRACT NUMBER: 3402   Poster Board Number: P195

TITLE: Alcohol Monitoring in Patients Seeking a Transplant for Alcohol Related Liver Disease: Removal from the Transplant List Due to Repeated False Positive Urine Alcohol Secondary to Urinary Tract Colonization with Fermenting Yeast

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KEYWORDS: Clinical Toxicology; Infection

ABSTRACT: Purpose: Inquiry into the case of a poorly controlled diabetic patient (HA1C 8.6) with alcohol related cirrhosis, deactivated from and outside hospital transplant waitlist due to positive urine ethanol tests. She hoped to be relisted at our transplant center and denied alcohol consumption for over 2.5 years. Due to continued positive urine ethanol her team expressed skepticism about her sobriety and candidacy for listing. Of note, her blood glucose was over 300 at each visit and urinalysis revealed glucose over 1,000 mg/dl. We determined a full workup to rule out colonization by a fermenting organism was necessary. Methods and Data: We examined a urine sample found positive for ethanol in our lab, where the lower limit for detection is 20 mg/dl. The specimen was negative for alcohol metabolites ethyl glucuronide/sulfate and same day plasma ethanol was negative. From these discrepant results we hypothesized the result may not reflect drinking. The patient was instructed to give new urine and blood samples and produced ~400 cc of urine, which was immediately transported to lab. Serum Ethanol was again negative. The urine sample was positive for ethanol at 43 mg/dl. Many budding yeast were discovered in the specimen and urine glucose was again over 1000 mg/dl. We centrifuged an aliquot of the original sample and took the yeast free supernatant as specimen A and precipitate as specimen B. Samples were incubated for 24 hours at 4, 25 and 37 degrees C. We also took samples of the yeast containing resuspension and incubated them with the fermentation inhibitor NaF. Results: Compared to the baseline of 43 mg/dl, after 24 hrs, the alcohol level of the yeast resuspension (minus NaF, incubated at 37 degrees C) was impressive at 816 mg/dl. Surprisingly, even the resuspension tube left at 25 degrees C (room temp) had an alcohol level of 476 mg/dl. Culture confirmed non cryptococcal yeast, with speciation pending. Conclusion: We have strong evidence for a case of false positive urine ethanol with wrongful termination from the transplant list, due to urinary tract colonization with fermenting yeast. This argues against using urine ethanol to monitor abstinence, especially in diabetic or immunocompromised patients awaiting a life-saving liver transplant.
ABSTRACT NUMBER: 3404  Poster Board Number: P198

TITLE: Prolonged Exposure to Subconvulsive Doses of Domoic Acid Causes Mitochondrial Injury in Cerebellum and Kidney

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KEYWORDS: Excitotoxicity; Natural Products; Food Safety/Nutrition

ABSTRACT: Domoic acid (DA) is an algal-produced seafood toxin that causes profound neurotoxicity in mammals. Acute exposure to high doses of DA causes convulsive seizures and excitotoxicity via overactivation of glutamate receptors, and oxidative and mitochondrial injury. However, the risk associated with exposures to DA at low doses (non-convulsive) remains poorly understood. Here, we investigated whether repeated exposures to subconvulsive DA causes mitochondrial injury in the cerebellum and kidney, two susceptible tissues enriched with mitochondria and containing glutamate receptors. Mice were exposed to subconvulsive DA (0.42 or 1.7 mg DA/kg) via daily i.p. injections for one week. In cerebellum, exposure reduced respiration per mitochondria via inhibiting flux through electron transport system complex I. Mitochondrial dysfunction was concomitant with reduced concentrations of total glutathione (GSH) and elevated protein expression of superoxide dismutase isoform 1 (SOD1), consistent with elevated oxidative stress. By contrast, in kidney there were no differences in mitochondrial function or cellular redox status after one week of daily DA exposure. As an additional chronic exposure model, we conducted a 34-week weekly i.p. exposure study using one subconvulsive dose of DA (0.75 mg DA/kg). In cerebellum, the prolonged exposure reduced rates of respiration per mitochondria, lowered total glutathione, and elevated expression of SOD1, similar to the 1-week daily exposure results. Interestingly, the prolonged exposure also elicited mitochondrial toxicity in kidney, manifest as reduced rates of respiration per mitochondria. Our results provide further evidence that exposure to subconvulsive doses of DA are not benign, causing subcellular impacts more subtle than overt neurotoxicity.

ABSTRACT NUMBER: 3405  Poster Board Number: P199

TITLE: Examination of Microcystin Neurotoxicity Using Central and Peripheral Human Neurons


ABSTRACT: Microcystins (MC) are cyclic heptapeptides produced by cyanobacteria during algae blooms. Over 50 different congeners are known, and the liver is the main toxicological target organ upon acute exposure. Since microcystins can accumulate in contaminated water and in the food chain, some humans are chronically exposed to low doses that may affect also other organs. This study focuses on the effect of two microcystin congeners (MC-LF and MC-LR) on human neurons. As a model of central neurons, the well-established LUHMES cell line was used. In addition, peripheral neurons derived from induced pluripotent stem cells (iPSC) were studied to account for potential neurotoxicity, independent of blood brain barrier (BBB) passage. Both cultures were found to express the organic-anion-transporting polypeptides (OATP) 1A2 and 3A1, which are known transporters mediating cellular uptake of microcystins. In picomolar concentrations - realistic for human exposure - no effect of MC-LF and MC-LR was observed on peripheral neurons. At high concentrations (10 µM) and prolonged (4 days)
incubation times, cell death was observed for MC-LF but no effect was observed for MC-LR. None of the exposure conditions resulted in specific effects on neurites, as they are commonly observed with chemotherapeutic drugs triggering peripheral neuropathies. Valinomycin, a cyclic peptide produced by Streptomyces, was included as a positive control and showed neurite specific effects in the low nanomolar range. These data suggest that short-term exposures to microcystins do not result in a dying back peripheral neuropathy in man. Also for LUHMES neurons, no effect was observed at concentrations found in man. However, in the micromolar range (2.5-5 µM), a concentration-dependent and time-dependent neurite-specific toxicity was observed for MC-LF. This was not paralleled by a pronounced inhibition of proteinphosphatase 1 and 2A, or expected downstream consequences, such as tau hyperphosphorylation. Similar to peripheral neurons MC-LR did not show any effect in the tested micromolar concentrations in LUHMES. To identify potential alternative mechanisms of toxicity, an analysis of transcriptome wide changes of mRNA was performed, and the alteration pattern will be presented.

ABSTRACT NUMBER: 3406  Poster Board Number: P200
TITLE: Protective Effect of Pituitary Adenylate Cyclase Activating Polypeptide against Chlorpyrifos-Induced Neurotoxicity in Human Neuroblastoma Cells


ABSTRACT: Chlorpyrifos (CPF), an organophosphate insecticide, is widely used worldwide, and classified as a chemical threat agent. CPF represents a concern for the human population. The primary target of CPF toxicity is the central and peripheral nervous system due to its ability to inhibit acetylcholinesterase activity. Several studies identified associations between organophosphate (OP) exposures and neurologic disorders, including deficits in cognition, psychiatric illness, and neurodegeneration. Pituitary adenylated cyclase-activating polypeptide (PACAP) is a pleiotropic bioactive peptide that is widely distributed in the central and peripheral nervous systems. Recent studies have shown a neurotrophic and neuroprotective role of PACAP in many neurological disorders characterized by neurodegeneration, such as cerebral ischemia, traumatic brain injury, Parkinson’s disease and Alzheimer’s disease. This study was aimed to investigate whether PACAP38 prevents the neurotoxic effects of CPF in human neuroblastoma (SH-SY5Y) cells. Cells were treated with CPF, PACAP38 or PACAP antagonist (PACAP6-38) at various concentrations alone or in combination. Cell proliferation, viability and cell death were assessed. Results showed that CPF significantly reduced cell proliferation and viability in a concentration-dependent manner after 24h of exposure. Co-treatment of SH-SY5Y cells with CPF and graded concentrations of PACAP38 (0.01 to 100 nM) for 24h reduced the toxicity of CPF and restored cell viability. Longer incubation with PACAP38 (48h or 72h) did not enhance the effect of the peptide on cell viability. The augmentation of the cell viability and proliferation evoked by PACAP38 was significantly inhibited by the PACAP receptor antagonist PACAP6-38, suggesting that the neurotrophic activity of PACAP is mediated through activation of the adenyl cyclase/PKA pathway. Pretreatment of the cells with PACAP38 for 1h followed by CPF for 24h provoked a significant increase in cell proliferation and viability. The present results provide evidence that PACAP appears to function as a neuroprotective factor that attenuates the neural damage resulting from CPF. An understanding of the PACAP signaling pathways may lead to the development of new prophylactic and therapeutic drugs. 

Supported by Title III.
ABSTRACT NUMBER: 3407 Poster Board Number: P201

TITLE: Comparison of PMA-Induced Multinucleation of Primary Microglia and Microglial Cell Lines


ABSTRACT: As resident macrophages of the brain, microglia play a key role in neural inflammation of many central nervous system diseases. Both human and rat primary microglial cells are commonly used in in vitro models of neural inflammation and microglial multinuclearity is often observed. To obtain the optimal model of multinucleation in microglial cells, this study compared human microglial clone 3 (HMC3), murine microglia cell line, BV2, murine macrophage cell line, RAW 264.7 and primary rat microglia. To induce multinucleation, a PKC activator, phorbol myristate acetate (PMA) was used. Exposure parameters were set at concentrations of 10ng/ml, 100ng/ml and 1000ng/ml, for 24H, 48H and 72H at each concentration. Multinucleation was induced in all these cell types, with increasing multinuclearity as PMA concentration and exposure time increased. It was determined that optimal multinuclearity was induced at a concentration of 100ng/ml and at 72H exposure. Ideal nuclear visibility and multinuclearity was seen in the primary rat microglia. Of the microglial cell lines, the BV2 cell line has a better nuclear visibility but the HMC3 cell line has a better multinuclearity. This microglial multinuclearity will then be compared to their phagocytic ability.

ABSTRACT NUMBER: 3408 Poster Board Number: P202

TITLE: Functional Assay of Neural Activity with Cell-Based Neural Culture Models and Microelectrode Array Technology for Proconvulsant Risk Assessment in the Neutox Pilot Study


KEYWORDS: Cell Culture; Safety Pharmacology; Toxicity; Acute

ABSTRACT: The occurrence of drug-induced seizures is an important liability in drug discovery and development that can result in attrition for candidate drugs or withdrawal from the market. Thus, the pharmaceutical industry is motivated to detect proconvulsant risk early in the drug discovery and development process for CNS and non-CNS targets. Current approaches for evaluating proconvulsant risk, such as seizure threshold assays or EEG monitoring, rely on rodent animal models, which can be expensive and are often limited to later stages in drug development. Emerging methods utilizing in vitro cell-based assays of functional neurophysiology have shown promise in identifying neuro-active agents and may demonstrate value for proconvulsant risk assessment earlier in drug discovery. Specifically, the electrophysiological activity of networked neuronal cultures can be measured and quantified using multiwell microelectrode array (MEA) instrumentation. Here, we describe the recent progress for a consortium of stakeholders aiming to improve and standardize proconvulsant risk assessment using in vitro methods. The NeuTox consortium, organized by the Health and Environmental Science Institute (HESI), comprises representatives from the pharmaceutical industry, cell and laboratory instrumentation providers, contract research organizations, and academic researchers. Broadly, the NeuTox mission is to characterize the reliability and accuracy of detecting drug-induced seizurogenic activity using MEA technology and neuronal cultures. Towards this end, the group has reviewed retrospective data, developed a standardized assay protocol, selected a 12 compound training set, and coordinated an international multi-site pilot study. Here, we present results of one complete entry to the NeuTox pilot...
study. Specifically, we evaluated the predictive nature of 10 quantitative measures of functional neural network activity to discriminate between proconvulsant and control compounds within the training set. We find that non-parametric measures of activity (mean firing rate), synchrony (synchrony index), and oscillations (inter-spike interval coefficient of variation) provide a reliable and effective view into the proconvulsant risk of compounds in the training set.

ABSTRACT NUMBER: 3409  Poster Board Number: P203
TITLE: Profiling of Seizuregenic Liability Compounds Using High-Throughput Human iPS-Derived Neuronal 3D Cultures
KEYWORDS: Alternatives to Animal Testing; Toxicity; Acute; Cell Culture
ABSTRACT: Spheroid-based cellular platforms are considered to enable more complex, biologically relevant, and predictive assays for compound screening, safety evaluation and toxicity studies. Thus, here we deployed a high throughput spheroid co-culture of cortical glutamatergic and GABA-ergic neurons as well as astrocytes, more closely resembling the tissue constitution of native human brain tissue. High content imaging indicated that this platform shows robust well-to-well size homogeneity, as well as the neural networks established in this model express typical neuronal and astrocytic identity and functional markers, which altogether enable a highly functional neuronal circuitry. High throughput kinetic fluorescence imaging of Calcium-sensitive dyes indicated robust spontaneous, synchronized, readily detectable calcium oscillations, with reproducible baseline activity patterns across wells and inter-plates. In order to validate the capabilities of the platform in toxicology and safety pharmacology, a set of 12 compounds including drugs known to cause seizures in animal models through independent mechanisms was evaluated. The compounds consisted of Pentylenetetrazole (PTZ), Picrotoxin, Strychnine, Pilocarpine, Cholopromazine, Amoxapine, Enoxacin, Phenytoin, Linopirdine, 4-aminopyridine, Amoxicillin and Acetominophen. In order of potency, 4-aminopyridine, Strychnine, Linopirdine, Cholopromazine, Phenytoin, and Pilocarpine induced drastic changes in calcium oscillation patterns in the natural network bursting observed in this model. 4-aminopyridine was the only compound that increase the frequency in calcium oscillation patterns whereas the remaining active compounds all reduced the frequence calcium oscillation. The negative controls (Amoxicillin and Acetominophen) had no significant effect when compared to DMSO/vehicle. In conclusion, high throughput functional assays using the human iPS-Derived 3D neuronal spheroids platform deployed in this study provided robust data for assessment of functional neurotoxicity and seizure liabilities.

ABSTRACT NUMBER: 3410  Poster Board Number: P204
TITLE: Effect of Marijuana on Individuals Exposed History of Toxin Exposure
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KEYWORDS: Neurotoxicity; Metals; Behavior; Exposure Assessment
ABSTRACT: Rationale: With legalization of marijuana its usage is increasing. It has been theorize that marijuana could have some effect on mental health. Methods: The medical program takes place in
Weimar, California. The study documented 3 years of data (n=5003) of unique individuals that took the Depression and Anxiety Assessment Test (registration TX 7-398-022). It assessed depression, anxiety, emotional intelligence (EQ) demographics and asked about cannabis/marijuana usage and possible toxin exposure (intake of toxin-prone fish, lead exposure, etc). The program uses toxic exposure as one the many triggers of depression. The participants qualified as having the toxic exposure if they have: high lead levels, high mercury levels, high arsenic, bismuth, or other toxin levels or by having a high risk of exposure to these toxins. The depression was classified according to the DSM-5 [The Diagnostic and Statistical Manual of Mental Disorders Volume 5] into 4 categories as none (0-6), mild (7-10), moderate (11-19) or severe (20 or more). Anxiety level was based on DSM-5. The anxiety was classified according to DSM-5 into 4 categories as none (0-4), mild (5-8), moderate (9-12) or severe (13 or more). Results From the n=5003 individuals that took the test n=1191 had one or more toxin exposure. From those n=1191, n=98 was using marijuana at least one time per week. From those n=98 that use the marijuana they had a mean depression score of 15.5, ST Dev 7, median 16, mode 23, they had a mean anxiety score of 8.9, St Dev 4, median 9, mode 15 and mean EQ of 89.1, St Dev 13.6, median 88, mode 88. Those not using marijuana had a mean depression score of 11.7, 7.8, median 11, mode 0, they had a mean anxiety score of 6.4, St Dev 4.6, median 6, mode 0 and mean EQ of 103.5, St Dev 17.2, median 102, mode 94. Conclusions: Marijuana seems to have a pernicious effect on all the mental health markers. The group is being follow up to see the long term effect on both groups.

ABSTRACT NUMBER: 3411  Poster Board Number: P205

TITLE: Neurotoxicity of Perfluorooctanesulfonic Acid (PFOS) and Perfluorooctanoic Acid (PFOA) in Human Neuroprogenitor Development

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KEYWORDS: Neurotoxicology; Perfluorinated Agents; Neurotoxicity; Developmental

ABSTRACT: Perfluoralkyl substances (PFAS) are persistent fluorinated carbon chain compounds that are used in numerous products, including non-stick cookware and stain- and water-resistant fabrics. Perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are two of the most notable PFAS and have been associated with numerous adverse health effects like thyroid disease and testicular cancer. Epidemiological studies recently associated developmental PFAS exposure with neurocognitive and neurobehavioral deficits, pointing to a potential neurodevelopmental toxicity. Additionally, several in vivo studies have suggested that PFAS, primarily PFOS, modulate dopaminergic (DAergic) function. Therefore, we hypothesized that PFAS disrupt DAergic neurodevelopment, with PFOS showing greater toxicity than PFOA. To test this hypothesis, we treated human induced pluripotent stem cells (hiPSCs) with various concentrations of PFOS or PFOA during early differentiation of floorplate neuroprogenitors (NPs), the precursors of midbrain DAergic neurons. The CellTiter-Blue assay was used to assess cell viability upon exposure to 0, 0.1, 10, 100, 1000, and 10,000ppm PFOA or PFAS. Exposure at 10,000ppm PFOA or PFOS was shown to reduce cell viability, with no cytotoxicity seen at 1000ppm or lower levels (n=3). To analyze the impact on neurodevelopmental lineage specification, gene expression analysis by qRT-PCR was performed on early developing human DAergic floor plate NPs after a 5-day exposure to 0, 4, 40, 400, or 4,000ppb PFOA or PFOS, or a 24-hour exposure to 4,000ppb PFOS or PFOA. Expression of markers for hiPSCs, early NPs, and lineage-specific markers were assessed. Concentrations of 400 and
4,000ppb PFOA significantly reduced expression of FOXA2, a DAergic specific lineage marker, with minimal effects for PFOS. No significant changes were observed in the general neural precursor marker OTX2 (n=6). Our results show that doses of PFOA that alter the expression of early stage of DAergic neuronal markers are substantially lower than the dose causing reduction in cell viability. This indicates non-lethal doses of PFOA are giving rise to gene expression changes in NPs. Lastly, contrary to our hypothesis, PFOA was more toxic than PFOS to DAergic neural development.

ABSTRACT NUMBER: 3412  Poster Board Number: P206

TITLE: Modeling of De- and Remyelination in 3D Brainspheres by Cuprizone and PAR1 Antagonist, Vorapaxar

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ABSTRACT: (Developmental) Neurotoxicity remains among the least studied parts of toxicology. It is not possible to test the high numbers of untested chemicals using current guideline studies, which are based on time-consuming, resource-intensive, and expensive animal testing. Additionally, there are concerns about the extrapolating the results from animal studies to human health effects. Therefore, the use of alternative methods to animal testing will allow more cost-effective and human-relevant substance screening for neurotoxicity. Currently existing in vitro models are mainly focus on neurotoxicity, overlooking gliotoxicity, especially oligodendrocyte toxicity. Myelination is one of the key events of neural development and its disruption is involved in neurological diseases, such as MS, but it is difficult to model in vitro. The induced pluripotent stem cells (iPSC) derived human BrainSphere model is an emerging technology to tackle it, since it has different types of neurons, astrocytes and oligodendrocytes present, with 40% of axons myelinated. Proteinase-activated receptor(PAR) 1 and PAR2, which are widely expressed in the CNS are suggested to play a significant role in myelination process. First, we treated BrainSpheres during the entire differentiation period with agonists and antagonists of PAR1 and PAR2. We confirmed mice in vivo findings: activation of PAR1 and PAR2 lead to decreased myelination, while inhibition of PAR1 enhanced myelination after 8 weeks of treatment. Treatment of BrainSpheres with Cuprizone during critical window of olygodendrogenesis and myelination significantly reduced percentage of myelinated axons in comparison to negative controls (DMSO and ibuprofen). Currently, we are exploring re-myelination process after cuprizone withdrawal and whether PAR1 antagonist, vorapaxar, can enhance the process of re-myelination. Taking together, we established a method of myeline quantification in 3D brain model and demonstrated how this model can be used in studying demyelination. In addition, for the first time in human 3D brain model, we have shown, that PAR1 and 2 activity is important for olygodendrogenesis and myelination process during development.
ABSTRACT NUMBER: 3413    Poster Board Number: P207
TITLE: CHD8 Knockout Brainspheres and Chlorpyrifos to Study Gene Environmental Interactions in Autism

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ABSTRACT: Autism spectrum disorders (ASD) is a complex developmental syndrome. A wide spectrum of mutations has been related to ASD. Loss of function mutations of the chromodomain helicase DNA-binding protein 8 (\textit{CHD8}), is one of the main genetic risk factors associated with ASD. In addition to genetic factors, a potential correlation between the exposure to environmental chemicals and the higher ASD risk was suggested. However, the specific mechanisms through which the environmental factors contribute to ASD are still unknown. The aim of our study is to investigate the synergistic effects of genetic and environmental factors in ASD. We used a 3D BrainSphere model, derived from human induced pluripotent stem cells (iPSC) to test this interplay. We analysed the sensitivity of BrainSpheres with known ASD genetic background (CRISPR-Cas9-induced heterozygous knockout of CHD8 gene) and control cultures from the same donor to environmental chemical (chlorpyrifos (CPF) and its active metabolite - chlorpyrifos-oxon (CPO)). After 4 weeks of differentiation, we exposed BrainSpheres to 46.7 and 100 µM CPF and CPO for 24 h. Previously, by performing confocal imaging, we observed increase in reactive oxygen species production and decrease in mitochondria activity due to both, mutation and exposure. Recently, we optimized a protocol of BrainSphere dissociation and flow cytometry, that allowed us to confirm and quantify those events. Interestingly, we observed a significant increase in tyrosine hydroxylase (an enzyme involved in dopamine synthesis) expression in knockout BrainSpheres and due to exposure. To quantify dopamine and other neurotransmitters in control and CHD8 knockout BrainSpheres upon exposure to CPF and CPO, we have been developing a method for neurotransmitter detection using mass spectrometry (LC-MS/MS). Finally, by performing untargeted metabolomics, we observed a stronger perturbations of metabolic network involving purine and pyrimidine metabolism, after exposure to CPO than to CPF with more profound effects in knockout line. Our findings correlate with known ASD facts, such as increased oxidative stress and perturbed dopamine synthesis.

ABSTRACT NUMBER: 3414    Poster Board Number: P208
TITLE: Endocrine-Epigenetic Mechanisms of Sex-Specific Behavioral Toxicity following Developmental Exposure to an Endocrine Disrupting Chemical Mixture


KEYWORDS: Neurotoxicology; Endocrine Disruptors; Epigenetics

ABSTRACT: Recent research shows that developmental exposure to endocrine disrupting chemical (EDC) mixtures can disrupt androgen production resulting in enhanced male reproductive disease, yet, little is known about any extension to the central nervous system (CNS). Our previous research revealed that exposure to a mixture of EDCs (MIX) induced male-specific behavioral deficits across broad behavioral domains, including reduced attention/memory, increased impulsivity, and reduced social preference conditioning. Further, developmentally, MIX increased serum testosterone (T) at birth, a critical window of CNS sensitivity to androgens. It is increasingly being recognized that neuroendocrine and
Neuroepigenetic mechanisms play an integral role in sex-specific neurodevelopment, including neurochemical and behavioral function. Androgens, specifically T, reprogram epigenetic profiles in the CNS. Administration of T at birth to females has been shown to induce late emerging alterations in methylation patterns in striatum into adulthood. To address the possibility that methylation patterns would be altered, mouse dams were exposed to relatively low doses of four EDCs that share consequences on brain and behavioral function: Atrazine (ATR – 10mg/kg), Perfluorooctanoic acid (PFOA – 0.1 mg/kg), Bisphenol-A (BPA - 50µg/kg), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD – 0.036µg/kg) and their mixture, from gestational day 7 until weaning. Given the behavioral sensitivity of males to MIX exposure and importance of striatal function in these behaviors, male striatum was used to test the hypothesis that developmental MIX exposure would alter methylation profiles in adulthood. MIX exposed males show hypomethylation across Esr1, Igf2/H19 and Kcnq1ot1 genes. Methylation profiles on Bdnf and Th were not altered. Total methylation values for imprinted genes correlate with adult serum T concentrations. Taken together, these data suggest developmental EDC exposure reduced methylation profiles in the CNS into adulthood. Future research is needed to directly test if elevation of T at birth results in similar patterns of hypomethylation into adulthood and if these changes mediate behavioral variability. This work highlights the need to investigate the role imprinted genes play in sex-differentiated mechanisms of neurotoxicity. Supported by P30 ES001247 and T32 ES007026-36.

**ABSTRACT NUMBER:** 3415  
**Poster Board Number:** P209  
**TITLE:** Manganese Induces Lysosomal Degradation of Zip14  
**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** K. Thompson, and M. Wessling-Resnick.  
**Harvard T.H. Chan School of Public Health, Boston, MA. Sponsor:** J. Kim  
**KEYWORDS:** Metals; Liver; Neurotoxicity; Metals  
**ABSTRACT:** Manganese (Mn) is an essential element necessary for proper development and brain function. Mn blood levels, and toxicity, are controlled by hepatobiliary clearance. The goal of this study was to determine the transporters involved in Mn uptake at the plasma membrane in hepatocytes and investigate the effect of Mn on their trafficking and function. Zip14 has been identified as a major component of Mn uptake into hepatocytes. This study tested the hypothesis that Mn exposure alters Zip14 transporter levels and subsequently changes Mn uptake kinetics. HepaRG cells, a hepatocyte cell line, were grown on 24-well plates or 13mm coverslips to confluence for 12 days to allow for maximal polarity. Cells were exposed to a range of Mn (0, 5, 50, 100µM) for 4 and 16 hours in standard culture conditions. Uptake parameters were determined using 54Mn. Protein levels were determined by Western analysis and indirect immunofluorescence microscopy. The effects of zinc (Zn) on Zip14 expression and Mn uptake were also determined to test for specificity of a Mn effect. A subset of cells were pretreated with the lysosomal inhibitor Bafilomycin A1 prior to Mn exposure and co-stained with Lamp1 to determine if Zip14 targeted to lysosomes for degradation. Zip14 levels were reduced in response to Mn and decreased cellular 54Mn uptake was observed. Both occurred in a dose dependent manner. Zinc treatment did not reduce Zip14 protein levels but did decrease 54Mn uptake suggesting a non-specific metal effect on uptake. Bafilomycin A1 treatment prevented the degradation of Zip14 and increased the colocalization of the transporter with Lamp1 positive vesicles. Together these results indicate that high Mn exposure decreases Zip14 protein levels and the ability of hepatocytes to handle additional Mn. The decrease in Zip14 protein levels was due to lysosomal degradation. The liver protects the body from excess Mn through the first-pass-hepatic clearance mechanism. This study reveals this
essential mechanism is compromised when hepatocytes are exposed to uM amounts of Mn. This seemingly protective mechanism for the hepatocytes would raise circulating Mn levels. As a result, toxic Mn levels could be maintained in the circulation leading to neurotoxicity.

ABSTRACT NUMBER: 3416  Poster Board Number: P210
TITLE: Lead Induces Dose-Dependent Alterations in Microglial Chemotaxis

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KEYWORDS: Metals; Neurotoxicity; Metals; Neurotoxicology

ABSTRACT: Microglia are macrophages of the central nervous system (CNS) that play a critical role in inflammation and tissue repair in the brain. Following brain injury, microglia migrate to accumulate in damaged sites. Lead (Pb) is highly toxic to the CNS, but its intervention in the regulation of microglia chemotaxis, as well as the intercellular signals that underlie this process, are not well understood. This study evaluated the effects of Pb exposure on chemotaxis of microglia and identified possible molecular mechanisms underlying this process. Primary microglia cells were exposed to Pb acetate at 10^{-5}-10^{-8} M for 24 h and 72 h, respectively. Following Pb washout, the microglia were transferred to Transwell inserts. Inserts were then placed in 50 μM ATP for 8 h to observe migration. The Pb treatments generated dose- and time-dependent reductions in chemotaxis, as the number of migrated cells decreased significantly with increasing Pb concentrations. The 24 h exposure group also migrated significantly more than the 72 h exposure group, with half maximal migration occurring at 10^{-6} M Pb treatment in the 24 h exposure group and 10^{-7} M Pb treatment in the 72 h exposure group. This suggests that Pb progressively reduces the chemotactic ability of microglia to respond to ATP. Pb-exposed microglia were further examined by scanning electron microscopy. Microglia exposed to 10^{-5} M Pb revealed prominent membrane blebbing greater than that of the lower exposed groups. The next question was whether Pb-exposed/injured microglia attract healthy, unexposed microglia. Microglia were exposed to Pb at 10^{-5}-10^{-8} M for 24 h, washed out, and placed in the receiving well of the Transwell plates. Unexposed microglia were placed in the inserts, and an 8 h migration toward Pb-exposed microglia was measured. Pb-treated microglia demonstrated a dose-dependent increase in migration of unexposed microglia to Pb-exposed microglia, with half maximal migration occurring at 10^{-6} M Pb treatment. This indicates that injured microglia release chemotactic factors that will trigger chemotaxis of healthy microglia to the site of injury.

ABSTRACT NUMBER: 3417  Poster Board Number: P211
TITLE: Developmental Neurotoxicity Due to Nickel Exposure In C. elegans

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KEYWORDS: Neurotoxicology; Metals; Neurotoxicity; Developmental

ABSTRACT: Nickel (Ni) is an ubiquitous metal in the environment that has had increased industrial application. Environmental and occupational exposure to Ni compounds has been documented to give rise to toxicities in a variety of organs, including liver, kidney, lungs, and skin. However, neurotoxic
effects have not been extensively investigated. In this present study, we utilized a *C. elegans* model to investigate specific neuronal susceptibility to acute nickel neurotoxicity. Wild-type worms and worms expressing green fluorescent protein (GFP) in cholinergic, dopaminergic or GABAergic neurons were treated with NiCl₂ for 1h at the first larval (L1) stage. The median lethal dose (LD₅₀) was calculated to be 5.88 mM in this paradigm. Morphological studies of GFP-expressing worms showed significantly increasing degeneration of cholinergic, dopaminergic and GABAergic neurons with increasing Ni concentration. Significant changes in locomotion and basal slowing response assays reflected that cholinergic and dopaminergic neuronal function, respectively, was impaired due to Ni treatment. Interestingly, a small but significant amount of worms exhibited shrinker phenotype upon Ni exposure but no loopy head foraging behavior was observed suggesting that function of D-type GABAergic neurons of *C. elegans* may be specifically attenuated while the RME subset of GABAergic neurons are not. GFP expression due to induction of glutathione S-transferase 4 (*gst-4*), a target of Nrf2 homolog skn-1, was increased in a Pgst-4::GFP worm highlighting Ni-induced oxidative stress. RT-qPCR verified upregulation of this expression of skn-1 immediately after exposure. These data suggest that oxidative stress is associated with neuronal damage and altered behavior due to developmental Ni exposure.

**ABSTRACT NUMBER:** 3418  
**Poster Board Number:** P212  
**TITLE:** Persistent Thalamic Neurodegeneration and Neuroinflammation following Successful Termination of Nerve Agent-Induced Status Epilepticus.  

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** M. Anderson, D. Palmer, E. Johnson, and J. Skovira. USAMRICD, Aberdeen Proving Ground, MD.  

**KEYWORDS:** Organophosphates; Chemical & Biological Weapons; Inflammation  

**ABSTRACT:** Status epilepticus (SE), defined as persistent, continuous and unremitting seizure lasting 5 min or longer, is a condition of great medical concern that can occur rapidly following organophosphate (OP) nerve agent exposure. If not controlled with pharmacological interventions, SE can lead to extensive and progressive brain damage and neuroinflammation. Although great focus has been placed on interventions to control OP-induced SE, few studies have examined the pathological processes that continue after SE has been abated. This study evaluated (1) whether neurodegeneration and neuroinflammation continue in the thalamus of C57BL/6 mice following cessation of prolonged OP-induced SE and (2) whether the cyclin-dependent kinase (CDK) inhibitor CR8 can mitigate the extent of the neurodegenerative and neuroinflammatory processes. Mice were surgically prepared two weeks prior to the experiment with electrodes to record brain activity. The oxime HI-6 (50 mg/kg; IP) was given prior to nerve agent exposure to increase survival without affecting the onset of seizure activity. Mice were exposed to the nerve agent sarin to elicit seizure activity (256 µg/kg; SC) followed by the anti-muscarinic atropine (1 mg/kg; SC). The CDK inhibitor CR8 (5 mg/kg, IP) was administered 5 min following seizure onset. SE was terminated with midazolam (5 mg/kg; IP) and the centrally active oxime monoonitrosoacetone (MINA) (50 mg/kg; SC) at 60 min following onset. Brain tissue was collected at 7 d or 30 d following exposure for analysis of microglia activation and neuronal degeneration. The results suggest that nerve agent casualties may still be at risk for progressive neurodegeneration in the thalamus from the continuation of inflammatory processes, even if SE is successfully terminated. CR8 treatment significantly reduced the extent of the neuroinflammatory microglial response, thus aiding in neuroprotection following OP-induced seizures.
ABSTRACT NUMBER: 3419    Poster Board Number: P213
TITLE: A Network Approach to Phosphoprotein Signaling in a Mouse Model of Gulf War Illness Using Corticosterone and Diisopropyl Fluorophosphate

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KEYWORDS: Organophosphates; Neurotoxicity; Pesticides

ABSTRACT: An estimated 3 million people are exposed to organophosphates each year; however, many do not report acute effects, but report symptoms of adverse neurological effects years later, as is the case for the 250,000 veterans from the 1991 Persian Gulf War who suffer from Gulf War Illness (GWI). Our previous GWI research has focused on developing a model of GWI using organophosphate acetylcholinesterase inhibitors (OP AChEI, e.g. chemical warfare agents and pesticides) and exogenous corticosterone (CORT), to simulate high stress, in an effort to mimic several of the conditions experienced in theater and emulate the chronic neuroinflammation hypothesized to underlie GWI symptomology. In these studies, we uncovered a lack of correlation between OP AChEI-associated neuroinflammation and the levels of ACh or enzyme inhibition, suggesting that GWI and its associated neuroinflammation may result from the phosphorylation of other targets. Thus, an investigation into early phosphoprotein responses in the hippocampus and striatum was performed to better understand the signaling changes involved in this etiology. Using our validated mouse model, adult male C57BL/6J mice were exposed to CORT in the drinking water for 7 days followed by a single injection of diisopropyl fluorophosphate (DFP; 4.0 mg/kg, i.p.) on day 8. Mice were euthanized 30 min and 2 h post-injection via focused microwave irradiation. To evaluate region-specific effects, 20+ post-translationally modified protein targets were measured using multiplex ELISA (e.g., ERK1/2, GSK3, IkB-a, JNK, MEK1). To then optimize analysis of the specific data sets, a network parameter approach corresponding to radiality was used to assess the response of the phosphoprotein targets in relation to all other responses. This approach identified specific proteins (RPS6, CREB, p90RSK, and IkB-a) that were substantially activated or inhibited within the network, and is informative with regard to the mechanisms of interactions that are occurring as a result of this GWI exposure. These significant proteins suggest new potential biomolecular drivers and therapeutic targets of GWI symptomology.

ABSTRACT NUMBER: 3420    Poster Board Number: P215
TITLE: Cadmium-Induced Renal Toxicity through GATA Family Suppression


KEYWORDS: Metals

ABSTRACT: Cadmium (Cd) can cause renal toxicity through the proximal tubular cell damage. Our previous study demonstrated that Cd changed the activities of various transcription factors in human proximal tubular HK-2 cells. Interestingly, several GATA families were included in the transcription factors whose activities were decreased by Cd treatment. GATA family has diverse roles in the proliferation of cells, development of tissues, disease regulation, oncogenic effect, and so on. However, the effect of GATA family on Cd renal toxicity has remained unclear. In this study, we examined whether
knockdown of GATA family may affect the viability of HK-2 cells. The single knockdown of GATA1, GATA3 or GATA6 using siRNA significantly decreased the viability of HK-2 cells. Especially, GATA6 knockdown exerted most-suppressing effect on the viability of HK-2 cells. GATA family can be divided into two subfamilies, GATA1/2/3 and GATA4/5/6, concerning their phylogenetic and expression profiles. Therefore, the effect of multi-knockdown of GATA1/3/6 on the viability of HK-2 cells was examined. As a result, the multi-knockdown of GATA family showed similar suppressing effect as when GATA6 was knockdowned on the HK-2 cell viability. These results suggest, therefore, that GATA family may be involved in the Cd renal toxicity through same pathway.

ABSTRACT NUMBER: 3421    Poster Board Number: P216

TITLE: Comparison of Speciated Urinary Arsenic and Depression in US Population from 2011-2012 and 2015-2016

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KEYWORDS: Kidney; Risk Assessment; Epidemiology

ABSTRACT: Introduction: arsenic is a neurotoxin and can cause memory dysfunction and depression. Very few studies have been conducted on exposure to arsenic and association of depression in human. Objective: The objective of this study was to assess and compare the association between urinary speciated arsenic and depression among adults from 2011-2012 and 2015-2016 population from NHANES III data set. To identify whether the association of urinary speciated arsenic and depression pattern from 2011-2012 and 2015-2016 NHANES III population among male and female. Methods and Materials: We used data from the National Health and Nutrition Examination Survey 2011-2012 and 2015-2016. Depression was measured using 9 item patient questionnaires (PHQ9). We computed total score PHQ A, and a score of ≥10 is categorized as depressed. Six speciated arsenic concentration were the exposure from both 2011-2012 and 2015-2016 NHANES III laboratory result. We conducted crude and multivariate logistic regression analysis using complex survey procedures as well as stratified by gender. Results: The sample consisted of 963 and 2067 adults from 2011-2012 and 2015-2016 NHANES III population respectively. 54.0% were female from the 2011-2012 dataset, and 50.26% were female from the 2015-2016 dataset. The majority had high school education for both dataset, and 47.7% and 50.5% were married from 2011-2012 and 2015-2016 dataset respectively. From 2011-2012 dataset, only urinary arsenobetaine was significantly associated with depression (Odds ratio =1.89, Confidence Interval= 1.32-5.24). Also, from the 2015-2016 dataset, only urinary arsenous acid was significantly associated with depression (Odds ratio= 1.64, CI= 1.10-2.45). The female population showed a borderline association with depression in comparison to the male population from the 2011-2012 dataset. In contrast, no association among female than male population observed with depression from the 2015-2016 dataset.
ABSTRACT NUMBER: 3422  Poster Board Number: P217
TITLE: Effects of Uranium and Arsenic on T-Cell Differentiation

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KEYWORDS: Metals; Immunotoxicology

ABSTRACT: Communities in the western region of the United States are frequently exposed to metal mixtures from numerous unremediated abandoned uranium mines. Metals including Arsenic (As) and Uranium (U) co-occur in and around these sites at levels higher than the USEPA maximum contaminant levels (MCL) and pose unknown health risks. Chronic exposure of populations living in the proximity to mining and milling sites and waste piles, through inhalation of dust, contact with skin and/or consumption of contaminated food and water, has continued to create concerns about the risk of adverse health outcomes. Some research suggests that uranium exposure increases the risk of immune dysregulation although the mechanism is unknown. T-cells are the regulators of the immune system and play a central role in immune system function. Environmental chemicals are thought to influence T-cell subpopulations by stimulating or suppressing their differentiation and/or activation. Analysis of RNA sequencing (RNA Seq) data conducted on naïve CD4+ T-cells exposed to As, U or As + U for 24 hours before activation by CD3/CD28 antibodies showed U modifies As response. In combination U amplifies the number of genes with a significantly positive log-2 fold change and decreases the number of genes with a negative log-2 fold change compared to arsenic treatment alone, despite no evidence of significant gene expression changes with U alone (FDR = 0.05, p-adj = ≤ 0.05). Preliminary analysis of T-cell subtype specific expression markers, cytokines and microenvironment cues indicate a possible reduction in the Th1 and Th17 subtypes and no significant change in the Th2 subtype in the presence of 10uM As. The data indicates that As and U cause distinct alterations in overall gene expression in CD4+ T-cells. Despite causing no significant changes in gene expression alone, U was able to modify the As response suggesting an interaction requiring further examination.

ABSTRACT NUMBER: 3423  Poster Board Number: P218
TITLE: Protection of Dihydrolipoic Acid on Mercury Induced Cytotoxicity in PC12 Cells

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ABSTRACT: Mercury (Hg) is a highly toxic heavy metal. Exposure to Hg in humans and animals causes damage in several organs or systems. However, the mechanisms of inorganic Hg-induced cell death and toxicity are still incompletely understood. Dihydrolipoic acid (DHLA) is the reduced form of a naturally occurring compound lipoic acid, which act as a potent antioxidant through multiple mechanisms. To investigate mechanism/s of cyto-protection of DHLA against Hg induced toxicity PC12 cells were used. Treatment of PC12 cells with HgCl2 (0-2.5 µM) for 24 h resulted in toxic effects, dose dependently. Exposure of Hg2+ resulted in cell viability loss, high level of lactate dehydrogenase (LDH) release, DNA damage and cellular glutathione (GSH) level decrease. In addition, protein level expressions of akt and mTOR in cells were downregulated after Hg2+ exposure. However, pretreatment with DHLA (50 µM) for 0.5 h before Hg2+ exposure showed inhibition against Hg2+-induced cytotoxicity in PC12 cells. Pretreatment Of DHLA significantly increased cell viability, decreased lactate dehydrogenase (LDH)
release, reduced DNA damage and increased cellular glutathione level. Moreover, significant upregulation was observed in protein level expressions of akt and mTOR. In conclusion, present results showed that DHLA could mitigate Hg$_{2+}$-induced cytotoxicity via enhancing antioxidant defense in PC12 cells.

**ABSTRACT NUMBER:** 3424  **Poster Board Number:** P219

**TITLE:** Chronic Arsenic Exposure in a HaCaT Cell Model of Squamous Cell Carcinoma: Altered Splicing Events as Regulatory Mechanisms?

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**KEYWORDS:** Metals; Carcinogenesis; Environmental Toxicology

**ABSTRACT:** Background: Chronic exposure to arsenic through contaminated drinking water plagues millions worldwide and causes multiple cancers including skin cancer. Several mechanisms have been proposed including aberrant alternative splicing events. RNA-seq data from human keratinocytes chronically exposed to arsenite shows >600 significant alternative splicing events as each time point tested (7, 19 and 28 weeks) lending credence to the global aberrant alternative splicing hypothesis. Objective: This project aims to examine and validate putative alternative splicing events in a HaCaT cell line model of chronic arsenic exposure induced skin carcinogenesis. Methods: Total RNA was isolated at 7, 19 and 28 weeks from independent quadruplicate cultures of HaCaT cells chronically exposed to 0 or 100 nM Na$_2$AsO$_3$ and cDNA was prepared. Based on analysis of RNA-seq data by rMATS algorithm and known carcinogenic association, two genes, ELK4 and MINOS1, were selected for validation of predicted alternative splicing events at 7 weeks by RT-PCR. Primers were designed on the flanking exons as predicted by rMATS, followed by amplification, densitometric analysis, cloning and sequencing. Densitometric data was analyzed using Image J and subsequently difference in percent spliced in between the exposed and unexposed groups was computed using one tailed Mann-Whitney test and one-tailed unpaired t-test with Welch correction. Results: For both ELK4 and MINOS1, statistical analysis of densitometric data from RT-PCR corroborated the RNA-seq alternative splicing predictions. For each gene, several novel isoforms were also identified, some of which were confirmed by cloning and sequencing. All ELK4 isoforms detected had altered 3'UTR structure, but skipped MINOS1 isoform had premature stop codon. Such alterations could be critical for structure, function and interactions. Conclusions: The current data imply that proportions of different isoforms vary significantly between control and arsenic treated HaCaT cells. Alternative splicing events could play a regulatory role, altering cellular function possibly contributing to carcinogenesis. **Funding:** NIH grants R01ES027778, R21ES023627, P20GM103436, R15GM126446.
ABSTRACT NUMBER: 3425       Poster Board Number: P220

TITLE: Effect of Polymorphisms of Glutathione Metabolism Genes on Mercury Nephrotoxicity in Artisanal and Small-Scale Gold Mining

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KEYWORDS: Metals; Glutathione; SNPs

ABSTRACT: Inhalation of mercury (Hg) vapor can produce harmful health effects in several organs, including the kidneys. However, in a historically gold-mining town in Colombia, we reported that, despite statistically significant differences in blood and urinary Hg levels, kidney function parameters were found unaltered in miners compared to a control group. To investigate whether genetic variation in detoxification pathways accounts for the modulation of Hg toxicity, single nucleotide polymorphisms (SNPs) derived from genes involved in the glutathione metabolism were analyzed in our study population, both mining (n= 160) and non-mining (n= 121) towns. All the SNPs included were genotyped by TaqMan method: GCLC rs1555903, GCLM rs41303970, GSS rs3761144, GSTA1 rs3957356, and GSTP1 rs4147581. A multivariable linear regression model was used to assess the effect of SNP on Hg levels and on estimated glomerular filtration rate (eGFR) while adjusting for possible confounders. Empirical significance (P < 0.05) was determined based on 5,000 permutations. Statistical analysis was performed with R. Of SNPs analyzed, rs41303970-T allele was significantly associated with increased urine-Hg levels (β = 0.06, P = 0.009), whereas rs1555903-C allele was significantly associated with reduced blood-Hg levels (β = -0.04, P = 0.028) and eGFR (β = -2.19, P = 0.004) when both groups exposure and non-exposure groups were pooled. The rs41303970-T allele was significantly found more frequently in miners than in controls (34.5% vs 17.4%; P = 0.001), although no significant difference in rs1555903 allelic distribution was found between groups. Regarding kidney function assessment, decreasing eGFR was significantly associated with rs1555903-C allele in controls (β = -3.42, P = 0.005) but not in miners (β = -1.53, P = 0.110). There was no significant interaction between these two SNPs in either miners or controls. Taken together, these findings suggest that GCLM and GCLC genes may modulate the pathogenesis of Hg nephrotoxicity. Thus, the decrease gene expression associated to rs41303970-T allele could play a key role in Hg detoxication by reducing its reabsorption, while the increased Hg retention in tissue associated to rs1555903-C allele could contribute to kidney damage.
ABSTRACT NUMBER: 3426    Poster Board Number: P221
TITLE: Co-exposure to Mercury (Hg) and 2,3,7,8 Tetrachlorodibenzo-p-dioxin (TCDD) Induces Unique Transcriptional Changes through Deregulation of the Epigenome

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KEYWORDS: Metals; Dioxin; Epigenetics

ABSTRACT: Environmental exposures to hazardous substances often involve mixtures of chemicals. Heavy metal, mercury (Hg) and dioxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which accumulate in the atmosphere due to both natural and industrial processes, are among the most common environmental co-contaminants. Health hazards of Hg and TCDD exposure include diseases of neuronal, respiratory, cardiovascular, endocrine and immune systems. Growing evidence indicates that the combined effects of environmental co-contaminants could be significantly different from that of the effects of individual contaminants. However, most of the earlier studies have investigated the adverse effects of Hg and TCDD individually. Consequently, there is a significant knowledge gap in the understanding of the deleterious effects of Hg and TCDD. Our studies on Hg and TCDD co-exposure show that a number of genes are differentially expressed in a co-exposure-specific manner in lung and neuronal cells. Moreover, several biological processes and pathways that were not impacted or only mildly impacted by single-exposures were significantly impacted by co-exposure. Interestingly, we found several neuronal pathways including CREB signaling to be significantly inhibited only in the co-exposed cells. To obtain insights into the mechanisms underlying co-exposure-specific transcriptional changes, we examined the co-exposure specific differentially expressed genes using BART (Binding Analysis for Regulation of Transcription), a computational method that uses a novel semi-supervised learning integrated with public ChIP-Seq data mining approach, to predict functional transcription factors that regulate any given gene set. Our analysis suggests that deregulation of PRC2- and BRD4- associated chromatin regulation could underlie co-exposure specific gene up- and down- regulation, respectively. Collectively, our results suggest that Hg+TCDD co-exposure alters the epigenome.

ABSTRACT NUMBER: 3427    Poster Board Number: P222
TITLE: An Increased Dietary to Environmental Copper Ratio Reduces Toxicity Endpoints in C. elegans


KEYWORDS: Metals; Exposure, Environmental; Bioavailability

ABSTRACT: Caenorhabditis elegans, a free-living nematode that’s widely used in biological research, is typically grown in laboratory conditions using a strain of uracil-auxotrophic Escherichia coli (OP50) as its food source. Advances in imaging, neurobehavioral, and small-molecule analysis make this model system ideal for examining questions related to complex xenobiotic exposures like Cu. In the case of essential trace elements like Cu, where too much (excess) or too little (deficiency) can be detrimental, C. elegans respond to inappropriately by initiating conserved mechanisms to maintain homeostasis. When these checks and balances are not enough to compensate for increasing levels of Cu in the media, Cu-toxicity endpoints like 1) reduced lifespan 2) decreased adult length 3) increased bagging 4) reduced

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brood size and 5) increased Cu body burden have been reported. However, when toxicity is established
by adding varying molarities directly in the media, this exposure method is best described as
environmental because the nematode is initially presented with excess Cu cutaneously (from its
environment) before it reaches the intestinal lining. The limitation of this experimental design is that it is
at odds with the predominant exposure route of Cu in higher organisms where the majority of Cu intake
is derived from dietary sources. To address the most relevant route of Cu exposure, N2 C. elegans were
grown with genetically manipulated E. coli that accumulate 60-90x Cu to artificially increase the ratio of
dietary to environmentally derived Cu. Despite having the same molarity of Cu introduced to the media,
an increased dietary to environmental ratio reduced or eliminated all Cu-toxicity endpoints tested
compared to control exposures. Relative to WT E. coli, reduced lethality, increased length, decreased
bagging and increased brood size is observed when C. elegans are grown on Cu-accumulating E. coli.
Importantly, these phenotypic discrepancies were only observed when excess Cu (100µM) was added to
the system. Future studies will examine the mechanisms behind these altered toxicity endpoints by
testing 1) the extent of reactive oxygen species (ROS) resistance and 2) Cu body burden/localization in C.
elegans to distinguish between an increased response to ROS (produced by Cu) and reduced
uptake/bioavailability of dietary Cu.

**ABSTRACT NUMBER:** 3428  **Poster Board Number:** P223

**TITLE:** Arsenic and Uranium Exposure Elicits Differential Responses in Human Immune Cells

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** E. J. Dashner-Titus, J. R. Schilz, K. Simmons,
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**KEYWORDS:** Immunotoxicity; DNA Repair

**ABSTRACT:** Thousands of unremediated abandoned uranium mines are located in the western region of
the United States, leaving some communities vulnerable to adverse effects of metal mixtures through
inhalation of dust, contact with skin and/or consumption of contaminated food and water. Metals
including arsenic and uranium co-occur in and around these sites at levels higher than the USEPA
maximum contaminant levels (MCL) and pose unknown health risks. Evidence of uranium’s chemical
toxicity in immune cells is limited however, research suggests that exposure to uranium increases the
likelihood of immune dysfunction. We compared the toxicity of uranium as uranyl acetate in Jurkat
(human T-lymphocytic) cells to the known mechanisms of arsenic toxicity including decreased cell
viability, initiation of oxidative stress, induction of DNA damage and alteration to the production of DNA
repair protein expression. Arsenic induces cytotoxicity at 3 uM. In contrast UA caused no toxicity in
Jurkat cells up to 100 uM. Proposed mechanisms of metal toxicity include generation of oxidative stress.
Uranium increased oxidative stress at 2 and 4 hours as measured by 2’,7’-dichlorofluorescin diacetate
(DCFDA) however it was not sufficient to initiate changes in the expression of the oxidative stress
markers Heme oxygenase I (HMOX1) and NAD(P)H Quinone Dehydrogenase 1 (NQO1), whereas arsenic
was able to initiate an increase in these markers. Cellular oxidative stress can cause DNA damage;
arsenic, but not uranium stimulated DNA damage as measured by an increase in pH2AX. Arsenic
enhanced cytotoxicity induced by the DNA damaging agent etoposide suggesting an inhibition of DNA
repair, however uranium did not modify the etoposide effect. Arsenic, but not Uranium, inhibits PARP-1
activity as measured by the PAR ELISA. Despite not having an effect on PARP-1 activity, uranium
decreased expression of XPC and XRCC1 DNA repair proteins. The marked differences in the direct and
augmented cytotoxicity of Arsenic and Uranium, and the lack of inhibition of PARP activity by uranium
despite inhibition of DNA repair by both metals, suggest distinct mechanisms of uranium vs arsenic toxicity in human T-lymphocytic cells.

ABSTRACT NUMBER: 3429    Poster Board Number: P224
TITLE: Association of Lead, Cadmium, and Mercury with PON1 Activity and MDA in a General Population in Southern Brazil

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ABSTRACT: Metal exposure is associated with increased oxidative stress (OS). Malondialdehyde (MDA) is a final product of lipid peroxidation, and it has been extensively used to evaluate metal-induced OS. Pro-oxidant effects produced by metals can be mitigated by paraoxonase 1 (PON1), an antioxidant enzyme known to prevent cardiovascular disease and atherosclerosis. Here, we evaluated the association between blood lead (Pb), cadmium (Cd) and mercury (Hg) levels with PON1 activity, and with MDA concentrations in a randomly selected sample of Brazilian adults aged 40 years or older, living in an urban area in Southern Brazil. A total of 889 subjects were evaluated for blood Pb and Cd levels, and 832 were tested for Hg. Geometric mean of blood Pb, Cd and Hg was 1.93 μg/dL, 0.06 μg/L and 1.40 μg/L, respectively. PON1 activity was significantly different among various genotypes: QQ (PON1=121.4 U/mL), QR (PON1=87.5 U/mL), and RR (PON1=55.2 U/mL), at p<0.001. However, PON1 activity was not significantly associated with blood metal concentrations. Cluster analysis showed that men who reported to be current smokers and drinkers with higher blood Pb and Cd levels, had significantly lower PON1 activity than non-smokers or drinkers, and women with lower Pb and Cd levels. RR genotype carriers had lower PON1 activity than those with the QR genotype, and had higher levels of Pb and Cd compared with other genotype carriers. For blood Hg, no association with PON1 activity or genotype was noted. The results of cluster analysis suggested that smoking status exerts a significant influence on PON1 activity. Other studies with environmentally exposed populations are required to further clarify whether low blood levels of metals influence OS biomarkers.

ABSTRACT NUMBER: 3430    Poster Board Number: P225
TITLE: Acute Lead Exposure Alters Phagocytic Activity and Morphology in Primary Microglia

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: R. N. Khan, S. M. Nicholson, and F. A. X. Schanne. St. John’s University, Queens, NY.

ABSTRACT: Adverse effects of lead (Pb) on neurons have been previously studied; however, current research examines effects on the supporting cells of the nervous system, microglia. Previous lead exposure studies have demonstrated altered expression of toll-like receptor 4, a known activator of microglial phagocytosis. In the present study, primary microglia were isolated from mixed glial cultures derived from 14 day post-natal rats. Microglia were then exposed to physiologically relevant (10⁻⁷-10⁻⁵ M Pb) concentrations of lead acetate for 24 hours and observed for phagocytic activity, toll-like receptor expression, and morphological changes. Cells were washed to remove lead and starved with serum-free media for two hours. Microglia were then incubated with opsonized 1.75 μm fluorescent latex beads.
Ingestion was quantified by total fluorescence observed via flow cytometry and microplate reader. Maximal phagocytic activity was observed in the low exposure groups (10^{-8} - 10^{-7} M Pb) and decreased by 40% in the 5 μM and 10 μM groups. Additionally, immunofluorescence revealed an increase in toll-like receptor 4 expression at the 5 μM and 10 μM concentrations. Scanning electron micrographs revealed a gradual loss of finger-like projections and increase in membrane blebs in a seemingly dose-dependent manner. Treated microglia also displayed a decrease in retraction fibers and loss of adherence in culture beyond 24 hours. The evident changes to microglial structure and function have implications for neuroimmunity following acute Pb exposure.

**ABSTRACT NUMBER:** 3431  
**Poster Board Number:** P226  
**TITLE:** A Whale of a Tale: A One Environmental Health Approach to Study Metal Pollution in the Sea of Cortez  
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**KEYWORDS:** Metals; Environmental Toxicology  
**ABSTRACT:** Recent data, using a One Environmental Health approach, have identified marine metal pollution as an emerging concern for human, animal and ecosystem health in the Atlantic Ocean. Here we extend this approach to consider to the Sea of Cortez, a relatively isolated marine body of water rich in biodiversity. Most human activities in this area center on tourism, and there are also potentially significant inputs of pollution from agriculture, fishing practices, and metal mining. We consider the levels of heavy metals in seven distinct cetacean species found in the area. Our efforts considered two different periods of time. The first time period was in 1999 and was part of a global voyage focused on sperm whales (*Physeter macrocephalus*). The second time period started in 2016 and is part of an ongoing series of voyages focused on the Sea of Cortez. The second time period considered sperm whales, blue whales (*Balaenoptera musculus*), humpback whales (*Megaptera novaeangliae*), fin whales (*Balaenoptera physalus*), pilot whales (*Globicephala macrorhynchus*), minke (*Balaenoptera acutorostrata*) and Bryde’s whales (*Balaenoptera edeni*). We measured the levels of 23 metals and selenium in the skin of these whales in separate voyages during the spring seasons of 2016 and 2017. We considered the metal levels found in (1) all species together across years, (2) each species individually across years, and (3) each species by gender across years. We further compared metal levels found in sperm whale skin samples collected during these voyages to a previous voyage in 2000, to assess changes in metal levels over a longer time scale. The metals Mg, Fe, Al, and Zn were found at the highest concentrations across all species and all years. Within sperm whales, we observed decreased metal levels from 1999 to 2016/2017, except for nickel (Ni) and chromium (Cr), which increased during this time period. These results indicate a recent change in the pollution input to the Sea of Cortez, which may indicate a decreased potential concern for human, animal and ecosystem health for some metals, but an increased concern for the genotoxic metals Cr and Ni. *This work was supported by NIEHS grant ES016893 (J.P.W.) and numerous donors to the Wise Laboratory.*
**ABSTRACT NUMBER:** 3432       **Poster Board Number:** P227

**TITLE:** The Cytotoxic and Genotoxic Effects of Prolonged Particulate Hexavalent Chromium Exposure on Human Lung Epithelial Cells

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** T. J. Croom-Perez, C. R. Ziemba, C. T. Anglin, and J. P. Wise. *University of Louisville, Louisville, KY.*

**KEYWORDS:** Metals; Carcinogenesis; Genotoxicity

**ABSTRACT:** Hexavalent chromium (Cr(VI)) is a well-established human lung carcinogen and a major public health concern with widespread exposure; however the mechanisms of Cr(VI) carcinogenesis are not well understood. Pathology data from Cr(VI) workers indicate that accumulation of chromium in the stroma and not the years of exposure was a key factor in the Cr(VI) induced lung cancer. In addition, while chromium accumulates in the fibroblasts and not epithelial cells, the tumors are all of epithelial origin. These data suggest that the interaction between fibroblasts and epithelial cells is an important consideration in the mechanism of Cr(VI) carcinogenesis. Thus, our research is focused on characterizing the impact of Cr(VI) in a fibroblast-epithelial co-culture model. One of the first steps to studying this was to determine the cytotoxicity and genotoxicity of particulate Cr(VI) in human bronchial epithelial cells mono-cultured in media that would support the growth of both fibroblasts and epithelial cells. Using BEP2D cells as a model cell line, the cytotoxicity, amount of intracellular chromium, the amount of sister chromatid exchange, and a mitotic stage analysis after a 24h or 120h exposure to particulate Cr(VI) was determined. It was determined that particulate Cr(VI) induced concentration and time-dependent cytotoxicity in BEP2D cells. The amount of sister chromatid exchange increased with concentration after a 24h exposure, but decreased after a 120h exposure, consistent with previously published results in human lung fibroblasts. It was also shown that the mitotic index decreased in a concentration-dependent manner and the number of aberrant mitotic cells increased after exposure to particulate Cr(VI). These results suggest that particulate Cr(VI) is cytotoxic and genotoxic to human lung epithelial cells cultured in media that supports the co-culture of fibroblasts and epithelial cells.

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**ABSTRACT NUMBER:** 3433       **Poster Board Number:** P228

**TITLE:** Elucidating Differential Mechanisms of Cellular Toxicity among Lanthanide Series Metals Using Yeast Functional Toxicogenomics

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**KEYWORDS:** Metals; Mechanisms; Genomics

**ABSTRACT:** Lanthanide series metals have a range of uses in industrial and medical settings, but a comprehensive comparative analysis of their behaviors in biological systems has yet to be completed. In this study, the yeast deletion library was used to conduct a non-biased functional toxicogenomic study of the Lanthanide metals to identify the cellular and biochemical pathways relevant to the tolerance and metabolism of these rare earth metals. Bar-Seq and pathway enrichment analyses indicated that intracellular phosphate regulation was important for some but not all of the minerals studied. Further, some metals, such as Gadolinium, were seen to affect calcium-regulated processes - an observation in agreement with other test systems. Significantly, despite their proximity on the periodic table, the
Lanthanide metals did not all activate the same pathways, and demonstrated distinct biochemical effects.

**ABSTRACT NUMBER:** 3434  
**Poster Board Number:** P229  
**TITLE:** Obesogenic Effects and Metabolic Dysfunction in Individuals with Acute Pesticides Poisoning in a Remote Period of Time after the Incident  

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** N. Bubalo, M. Prodanchuk, O. Kravchuk, G. Balan, and P. Zhminko. L.I. Medved’s Research Center of Preventive Toxicology, Food and Chemical Safety Ministry of Health, Kyiv, Ukraine.  

**KEYWORDS:** Pesticides; Endocrine Disruptors; Clinical Toxicology  

**ABSTRACT:** In this study are 65 agricultural workers with the acute poisoning of 2,4-Dichlorophenoxyacetic acid (2,4-D). We observed patients for a long period of time from 2001 until nowadays. Along with neurological disorders, the toxic liver damage was detected, followed by the formation of hepatosteatosis in 35.8% of cases in patients with acute poisoning with 2,4-D herbicides, which was associated with the formation of metabolic disorders: dyslipidemia with a predominance of low-density lipoprotein (LDL), triglycerides and total cholesterol. At the last time, only 23 patients have been investigated. That way we will compere only this 23 patients (agricultural workers) with a control group (9 office workers). The aim of the study was investigating the frequency of metabolic disorders and obesogenic effects and to give ground for evaluation methods and optimizing prevention. We focused on the investigation body mass index (BMI), lipid profile and investigation of adipose tissue hormones (leptin, adiponectin) in individuals with acute pesticides poisoning in a remote period of time after the incident. Then patients were divided into two groups: I group with toxic liver injury (hepatosteatosis), II group without toxic liver injury. Anthropometric and biochemical patients studies in the long-term period made possible to detect a significant increase human body weight among 14 patients (group I) of 23 patients who had toxic liver injury with the formation of light and moderate hepatosteatosis in the acute period of poisoning and in the delayed period in the structure of clinical syndromes was noted. BMI significantly increased to an average of 42.75 +/- 2.39, which corresponds to the II degree of obesity. There was a significant increase in triglyceride, cholesterol, LDL, and atherogenic index in group I. Patients who had acute poisoning 2.4-D herbicide with toxic liver injury were observed a significant increase of leptin and reliable minor decrease adiponectin level. Our data show that increase secretion of leptin and decreased a level of adiponectin not only support metabolic disorders and obesity effects but also contribute to their progression, which is due to their biological role in the human body apparently. Patients with acute poisoning 2.4-D herbicide have a potential risk to the formation of dislipoproteinemia, hepatosteatosis, overweight.
**ABSTRACT NUMBER:** 3435  **Poster Board Number:** P230
**TITLE:** Study on Biomarkers Exposed to 1-bromopropane


**KEYWORDS:** Neurotoxicology; Biomarkers; Inhalation Toxicology

**ABSTRACT:** 1-Bromopropane (1-BP) has been used in many industries with significant adverse effects on nervous system. Animal tests and occupational survey were performed to explore the exposure and effect biomarkers of neurotoxicity induced by 1-BP. *In vivo* study: Male Wistar rats were exposed to 0, 500 and 1000 ppm 1-BP (18/group), 6 h/day for 21 consecutive days. Six rats of each group were euthanized on the 7th, 14th and 21st days. The brain tissues, urine and serum were collected. Histopathological examination was applied. Epidemiological survey: The exposure and control groups were studied with 71 workers in each group. Serum and urine were collected after work. Concentrations of 1-BP in the work sites and individuals were tested. Changing of NSE, S-100β and COX-2 in the cerebral cortex of rats and the serum of exposed workers were measured respectively, changes of 1-BP and its metabolite AcPrCys in the urine were detected. *In vivo* study: Purkinje cell atrophy, lumbar gray matter vacuolar degeneration, tibiofibular nerve fibers swelling and thickening were tested at 1000ppm on the 21st day. cNSE and cS-100β at 1000ppm, sNSE at 500ppm were increased significantly at all checkpoints. cS-100β at 1000ppm on the 21st days was increased significantly. Compared with the control, cCOX-2 at 500 and 1000ppm was increased greatly as well as at 500ppm on the 14th and 21st days compared with the 1000ppm group. sCOX was increased greatly on the 14th days at 500ppm, and on the 7th and 14th days at 1000ppm. A correlation between the changes of COX-2 in the cerebral cortex and serum was found, and 1-BP and AcPrCys were detected in the urine, there was a correlation between the changes of sNSE and sCOX-2 and AcPrCys in the urine at 500ppm. Epidemiological survey: The concentration of 1-BP in the use enterprises was generally higher than that of the production enterprises. 1-BP was detected in some urine samples and AcPrCys was detected in all urine samples of exposed workers. Correlation of AcPrCys and exposure concentration was detected. Subacute nerve injury was induced by 1-BP in rats under the conditions of this test. Concentrations correlation of COX-2 between cerebral cortex and serum was found. The prototypes and AcPrCys of 1-BP were detected in urine in exposure groups, and a good correlation between AcPrCys and exposure concentration was found. Results indicated that AcPrCys was more sensitive than the prototype as the 1-BP exposure biomarker.

**ABSTRACT NUMBER:** 3436  **Poster Board Number:** P231
**TITLE:** Mixture Designs to Investigate Synergistic Effects upon Co-exposure to Environmental Cyanotoxins

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** R. M. Martin, M. S. Bereman, and J. Stallrich. North Carolina State University, Raleigh, NC.

**KEYWORDS:** In Vitro and Alternatives; Exposure, Environmental; Proteome

**ABSTRACT:** The goal of this study was to implement powerful mixture design techniques, commonly used in process optimization, to investigate the synergistic effects of exposure to cyanotoxic mixtures. Exposure to cyanobacteria, which are found ubiquitously in environmental water reservoirs, have been linked to several neurodegenerative diseases. Despite the known co-occurrence of various cyanotoxins,
the majority of studies investigating this link have focused on the investigation of a single cyanotoxin, a noncanonical amino acid called β-methylamino-L-alanine (BMAA), which poorly recapitulates an actual environmental exposure to cyanobacteria. To this end, we describe the use of a simplex axial mixture design to screen for synergistic effects of cyanotoxic mixtures. Using a combination of basic toxicity assays coupled with contemporary proteomic techniques, our results show the existence of a significant (ps0.01) interaction between BMAA and its isomers aminoethyl glycine (AEG) and 2,4-diaminobutyric acid (2,4DAB). Cyanotoxic mixtures significantly decreased cell viability by an average of 19% and increased caspases 3/7 activities by an average of 110% when compared to individual cyanotoxins (ps0.05). Cyanotoxic mixtures perturbed various biological pathways associated with neurodegeneration, including inhibition of protective autophagy and activation of mitochondrial dysfunction (z-score >|2|). Additionally, exposure to mixtures perturbed important upstream regulators involved in cellular dysfunction, morbidity, and development. Taken together, our results highlight: (1) the need to study combinations of cyanotoxins when investigating the link between cyanobacteria and neurodegenerative disease and (2) the application of design of experiment (DoE) as an efficient methodology to study mixtures of relevant environmental toxins.

ABSTRACT NUMBER: 3437   Poster Board Number: P232
TITLE: Microfluidic Small Airway Lung Model for Toxicity Screening of Tobacco Products

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: P. Makena¹, D. Gordan², D. Haithcock², B. Prabhakarpandian², K. Pant², and G. L. Prasad¹. ¹RAI Services Company, Winston Salem, NC; and ²SynVivo Inc, Huntsville, AL.

KEYWORDS: Cytotoxicity; Biomarkers; Cell Culture

ABSTRACT: Physiologically relevant in vitro lung models are valuable tools to accurately assess the cytotoxicity of inhaled toxicants. Epithelial cells cultured in transwells do not completely represent the cellular architecture, physiology, and fluidic shear present in vivo. Recent advances in microfluidics have led to the development of bioengineered, three-dimensional human small airway models composed of human bronchial epithelial (NHBE) cells surrounded by human lung microvascular endothelial (HMVE) cells. In this study, we evaluated the potential of a human small airway lung-on-a-chip model to assess cytotoxicity and functional endpoints following exposure to cigarette total particulate matter (TPM). The microfluidic device is comprised of a central air channel and lateral vascular channels separated by porous architecture. Using an optimized co-culture protocol, NHBE and HMVE cells were co-cultured using a combination of air and fluidic pumps. The cells were exposed apically to different concentrations of TPM for 4 hours and 1) cell viability, 2) oxidative stress, 3) cell death, and 4) epithelial permeability were assessed after 20 hours post exposure. TPM induced a dose-dependent decrease in cell viability and increased oxidative stress, cell death, and epithelial permeability. These results 1) show the potential of human small airway in vitro lung model to mimic physiologically relevant conditions and 2) lay a foundation to study the effects of different tobacco products. This model enables our understanding of key molecular events that lead to early biochemical, toxicological, and/or physiological perturbations and will be useful in identifying relevant biomarkers of effect.
ABSTRACT NUMBER: 3438   Poster Board Number: P233
TITLE: Expression Patterns of Metallothionein Isomers in Various Subtypes of Prostate Cancer Cell Lines

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: S. Kim, S. Jin, and H. Jeong. Chungnam National University, Daejeon, Korea, Republic of.

ABSTRACT: The metallothioneins (MTs) are a family of low molecular weight, cysteine-rich intracellular proteins that bind transition metals, including Cu^{2+}, Zn^{2+}, Ag^{+}, and Cd^{2+} are involved in metal detoxification, chemoresistance, cell proliferation, apoptosis, and essential metal homeostasis. This study investigated the expression of 8 MT isomers, MT1A, MT1B, MT1F, MT1G, MT1H, MT1X, MT2A, and MT3, screened in human androgen-dependent prostate cancer cell lines (LNCap-FGC and LNCap-LN3) and human androgen-independent prostate cancer cell lines (DU-145 and PC3). Especially, this study focused on the mRNA expression of prostate cancer proliferation marker, such as prostate specific antigen (PSA), kallikrein2 (KLK2) and metastatic marker, such as matrix metalloproteases (MMPs), E-cadherin, N-cadherin, Vimentin, Twist, Snail, Slug, and ZEB1. MT1A, MT1B, MT1F, MT2A, and MT3 mRNA expression are highly expressed in LNCap-LN3 cells compared with the DU-145 and PC3. Also, LNCap-LN3 showed high mRNA expression in PSA and KLK2 compared with DU-145 and PC3. Furthermore, prostate proliferation and metastatic markers decreased by MT-2A-shRNA in LNCap-LN3. MT2A depletion decreased PSA and KLK2 mRNA expression and suppressed cell proliferation and migration. Thus, MTs expression may be involved in regulation of prostate cancer proliferation and migration.

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ABSTRACT NUMBER: 3439   Poster Board Number: P234
TITLE: Red Wine Consumption and the Effects on Paraoxonase 1 in a Healthy Population

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KEYWORDS: Biomarkers

ABSTRACT: Serum paraoxonase 1 (PON1) is a high-density lipoprotein (HDL)-associated enzyme capable of hydrolyzing a wide spectrum of substrates. The PON1 expression and enzymatic activities can be modulated by many factors such as physical activity, diet, alcohol and tobacco consumption and genetic polymorphisms. Aim: To evaluate the effects of red wine daily consumption for 6 weeks on PON1 concentration and enzymatic activity in a healthy population. A cohort pilot study was conducted in 44 volunteers from Nayarit, Mexico. The study was approved by the local Institutional Ethics Committee. A written informed consent was obtained from each patient in compliance with Good Clinical Practice, and the investigation conforms the principles in the Declaration of Helsinki. Anthropometric variables and family history were obtained using a structured and validated questionnaire. Blood samples were collected every two weeks after the first glass of wine to evaluate PON1 Lactonase and CMPAse activities according to Rock et al, (2008) and Ritcher et al, (2008), respectively. Basal and final blood samples were tested to evaluate lipid profile conducted by a certified clinical laboratory, and PON1 concentrations by ELISA. The population of the study showed an increased lactonase activity at the second week (10.98 ±0.25 U/mL) but decreased at the sixth week (9.96 ±0.22 U/mL). CMPAse activity
was low at the beginning, and then it increased at the sixth week (21.01 ±0.78 U/mL). Lineal regression analyses showed an association of lipid profile parameters and lactonase activity, where concentrations of PON1, HDL-c, triglycerides, low density lipoproteins and total lipids have a 16.47% (p=0.03) influence over the catalytic activity. Significant differences were observed at the sixth week in HDL-cholesterol levels and PON1 concentration (p=0.02). Red wine consumption has an hormesis effect on the PON1 concentration and activities in a healthy population.

ABSTRACT NUMBER: 3440  
Poster Board Number: P235  
TITLE: MicroRNAs as Biomarkers of Testicular Toxicity in Sprague Dawley Rats  

KEYWORDS: Biomarkers; RT-PCR; Testis  

ABSTRACT: Testicular toxicity (TT) is an important safety concern in drug development, but reliable and translatable biomarkers for predicting drug-induced TT are not available. The goal of this study, a collaborative project of the Critical Path Institute’s Predictive Safety Testing Consortium, was to determine whether circulating miRNAs (cmiRs) can serve as biomarkers of TT. Three known testicular toxicants, 1,3-dinitrobenzene (DNB, 10 mg/kg, oral gavage [po]), methoxyacetic acid (MAA, 250 mg/kg, po), or cadmium chloride (CC, 1.5 mg/kg, intraperitoneal) were administered for 3 consecutive days to male Sprague Dawley rats. Doses were selected to cause TT without toxicity to other tissues. Serum and tissues were collected from toxicant- and vehicle-treated rats at various times up to 7 days post-dose for cmiRs quantification and for microscopic evaluation of testes, epididymides, and 5 other major organs. All three toxicants caused microscopic lesions of varying severity (depending on the toxicant) in testes and epididymides but not in any other tissue. The exception to this was CC, which caused injection site related findings in the liver and heart at later timepoints. Following RNA isolation, 3 target cmiRs (miR-202-5p, miR-471-5p, miR-741-3p) were evaluated by RT-qPCR. C. elegans miR-39-3p was used as a spike-in normalization control. Quantitative analyses using the standard curve method showed greatly elevated levels of all three cmiRs in serum from CC-treated rats compared with controls on days 2–4; however, miR-202-5p elevation persisted until day 8. There were minimal to no increases in cmiR levels in DNB- and MAA-treated rats. Assay reproducibility was shown by having several independent laboratories quantify the cmiRs in serum aliquots from the same study. These data suggest that the rise in cmiRs was specific to CC-induced TT since CC-induced damage to other tissues was not observed. In conclusion, the three cmiRs were evaluated and reliably detected in serum concurrent with CC-induced TT; however, cmiRs could not be detected in mildly induced TT. Future studies will define the context of use and translatability of these cmiRs as testicular injury biomarkers.
Abstract Number: 3441  
Poster Board Number: P236  
Title: Development of LC-MS/MS Assays to Measure Thyroid Hormones in Rat Serum  

Authors (First Initial, Last Name) and Institutions: E. A. Groeber, H. Wang, S. R. Bell, J. Guo, J. Kooistra, P. S. Coder, and L. B. Moran. Charles River Laboratories, Ashland, LLC, Ashland, OH.

Abstract: Purpose: Thyroid hormones are of prime importance in the regulation of metabolism and protein synthesis. During development, they play a critical role in fetal growth and neurodevelopment. The determination of serum concentrations of thyroid stimulating hormone (TSH), triiodothyronine (T3) and thyroxine (T4) in adult, late-fetal and neonatal animals is required by US EPA and OECD. The roles of reverse T3 (rT3) and T2 hormones are less understood, but may have value as supplementary indicators of endocrine disruption. In our laboratory, adult rat TSH was traditionally analyzed by radioimmunoassay (RIA) while T3 and T4 were analyzed by a sensitive LC-MS/MS assay. The RIA method is not feasible in fetal and neonatal rat studies as it requires larger sample volumes (200 µL) and has poor robustness and specificity. To improve feasibility and simplicity, it is preferred to measure TSH and other thyroid hormones by LC-MS/MS methods. Methods: Two parallel LC-MS/MS assays were developed for thyroid hormone analysis. For TSH, a biotinylated mouse anti-rat TSH beta mAb immobilized on magnetic beads was used to extract rat TSH from 50 µL rat serum. The extracted TSH was digested on-bead by trypsin. A unique peptide in TSH was analyzed by UHPLC-high resolution Q-Exactive mass spectrometer for quantification. A stable isotope labeled peptide was used as internal standard. For T3, T4, rT3 and T2, the surrogate analytes were used for quantitation. All four hormones were co-extracted from 50 µL of rat serum by an SLE extraction method and analyzed in a single LC-MS/MS run. Results: The LC-MS/MS method of TSH has a linear dynamic range of 1.8-360 ng/mL, which is comparable to the previously qualified radioimmunoassay (2-64 ng/mL) with only 50 µL sample volume. The calibration curve has a linear regression at r²=0.9937 in 1/x² weighting. The QC accuracy was between 87 to 111% and an overall CV was within 10% for all four concentration levels (n=4). Assay selectivity and carryover was assessed and met acceptance criteria. For the four other thyroid hormones, the curve ranges of T3, rT3, T4 and T2 on LC-MS/MS were determined to be 5-2500, 125-62500 and 10-2500 pg/mL, respectively. Conclusion: Two sensitive LC-MS/MS assay were developed to quantify five thyroid hormones in rat serum with a small sample volume. This all-in-one mass spectrometric analysis approach will simplify the sample volume needs and procedures used for developmental endocrine toxicity studies.

Abstract Number: 3442  
Poster Board Number: P237  
Title: Evaluation of Circulating MicroRNA Biomarkers in the Acute Pancreatic Injury Dog Model

Authors (First Initial, Last Name) and Institutions: H. Lee, H. Park, H. Choi, S. Lee, J. Lee, E. Cho, H. Han, J. Seok, and W. Son. Ulsan University, Seoul, Korea, Republic of.

Abstract: This study aimed to evaluate the usefulness of four microRNAs (miRNAs) in an acute pancreatic injury dog model. Acute pancreatitis was induced by infusion of cerulein for 2 h (7.5 μg/kg/h). The levels of well-known miRNAs, microRNA-216a (miR-216a) and microRNA-375 (miR-375), and new candidates microRNA-551b (miR-551b), and microRNA-7 (miR-7), were measured at 0, 0.5, 1, 2, 6, 12, and 24 h with serum amylase and lipase, and histopathological examination was performed. Among the four miRNAs, miR-216a and miR-375, and serum enzymes were significantly increased by cerulein treatment. The expression levels of miRNAs and serum enzymes peaked at 2-6 h with a similar pattern;
however, the overall increases in miR-216a and miR-375 levels were much higher than those of the serum enzyme biomarkers. Increased levels of miR-216a and miR-375 were most highly correlated to the degree of individual histopathological injuries of the pancreas, and showed much greater dynamic response than serum enzyme biomarkers. Twenty-four-hour time-course analysis in this study revealed time-dependent changes of miRNA expression levels, from initial increase to decrease by predose level in acute pancreatitis. Our findings demonstrate that, in dogs, miR-216a and miR-375 have the potential to sensitively detect pancreatitis and reflect well the degree of pancreatic injury, whereas miR-551b and miR-7 do not.

ABSTRACT NUMBER: 3443    Poster Board Number: P238
TITLE: The Extended Effects of Thapsigargin on Human Neutrophils’ Calcium Pathways
KEYWORDS: Pharmaceuticals; Mechanisms
ABSTRACT: Intracellular free calcium levels [Ca^{2+}]i have an important role in the pro-inflammatory activity and life span of neutrophils. For these reasons, the mechanisms by which [Ca^{2+}]i are regulated in neutrophils have been comprehensively discussed and studied along the years. However, the exact mechanisms through which it occurs are still unclear. Thapsigargin, a plant-derived sesquiterpene lactone, is a highly specific and essentially irreversible SERCA [sarco(endo)plasmic reticulum Ca^{2+}-ATPase] inhibitor, that has been extensively used in the study of intracellular Ca^{2+} flux, but its effects on neutrophils are poorly known. In this sense, this work has its focus on the thapsigargin effects in the [Ca^{2+}]i flux, evaluated in freshly isolated human neutrophils, using a microplate reader for monitoring fluorimetric kinetic readings. The [Ca^{2+}]i flux was measured recurring to the FLUO-4 AM probe and cellular viability was evaluated by the trypan blue exclusion assay. To better understand the mechanism through which thapsigargin exerts its effects in the [Ca^{2+}]i flux, a broadly described inhibitor of SOCE (store-operated calcium entry), ML-9, and a chelating agent of extracellular Ca^{2+}, EGTA, were used. The obtained results corroborate the general thapsigargin-induced intracellular pattern of [Ca^{2+}]i fluctuation, but a much more extended effect in time and a clearly sustained increase of [Ca^{2+}]i due to the influx by SOCE were observed. Finally, in human neutrophils, thapsigargin acts in another Ca^{2+} influx pathway than SOCE, with the consequent stimulation of neutrophils’ death. Acknowledgements: This work was financed by FEDER-Fundo Europeu de Desenvolvimento Regional through the COMPETE 2020-Operacional Programme for Competitiveness and Internationalisation (POCI), and by Portuguese funds through FCT-Fundação para a Ciência e a Tecnologia in the framework of the project POCI-01-0145-FEDER-029253. We gratefully acknowledge Margarida Amil and Graça Porto and the nursing staff of the Centro Hospitalar do Porto-Hospital de Santo António blood bank for the collaboration in the recruitment of blood donors to participate in the study.
ABSTRACT NUMBER: 3444    Poster Board Number: P240

TITLE: COVER: Conformational Oversampling of Datasets for Deep Learning in In Silico Toxicology

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KEYWORDS: Computational Toxicology; QSAR; Predictive Toxicology

ABSTRACT: Traditional Machine learning (ML) approaches are commonly used for in silico toxicology. In 2012 the Merck Kaggle competition and the Tox21 Challenge in 2014 cast light upon the use of neural networks for biological activity prediction. Both challenges were won by deep neural network architectures, showing that these methods are viable alternatives to traditional ML approaches. The results of the two challenges indicate that deep learning could improve the predictivity, especially for complex endpoints such as organ toxicity. Deep learning is widely researched but only very limited applications for in silico toxicology have been published, especially because datasets are sparse. To show that a combined database of published QSAR data would enhance model building and facilitate data collection, a python library named JUBioactivities containing 119188 compounds with 476 reported endpoints was developed. A drawback is that, although the library contains a high number of compounds, and can be used for multi-task learning, single endpoints still have a low number of compounds and are mostly imbalanced. This leads to overtraining and disregard of the minority class. To aim at balanced training and creation of larger datasets a technique called Conformational OVERSampling (COVER) was established. COVER is generating a given number of molecular conformations for each compound. With the help of 3D descriptors the enlarged dataset is used to train deep neural networks. This increases the size of the dataset without creating artificial samples. In first trials the method was validated on two class labels from two distinct datasets from the JUBioactivities library. The first dataset is part of the Tox21 dataset (imbalance ratio 1:16) the second dataset is part of the hepatotoxicity dataset published by Mulliner et al. (imbalance ratio 1:2). Both were balanced with COVER and models were validated on an external test set. For the Tox21 dataset performance increased from a mean sensitivity of 0.18 to 0.42 and a mean balanced accuracy of 0.57 to 0.67. For the Mulliner dataset the mean sensitivity increased from 0.19 to 0.51 and the balanced accuracy increased from 0.56 to 0.61. We acknowledge financial support provided by eTRANSAFE.

ABSTRACT NUMBER: 3445    Poster Board Number: P241

TITLE: Toxicity Prediction for Human Mitochondrial Respiratory Complex I: Combining Structure-Based Methods and Machine Learning


KEYWORDS: Computational Toxicology; Predictive Toxicology; QSAR

ABSTRACT: Inhibition of the mitochondrial respiratory complex is one of the factors leading to toxicity of drugs and drug candidates. The increasing amount of high resolution crystal-structures of proteins from the mitochondrial respiratory complex enables structure-based approaches to predict mitochondrial toxicity. In the work presented here, a common binding mode for rotenone and deguelin, two complex I inhibitors, could be proposed. Therefore, induced-fit docking and evaluation of the docking poses, using
common scaffold clustering and RMSD calculations have been performed. Subsequently, two pharmacophore models were created and used for virtual screening of Drugbank and the Chemspace database. The retrieved hits were subjected to a set of machine learning based models for mitochondrial toxicity. The machine learning models comprised of a random forest, a gradient boosting, and a deep learning model. All were trained on a dataset based on 5759 compounds mined from various databases and literature. After re-docking, ten compounds have been chosen for subsequent experimental testing. Overall, characterizing the binding-mode of known inhibitors of respiratory complex I improves our molecular understanding of the interactions leading to toxic events. This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 681002 (Eu-ToxRisk) and No. 777365 (eTRANSAFE)

**ABSTRACT NUMBER:** 3446  
**Poster Board Number:** P242  
**TITLE:** Whole-Brain R1 Mapping Predicts Mn Accumulation in the Human Brain: A Support Vector Machine Approach  
**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** D. A. Edmondson, S. Hélie, and U. Dydak, Purdue University, West Lafayette, IN.  
**KEYWORDS:** Computational Toxicology; Neurotoxicity; Metals; Biological Modeling  
**ABSTRACT:** Exposure to welding fume leads to accumulation of Manganese (Mn), a MRI contrast agent, in the brain. Although we may safely assume that the MRI relaxation rate (R1) is proportional to Mn accumulation, previous studies suggest that the relationship between exposure and changes in R1, in contrast, is likely non-linear, especially at low exposure levels. To better understand the relationships between Mn exposure, accumulation, and changes in R1, we used whole-brain R1 maps to predict the Mn exposure and Mn deposition in the brain using a series of computational models. Data was used from a prior study involving 89 subjects (57 welders, 32 controls). Scans had been performed on a 3T GE Signa MRI scanner. Whole brain 3D R1 relaxation maps were calculated from two spoiled gradient echo images resulting in a quantitative measure of Mn deposition in the brain. R1 maps were then segmented into 192 regions of interest using FreeSurfer. R1 in each region was summarized by five statistics: mean, variance, skew, minimum, and maximum. Four targets were predicted using R1: group (welder or control), years welding, Mn air exposure (MnAir), and excess Mn (MnEX) in the brain. MnAir was calculated using air samples from the workplace and adjusting for other factors (e.g. respirators). MnEX was calculated from a biokinetic model that predicted excess Mn accumulation in the brain given MnAir and a detailed work history for each subject. A support vector machine was used for classification (SVC) at increasing thresholds in years welding, MnAir, and MnEX for each summary statistic using a penalty term to account for unbalanced classes. For each threshold, SVCs for each summary statistic were aggregated and the final prediction was determined by a majority rule. Leave one out cross-validation was used to measure accuracy of each model. We found that while individual statistics performed better than chance, the aggregated SVC model for determining welder or control (group) was about 74%. However, other targets were more accurate. For MnAir, accuracies greater than 80% were found for air exposure levels of greater than 0.1 mg/m³. Whereas, MnEx performed well across all levels of brain accumulation with accuracies approaching 90% at MnEx higher than 7 mg. Finally, an aggregated SVC targeting years welding scored accuracies greater than 80% for greater than 8 years of welding. Results
Indicate that we can predict Mn exposure and excess Mn in subjects’ brains with reasonably high accuracy using R1 maps.

**ABSTRACT NUMBER:** 3447  **Poster Board Number:** P243  
**TITLE:** Physiologically Based Pharmacokinetic (PBPK) Modeling of Variability in Perchloroethylene Metabolism across Multiple Strains of Mouse  
**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** C. Dalaijamts, J. A. Cichocki, Y. Luo, I. Rusyn, and W. A. Chiu. Texas A&M University, College Station, TX.  
**KEYWORDS:** Physiologically-Based Pharmacokinetics; Dosimetry; Predictive Toxicology  
**ABSTRACT:** Quantifying inter-individual variability in perchloroethylene (perc) induced organ-specific toxicity is a challenge in risk assessment. Our previously updated Bayesian population physiologically-based pharmacokinetic (PBPK) model has provided evidence for inter-strain variability in oxidative metabolism of perc in three mouse strains, suggesting the need to better characterize toxicokinetic uncertainty and variability. To address this need, 45 strains of mice from the Collaborative Cross were administered perc, and toxicokinetic time course data on parent and metabolites in blood and tissues were collected for analysis and modeling. Specifically, these data are used to refine the previous PBPK model in mice and characterize inter-strain variability and uncertainty in the toxicokinetics of perc and its oxidative and conjugative metabolism. We first identified the most influential PBPK parameters based on global sensitivity analysis. Hierarchical Bayesian population analysis using Markov chain Monte Carlo simulations was conducted to characterize uncertainty and inter-strain variability in perc metabolism. The refined PBPK model provides accurate estimates in tissue-specific inter-strain variability and uncertainty in oxidative and GSH conjugation metabolism after exposure to perc in the mouse population. Both oxidative and conjugative metabolism were predicted to varied across multiple inbred strains, consistent both qualitatively and quantitatively with in vivo measurements. This refined PBPK model improves quantitative risk assessment by predicting the internal dosimetry of perc and its metabolic pathways, and characterizing organ-specific toxicokinetic variability among individuals.

**ABSTRACT NUMBER:** 3448  **Poster Board Number:** P244  
**TITLE:** Meta-Analysis Approach for Developing Predictive Toxicity Models of Metal Oxide Nanoparticles  
**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** R. Garg, and D. Stover. Charter School of Wilmington, Wilmington, DE. Sponsor: N. Yanamala  
**KEYWORDS:** Computational Toxicology; Metals; Environmental Toxicology  
**ABSTRACT:** Metal nanoparticles are particles ranging in size from 1 to 100nm that are increasingly found in consumer products, electronics, medications, construction, and other industrial applications. In this study, a generalized classification model for predicting toxicity of metal and metal oxide nanoparticles was developed using meta-analysis approaches. The physical, chemical, and structural properties of the seven different nanoparticles along with the in vitro experimental conditions were considered to evaluate the role of these factors in predicting toxicity. Data preprocessing techniques such as scaling, and oversampling were implemented to address issues associated with skewness and imbalanced data. A number of unsupervised learning algorithms such as hierarchical clustering, principal component analysis, factor analysis, and outlier analysis were employed to guide the development of the
classification model. Following this, several supervised learning algorithms, including generalized linear model, standard vector machine, gradient boosting, and random forest were evaluated to develop the predictive models. Both external and internal validation methods were employed to overcome sources of bias and overfitting. Of the models developed, the random forest model and gradient boosting were best at predicting toxicity. The variables of dose, formation enthalpy, and time had the greatest importance, suggesting a correlation between physical characteristics of nanoparticles and the biological response of a cell. In summary, this study highlights the need for data preprocessing techniques to reduce bias and noise during the development of the model.

ABSTRACT NUMBER: 3449   Poster Board Number: P245

TITLE: The Danish (Q)SAR Database with Predictions for More Than 650,000 Substances

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: N. Nikolov, and E. Wedebye. Technical University of Denmark, Kongens Lyngby, Denmark. Sponsor: D. Woolley

KEYWORDS: QSAR; Computational Toxicology

ABSTRACT: The Danish (Q)SAR database is a free searchable online repository of structural information and (Q)SAR predictions for more than 650,000 substances, including more than 80,000 substances registered and/or pre-registered under the EU chemicals legislation, REACH. The predicted properties cover many physical-chemical, environmental fate, bioaccumulation, eco-toxicity, absorption, metabolism and toxicity endpoints. The applied (Q)SAR models are developed in-house or obtained from external sources. To further increase prediction accuracy and/or model coverages of the chemical universe, three software systems, namely Leadscope, CASE Ultra and SciQSAR, were applied when possible to develop models for the same endpoint and an overall battery prediction was made as a majority vote between the three systems. To ensure transparency up against the OECD (Q)SAR Validation Principles, documentation for all models developed in-house as well as DTU-licensed commercial models is available from the database in the internationally agreed QSAR Model Reporting Format (QMRF). The database is freely available via a web portal (http://qsar.food.dtu.dk) with an extensive search system including search by predictions, structure, similarity and experimental data from training sets. A full chemical profile is downloadable for any of the database substances. The database is developed by the QSAR team at the DTU Food Institute, with financial support from the Danish EPA and Nordic Council of Ministers, and is furthermore supported by the European Chemicals Agency. Scientific and user inputs from an international board including representatives from regulators, Industry, NGO and Academia during the development phase is likewise acknowledged. Since the publication of the database in November 2015, it has been used by more than 5,400 unique IP addresses running more than 77,000 searches and requesting download of over 50,000 (Q)SAR profiles by users in more than 25 countries. The database was recently linked to the OECD QSAR Toolbox via a hotlink for dynamic data retrieval. The database was expanded in 2018 with more than 10,000 new structures and predictions from many new models, including predictions from a number of OECD QSAR Toolbox profilers.
ABSTRACT NUMBER: 3450   Poster Board Number: P246

TITLE: Danish (Q)SAR Models: A Free Online DTU QSAR Predictor Powered by Leadscope

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ABSTRACT: The Danish (Q)SAR Database (http://qsar.food.dtu.dk) is a freely available online database with pre-generated predictions from a large number of QSAR models for over 650,000 chemical substances. A selection of >30 of these QSAR models of diverse endpoints encompassing metabolism, endocrine activity, genotoxicity and sensitization have been implemented in the new Danish (Q)SAR Models real-time online predictive system, accessible from the Danish (Q)SAR Database. The new system generates predictions on the fly for user-submitted structures and outputs detailed reports including probability for activity, structural alerts and training set analogs. All the provided QSAR models have undergone robust cross-validation and documentation in the international QMRF format is available from the site. The client and server software for the new system as well as the QSAR models are developed by DTU. The models are developed using the Leadscope Enterprise software. These models hosted in the Leadscope software are made freely available online under a special agreement between Leadscope and DTU. The Danish (Q)SAR Models system uses secure communication and works in a browser without need to install programs, plugins or add-ons. All major browsers and operating systems are supported.

ABSTRACT NUMBER: 3451   Poster Board Number: P247

TITLE: Evaluation of the Global Performance of Eight In Silico Skin Sensitization Models Using Human Data

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KEYWORDS: QSAR

ABSTRACT: Skin sensitization, or allergic contact dermatitis, is a leading human health hazard in occupational settings. Consequently, several testing approaches exist, including in chemico, in vitro, and in silico models, to assess skin sensitization. While all these approaches offer unique advantages in identifying potential dermal sensitizers, in silico models are perhaps the most advantageous due to their high speed and low-cost results. Many in silico skin sensitization models are available, though many have only been validated against results from animal studies, most commonly the local lymph node assay (LLNA). Consequently, this creates uncertainty in their accuracy when used to predict skin sensitization in humans and, ultimately, limits their use in response to regulatory requirements. This assessment evaluates the accuracy of eight in silico skin sensitization models against two human data sets: one highly curated (Basketter et al. 2014, n=131) and one created for this project, a screening level data set drawn from the Hazardous Substances Data Bank (HSDB, n=375). Most models performed comparably, with accuracies ranging from approximately 60% to almost 90% (balanced accuracies: 55% to almost 90%) for the highly curated data set and approximately 55% to 80% (balanced accuracies: 50% to almost 80%) for the screening level data set. Yet, some offer advantages over others, such as near perfect sensitivity (at the expense of specificity) or near perfect specificity (but with compromised sensitivity). Some models include probability of accuracy, which makes the confidence in the prediction
transparent. Others include a metabolism feature: this highlights the role of metabolism in model accuracy. Importantly, several models performed as well as an LLNA assay (i.e., 80%). In conclusion, in silico skin sensitization models offer accurate and useful insights in a screening context; however, additional work is needed to further improve these models so that they may be considered fully reliable for regulatory applications.

**ABSTRACT NUMBER:** 3452  **Poster Board Number:** P248

**TITLE:** Thorough QT/QTc in a Dish: An In Vitro Human Model That Accurately Predicts Clinical Concentration-QTc Relationships

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**KEYWORDS:** Computational Toxicology; In Vitro and Alternatives; Cardiovascular System

**ABSTRACT:** Thorough QT/corrected QT (QTc)” (TQT) studies are cornerstones of clinical cardiovascular safety assessment. However, TQT studies are resource intensive, and preclinical models predictive of the threshold of regulatory concern are lacking. We hypothesized that an in vitro model using induced pluripotent stem cell (iPSC)-derived cardiomyocytes from a diverse sample of human subjects can serve as a “TQT study in a dish.” For 10 positive and 3 negative control drugs, in vitro concentration-QTc, computed using a population Bayesian model, accurately predicted known in vivo concentration-QTc. Moreover, predictions of the percent confidence that the regulatory threshold of 10 ms QTc prolongation would be breached were also consistent with in vivo evidence. This “TQT study in a dish,” consisting of a population-based iPSC-derived cardiomyocyte model and Bayesian concentration-QTc modeling, has several advantages over existing in vitro platforms, including higher throughput, lower cost, and the ability to accurately predict the in vivo concentration range below the threshold of regulatory concern.

**ABSTRACT NUMBER:** 3453  **Poster Board Number:** P249

**TITLE:** Predictions of Respiratory Tract Uptake of High Vapor Pressure Constituents in Puffs from Electronic Nicotine Delivery Systems (ENDS)


**ABSTRACT:** ENDS aerosols can contain harmful aldehyde byproduct constituents (e.g., formaldehyde, acetaldehyde, acrolein), which can be carcinogenic or lead to adverse health effects upon inhalation. Additionally, ENDS aerosols may also contain high vapor pressure flavor constituents (e.g., diacetyl, 2, 3-pentanedione, acetoin). High vapor pressures make these constituents readily available in the vapor phase. Assessment of the uptake of these constituents during ENDS use can facilitate better understanding of the relationship between exposure and tissue deposition, which can be informative for risk evaluations. Vapor uptake from lung airways may depend on lung tissue concentrations; thus, vapor concentration in the air and tissue phases is coupled and will depend on the vapor concentration at the air-tissue interface. An uptake model for high vapor pressure constituents of ENDS was developed for the human lung. Vapor concentrations in the air and tissue were considered as a function of interface concentration. Interface concentration was determined from the flux of vapor across the interface and
substituted into expressions for concentrations in air and tissue. Mass of vapor absorbed by the tissue and the amount travelling through the tissue was determined. Model predictions for three aldehyde byproducts and three flavor constituents indicated significant uptake in the oral cavity (60% to 80%). Tissue absorption in the lower respiratory tract was highly dependent on the reactivity, solubility, and diffusivity of the vapor in the tissue. Formaldehyde, a highly tissue-soluble compound, was absorbed by lung tissues (~15%) during the first 8 generations of the respiratory tract. Diacetyl, which has a lower tissue-solubility, deposited farther into the lung with 27% of the amount inhaled being absorbed in the tracheobronchial airways and <5% absorbed in the alveolar region. Thus, the results showed that vapor properties play a significant role in their absorption and subsequent health effects. The model developed will be a useful tool in evaluating risks of high vapor-pressure constituents of ENDS.

ABSTRACT NUMBER: 3454    Poster Board Number: P250
TITLE: Translating an Existing Physiologically-Based Pharmacokinetic Model for Isopropanol from a Scalar to an Array Format

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KEYWORDS: Physiologically-Based Pharmacokinetics; Biological Modeling

ABSTRACT: Physiologically-based pharmacokinetic (PBPK) models often contain sub-models for not only the parent, but also one or more metabolites, which may mirror the structure of the parent sub-model (e.g., the same compartments included) as well. In these cases, modifications made to the sub-model for the parent need to be duplicated in the one or more sub-models for the metabolites. This is not such an overwhelming task if there is only one metabolite sub-model; however, if there are multiple sub-models, the task can be daunting to define the additional parameters and ensure consistency with each sub-model. One simple example of a parent/metabolite PBPK model is the published one for isopropanol and its metabolite acetone. Using the published version of the isopropanol model and acslX, the model was recoded to use arrays for each compartment in the model as well as the parameter values needed for the equations describing each compartment. Switch parameters (i.e., parameters with either a value of 0 or 1 for each chemical) are also included in the equations to include/exclude any terms or factors within a term that are needed for one chemical but not another. The resulting model was then run to demonstrate that the array version of the model duplicates the published predictions from the scalar version of the model. Additionally, a parameter was included to define the dimension of the arrays such that a model for two chemicals can quickly and easily be changed to include additional chemicals. The array concept was then carried one step further by combining the arrays for each tissue into a single array (i.e., describing all tissue compartments with a single set of equations), thus decreasing the number of equations that might have to be modified even further while still accurately duplicating predictions from a scalar model. This poster will present the predictions of both array versions in comparison to predictions from the published model as well as issues that were encountered in the translation process. This array model structure is also very flexible in that the sub-models defined by the arrays do not have to be linked (e.g., parent and metabolite), but may instead be for separate and unrelated chemicals. This concept could greatly help streamline model development as well as execution of models, as multiple chemicals could be simulated simultaneously.
ABSTRACT NUMBER: 3455    Poster Board Number: P251
TITLE: Novel Array Approach to Mixtures Model Streamlines Predictions of Cochlea and Brain Region Tissue Concentrations from JP-8 Inhalation Exposure

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KEYWORDS: Physiologically-Based Pharmacokinetics; Biological Modeling

ABSTRACT: Physiologically-based pharmacokinetic (PBPK) models of mixtures are typically coded in parallel and run simultaneously. The combined code may be thousands of lines long, giving the modeler countless opportunities to introduce error. The novel array approach allows each mixture component to be simulated through one unified model structure. This approach has been applied to time course predictions for five JP-8 jet fuel components: toluene, ethylbenzene, xylenes, nonane and decane. The model structure includes brain regions (brainstem, temporal lobe, remainder of brain), cochlea, fat, liver, rapidly perfused and slowly perfused tissues. Published models of toluene, ethylbenzene and xylenes utilize simple flow limitation to each tissue. In contrast, models simulating nonane and decane require diffusion limitation to certain tissues. The array model was constructed with optional diffusion limitation in each tissue compartment; this feature can effectively be turned off for flow limited chemicals. The model was validated through successful simulation of literature based time course data sets for the five individual JP-8 components. The model was then utilized to predict hearing pathway tissue concentrations for a study published by Guthrie et al. (2014) in which rats were exposed to 1000 mg/m³ JP-8 for 28 days (6 hours/day, 5 days/week). The study found central auditory processing defects without peripheral hearing loss. Model predictions indicate the concentration of decane, which constitutes about 2.5% of JP-8, is 4 times higher in the brain stem than in the cochlea. All five JP-8 components are predicted to peak at higher concentrations in the brain stem than in the cochlea or blood, supporting the finding that rats are more sensitive to central auditory pathway effects than peripheral hearing deficits when exposed to JP-8.

ABSTRACT NUMBER: 3456    Poster Board Number: P252
TITLE: Quantification of Sertraline Dosimetry in Pregnancy Using Physiologically Based Pharmacokinetic Approaches

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KEYWORDS: Pharmaceuticals; Physiologically-Based Pharmacokinetics; Biological Modeling

ABSTRACT: Pregnancy is a high-risk period for women with psychiatric illness such as depression; therefore, treatment with antidepressants may be unavoidable when indicated. Physiological changes that occur during pregnancy can affect the pharmacokinetics (PK) of many drugs including antidepressants. PK variations may translate into undesirable pharmacodynamic (PD) consequences such as decreased maternal drug efficacy and safety and present unknown risk to the unborn child. Hence, the ability to quantify maternal and fetal exposure to antidepressants can provide an evidence-
based approach to improving drug efficacy and safety during pregnancy. Sertraline is among the most frequently used antidepressant during pregnancy. We have used a deterministic physiologically based pharmacokinetic (PBPK) model to understand changes in exposure to sertraline. We utilized knowledge of sertraline disposition in nonpregnant women, physiological changes during pregnancy and in vitro metabolism studies. Simulations were performed to predict sertraline dosimetry in non-pregnant women and during the second and third trimesters of pregnancy. The average (min - max range) predicted-to-observed sertraline area-under-the-curve (AUC) ratio was 1.2 (0.6 - 2.2) in second trimester and 1.1 (0.8 - 3.3) in third trimester of pregnancy. The average (min - max range) predicted-to-observed sertraline maximum plasma concentration (Cmax) ratio was 0.98 (0.5 - 1.6) in second trimester and 0.9 (0.6 - 1.5) in third trimester of pregnancy. The model performance was similar in nonpregnancy with the predicted-to-observed ratio for AUC and Cmax averaging 0.98. The model we have developed can be used for maternal sertraline dose adjustment during pregnancy. Ongoing research includes extending the current model to predict fetal exposure to maternal sertraline.

ABSTRACT NUMBER: 3457    Poster Board Number: P253
TITLE: A PBPK Model Describing the Pharmacokinetics Of γ-HBCD Exposure in Mice
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KEYWORDS: Biological Modeling; Persistent Organic Chemicals; Toxicokinetics
ABSTRACT: The brominated, flame-retardant 1,2,5,6,9,10-hexabromocyclododecane (HBCD) is added, but not bound in consumer products, then found eventually in the environment and human tissues. Commercial HBCD mixtures contain three major stereoisomers, alpha (α), beta (β), and gamma (γ), that are typically at ratios of 1.2:0.6:8.2, respectively. Although HBCD is widely used, the toxicological effects from its exposure in humans are not clearly understood. A better understanding of the pharmacokinetics via a physiologically based pharmacokinetic (PBPK) model may help to understand the toxicity of HBCD. The objective of this work was to develop a PBPK model to evaluate the pharmacokinetics of γ-HBCD in C57BL/6 mice. This PBPK model consists of six compartments: brain, kidneys, liver, skin, adipose tissue, and rest of the body. All compartments are described as flow limited except liver and adipose tissue which are diffusion limited. Physiological parameters related to body size, organ weights and blood flow were taken from the literature. All partition coefficients were calculated based on the log Kow. All other parameters were optimized to tissue dosimetry available in the literature. Urinary elimination is described as a saturable function mediated by a protein transporter in the renal proximal tubules. The elimination in urine and feces was optimized to reflect the % dose eliminated from the literature. Unlike most persistent organic pollutants, data in the literature indicates that the liver accumulates HBCD. This accumulation in the liver is similar to dioxin-like chemicals, so an inducible hepatic HBCD binding protein was included in the model, as has been done with PBPK models for dioxin-like chemicals. Compared with data from the literature for liver, blood, and adipose tissue, the PBPK model simulation provided a good fit. The model accurately describes the mouse data set within 1.5-fold of the data. While this version of the PBPK model only describes γ-HBCD, more efforts are required to clarify and improve the model to discriminate between the γ, α, and β enantiomers. This
ABSTRACT NUMBER: 3458   Poster Board Number: P254
TITLE: In Silico Prediction of Structure and Compound Affinity of Multidrug Resistance-Associated Protein 2 (MRP2) for Predicting Hepatotoxicity

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KEYWORDS: Biliary Excretion; Xenobiotic Transporters; Systems and Integrative Toxicology

ABSTRACT: Background: MRP2 is an unidirectional efflux transporter mainly present in liver, that primarily transports organic anions, including drug conjugates and conjugated bilirubin [1]. Due to the lack of a X-ray structure of MRP2, there is no detail information about interaction of compounds with MRP2. Objective: The objectives are to develop 3D model for MRP2 using homology modeling and analyze affinity with compounds using molecular docking approach. Methods: The 3D model of MRP2 was determined by homology modeling and validated by Ramachandran plot, Z-score. Molecular Dynamics was used to optimize MRP2 model which later used for compound affinity prediction using docking. Results: The 3D model of MRP2 is derived by homology modeling using bovine MRP1 as template with more than 50% sequence identity. The model of MRP2 was validated by Ramachandran plot with 90.4% of amino acids in the most favored regions and 8.3% in additional allowed regions. Molecular docking between MRP2 model and compounds shows a very good affinity with CDFDA (docking energy of -10.5 kcal/mole) which is also known as strong MRP2 substrate. Caffeine (Cf) and chlorpromazine (CPZ) are chosen as our negative control (respectively: IC50 >133 and IC50=260 µM), with higher docking energy of -6.6 kcal/mole and -6.3 kcal/mole. Cyclosporine A (IC50=10µM, docking energy = -8.8 kcal/mole) predicts lower docking energy than Cf and CPZ and found to be a good inhibitor of MRP2. We will present more affinities of compounds against MRP2 in our poster. Reference: [1]: Colombo F, Armstrong C, Duan J, Rioux N. A high throughput in vitro mrp2 assay to predict in vivo biliary excretion. Xenobiotica. 2012 Feb;42(2):157-63.

ABSTRACT NUMBER: 3459   Poster Board Number: P255
TITLE: Comparing Regulatory Networks between Breast Cancer Cell Line MCF-7 and Human Breast Cancer Tissues

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KEYWORDS: Bioinformatics; Transcription Factors; Cell Culture

ABSTRACT: Lack of characterization of cell lines can have serious consequences on the translatability of in vitro scientific studies to human clinical trials. This project focuses on the Michigan Cancer Foundation-7 (MCF-7) cells, a human breast adenocarcinoma cell line that is commonly used for in vitro cancer research, with over 33,000 publications in PubMed. Previously, our center has showed that even MCF-7 cells obtained from the same cell batch at the same cell bank can display cellular and phenotypic heterogenicity, which affected reproducibility of experiments using this cell line. As a follow-up study,
we compare a large mRNA microarray data set of MCF-7 to a large mRNASEq breast cancer tissue data set. The MCF-7 dataset includes 351 samples obtained from Gene Expression Omnibus, in which cells were treated with a dose-response curve of xenoestrogens and estradiol; the human breast cancer tissue dataset came from the Cancer Genome Atlas, including 1098 breast tissue samples from individuals with breast invasive carcinoma. We used Weighted Gene Correlation Network Analysis (WGCNA) – a method that takes advantages of graph theory – to explore similarities and differences in key transcription factors and signaling mechanisms in MCF-7 cell line and human breast cancer tissues. To our knowledge, this is one of only few studies that use publicly available databases and network analysis to compare an immortalized cell line to its tissue of origin. Since cancer cell lines are commonly used in both basic and translational research to understand biological mechanisms, drug effects, and toxicology pathways, our comparison of the regulatory networks of MCF-7 and breast cancer tissues can address some concerns over the validity of using cancer cell lines as standard models for humans.

ABSTRACT NUMBER: 3460  
Poster Board Number: P256

TITLE: Upregulation of Glutathione in Hepatocytes by the Antibiotic Nitrofurantoin

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KEYWORDS: Glutathione; Hepatocytes; Predictive Toxicology

ABSTRACT: The systems biology of liver toxicity prediction (SysBioToP) collaborative project was established to develop a high-throughput dynamic imaging test platform for the quantitative assessment of different cellular stress response pathways to allow testing and predictions on the liver-damaging potential of large sets of compounds. The HepG2 human liver cell line was chosen as platform for the expression of fluorescent stress pathway reporters, based on bacterial artificial chromosome gene editing technology. The results obtained from pathway analyses were compared with data from cytotoxicity assays (e.g. LDH release, ATP, GSH). In the course of these investigations, we observed that the antibiotic Nitrofurantoin (NFT), when applied in a subtoxic concentration range (10-100 µM), evoked an increase in cellular glutathione (GSH) levels. This elevation was a consequence of an upregulation in the expression of glutamate cysteine ligase (GCL), the rate-limiting enzyme in GSH synthesis. Pathway analysis and knockdown studies revealed that GCL induction was largely under control of the Nrf-2 pathway. In addition, NFT lead to the activation of several components of the unfolded protein response (UPR) pathway. Cells, pretreated with NFT to trigger GSH levels, demonstrated an increased resistance against secondary stressors such as experimentally evoked mitochondrial impairment (rotenone), oxidative stress (paraquat), or proteasomal stress (bortezomib, MG-132). The observations made in the HepG2 model could be confirmed in primary human hepatocytes. In contrast to the well-known liver-damaging potential of NFT when applied in higher doses, low concentrations of NFT could serve as novel strategy to boost the antioxidant capacity of liver cells.
ABSTRACT NUMBER: 3461    Poster Board Number: P257
TITLE: Synergistic In Vitro Hepatotoxicity of Ketoconazole and Cyclophosphamide in Primary Cultured Human Hepatocytes

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: H. Wei, and A. P. Li. In Vitro ADMET Laboratories LLC, Ellicott City, MD.

KEYWORDS: Hepatocytes; Methods/Mechanism; Hepatic

ABSTRACT: We report here an unexpected observation of synergistic hepatotoxicity between cyclophosphamide (CP), an anticancer drug, and ketoconazole (KZ), an antifungal drug and CYP3A4 inhibitor. Primary cultured human hepatocytes were treated with a combination of CP and KZ as an attempt to evaluate the role of CYP3A4 in CP hepatotoxicity. Plateable cryopreserved human hepatocytes were used in the study. The hepatocytes were thawed and cultured for approximately for 4 hrs, followed by treatment with 0, 0.31, 0.63, 1.25, 2.5, 5, 10, and 20 mM of CP and for each of the CP concentrations, co-treatment with 0, 1.25, 2.5, 5, and 10 uM of KZ. After a treatment duration of 48 hrs, viability was determined via quantification of cellular ATP contents. The KZ concentrations chosen were noncytotoxic, while the CP concentrations chosen yielded dose-dependent cytotoxicity. At 1.25 uM ketoconazole, CP cytotoxicity was reduced, an observation consistent with the inhibition of CYP3A4 activity by KZ, leading to decreased level of metabolic activation of CP to cytotoxic metabolites. The unexpected observations were made at the higher concentrations of 5 and 10 uM, where the apparent CP cytotoxicity was increased rather than decreased. For instance, in one of the experiments, the relative cytotoxicity values upon treatment of the hepatocytes with 5 mM CP in the presence of 0, 1.25, 2.5, 5 and 10 uM of KZ were 60.61%, 118.7%, 70.5%, 32.6%, and 14.4%, respectively. It is to be emphasized that the concentrations of KZ used were noncytotoxic. This observation of the apparent enhancement of CP cytotoxicity by KZ at 5 and 10 uM was reproduced in multiple independent experiments using hepatocytes from 6 donors. Interestingly, no synergistic cytotoxicity was observed when HEK293 cells were used, suggesting that the observation may require competent drug metabolizing enzymes that are present in human hepatocytes but not in HEK293 cells. As both CP and KZ are known to be hepatotoxic in humans in vivo, the observed synergism may be one of the exacerbating factors for their potential to cause liver damage. Our observation may have significance in the care of cancer patients as antifungals such as ketoconazole are often co-prescribed with anticancer agents such as cyclophosphamide. The synergistic effects of KZ and CP also provide evidence that idiosyncratic drug induced liver injuries may be a result of synergistic hepatotoxic effects of multiple drugs and xenobiotics.

ABSTRACT NUMBER: 3462    Poster Board Number: P258
TITLE: Exogenous Ah Receptor Activation Dysregulates Bile Acid Homeostasis in the Liver of Bile Duct-Ligated Mice

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: S. E. Kobernat, A. L. Celedon, B. Heidemann, K. A. Cornell, and K. A. Mitchell. Boise State University, Boise, ID.

KEYWORDS: Receptor; Aryl Hydrocarbon; Dioxin; Hepatic

ABSTRACT: A growing body of evidence supports a role for aryl hydrocarbon receptor (AhR) activation in the development of liver fibrosis, which is a pathological condition characterized by the excessive
accumulation of extracellular matrix. Liver fibrosis occurs in response to hepatic injury and inflammation, which promote the conversion of quiescent hepatic stellate cells (HSCs) to an activated, myofibroblast-like phenotype characterized by the synthesis of collagen type I. We previously reported that AhR activation by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) increased liver damage, inflammation, and HSC activation in the liver of mice subjected to bile duct ligation (BDL), which is a well-established model of experimental liver fibrosis. In response to BDL, bile acids accumulate in the liver and elicit hepatocyte damage and inflammation, leading to myofibroblast activation and periportal fibrosis. Recent reports in the literature indicate that AhR activation by TCDD dysregulates bile acid homeostasis, which led us to speculate that dysregulated bile acid homeostasis may contribute to the increased liver damage and inflammation observed in TCDD-treated BDL mice. To test this, male C57Bl/6 mice were treated with TCDD (20 μg/kg) or peanut oil (vehicle) one day prior to BDL or sham surgery. Mice were euthanized 3, 7, or 14 days after surgery. Histological and biochemical analysis revealed that TCDD treatment exacerbated liver injury, inflammation, and myofibroblast activation 14 days post-BDL, and it also produced a 3-fold increase in the level of total hepatic bile acids. In addition, exposure to TCDD was found to decrease the expression of genes involved in bile acid synthesis and export and increase expression of genes involved in alternative trafficking and synthesis pathways. This raises the possibility that TCDD treatment may exacerbate liver damage, inflammation, and HSC activation by a mechanism that involves modulating bile acid homeostasis.

ABSTRACT NUMBER: 3463        Poster Board Number: P259
TITLE: Research of FSH as a Biomarker of Susceptibility for Reproductive Damage Induced by Vinyl Chloride


KEYWORDS: Reproductive and Developmental Toxicology; Endocrine; Androgens; Biomarkers

ABSTRACT: To investigate the effect of vinyl chloride exposure on the damage of male reproductive endocrine system, and the changes of various reproductive hormone with the damages.

Experimental study: male rats were randomly divided into high-dose and low-dose treatment groups, and control group. 28 days after exposure, serum and testis samples were collected for testing testosterone (T), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2) and inhibin (InhB) levels. Epidemiological survey: 22 TCE exposed workers and 22 non-exposed controlled subjects were enrolled in the study. The serum levels of FSH, LH, E2, T and InhB were analyzed using enzyme-linked-immunosorbent-assay (ELISA). Experimental study: Compared with the control group, after 14-day exposure serum levels of T and inhibit B were decreased (P>0.05), E2 and LH levels were increased (P>0.05), and testis level of T was decreased (P>0.05); after 28-day exposure, serum levels of T and inhibit B were decreased (P<0.05), FSH was increased (P<0.05), testis level of T and inhibit B were decreased (P<0.05). It was found that Leydig cell and Sertoli cell were damaged according to histopathological examinations. Epidemiological survey: Adjusted by age, work age, gender, smoke and drink adjusted, serum E2 levels were decreased (P<0.05) in of VCM-exposed workers, whereas FSH and LH levels were increased (P<0.05) compared with unexposed workers. In addition, E2 level in exposed workers younger than 38 yr-old was lower than that of unexposed workers at the comparable age (P<0.05). FSH and LH levels of exposed workers older than 38 yr-old were higher than those of unexposed workers older than 38 yr-old (P<0.05) Both results of experimental studies and occupational
epidemiology survey indicated that VCM had reproductive and endocrine toxicity on male rats and male human subjects. Although changes of serum effect indexes in the two stages were not uniformly the same, FSH might be a sensitive and effective early warning indicator for reproductive function damage.

ABSTRACT NUMBER: 3464    Poster Board Number: P260
TITLE: A Single-Cell Quantification of DNA Methylation across the Cell Cycle by High-Content Imaging: Impact of Heavy Metals in Spermatogonial Cell

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: L. Yin¹, J. Siracusa², and X. Yu². ¹Reprotox Biotech, Athens, GA; and ²University of Georgia, Athens, GA.

ABSTRACT: DNA methylation is the most well-studied epigenetic alteration and could be influenced by a variety of environmental factors, and its dysregulation has been implicated in various diseases. A large assortment of techniques for DNA methylation analysis has been developed, including electrophoretic, chromatographic, PCR-based, array-based, and sequencing technologies, but these assays are the population-average response with high cost and low throughput. Recent image-based quantification of DNA methylation and topology demonstrated as a valuable tool to determine the methylation in situ at a single cell level. The purpose of this study was to establish a single cell-based high-throughput/high content imaging platform (HCA) to quantitatively evaluate the effect of heavy metals on both DNA methylation level and topology in spermatogonial cell. We treated the C18-4 spermatogonial cell with a range of doses of metals for 48h, including arsenic (As), cadmium (Cd), mercury (Hg) and nickel (Ni). Multiparametric HCA was applied to examine the phospho-histone H3 and nuclear DNA (Hoechst 33342) to stage individual cells in the cell cycle and quantify the spatial distribution of DNA methylation (5-MeC). Single-cell-based quantification of topological features was conducted using CellProfiler, and a machine-learning tool in the Cellproflier Analyst was applied to categorize phenotypic classification. We found there were significant variations of DNA methylation from cell to cell in the different stage of cell cycle, and heavy metals differentially altered the intensity, distribution, and texture of 5-MeC. Cd and Hg showed a dose-dependent decrease of intensity of 5-MeC, while As and Ni showed an inverted U-shaped dose-effect curve of 5-MeC. Topological analysis revealed that Cd uniquely increased the Texture_difference_variance, a marker reflecting the topological heterogeneity of 5-MeC. Our results demonstrated that this single-cell based HCA approach allows to determine DNA methylation and cell cycle simultaneously and provides a rapid, high-throughput and cost-efficient tool to examine the correlation of methylation across the cell cycle and to study the heterogeneity of cellular responses.

ABSTRACT NUMBER: 3465    Poster Board Number: P261
TITLE: Mechanisms of Transgenerational Reproductive Dysfunction through Comprehensive Examination of Germline Target Genes in C. elegans

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: C. McClure, J. Camacho, and P. Allard. University of California Los Angeles, Los Angeles, CA.

ABSTRACT: The processes by which organisms inherit effects from environmental exposures across generations is a phenomenon that is still poorly understood. The Allard lab has recently shown that exposure to the environmental toxicant BPA (Bisphenol A) in C. elegans can reduce the levels of repressive histone marks H3K9me3 and H3K27me3, regulated by histone demethylases jmjd-2 and jmjd-3/utx-1. This disruption causes a de-silencing effect and reproductive dysfunction that passed through
the germline, as effects continue to be observed for generations after direct exposure (Camacho et al.,
2018). Currently, our research aims to further characterize the transgenerational mechanisms by which
BPA can elicit reproductive defects, including increased embryonic lethality and germline apoptosis. We
plan to characterize how BPA affects the kinetics of various markers for meiotic processes such as
pairing, synapsis, and recombination. Previous work in our lab has shown that BPA causes a profound
alteration of meiotic recombination in worms directly exposed (Allard et al., 2010). In C. elegans, meiotic
recombination depends on several proteins such as COSA-1 and ZHP-3. Both appear in late pachytene,
presumably marking crossover position as one focus per chromosome pair with the standard being 6
chromosome pairs per nuclei (Yokoo et al., 2012). COSA-1 and ZHP-3 analysis indicated a difference in
distribution of foci per germ cell nuclei in the adult germline of BPA exposed worms both directly and
transgenerationally (COSA-1 F1: DMSO vs BPA p<0.001, F3: DMSO vs BPA p<000.1, N=250 nuclei in 18-
25 worms per group,Chi-square test, ZHP-3 F1: DMSO vs. BPA p=0.1 , F3: DMSO vs. BPA p<0.01, N= 170
nuclei, 7-10 worms per group, Chi square test). This variation shows that meiotic recombination is
possibly affected by altering homologous crossover dynamics. Our current work seeks to further
characterize BPA’s transgenerational effects on recombination processes, focusing on other important
events like checkpoint activation (pCHK-1), double strand break formation (SPO-11), strand invasion
(RAD-51), and downstream effects on diakinesis (bivalent number). Together, our future experiments
will help us determine the molecular mechanisms connecting ancestral exposure and deregulation of
the recombination process.

ABSTRACT NUMBER: 3466   Poster Board Number: P262
TITLE: Exploring the Role of the Maternal Environment in Dioxin Mediated Placental Adaptations
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KEYWORDS: Reproductive and Developmental Toxicology; Reproductive System
ABSTRACT: The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor controlling
the biological responses to environmental pollutants such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a
dioxin. The hemochorial placenta serves an essential role in fetal health and is potentially susceptible to
environment exposures. Placenta formation depends upon an intrinsic tropoblast cell differentiation program
and interactions of specialized tropoblast cells with uterine versus allantoic structures. The purpose of
this study was to explore how exposure to TCDD acting through AhR shapes placental development.
Gestational TCDD exposure resulted in a significant upregulation of transcripts for Cyp1a1 in liver,
placental, and adjacent maternal and fetal tissues at gestation day (gd) 13.5. Immunostaining of CYP1A1
in gd 13.5 placenta sites revealed activation of AhR signaling in decidua/metrial gland and in
mesenchymal components of the labyrinth zone but not in the junctional zone of the chorioallantoic
placenta. TCDD treated gd 13.5 placenta sites exhibited deep intrauterine trophoblast invasion,
unlike oil treated controls. We next generated an Ahr null rat model that failed to express AhR and to
induce CYP1A1 enzyme expression following TCDD exposure. TCDD-induced placental adaptations were
AhR dependent. Additionally, we observed TCDD-activated placental adaptations in CYP1A1 null rats. As
a first step in determining the site(s) of TCDD actions, we evaluated the effects of TCDD administration
during pregnancy in wild type females mated with wild type males, Ahr null females mated with Ahr null
males, and Ahr null females mated with wild type males. Mating schemes that resulted in disruption of
AhR activity in maternal tissues interfered with TCDD-activated placenta site adaptations. RNA-Seq,
qPCR and immunostaining of metrial glands revealed that TCDD dysregulated the uterine natural killer (NK) cell specific transcripts without affecting overall NK cell numbers in the metrial gland, indicating that TCDD affected the uterine NK cell phenotype. Collectively, these findings indicate that at least some of TCDD effects on placental development are mediated through its actions on the mother. In summary, we have identified a developmental window of sensitivity to environmental pollutants affecting hemochorial placentation with the potential of impacting fetal and postnatal health. (Supported by ES028957, ES029280; Sosland Foundation)

ABSTRACT NUMBER: 3467        Poster Board Number: P263
TITLE: Placental Hypoxia: The Trigger for the Release of Toxic Messengers in Preeclampsia?

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KEYWORDS: Antioxidants; Oxidative Injury; Reproductive and Developmental Toxicology

ABSTRACT: Preeclampsia is a multifactorial hypertensive disorder severely complicating 5-8% of all pregnancies. The complication is associated with placental hypoxia causing a release of toxic placental messengers into the maternal circulation, initiating the clinical symptoms (e.g. hypertension and edema). Our objective is to study placental hypoxia induced hypertension and associated endothelial damage and to test the protective effect of the dietary flavonoid quercetin. Placental explants (n=3) obtained from healthy mothers after a delivery in Maastricht University Medical Center were obtained and incubated for 3 hours under a hypoxic (1% O\(_2\)) or standard culture condition (21% O\(_2\)). After 3 hours the medium was removed and added to chorionic arteries isolated from human term placentas (>38 weeks gestation) and mounted into a wire myograph or onto two glass cannulas for pressure myography. The effect on endothelial permeability, contractile response and long-term sensitivity to tromboxane-A2 was examined. Additionally, the protective capacity of the food derived flavonoid quercetin was tested on all parameters. Media obtained from hypoxic treated explants increased the vascular contractile response in the arteries compared to controls (78 ± 33 vs 20 ± 9%, n=8, p<0.001), a sustained increase in vascular sensitivity to tromboxane-A2 (66 ± 27 vs 2 ± 8%, n=8, p<0.001) and increased permeability for intraluminal potassium chloride (40 ± 9 vs 5 ± 1%, n=8, p<0.001). Quercetin showed a dose dependent (1-10 μM) protection for all 3 parameters (-73 ± 31, -98 ± 16 and -91 ± 3%, n=8, p<0.001). By pre-incubation of specific antagonists (Losartan and Bosentan) both angiotensin-II and endothelin-1 were identified as the main contributors to the increased vascular response. In an \textit{in vitro} model of placental hypoxia we proved the release of toxic placental messengers inducing a hypertensive like response in arteries as well as endothelial leakage, phenomena also observed in preeclampsia. Vascular effects of these messengers could be dose-dependently normalized by the flavonoid quercetin. The next step of our study is to identify these messengers in more detail.
ABSTRACT NUMBER: 3468       Poster Board Number: P264

TITLE: Reproductive Toxicity of Eight Per- and Polyfluoroalkyl Substances in Murine C18-4 Spermatogonial Cells

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KEYWORDS: Perfluorinated Agents; Testis; Reproductive and Developmental Toxicology

ABSTRACT: Per- and Polyfluoroalkyl substances (PFAS) are a set of anthropogenic chemicals known for their chemical stability, persistence in the environment and human body and potential for biomagnification in the food chain. PFAS are widely used in industrial and consumer applications to increase the water-, oil- and stain-resistance of fabrics, leathers and carpets. The production and use of Perfluorooctanoic Acid (PFOA) and Potassium Perfluorooctanesulfonate (KPFOS) has been phased out in the United States. Studies on rodents illustrate these compounds induce hepatotoxicity, immune toxicity, and developmental toxicity and play a role in thyroid hormone disruption as potential endocrine disrupting compounds. Epidemiological studies illustrate exposure to PFOA and KPFOS leads to increased infertility, as well as the development of testicular cancer. Thus, alternatives to PFOA and KPFOS were introduced into industry with limited toxicological testing: Perfluorobutyrate (PFBA), Perfluorononanoic Acid (PFNA), Perfluorodecanoic Acid (PFDA), Perfluoroundecanoic Acid (PFUA), Perfluoroheptanoic Acid (PFHpA) and Perfluorohexanoic Acid (PFHxA). The mechanisms of toxicity for PFOA, KPFOS and these alternatives has not yet been fully elucidated regarding reproductive toxicity. The objective of the study is to evaluate the reproductive toxicity of these eight compounds in murine C18-4 spermatogonial cells, a major target cell in the testis. We characterized the cytotoxicity of these compounds using the Neutral Red Uptake Assay. The C18-4 spermatogonial cells were treated with a range of doses of the compounds (0-200 µM) for 24, 48 and 72 hours, and the half-maximal inhibitory concentration (IC50) of each compound was obtained from an experimentally derived dose-response curve. We found treatment of PFUA at a dose of ≥ 100 µM resulted in ~ 100% cell death, while PFHxA induced non-monotonic cell proliferation across all three time points. Specifically, PFDA (IC50: 10.21 µM, 48 h) and PFUA (IC50: 99.5 µM, 48 h) induced cytotoxicity at lower or equal concentrations as KPFOS (IC50: 98.30 µM, 48 h), respectively. In summary, the results illustrate that PFDA and PFUA were more cytotoxic or approximately as cytotoxic as KPFOS, followed by PFOA, PFNA, PFBA, PFHpA and PFHxA in spermatogonial cells based on the IC50 values after 48 hours of exposure. Future studies will delve in to the molecular mechanisms to examine phenotypic, nuclear and cell cycle perturbations. Funding supported by R44ES027374-02.
TITLE: Pubertal Development and Fertility of Female Wistar Rats after Juvenile Exposure to the Hypolipidemic Agent Rosuvastatin

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KEYWORDS: Juvenile Toxicity; Reproductive Tract; Female; Developmental Toxicity; Post-Natal

ABSTRACT: Statins are drugs used to reduce cholesterol. The use of statins by young people is increasing due to poor eating habits and sedentary lifestyle. Studies conducted in our Laboratory reported that exposure to rosuvastatin, a last generation statin, impairs reproductive function of male rats. The effects on the female reproduction remains unknown. This study aimed to evaluate the effects of rosuvastatin in reproductive development and function of female rats exposed to this drug since pre-puberty. Female Wistar rats were allocated into three experimental groups: control, treated with saline (vehicle); and R3 and R10 groups, which were treated with rosuvastatin at doses of 3 or 10 mg/Kg/Day. Treatments were performed daily, gavage, starting on postnatal day / PND 22 and ended at two different ages: at puberty (around PND 42) and at adulthood (PND 75), when the rats were euthanized in estrus and evaluated for body and organ weights, and steroid hormone levels. At adulthood part of the rats from each group was tested for sexual behavior and mated with no treated males for fertility assessment. Pregnant females were euthanized on gestational day 20. The following parameters were also evaluated: age of puberty onset; estrous cyclicity starting on PND 60; uterine motility, both on gravidic and non-gravidic rats. Statistics: ANOVA and Kruskal-Wallis, p<0.05. Puberty onset was not altered by juvenile rosuvastatin exposure. However, R10 group showed shorter reproductive cycles than control group. Body and organ weights were not affected by the treatment, except for liver and hypophysis of animals at PND 42, which were reduced in R10 group. Hormone levels were similar among groups at all ages. On the other hand, uterine motility, either in non-gravid and gravid uterus showed alterations in R3 and R10 animals. Rosuvastatin was also associated with altered reproductive performance, once R10 animals were less receptive during sexual behavior and placental weight was decreased compared with control group. Our results showed that pre-pubertal exposure to rosuvastatin altered female rat reproductive development and function, raising concern for women reproductive health after using this statin since young ages.

Funding: CNPq.
ABSTRACT NUMBER: 3470    Poster Board Number: P266
TITLE: Congenital Heart Defects Are Not Increased in Rats Exposed In Utero to Trichloroethylene in Drinking Water

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KEYWORDS: Developmental/Teratology

ABSTRACT: A single laboratory reported increased congenital heart defects in rats exposed to TCE in drinking water. In contrast, two other laboratories conducted studies by inhalation exposure and oral gavage using internationally accepted guidelines and failed to replicate these defects. The current study concentrated on the development of the heart and great vessels and was similar in design to the original drinking water studies but was enhanced to meet current EPA test guidelines and data quality standards. Pregnant SD rats were given drinking water with 0, 0.25, 1.5, 500, or 1,000 ppm TCE from Gestational Day (GD) 1 through GD 21. The mean daily intakes of TCE were 0, 0.04, 0.21, 58.03, and 113.45 mg TCE/kg-day for the respective dose groups, based on the analytically measured concentrations of TCE in the water formulations. There were no deaths or treatment-related clinical signs. Mean water consumption for the 500 and 1,000 ppm groups was significantly lower than the control groups throughout the exposure period. However, maternal body weights, body weight gain, and feed consumption were similar between the TCE-treated and control groups. There were no significant maternal macroscopic findings in the TCE-treated groups, and fetal growth and lethality were unaffected by treatment. There was no evidence of an increased incidence of cardiac malformations in the TCE-treated groups compared to the control group. The incidences of membranous interventricular septal defects (VSDs) were not statistically significantly different between the TCE-treated and control groups. The incidence values for all groups were within the range of spontaneous background occurrences for rats reported in the published literature that had a similar focus on cardiovascular development and that used examination methods that were standard at the time. In contrast, fetal VSDs were markedly increased in the retinoic acid-treated group that was included as a positive control. This GLP study supports previous guideline results demonstrating no developmental cardiac effects in rats following in utero exposure to TCE.

ABSTRACT NUMBER: 3471    Poster Board Number: P267
TITLE: Immunotoxicology Evaluation in the Juvenile Göttingen Minipig

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: L. Allais, A. Perbet, and F. Condevaux. Charles River Laboratories, St Germain Nuelles, France. Sponsor: A. Hoberman

KEYWORDS: Immunotoxicity; Developmental Toxicity; Post-Natal

ABSTRACT: Safety evaluation of new pediatric medicines is performed by the conduct of toxicology studies using juvenile animals. The minipig is now considered as a useful alternative non-rodent species for safety testing of pharmaceuticals. Human parallels in many features of its anatomy, physiology and biochemistry make the minipig a good model for man. For use in juvenile toxicology studies, the
development of main organs or systems of the minipig still requires further characterization, the
immune system being one of the main areas to be explored. Although the immune system of the adult
pig has been studied, particularly in relation to different infectious diseases in pigs and
xenotransplantations, the development of the immune system in juvenile pigs is still unknown or was
evaluated with very limited immune developmental endpoints. There is a real need to better
understand the immune system organization and response in the Göttingen minipig to better evaluate
the toxicological effect of new pharmaceuticals in development in this species. A well-known
immunosuppressive compound, cyclosporin, was given at the dose level of 10 mg/kg/day to 6 juvenile
Göttingen minipigs (3 males and 3 females) by the oral route (gavage) from postnatal day 3 to
postnatal day 28. A negative control group was given sterile water throughout the same treatment
period. Blood immunophenotyping (CD45, CD21, CD3, CD4, CD8, γδ-TCR, CD16, NKP46) as well as
lymphoproliferation activity (following ex-vivo induction by Concanavalin A) were evaluated in 2-week
old and 4-week old minipigs exposed to either cyclosporin or water. These endpoints were also
evaluated one or five months after the end of the treatment period, i.e. in 2- or 6-month old minipigs,
respectively. The Immunosuppressive effect of the cyclosporin was demonstrated in the juvenile
Göttingen minipig and was still observed one month after the end of the treatment period, although
being much less pronounced than during the treatment period. There were no relevant differences
between males and females at any age. The proportion of main peripheral immune cell populations was
comparable between age (2-week, 4-week, 2-month and 6-month old minipigs).

ABSTRACT NUMBER: 3472    Poster Board Number: P268
TITLE: Maternal Cigarette Smoke Exposure Induces Alterations in the Transcriptome and Methylome of
Human Placenta

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: C. A. Vyhlidal, A. Kietzman, W. A. Cheung, J.
Johnston, A. Walter, R. Biswell, and E. Grundberg. Children’s Mercy Kansas City, Kansas City, MO.

KEYWORDS: Gene Expression/Regulation; Receptor; Aryl Hydrocarbon; Epigenetics

ABSTRACT: Maternal cigarette smoke exposure (CSE) continues to be a common prenatal exposure with
approximately 10% of babies exposed in utero during the third trimester in the U.S. and is the
predominant risk factor for having an infant that is small for gestational age (SGA). Cigarette smoke is a
complex mixture of thousands of chemical compounds that may directly affect the developing fetus or
indirectly affect growth by disrupting placenta development and function. The individual components of
maternal cigarette smoke and the biological pathways which they perturb to adversely affect the
developing fetus and placenta have not been fully explained. We investigated changes in the
transcriptome and methylome using RNA sequencing (RNA-Seq, n=35) and whole genome bisulfite
sequencing (WGBS, n=10) on early pregnancy placenta samples with or without maternal CSE. Overall,
204 transcripts (100 up-regulated, 104 down-regulated) were differentially expressed with maternal CSE
(nominal p-value <0.01). As expected, CYP1A1 expression was induced in samples with maternal CSE.
Other differentially expressed genes included CGB (p<10^{-3}) which encodes the β subunit of human
chorionic gonadotropin (hCG), important for the establishment and maintenance of pregnancy. WGBS
identified 458 differentially methylated regions (each containing CpGs) enriched for binding sites of
transcription factors with known function in trophoblast development and response to CSE including
Oct1 and AhR. Regulatory networks of the methylation signatures were obtained by applying weighted
correlation matrices which identified three modules (R=0.8, p<10^{-3}) that associated with CSE. Targeted
genes mapping to these modules were identified by GREAT and pathway analysis by DAVID was applied. Strikingly, Tobacco Use Disorder (N=69 genes, p=1.16x10-5) was the top associated disease. Among the genes with altered methylation was NRG1, which promotes extravillous trophoblast formation in placental explants. In conclusion, maternal CSE alters the transcriptome and the methylome of the placenta that provides insights into the mechanisms that may lead to the increased risk of SGA of infants exposed to maternal CSE in utero.
ABSTRACT NUMBER: 3474   Poster Board Number: P270

TITLE: Developmental Delays and Neurotoxicological Effects of Bisphenol A and Its Analogs on Schmidtea mediterranea Planaria


ABSTRACT: The vast majority of chemicals used in the United States have not been systematically evaluated for their risks to human health. The potential consequences include an increasing trend in birth defects that could stem from developmental toxicity from chemical exposure. Thus, there is a crucial need to assess toxicity on growing brains during fetal development, which requires an alternative in vivo model. Schmidtea mediterranea (Smed) planaria flatworms have previously revealed exposure-induced behavioral effects in neuroregeneration and were thus chosen to showcase anatomical, molecular, and cellular differences following exposure to ethanol, bisphenol A (BPA), and BPA analogs (e.g. BPF and BG). We conducted an immunostaining study of Smed head regeneration that validated behavioral data among head regenerating worms that were either naïve or continuously exposed to trace amounts of these chemicals. The anatomical data shows a dramatic delay in head reacquisition for worms exposed to BPA when compared to naïve and all other exposed worms. BPA exposed worms showed fewer neural connections that developed later in the head regeneration process. Novel chemicals such as BG provide a possible plasticizer alternative without negative developmental effects. Through anatomical studies in Smed, we are beginning to evaluate these chemicals for their neurodevelopmental toxicity in order to ultimately optimize chemical design.

ABSTRACT NUMBER: 3475   Poster Board Number: P271

TITLE: Gestational and Lactational Exposure to an Environmentally-Relevant Mixture of Brominated Flame Retardants Disrupts Cell-Cell Interactions, Thyroid Homeostasis, and the Proliferation-Apoptosis Balance in Rat Mammary Glands at Puberty

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KEYWORDS: Endocrine Disruptors; Reproductive and Developmental Toxicology; Exposure, Environmental

ABSTRACT: Mammary gland development occurs mainly during puberty, pregnancy and lactation and is tightly regulated by hormones. Cell-cell interactions via intercellular junctions are also essential for proper mammary development and homeostasis. Exposure to endocrine disruptors, such as brominated flame retardants (BFRs), is ubiquitous and may alter mammary development and function. BFRs are added to consumer products to satisfy flammability standards. We showed that exposure of rat dams during gestation and lactation to an environmentally relevant mixture of BFRs, based on the mixture observed in house dust, disrupted proteins of the adherens junctions in the mammary glands. Here, we hypothesized that gestational and lactational exposure to BFRs will have similar effects in the offspring. Prior to mating and during pregnancy and lactation, female Sprague-Dawley rats were exposed to this BFRs house dust mixture in diets designed to deliver 0, 0.06, 20 or 60 mg/kg/day. Female offspring were euthanized on post-natal day 46 (post-pubertal) and mammary glands were collected. Exposure to BFRs...
(0.06 mg/kg/day) significantly down-regulated the levels of adherens junction proteins E-cadherin and β-catenin and of the gap junction protein p-Cx43. Exposure to this low dose of BFRs, but not higher doses, reduced thyroid hormone receptor alpha (TRα) protein levels in the mammary glands of the offspring. Interestingly, the same low dose of BFRs induced a significant decrease in the protein levels of cleaved caspase-3, a marker of apoptosis, while PCNA, a marker of proliferation, tended to increase. Together, our results suggest that gestational and lactational exposure to an environmentally relevant mixture of BFRs disrupts cell-cell interactions as well as thyroid hormone receptor levels and tissue homeostasis during puberty. Since puberty is a sensitive period for mammary gland development and dysregulation of signaling during this period is strongly associated with cancer and metastatic progression in the breast, our results raise important questions about the long-term consequences of this dysregulation on breast carcinogenesis.

**ABSTRACT NUMBER:** 3476  
**Poster Board Number:** P272  
**TITLE:** Identification of a Common Pathway Related to Metabolism Altered by Different Nuclear Receptor Ligands  

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**KEYWORDS:** Endocrine Disruptors; Endocrine Toxicology; Halogenated Hydrocarbon  

**ABSTRACT:** Obesity remains a leading health condition worldwide, with increasing prevalence both in adults and children. The causes for obesity are multifactorial; however, one suggested cause is exposure to obesogens, which are compounds that affect metabolic endpoints. Obesogens are known to be ligands for members of the nuclear receptor family. Examples of obesogens are BPA and DES acting on the estrogen receptor, and rosiglitazone and TBBPA acting on PPARγ. In addition, ligands for LXR are known to alter metabolic endpoints. We have previously shown that compounds activating ERs, PPARγ and LXR induce adipogenesis or regulate lipid metabolic processes in zebrafish. The aim of the current project is to investigate whether ligands for ERs, PPARγ and LXR act through similar downstream pathways to affect metabolism. We analyzed whole organism mRNA expression in zebrafish treated with different nuclear receptor ligands by RNA sequencing, and identified that 503 genes were regulated in common. We also identified a common pathway related to metabolism that is altered by these 3 different nuclear receptor ligands. To investigate the potential obesogenic effect of these ligands, we treated zebrafish larvae with the ligands in combination with a high lipid diet and visualized lipid accumulation by Oil Red O Staining. We propose that different obesogens acting through different nuclear receptors affect a common set of genes and pathways that alter the metabolism.
**TITLE:** In Vivo Measurement and In Silico Prediction of Estrogen-Like Potency of Bisphenol A and Its Analogues in Zebrafish

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** A. Kubota¹, J. S. Lee¹, Y. Wakayama¹, M. Nakamura¹, Y. Yoshinouchi², H. Iwata², M. Hirano³, and Y. Kawai¹. ¹Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan; ²Ehime University, Matsuyama, Japan; and ³National Institute of Technology, Kumamoto College, Yatsushiro, Japan.

**KEYWORDS:** Endocrine Disruptors; Endocrine; Receptor; Environmental Toxicology

**ABSTRACT:** Bisphenol A (BPA) is a monomer commonly used in polycarbonate plastic products such as food and beverage containers. Since the effects of BPA and its risks have been controversial, the use of BPA has been partially replaced with bisphenol A analogues (BPs) such as BPS and BPF. The objective of this study is to evaluate the estrogenic potency of a wide array of BPs by *in vivo* and *in silico* approaches using zebrafish. Exposure of zebrafish embryos to 16 BPs resulted in the induction of CYP19A1b by most of BPs in a concentration-dependent manner. BPs induction of CYP19A1b was suppressed by fulvestrant, a specific antagonist for estrogen receptor (Esr) subtypes. For BPs that exhibited concentration-dependent CYP19A1b induction, EC50 (or LOEC) and BPA and 17β-estradiol (E2) relative potencies (REPs) were estimated. Based on the estimates of the REPs, BPA and E2 induction equivalency factors (IEFs) were determined. The results showed that different BPs have distinct induction efficacy and potency, as revealed by the fold-induction and by the EC50/LOEC and IEFs, respectively. We constructed *in silico* homology models of the ligand binding domains of zebrafish Esr subtypes, including Esr1, Esr2a and Esr2b. Molecular docking simulations of 16 BPs with Esr subtypes using ASEDock revealed that the interaction energy for many of BPs was lower than that of BPA. The interaction energy of BPs to each of Esr subtypes showed a significant correlation with EC50/LOEC for *in vivo* CYP19A1b induction by BPs. To the contrary, IEFs were significantly correlated only with the interaction energy to Esr2b, but not Esr1 or Esr2a, indicating that Esr-ligand binding are linked more closely to EC50/LOEC rather than REPs. The present study demonstrates that some BPs showed a partial agonistic feature while others showed a full agonistic feature *in vivo* in zebrafish. The current results also suggest that *in silico* simulations of interaction between BPs and Esr subtypes can predict *in vivo* sensitivity of the Esr-mediated response by untested chemical substances.

**TITLE:** Weight of Evidence Analysis to Assess the Potential of PFOA to Act as a Steroidogenesis Inducer and Inhibitor.

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** E. M. Beckett¹, S. E. Brown², D. R. Cheatham³, H. A. Reamer³, and M. L. Kreider⁴. ¹Cardno Chemrisk, Boston, MA; ²Cardno Chemrisk, Boulder, CO; ³Cardno Chemrisk, Chicago, IL; and ⁴Cardno Chemrisk, Pittsburgh, PA.

**KEYWORDS:** Perfluorinated Agents; Endocrine Disruptors

**ABSTRACT:** Perfluorooctanoic acid (PFOA) is a synthetic industrial chemical historically used in the production of fluoropolymers. Despite inconsistent results in the literature, some researchers have identified PFOA as an endocrine disruptor; however, the U.S. EPA maintains that chemical endocrine disruption must be evaluated using a weight-of-evidence (WoE) approach. Borgert et al. (2011, 2014)
developed a three-tiered endpoint-ranking framework to assess chemicals for endocrine disruption via 
eight modes of action (MoA) using specific assay methodologies. Within this framework, Rank 1 
endpoints encompass in vivo observations specific and sensitive for a MoA; Rank 2 endpoints are 
interpretable, but less specific, for a MoA and may be confounded, while Rank 3 endpoints are relevant 
to a MoA, but only when corroborative of Rank 1 and 2 endpoints. This analysis evaluates the effect of 
PFOA on two MoAs: 1) induction, and 2) inhibition of enzymes involved in steroidogenesis. A literature 
review yielded 6 studies that evaluated endpoints relevant to steroidogenesis induction, and 9 relevant 
to inhibition. Borgert et al. (2014) determined that there were no endpoints relevant to steroidogenesis 
induction that warranted Rank 1 classification. In addition, we did not identify any studies that 
evaluated Rank 1 endpoints for steroidogenesis inhibition. For each MoA, 8 studies evaluated Rank 2 
endpoints, most of which utilized methodologies comparable to the EPA Endocrine Disruption Screening 
Program (EDSP) assay protocol; however, the results reported for these endpoints, while statistically 
significant, were inconsistent and often incompatible with the MoAs evaluated. In addition, there were 
no studies that evaluated Rank 3 endpoints for the steroidogenesis induction MoA, while only one study 
evaluated Rank 3 endpoints for the steroidogenesis inhibition MoA, the results of which were also 
inconclusive. Thus, due to the lack of Rank 1 endpoints available for analysis, combined with 
inconsistent results observed in Rank 2 and 3 endpoints, the current evidence does not support the 
hypothesis that PFOA can act as an endocrine disruptor via the induction or inhibition of enzymes 
involved in steroidogenesis.

**ABSTRACT NUMBER: 3479**
**Poster Board Number: P275**

**TITLE:** Investigating the Toxicity of Biotransformation Products of Limonene in *Aspergillus flavus* Culture

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**ABSTRACT:** *Aspergillus flavus*, a well-known toxigenic fungus that produces aflatoxins, has been used for 
many different biotransformations of organic compounds. Cultures that add hydroxyl, carbonyl, and 
other groups at specific positions or reduce double bonds have resulted in the production of valuable 
new compounds. In this study, the biotransformation of the terpenoid (+)-limonene by *A. flavus* and the 
toxicities of the products and aflatoxin B₁ to HEK293 and HepG2 recombinant human cells were 
investigated. The culture extracts were analyzed by using GC-MS and trans-p-mentha-2,8-dienol 
(Rt=6.82 min) was found to be the major biotransformation product of limonene produced by *A. flavus*. 
To examine the effects on toxicity, cultures of the test cells first were treated for 3 hours with different 
amounts of the biotransformation products of limonene. The cells were then treated for 1 hour with 0.1 
µg/mL of aflatoxin B₁. HEK293 cells showed 70.6% viability with 1 µM of the biotransformation products, 
although some cytotoxicity was apparent at 5 µM. After addition of 1 µM of the products plus 0.1 µg/mL 
aflatoxin B₁, HEK293 cell viability decreased to 49.1%. HepG2 cells showed 56.1% viability with 0.001 µM 
of the biotransformation products, but only 38.6% viability at 0.1 µM. After addition of 0.001 µM of the 
products plus 0.1 µg/mL aflatoxin B₁, HepG2 cell viability decreased to 29.4%. At 0.01, 0.1, 0.5 and 1 µM 
concentrations of the biotransformation products plus 0.1 µg/mL aflatoxin B₁, HepG2 cells showed 
viability of 29.8, 35.2, 41.8 and 49.2%, respectively. To demonstrate the presence of cytochrome P450 
during biotransformation of limonene by *A. flavus*, carbon monoxide difference spectra of microsomal 
fractions from limonene-induced cultures of *A. flavus* with sodium dithionite were recorded. The UV
absorption spectra of microsomal fractions with CO showed peaks at 450 nm indicating the occurrence of cytochrome P450, which represented 90 pmol cytochrome P450 per mg protein. As a result of this study, biotransformation of limonene was achieved by A. flavus, and the products showed moderately inhibition activity against aflatoxin B1 and the cytotoxic effects of those were investigated on the both healthy and carcinoma cells.

ABSTRACT NUMBER: 3480        Poster Board Number: P276
TITLE: Determining Mechanism of Thiazole Metabolically Activated Toxic Outcome through Experimental and Computational Techniques

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: D. A. Barnette. University of Arkansas for Medical Sciences, Little Rock, AR. Sponsor: G.P. Miller

KEYWORDS: Biotransformation; Cytochrome P450

ABSTRACT: Thiazoles are biologically active aromatic heterocyclic five-membered rings occurring frequently in both natural products and drugs. In most cases, thiazole-containing molecules undergo harmless elimination; however, the structural motif elicits a hepatotoxic response often due to epoxidation of the 4,5 carbon-carbon double bond that ultimately leads to reactive thioamide. The structural diversity of the molecules plays a role in determining whether the molecule undergoes elimination or bioactivation, yet the determinants of those competing possibilities remain unknown. The very different toxic potential between the nonsteroidal anti-inflammatory drugs meloxicam and sudoxicam provide a typical example of the effect of structure on bioactivation. The presence of a single methyl group on the thiazole of meloxicam, but not sudoxicam, led to meloxicam approval for market while sudoxicam was discontinued due to hepatotoxic outcomes in clinical trials. The metabolic mechanisms differentiating between bioactivation and detoxification for the drugs have not been clearly defined, as the pathways contain multiple, unstudied reaction steps. We hypothesize that the substituent on the thiazole group affects the efficiency of bioactivation and/or detoxification pathways, and hence the toxic risk of the drugs. We are employing computational and experimental approaches to test the hypothesis. Although qualitative, our modeling suggested the methyl group does not impact the likelihood for epoxidation of the ring on the path to reactive metabolite formation. As a complement to that work, we are carrying out quantitative kinetic experiments to characterize competing metabolic pathways for the drugs. As a first step, we developed analytical methods to measure metabolites from each reaction step in pathways using in vitro metabolism assays. We are currently measuring the kinetics describing the reaction steps to determine the impact of the methyl group on metabolic flux through bioactivation and detoxification pathways. Knowledge of the mechanistic details for these pathways will provide insight for understanding the difference in their toxic outcomes as determined by the substituent. Findings from this study will be transformative in advancing an understanding of what governs thiazole bioactivation, and the knowledge gained could inform drug design of compounds containing the frequently used thiazole scaffold.
ABSTRACT NUMBER: 3481 Poster Board Number: P277

TITLE: Toxicokinetics and Dynamics of Microcystin Congeners Are Structure-Dependent


KEYWORDS: Toxicokinetics; Cell Lines, Transfected; Mechanisms

ABSTRACT: Microcystins (MCs) are cyclic heptapeptides comprised of 2 variable L- and 5 D-amino acids, thus amounting to approx. > 200 congeners, produced by cyanobacteria. Due to mass occurrences, primarily in freshwaters, cyanobacterial blooms can lead to acute or chronic exposure of humans to MCs. Resulting from the known hepato-, neuro- and nephrotoxicity of some very few MC congeners, considerable interest resides with elucidating structural determinants that govern the parts of the toxicokinetics and -dynamics of MC variants. While organic anion transporting peptides (OATPS) have been shown to be responsible for MC uptake, MC-mediated ser/thr protein phosphatases (PP) inhibition was identified as the primary mechanism underlying the observed MC cytotoxicity. Moreover, the ADDA side chain of the MC principle structure is a highly conserved moiety found in all MC congeners and was shown to play a major role in the functional inhibition of PPs. We thus employed the de novo synthesis of MC-LF to generate several structural modifications of the ADDA side chain. We used the latter to determine cellular uptake (via stably transfected OATP1B1- or OATP1B3-HEK-293 cells), protein phosphatase inhibition (in recombinant PP1 and PP2A) and cytotoxicity in OATP1B1- or OATP1B3-HEK-293 cells. The MC-LF variant lacking the terminal benzyl group in the ADDA side chain was transported more rapidly than the parent MC-LF, but had a much lower PP inhibition activity yet comparable cytotoxicity than the parent MC-LF. In contrast, a stereoisomer of MC-LF containing an enantiomeric ADDA residue showed slower OATP transport, lack of PP inhibition and no cytotoxicity. These results demonstrate the importance of the ADDA side chain for PP inhibition, as well as the fact that more rapid uptake via OATPs can compensate for a lower PP inhibition when apical cytotoxicity, being the sum of the kinetic and dynamics, is considered.

ABSTRACT NUMBER: 3482 Poster Board Number: P278

TITLE: Bioactivation of Halogen-Containing Drugs as Precursors to Drug-Induced Hepatotoxicity

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KEYWORDS: Metabolic Activation; Pharmacokinetics; Cytochrome P450

ABSTRACT: Though halogens are utilized in drug development, their impacts on toxic risk remain understudied. Halogen substituents affect drug metabolic clearance and bioactivation into reactive metabolites and consequent causation of adverse drug events. The halogen effect on metabolism has been studied using model molecules with human liver microsomes. However, these studies explored only the impact of halogens on chemical reactivity and not on specificity and affinity of halogenated drugs for metabolizing enzymes. We hypothesize that the presence, type, and location of halogens on a drug aromatic ring impacts the chemical step toward reactive oxidative product formation (Vmax) based on electronic effects, while enzyme specificity and affinity (Km) determine the concentration-dependent conditions in which the reaction occurs. Further, we hypothesize that compounds with known DILI risk
and unknown causative factors are due to formation of reactive quinone metabolites. We will establish trends across drug classes that link metabolism and reactive quinone metabolite formation, ascertain reaction kinetics, and identify responsible cytochromes P450 to understand the role of halogens in metabolic clearance and bioactivation contributing to drug-induced liver injury (DILI). We identified 72 lead compounds with degrees of DILI concern from the FDA DILIRank database. We have 13 negative controls (no DILI risk), 17 positive controls (varied DILI risk) and 42 halogenated test drugs (unknown contributors to DILI risk). Our computational model predicts metabolic reactivity and sites of metabolism but fails to predict halogen effects as a function of halogen identity. To experimentally assess drug metabolism and address model shortcomings, we are developing a novel, fluorescent metabolite-trapping method using dansyl amidoethylmercaptan to stabilize and quantify reactive quinone metabolites. Follow-up studies will define metabolic pathways and kinetic parameters, identify responsible cytochromes P450, and results produced will be used to improve computational modeling efforts. These findings will provide critical insights on the impact of halogenation on reactive oxidative metabolite formation as a precursor to DILI and thus, provide a foundation for better risk assessment in drug discovery and development.

ABSTRACT NUMBER: 3483    Poster Board Number: P279

TITLE: Predicting the Pharmacokinetics in Man of a Locked Nucleic Acid Oligonucleotide Targeting miR-221

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KEYWORDS: Pharmacokinetics; SiRNA; Reference Dose

ABSTRACT: Locked Nucleic Acid (LNA)-i-miR-221 is a phosphorothioate 13-mer oligonucleotide (PS-ODN), targeting microRNA (miR)-221, intended for the treatment of refractory multiple myeloma and advanced solid tumors. A dose-escalation phase I clinical trial in patients has been approved (EudraCT:2017-002615-33) requiring estimation of safe starting and escalation doses. The pharmacokinetic (PK) profile of LNA-i-miR-221 has been evaluated in different animal species including mice, rats and monkeys, with similar results in all the tested species. The pharmacokinetics of LNA-i-miR-221 is characterized by a large systemic volume of distribution and broad tissue penetration. This certainly involves binding to circulating plasma proteins, cell surface protein interactions and endocytosis, as has been well documented for other PS ODN therapeutics. Furthermore LNA-i-miR is strongly bound to plasma protein, thus preventing rapid renal clearance. To support prediction of Human pharmacokinetics using allometric interspecies scaling, the measurement of the free fraction of PS ODNs in plasma provides additional information for species comparison necessary for the estimation of (i) unbound versus total exposure and (ii) unbound clearance. For this purpose we developed a suitable ultrafiltration method to determine the binding of LNA-i-miR-221 to plasma proteins. Using this technique, the fraction of LNA-i-miR-221 (at 1 and 10 µM) bound to human, monkey and rat plasma proteins ranged between 98.2 and 99.05%. These results suggested that the LNA-i-miR-221 PS-ODN is similarly highly bound to the plasma proteins of the three species tested. Finally these results were integrated into multiple allometric interspecies scaling approaches that were used to draw inferences about LNA-i-miR-221 PK and safe dose selection in man.
ABSTRACT NUMBER: 3484        Poster Board Number: P280

TITLE: Assessment of Nicotine Kinetics and Subjective Effects of Two Tobacco Heating Products Compared to Cigarettes and a Nicotine Replacement Therapy


ABSTRACT: Studies have shown that when smokers switch from smoking combustible cigarettes to using a tobacco heating product (THP), their exposure to smoke toxicants decreases, in many cases, to similar levels as cessation. Nicotine pharmacokinetics (PK) and subjective effects of potentially reduced risk products (PRRPs) relative to combustible cigarettes and other nicotine products, such as nicotine replacement therapy (NRT; e.g. nicotine inhaler), may determine the likelihood of switching success and provide data on potential abuse liability. This study aimed to test the hypotheses that glo THP with consumables of two different nicotine yields (THP1.0 and THP1.1) have a closer nicotine PK profile to combustible cigarettes compared to NRT, and subjective effects are more positive compared to NRT. To test these hypotheses, 32 healthy smokers were recruited in a clinical study conducted in Verona, Italy (ISRCTN13439529), run in accordance with ICH-GCP following a research protocol approved by the local Research Ethics Committee. In accordance with pre-defined randomization sequences, subjects were assigned a different product for assessment during each of four PK periods, following overnight (minimum 12-hours) in-clinic nicotine abstinence. Subjective effects (product liking, urge to smoke a usual-brand cigarette, urge to use the study product, overall intent to use the product again) were also assessed at various timepoints during each PK period via single-item questionnaires. Systemic nicotine exposure, based on Cmax and AUC0-240min, was greater for the THPs than for the nicotine inhaler, but lower than the usual-brand cigarette. Median Tmax for the THPs (4 min) was closer to that observed for the cigarette (6 min) than for the nicotine inhaler (15 min). Product liking and overall intent-to-use again was greater for the THPs than for the nicotine inhaler, but lower than for cigarettes. Urge to smoke was reduced to the greatest extent when smoking a cigarette, and to the least extent when using the nicotine inhaler. These findings demonstrate the glo THPs assessed had a closer nicotine PK profile to subjects’ usual-brand cigarettes than the nicotine inhaler, and that subjective effects of glo THPs were more positive than those for the nicotine inhaler.

ABSTRACT NUMBER: 3485        Poster Board Number: P281

TITLE: Applications of Organ on a Chip Systems in DMPK

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KEYWORDS: Metabolism; Pharmacokinetics; Cytochrome P450

ABSTRACT: A large fraction of approx. 70% of medicines administered to patients are metabolized through biochemical processes in the liver. This metabolism decreases the concentration of the active compound over time. Therefore, the therapeutic concentration of drugs with high metabolic turnover is only maintained for a short period of time, which requires frequent administration to ensure therapeutic effects. To address this issue, the pharmaceutical industry is developing so-called low
clearance compounds, which are more stable and require less frequent dosing. Key for this drug optimization process is an accurate estimate of the drug metabolism in the liver. Current assays relying on primary hepatocytes in suspension or monolayers are short-lived and, therefore, not suited to assess metabolic effects over longer periods of time (>24h). The prediction of in vivo clearance based on in vitro data is central in drug discovery and important to set drug doses in the clinic. Here, we present an in vitro assay, based on 3D primary human liver microtissues showing long-lasting and stable metabolic activity over more than 4 weeks. Microtissues are cultured in a new microfluidic system with the following features: (a) Up to 10 microtissues are inter-connected through microfluidic perfusion and allow for obtaining a substantially higher cell-to-medium volume ratio as compared to conventional assays; (b) Continuous flow and the appropriate microenvironment provide more physiological conditions; (c) Downscaling of the system by using microfabrication techniques reduces the use of human donor material and reagents; (d) Microtissue handling in microfluidic devices allows for parallelization, automation and is compatible with higher throughput screening. By using the microfluidic device, we could show an up to two fold increase in metabolic activity of the liver microtissues compared to standard well-based culturing. Moreover, metabolic activity per microtissue was not decreased by a 10-fold higher cell-to-media ratio. Viability and functionality of the hepatocyte microtissues remained stable over at least 7 days without any medium exchange. In summary, we were able to substantially increase the metabolic competence of a microtissue-based assay, which turned out to be suited DMPK application among them for predicting intrinsic clearance of stable compounds.

ABSTRACT NUMBER: 3486        Poster Board Number: P282

TITLE: Hormetic Sex-Dependent Micronuclei Induction in Bone Marrow after Exposure to Low Dose of a Mixture of 13 Chemicals

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KEYWORDS: Organophosphates; Exposure Assessment; Genotoxicity

ABSTRACT: Introduction: Exposure to chemical mixtures in low doses represents a challenge for researchers as it relates to potential risk for human health. The current study aimed to evaluate the genotoxic effects of long term exposure (18 months) to low doses of 13 chemicals mixture using a rat model. Material and Methods: 4 groups of 10 Sprague Dawley rats (5 males and 5 females) were treated with 0, 0.25x acceptable daily intake (ADI), ADI and 5xADI doses of mixture of methomyl, triadimefon, dimethoate, glyphosate, carbaryl, methyl parathion, aspartame, benzoic acid, calcium disodium ethylene diamine tetra-acetate (EDTA), ethylparaben, butylparaben, bisphenol and acacia gum by drinking water. After 18 months of exposure the animals were sacrificed and bone marrow was collected from femurs immediately following sacrifice. The frequency of micronuclei in bone marrow (MNPCE) was evaluated by a standard procedure with Giemsa coloration. This study was conducted in accordance with the EU Commission Directive 2010/63/EU for animal experiments and approved by the Ethical Committee of University of Medicine and Pharmacy Craiova, Romania. Results: After 18 months of exposure to a mixture of 13 chemicals a nonmonotonic genotoxic effect was observed in females. At low dose exposures, females showed an increase of 63±11.6% in the MNPCE frequency compared to the
control group (p<0.001). In the medium dose, females a decrease of 14.6±15.2% in the MNPCE frequency was noted compared to controls (p>0.05), but in the high dose group an increased of 45±4.2% in the MNPCE frequency was noted compared to control (p<0.05). In male groups the exposure to the mixture of 13 chemicals determined a decrease of MNPCE frequency with 26.1±3.9% in the low dose group compared to control (p<0.05), with 36.11±6.5% in the medium dose group (p<0.001) and with 20.3±2.5% (p>0.05) in the high dose group compared with control. Conclusions: Long term low dose exposure to a mixture of 13 chemicals induces hormetic micronuclei induction in bone marrow in female rats.

ABSTRACT NUMBER: 3487  Poster Board Number: P283
TITLE: Findings from the 2015-2016 NHANES Data on Risks from Combined Exposures to Six Phthalates
AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: J. Reyes¹, and P. Price². ¹US EPA/ORISE, Research Triangle Park, NC; and ²US EPA, Research Triangle Park, NC.
KEYWORDS: Phthalates; Biomonitoring; Children’s Health

ABSTRACT: Phthalates are used in a range of consumer goods, resulting in widespread exposures among the general population in the United States. Exposures to specific phthalates vary over time due to changes in patterns of phthalate use. A previously published study evaluated trends in exposures and associated risks to six phthalates from biomonitoring data collected under the National Health and Nutrition Examination Survey (NHANES) from 2005 to 2014 (Reyes and Price, 2018). This work extends that analysis to consider the most recent (2015-2016) NHANES data. Because the most recent survey expanded data collection to children ages 3-5, this work also includes findings for this age group. Doses for the phthalates were estimated for each surveyed individual using reverse dosimetry. The Hazard Quotients (HQs), Hazard Indices (HIs), and Maximum Cumulative Ratios (MCRs) were determined for individuals using the phthalates’ tolerable daily intakes. HQs are a measure of chemical-specific risks, HI is a measure of cumulative risk, and MCR quantifies the degree to which a single phthalate drives the cumulative risk of an individual. There was a 1.4-fold decrease in the mean HI between 2013-2014 and 2015-2016 (0.15 to 0.11) and a 1.2-fold decrease in the percentage of participants with an HI>1 (0.79% to 0.68%). Decreases in the HI over 2005-2014 were largely due to decreases in risks from two phthalates: diethylhexyl and dibutyl phthalate. Decreases in the most recent data were mostly due to decreases in diisononyl and diisodecyl phthalate. The trend of higher HIs with younger ages observed in prior data occurred in the newest data. Children ages 3-5 had higher HIs than older children and adults. Mean HI values for age ranges of 20+, 12-19, 6-11, and 3-5 years, were 0.10, 0.09, 0.18, and 0.23, respectively. Within the latest data, the fractions of the age groups with HI that exceeded one ranged from 0.0% in ages 12-19 to 1.5% in ages 6-11. However, the frequencies of exceedances were too small to determine if there were an age-related trend. MCR values in the new data were low and inversely correlated with HI indicating that a single phthalate usually drove the hazards for highly-exposed individuals. These findings indicated that phthalate exposures in the US continue to change over time.

The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the US EPA. Reyes JM, Price PS, 2018. ES&T, 52(21).
ABSTRACT NUMBER: 3488    Poster Board Number: P284
TITLE: Alternative Vehicles Allow the LuSens Test Method to Predict Dermal Sensitization of Mixtures


KEYWORDS: Cutaneous or Skin Toxicity; Alternatives to Animal Testing

ABSTRACT: Predicting dermal sensitization is an important component of the acute toxicity testing battery. OECD test guideline assays have been established to measure the four key events of the dermal sensitization Adverse Outcome Pathway (AOP), allowing for screening of delayed-type 4 hypersensitivity potential. The second key event in the AOP is characterized by keratinocyte activation, for which the OECD 442D guideline details the in vitro ARE-Nrf2 Luciferase-based LuSens Test, herein referred to as the LuSens Test. This test has predictive power to identify skin sensitizers through their activation of cultured human keratinocytes. In order to explore the utility of the LuSens Test for predicting UN GHS sensitizers (Category 1) or non-sensitizers, we performed a series of assays using the LuSens Test protocol under the OECD 442D guideline. The LuSens Test correctly identified 5 of 6 sensitizers and 4 of 4 non-sensitizers from the validation chemical list, yielding an overall intra-laboratory accuracy of 90%. One limitation of this methodology for testing a wider array of chemicals and mixtures is limited solubility of potential test substances in the three guideline-approved vehicles, Dimethyl Sulfoxide (DMSO), Media, and Water. We sought to validate the use of additional vehicles applicable in the LuSens Test in order to expand its applicability domain. Propylene Glycol (PG), Polyethylene Glycol 400, 50-70% Ethanol, DMSO:Acetone:Ethanol (4:3:3), DMSO:Ethanol (1:1), Methanol, and 50% Isopropanol all demonstrated compatibility as vehicles in the LuSens Test, as determined by their ability to appropriately promote positive and negative control luciferase induction responses. Using DMSO or PG, when test articles were insoluble in the guideline-recommended vehicles, the LuSens Test was able to predict the dermal sensitization potential of nine commercially available mixtures. LuSens Test predictions agreed in 6 of 6 mixtures tested where a safety data sheet had a dermal sensitization prediction. For 2 of 2 lotion mixtures tested, which were not expected to induce a dermal sensitization response, no induction was seen. The last mixture was a shampoo that had a positive dermal sensitization prediction in both the LuSens Test and the Human Cell Line Activation Test (h-CLAT). Together, these data demonstrates that the LuSens Test can be performed on mixtures using an expanded list of intra-laboratory validated alternative vehicles.

ABSTRACT NUMBER: 3489    Poster Board Number: P285
TITLE: Application of Generalized Concentration Addition to Predict Mixture Effects of Glucocorticoid Receptor Ligands

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ABSTRACT: Environmental exposures often occur in complex mixtures and at low concentrations. There is a need for improved prediction models that evaluate the toxic effect of simultaneous human exposures. Generalized concentration addition (GCA) is a method used to estimate the joint effect of receptor agonists that vary in efficacy. GCA models have been successfully applied to mixtures of aryl hydrocarbon receptor (AhR) and peroxisome proliferator-activated receptor gamma (PPARγ) ligands,
each of which can be modeled as a receptor with a single binding site. One requirement of GCA is specification of the mathematical form for the dose response curves. The glucocorticoid receptor (GR) is a homodimer nuclear receptor that is activated in response to stress and by synthetic glucocorticoids. GR has systemic effects on the endocrine, metabolic, cardiovascular, immune, reproductive, and central nervous systems. Here, we evaluated whether GCA could be applied to homodimer nuclear receptors, which have two binding sites, to predict the combined effect of full GR agonists with partial agonists or competitive antagonists. We measured transcriptional activation of GR using a recently developed cell-based bioassay that contains a stably transfected luciferase reporter gene under the control of three glucocorticoid responsive elements. Individual dose response curves for dexamethasone (full agonist), prednisolone (full agonist), medroxyprogesterone 17-acetate (partial agonist), and mifepristone (antagonist) were generated to predict binary mixture effects of GR ligands using the following approaches: GCA, effect summation (ES), and toxic equivalency factor (TEF). GCA and TEF yielded adequate predictions of the experimental data for two full agonists. For all other binary mixtures, GCA fit experimental data significantly better than ES and TEF. This work expands the application of GCA to homodimer nuclear receptors and improves prediction accuracy of chemical mixtures that represent cumulative human exposures.

**ABSTRACT NUMBER:** 3490  
**Poster Board Number:** P286  
**TITLE:** Introducing the Dose-Equivalence/Zero-Interaction (DE/ZI) Method for Quantifying Small Molecule Interactions: Nrf2/Antioxidant Response Element Pathway Activation by Sulforaphane and a Non-Arylating Redox-Active Phenol  
**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** E. M. Repash, P. Palenchar, and A. L. Eggler.  
**Villanova University, Villanova, PA.** Sponsor: A.L. Eggler, Society for Redox Biology and Medicine  
**KEYWORDS:** Oxidative Injury; Natural Products; Phase II Metabolism  

**ABSTRACT:** Electrophiles and reactive oxygen species (ROS) can cause cellular damage and induce and exacerbate chronic disease conditions. These effects can be counteracted by the induction of detoxifying and antioxidant enzyme genes through the transcription factor Nrf2 and its cognate antioxidant response element (ARE). Numerous small molecules have been identified that activate this system. Treating with a combination of compounds offers the potential to increase efficacy while decreasing toxicity. We find that the efficacy and potency of the clinically-studied electrophilic Nrf2/ARE activator sulforaphane are enhanced by 18 µM 2,5-di-tert-butyl hydroquinone (dtBHQ), a ROS-generating diphenol. We sought to determine if the sulforaphane-dtBHQ interaction is synergistic. However, models of drug-drug interactions typically use a Hill-slope dose-response curve, and Nrf2/ARE activators, including sulforaphane, exhibit a hormetic dose-response. Hormesis is characterized as a biphasic dose-response, with a period of activation followed by a period of inhibition, producing a U-shaped curve. Therefore, we propose a novel model to assess interactions that can be applied to any curve shape. This method, termed dose-equivalence/zero-interaction (DE/ZI), defines additivity not by adding the effects of doses of drug A and drug B, but by adding the dose of A and the equivalent dose of B and calculating the predicted effect. DE/ZI differs from the related Loewe Additivity model in that DE/ZI releases the constraint that interpolation off curve A and curve B must give the same predicted additive result. An advantage of DE/ZI is that the curves for drug A or drug B do not need to fit to a particular equation. Rather, a nearest neighbor approach is used for interpolation between data points. We find that most combinations of sulforaphane with dtBHQ elicit a synergistic interaction, although at
low (1 μM) sulforaphane and low (4 μM) dtBHQ an antagonistic relationship is identified. Interestingly, sulforaphane-induced Nrf2 protein levels are suppressed by dtBHQ. The use of DE/ZI as a medium-throughput method to evaluate the effects of other small molecule combinations on Nrf2/ARE-regulated gene expression is illustrated.

**ABSTRACT NUMBER:** 3491  
**Poster Board Number:** P287  
**TITLE:** Predict the Point of Departure with High-Throughput Data and Robust Learning  
**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** D. Wang, and Z. Liu. US FDA/NCTR, Jefferson, AR.  
**KEYWORDS:** Dose-Response; Biological Modeling; Bioinformatics  
**ABSTRACT:** The development and application of high throughput *in vitro* assays is an important development for risk assessment in the twenty-first century. However, there are still significant challenges to incorporate *in vitro* assays into routine toxicity testing practices. In this paper, a robust learning approach was developed to infer the *in vivo* point of departure (POD) with *in vitro* assay data from ToxCast and Tox21 projects. Assay data from ToxCast and Tox21 projects were utilized to derive the *in vitro* PODs for several hundred chemicals. These were combined with *in vivo* PODs from ToxRefDB regarding the rat and mouse liver to build a high-dimensional robust regression model. This approach separates the chemicals into a majority, well-predicted set; and a minority, outlier set. Salient relationships can then be learned from the data. For both mouse and rat liver PODs, over 93% of chemicals have inferred values from *in vitro* PODs that are within ± 1 of the *in vivo* PODs on the log10 scale (the target learning region, or TLR) and R2 of 0.80 (rats) and 0.78 (mice) for these chemicals. This is comparable with extrapolation between related species (mouse and rat), which has 93% chemicals within the TLR and the R2 being 0.78. Chemicals in the outlier set tend to also have more biologically variable characteristics. With the continued accumulation of high throughput data for a wide range of chemicals, predictive modeling can provide a valuable complement for adverse outcome pathway based approach in risk assessment.

**ABSTRACT NUMBER:** 3492  
**Poster Board Number:** P288  
**TITLE:** Application of Systematic Review and Quantitative Evidence Integration Methods to Support Risk Assessment: Characterization of the Dose-Response Relationship between Exposure to Dioxin-Like Compounds (DLC) and Sperm Count  
**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** C. L. Ring1, J. D. Urban1, D. S. Wikoff2, C. M. Thompson1, R. A. Budinsky4, and L. C. Haws1. 1ToxStrategies, Inc., Austin, TX; 2ToxStrategies, Inc., Asheville, NC; 3ToxStrategies, Inc., Katy, TX; and 4Dow Chemical Company, Midland, MI.  
**KEYWORDS:** Dioxin; Developmental Toxicity; Prenatal; Sperm  
**ABSTRACT:** Systematic review is being used globally to support the risk assessment process; however, best practices for integrating evidence are still being developed. There is particular interest in developing or refining approaches to quantitatively integrate heterogeneous toxicology data. As part of a systematic review to evaluate the dose-response relationship between DLCs and reduced sperm count, we explored the utility and feasibility of using meta-regression to quantitatively integrate dose-response data from experimental animal studies. Seven studies were selected for a pilot evaluation of
feasibility based on secondary reviews; these included studies with single and multiple dose groups, single exposures and repeated exposures, subcutaneous and oral exposures, as well as studies that reported a relationship and those that did not. Exposure and outcome metrics were standardized, and meta-regression models were fit using the R meta-analysis package “metafor.” Based on the best-fit models, points of departure (PODs) similar to benchmark dose (BMD) and lower-bound BMD (BMDL) metrics were generated. Heterogeneity was large for this pilot data set, which was expected, given that the studies were purposely chosen to bracket the full range of diversity in the data set. Results allow for the exploration of sensitivity in model selection and other parameters as they relate to POD determination. Depending on threshold, model, and fitting parameters, PODs ranged from < 1.5 ng/kg-day to > 15 ng/kg-day, thus highlighting the need for care in model selection during the full study. The pilot results demonstrate the utility of the technique to estimate an overall average dose-response relationship and the consistency of dose-response across studies, as well as to use data that could not be analyzed using a traditional BMD approach. In conclusion, the pilot analysis indicated that meta-regression is feasible and useful for this data set, thus allowing for consideration of the entire evidence base vs reliance on single datasets. Such methods provide important information to risk managers, allowing for greater accommodation of the totality of evidence as well as characterization of uncertainty.

ABSTRACT NUMBER: 3493   Poster Board Number: P289
TITLE: Probabilistic Pharmacokinetic Modeling of Airborne Lead Levels Corresponding to Toxicologically Relevant Blood Lead Levels in Workers

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: L. M. Sweeney. UES, Inc., assigned to US Air Force School of Aerospace Medicine,. Wright-Patterson AFB, OH.

KEYWORDS: Risk Assessment; Biological Modeling; Toxicokinetics

ABSTRACT: The National Research Council (NRC) assessed the potential health effects associated with exposure of Department of Defense (DoD) personnel to lead (Pb) at firing ranges (NRC, 2013). In that report, NRC concluded that the current Occupational Safety and Health Administration permissible exposure limit (PEL) and the blood lead levels (BLLs) on which it was based were not sufficiently protective of worker populations covered under the general industry standard. In support of future selection of an occupational exposure limit, the relationship of airborne Pb levels to BLLs is of interest to the DoD. A range of toxicologically relevant BLLs was selected as targets for extrapolation to equivalent airborne Pb values. An existing physiologically based pharmacokinetic model for Pb in humans was modified to facilitate probabilistic simulations of DoD worker population BLLs, after determining parameter distributions of model inputs that were sensitive determinants of final predicted BLLs (e.g., breathing rates). Under working lifetime exposure, workplace airborne Pb time-weighted average concentrations of 0.80, 3.6, 6.5, or 9.7 µg/m³ were predicted by the model to maintain BLLs below 5, 10, 15, or 20 µg/dL, respectively, in the 95th percentile full-time Pb-exposed DoD employee. Reference: National Research Council (2003). Potential Health Risks to DOD Firing-Range Personnel from Recurrent Lead Exposure. Washington, DC, National Academies Press.
ABSTRACT NUMBER: 3494       Poster Board Number: P290
TITLE: New Insights in the Chlorpyrifos Risk Assessment

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: M. Dourson, B. Gadagbui, C. Onyema, and P. McGinnis. TERA, Cincinnati, OH.

KEYWORDS: Children's Health; Pesticides; Safety Evaluation

ABSTRACT: Rauh et al. (2011) from the Columbia Center for Children’s Environmental Health (CCCEH) claimed statistically significant associations for two neurological effects in children at age seven after potential low exposure to chlorpyrifos (CPF) during pregnancy. Very low levels of chlorpyrifos (CPF) were detected in a single cord blood at the time of birth, with 42% non-detects. The claims are surprising in light of the extensive animal and human studies on chlorpyrifos that point to changes in a blood enzyme as its first biological effect, occurring at much higher levels. The chlorpyrifos-specific neurological findings reported by CCCEH have not been replicated in other epidemiology publications, nor have the data been made available to government scientists for independent confirmation, despite that the CCCEH was supported, in part, by public funds and the data have been requested by the US EPA. Specifically, Rauh et al. (2011) reported evidence of deficits in Working Memory Index and Full-Scale IQ in children at 7 years old as a function of prenatal CPF exposure, even though the various neurotoxic chemical exposures during the intervening 7 years were not addressed (e.g., lead, phthalates). Although the underlying data have not been made available, we were able to accurately extract data of Figures 1A and 1E in Rauh et al. (2011). Our analyses uncovered a surprising finding: data from ~35% of Figure 1A and ~15% of Figure 1E were missing from the 265 children described in the text of Rauh et al. (2011); missing in both figures were also high dose data. Although some of the missing data are possibly due to overlay of data points not observable in the published figures, such overlay cannot reasonably account for the extent of these missing data. The data extracted from the figures were analyzed in a number of ways using typical toxicological and risk assessment approaches. In contrast to Rauh et al. (2011), our analysis does not suggest any evidence of an effect on Full-Scale IQ (Figure 1E). We also found less of a negative association (reduction) in Working Memory Index (Figure 1A) as compared with the authors' findings. The unavailability of the raw data from these studies makes statistical analysis and confirmation of the results, a hallmark of scientific inquiry, impossible, and rules out adjusting responses for other confounding variables in our analyses. The published associations of CPF cord blood levels with Working Memory and Full Scale IQ in children need to be further analyzed using all the data for sound risk assessment.
ABSTRACT NUMBER: 3495  Poster Board Number: P291
TITLE: An Approach for the Skin Compatibility Assessment of Botanical Ingredients/Extracts to Be Used in Cosmetics

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: S. Kim1, E. Bauza2, I. Garcia2, G. Oberto2, Y. Ferreir3, C. Jantzen3, R. Hamilton4, M. Arcioni3, M. Brulas2, C. Coquet1, E. Oger2, L. Zhang5, J. Botto2, and A. Schatz3. 1Ashland LLC, Lincoln, CA; 2Ashland LLC, Sophia Antipolis, France; 3Ashland LLC, Bridgewater, NJ; 4Ashland LLC, Wilmington, DE; and 5Ashland LLC, Ossining, NY.

KEYWORDS: Risk Assessment; In Vitro and Alternatives; Safety Evaluation

ABSTRACT: Due to the rising consumer interest and demand for natural products, the botanical extracts that support the health and integrity of the skin are increasingly used in cosmetic formulations. Many botanical ingredients being isolated and prepared as cosmetic components, however, are new and novel and require evaluation for their safe use by consumers. We describe an approach for the skin compatibility assessment of botanical ingredients to be used in cosmetics. The approach involves: (a) review of documented historic use of botanicals in comparison to existing and/or intended products (taking into account the effect of processing/extraction), (b) characterization of the intended finished product categories and consequent exposure, (c) in vitro assessment of irritation potential, and (d) confirmation of skin compatibility with human volunteers. For addressing the skin sensitization potential, we have focused on: (1) review of history of use, (2) identification of potential sensitizer(s) of concern, and if practical, (3) selected elimination of identified potential sensitizer(s) of concern from botanical extracts. The typical in vitro test battery for irritation potential included, but not limited to: (1) Reconstructed human Epidermis (RhE)-based EpiSkin™ and EpiDerm™ models and (2) MatTek EpiOcular™ and SkinEthic™ corneocyte models. The utility of in vitro irritation battery testing, together with historic use data review, as a useful screening tool in the skin compatibility evaluation of botanical ingredients was demonstrated from confirmatory human patch testing (48-hr human patch and human repeat insult patch). More than 40 botanical extracts/formulations were identified and screened using this in vitro irritation assessment methodology, together with historic use data review. No irritation or sensitization potential was observed from any of these extracts/formulations when conducted confirmatory skin compatibility testing with human volunteers.

ABSTRACT NUMBER: 3496  Poster Board Number: P292
TITLE: Endocrine Disruptors Risk Assessment: From the Human-Wildlife Connection Towards the One-Health Strategy

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: A. J. García-Fernández1,2, E. Martínez-López1,2, P. Gómez-Ramirez1,2, S. Espín1,2, I. Navas1,2, P. Maria-Mojica1,3, P. Jiménez1,4, P. Sánchez-Virosta1, J. Peñalver1,5, and M. Aldeguer1. 1University of Murcia, Murcia, Spain; 2Biomedical Research Institute of Murcia (IMIB-Arrixaca), Murcia, Spain; 3Santa Faz Wildlife Rehabilitation Centre. Generalitat Valenciana, Alicante, Spain; 4Veterinary and Zoonoses Service, Murcia, Spain; and 5Aquaculture and Fisheries Service, Region of Murcia, Murcia, Spain. Sponsor: E. Martínez-López, EUROTOX

KEYWORDS: Risk Assessment; Endocrine Disruptors; Environmental Toxicology

ABSTRACT: ONE-HEALTH is a collaborative, multisectoral, and trans-disciplinary approach with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants,
and their shared environment. In this sense, the WHO is working with the FAO to promote responses for food safety hazards, risks from zoonoses, and other threats at the human-animal-ecosystem interface, and provide guidance on how to reduce these risks. However, this one-health approach is not new for environmental persistent contamination, especially endocrine disruptors (EDs). The exposure to EDs in humans and wildlife can cause developmental malformations, interference with reproduction, increased risk of cancer, and disturbances in the immune and nervous system function. Since 1992 the Toxicology group of the University of Murcia has been working in the development of analytical methods to detect EDs and other related chemicals in several biological matrices from different species, including human, but especially wildlife. Pesticides and plant protection products, industrial pollutants, metals, and pharmaceutical and personal care products have been tested for endocrine and other sublethal effects in our lab. These methods have been applied in biomonitoring studies of contaminants in sentinel species, especially in raptors, due to their high trophic level as human. A huge amount of useful and interesting data has been recorded for more than 25 years. E.g, spatial-temporal variations in blood levels of pesticides or metals related to restricting or banning legislation, or changes in human activities. In vivo and in vitro studies gave us a better understanding of some consequences of environmental exposure to these chemicals and their mixtures. In conclusion, with an adequate use of the sentinel wildlife species and a correct interpretation of the analytical and experimental results, it is possible to estimate risks associated to the exposure to EDs in all agents involved under the ONE-HEALTH perspective.

**ABSTRACT NUMBER:** 3497  
**Poster Board Number:** P293

**TITLE:** Electrocatlytic Detection for NADH Monitoring in Mouse Whole Blood of Bleomycin-Induced Pulmonary Diseases

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** H. Moon¹, S. Yoon¹⁻², and K. Lee¹⁻². ¹Korea Institute of Toxicology, Jeongeup, Korea, Republic of; and ²University of Science and Technology, Jeongeup, Korea, Republic of.

**KEYWORDS:** Cytokine, Signalling; Nanotechnology; Biomonitoring

**ABSTRACT:** Nicotinamide adenine dinucleotide (NAD) play a major role in enhancing in metabolic redox reactions and cellular energy metabolism in living cells. As reductive form of NAD, NADH is the primary carrier of electrons from glucose and lactate for ATP synthesis to activate redox signaling pathways. In detail, as compounds with Nicotinamide adenine dinucleotide (NAD⁺), they are key central charge carriers in living cells and are essential in energy metabolism, reductive biosynthesis, and antioxidation. However, the monitoring of NADH in living animals and cells still remains challenging because commercial methods including fluorescence spectra and electrochemical methods have invasive, time consuming, low sensitivity and complex. In this study, we developed a simple method for NADH monitoring in mouse whole bloods of bleomycin-induced pulmonary diseases via an electrocatalytic detection. Here, our sensor consists with a surface-modified screen-printed electrode (SPE) for forming a self-assembled monolayer (SAM). As a result, this sensing platform has a limit of detection (LOD) of 1 nM in pure PBS and in whole blood, respectively, leading to a highly sensitive detection for quantitative analysis. Such a dynamic approach for monitoring NADH represents a potentially powerful tool for diagnosing pulmonary diseases. This study was funded by Korea Institute of Technology (KK-1707 and KK-1905-02).
ABSTRACT NUMBER: 3498        Poster Board Number: P294
TITLE: Risk Assessment of Hexavalent Chromium (Cr(VI)) in Drinking Water by Food Safety Commission of Japan (FSCJ)


ABSTRACT: FSCJ conducted risk assessment of hexavalent chromium (Cr(VI)) in drinking water and released the final report in 2018 (FS/602/2018). In toxicological evaluation, major toxicities induced by Cr(VI) in experimental animal studies were damage to the gastrointestinal tract. Most of in vitro and in vivo genotoxicity studies gave positive results with the exception of some oral exposure studies. These suggested genotoxicity under oral exposure was unclear. We got the new information that negative results were obtained from gene mutation assays in gpt delta mice and Big Blue® Transgenic F344 rats treated with sodium dichromate dehydrate in drinking water as Cr(VI). In 2-year oral toxicity studies on Cr(VI) in NTP, tumors of the mouse small intestine and the rat oral mucosa and tongue were observed. Diffuse epithelial hyperplasia of the mouse duodenum was also observed at the lower dose than the tumorigenic. These results indicated that the diffuse hyperplasia of duodenum in male mice was the most sensitive endpoint. Mode of action (MOA) for the tumorigenicity in male mice was considered to consist of the following key events: (a) Cr(VI) is absorbed from the small intestinal lumen, (b) Cr(VI) damages the villous epithelial cells, (c) regenerative hyperplasia in the crypt occurs to remedy the damage and, (d) excessive cell proliferation in the crypts results in tumors. Therefore, the toxicological evaluation indicated that in oral exposure a genotoxic mechanism was unlikely to contribute to the tumor development, and this enabled us to establish a threshold for Cr(VI). Consequently, it was decided to derive a health-based guidance value for drinking water from the NTP study of mice. At the hazard characterization, benchmark dose (BMD) approach was applied. The tolerable daily intake (TDI) of Cr(VI) was specified as 1.1 μg/kg b.w./day applying an uncertainty factor of 100 to BMDL10 of 0.11 mg/kg b.w./day for the diffuse hyperplasia of duodenum in the NTP study. Since the MOA analysis suggested the hyperplasia as the key precursor event to the tumor development, the TDI was considered to be covered both non-cancerous and cancerous effects. The estimated mean and high daily intakes among Japanese population were 0.04 and 0.29 μg/kg b.w./day, respectively, which were below the specified TDI. Overall, we concluded that the risk of health effects from Cr(VI) was considered to be low at the current exposure via drinking water consumption in Japan.

ABSTRACT NUMBER: 3499        Poster Board Number: P295
TITLE: Internal Dose-Based Thresholds of Toxicological Concern for Occupational Inhalation Exposure to Systemically Acting Organic Chemical Vapors

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KEYWORDS: Volatile Organic Compounds; Disposition; Risk Assessment

ABSTRACT: The thresholds of toxicological concern (TTC), corresponding to the inhalation concentrations of chemicals that would not represent a safety concern, are beginning to be developed for workplace contaminants. Developing TTCs on the basis of internal dose (i) the area under the blood concentration versus time curve during a workshift, AUC; (ii) the maximum blood concentration at the end of an 8-hr workshift, CMAX; and (iii) the amount of parent chemical metabolized during a specified
period of time, RMET) is scientifically sound but has not been done for occupational contaminants. The present study derived TTCs for inhalation exposure to systemically-acting organic chemical vapors on the basis of internal doses simulated using a physiologically-based pharmacokinetic (PBPK) model. A dataset of 276 organic chemicals with 8-hr time-weighted average threshold limit values (TLV®-TWA) was compiled along with their molecular structure and Cramer class to which they belong (Class I: low order of toxicity, Class II: intermediate toxicity, Class III: suggestive of significant toxicity). A human PBPK model, based on integration of quantitative property-property relationships for organic chemicals was used in this study. Using this model, three internal dose metrics (i.e., AUC, RMET, CMAX) were predicted for an 8-hr occupational inhalation exposure to the TLV®-TWA of each chemical. Distributional analyses of the predicted dose metrics were performed to identify the various percentile values corresponding to the internal dose-based TTCs. No TTC could be derived for class II due to few chemicals in this category. Based on RMET, the proposed internal dose-based TTCs were 0.056 and 0.0009 mmol/d at the 10th percentile level for Cramer classes I and III, respectively. Similarly, the AUC-based TTCs derived in this study corresponded to 0.001 and 0.000006 mmol/L.hr at the 10th percentile level. The TTCs developed in this study are potentially useful for screening level assessment as well as for prioritization within an integrated occupational risk assessment framework.

**ABSTRACT NUMBER:** 3500  **Poster Board Number:** P296
**TITLE:** In Vitro Toxicity of Perfluorooctanoic Acid in Liver Cells
**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** M. Abudayyak¹, E. Oztas², and G. Ozhan².
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**KEYWORDS:** Perfluoronated Agents; Cell Culture; Liver

**ABSTRACT:** Perfluorooctanoic acid (PFOA) has been widely used in the industrial field since the 1950s. It has been identified in the environment and human as a result of direct or indirect exposure during its production, use and disposal. There are several studies suggesting that PFOA considered to be as an endocrine disrupter and teratogenic which might cause damage to the reproductive, cardiovascular and immune systems. According to the *in vivo* studies, PFOA could cause hepatic and pancreatic cancers. Although some toxicological studies have been done the toxicity mechanisms of PFOA have not been fully elucidated [1]. Therefore, we aimed to investigate cell viability and the potential effects on oxidative damage, cytokines and apoptotic induction in HepG2 cell line after 24 h PFOA exposure at 0-50 μM. It was observed that PFOA caused the dose-dependent cell death (IC₅₀ = 253.74 μM), and oxidative damage with increase on the levels of catalase and cellular ROS formation, and decrease on glutathione level. PFOA induced necrosis at high concentrations and apoptosis at low concentrations. Also, it was observed increase in the levels of IL-6 expression and decrease in the levels of IL-8 expression. According to the results, PFOA might cause liver toxicity as a result of changes in inflammatory factors and induced oxidative damage. It should be inevitable to be detailed the possible risks in daily and occupational exposure to PFOA. *This work was supported by Scientific Research Projects Coordination Unit of Istanbul University (Project number: TSA-2018-31731). Reference [1] Ma, Z., Liu, X., Li, F., Wang, Y., Xu, Y., & Zhang, M. (n.d.). Perfluorooctanoic acid induces human Ishikawa endometrial cancer cell migration and invasion through activation of ERK/mTOR signaling. Oncotarget, 7(41), 66558-66568.*
ABSTRACT NUMBER: 3501 Poster Board Number: P297
TITLE: Genetic Damage and Cell Killing Induction by Five Head Lice Treatments on HaCaT Human Skin Cells In Vitro

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: A. Alnuqaydan¹, and B. Sanderson². ¹Qassim University, Qassim, Buraydah, Saudi Arabia; and ²Flinders University, Adelaide, Australia.

KEYWORDS: Carcinogenesis; Genetic Toxicology; Cytotoxicity

ABSTRACT: Background: Chemical head lice treatments used by parents to treat head lice infestation in their children due to their rapid and reliable removal of head lice. However, those treatments can be absorbed through the skin. Children are more sensitive to absorbing chemicals than adults. We hypothesized that synthetic chemical-based head lice treatments cause cytotoxic and genotoxic damage to human skin cells in vitro. Objective: To determine the cytotoxic and genotoxic damage of synthetic chemical-based head lice treatments on HaCaT human skin cells in vitro. Methodology: Cytotoxicity measured by the methyl tetrazolium cytotoxicity (MTT) assay and the crystal violet assay. In addition, the mechanism of cell killing was identified by the apoptosis detection, via Flow cytometry assay. The cytokinesis block micronucleus (CBMN) assay detected the frequency of binucleated cells (BN) with micronucleus (MNi), to indicate genetic damage induced by head lice treatments. Results: Tea Tree Oil (TTO), Pure Lavender oil and Pyrethrum did induce significant cytotoxicity. Also, they enhanced both early apoptosis and late apoptosis/necrosis. However, two head lice treatments, Permethrin (Lice Breaker) and Maldison (Malathion) (KP24) did not induce cytotoxicity. Early apoptosis and necrosis were observed in Permethrin treatment and late apoptosis and early necrosis were measured in Maldison (Malathion) (KP24). Moreover, Permethrin (Lice Breaker) and Maldison (Malathion) (KP24) induced micronuclei (MNi) at a frequency significantly higher (range=15-25MNi/1000 binucleated cells, n=3) than the background frequency (media alone control; MNi range= 6 MNi /1000 binucleated cells, n=3). Conclusion: This study indicates that exposure to chemical based head lice treatments enhanced cell death by both early apoptosis and late apoptosis/necrosis also induced chromosomal damage in human skin cells.

ABSTRACT NUMBER: 3502 Poster Board Number: P298
TITLE: From Cattle to Swine: Generic Physiologically Based Kinetic Models for Animal Risk Assessment

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: L. S. Lautz¹, N. I. Kramer², J. L. Dorne³, A. J. Hendriks¹, and A. M. J. Ragas¹. ¹Radboud University, Nijmegen, Netherlands; ²Utrecht University, Utrecht, Netherlands; and ³European Food Safety Authority, Parma, Italy.

KEYWORDS: Risk Assessment; Biological Modeling; Food Safety/Nutrition

ABSTRACT: The assessment of animal health risks due to chemical exposure has been highlighted as an area which requires further development. We developed generic physiologically based kinetic models for 3 domestic animals, such as cattle, swine and sheep, based on a systematic literature search of data on physiological and biological parameters. Performance of the models were illustrated through predictions of tissue concentrations of contaminants eliminated via renal excretion (i.e. melamine and oxytetracycline) and validated through comparison with measured data. Globally, 74% of the model predictions were within a 3-fold change of the measured data for all species and only 5% of the model predictions were outside a 10-fold interval. These are the first multi-compartment models that were
created for the purpose of use in animal health risk assessment. The case studies of the two chemicals demonstrate the potential applications of the model in risk assessment of animals.

ABSTRACT NUMBER: 3503   Poster Board Number: P299
TITLE: DNA Damage in Indoor Swimming Pools Users Analysed by Comet Assay

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: F. Esteves¹², R. Amaro¹², S. Silva², S. Costa¹², J. P. Teixeira¹², and C. Costa¹². ¹EPIUnit – Instituto de Saúde Pública, Universidade do Porto, Porto, Portugal; and ²Environmental Health Department, National Health Institute, Porto, Portugal.
Sponsor: J. P. Teixeira, EUROTOX

KEYWORDS: Disinfection By-Products; Genotoxicity; Exposure, Environmental

ABSTRACT: Swimming has been considered through the years as a healthy activity across all age groups. However, concerns are being expressed about the possible chemical hazards associated with swimming pools disinfection process. By now, independent studies have shown that disinfection products, such as chlorine, essential to maintain hygiene and avoid waterborne infections may interact with organic matter present in the water to form disinfection by-products that include trihalomethanes (THMs) and haloacetic acids. To understand possible health outcomes of human exposure to these compounds, the present study aims to analyse the levels of DNA damage in indoor chlorinated swimming pool users exposed to THMs. For this purpose, one hundred and fifty (n=150) swimming pool users were recruited from seven municipal indoor chlorinated pools located in the north of Portugal. Relevant information was obtained by a self-administered questionnaire and peripheral blood samples were taken by venipuncture for lymphocyte isolation and further analysis with comet assay (alkaline and oxidatively generated damage detected with FPG enzyme). Individual exposure to different THMs compounds, namely bromodichloromethane, dibromochloromethane, bromoform and chloroform, was estimated using Swimmer Exposure Assessment Model² (SWIMODEL). Results obtained showed that both primary and oxidative DNA damage were directly associated with concentrations of total THMs in indoor chlorinated swimming pools water. Furthermore, it was observed that short-term exposure to bromoform and to bromodichloromethane lead to an increase of primary DNA damage, while exposure to bromoform and dibromochloromethane over the years was associated with an increase of oxidative DNA damage. In conclusion, data reinforces the importance of THMs monitoring in pools and the need of establish guidelines for water treatment, structure requirements and personal hygiene demands to enhance the positive health effects related to physical activity by reducing the potential adverse health risks. Acknowledgments: This work was supported by Project NORTE-01-0145-FEDER-000010 (HEBE), cofinanced by Programa Operacional Regional do Norte (NORTE2020), through Fundo Europeu de Desenvolvimento Regional (FEDER). Authors also acknowledge the support of the COST Action CA15132 (hCOMET).
ABSTRACT NUMBER: 3504    Poster Board Number: P300
TITLE: Human Health Risk Evaluation of Volatile Organic Compounds (VOCs) Emissions from Cosmetic Sprays

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: M. Oh, S. Kim, S. Park, G. Kim, and S. An. Amorepacific, Yongin-si, Korea, Republic of. Sponsor: K. Lim

KEYWORDS: Volatile Organic Compound; Inhalation Toxicology; Risk Assessment

ABSTRACT: The sources of volatile organic compounds (VOCs) are very diverse, including indoor and outdoor environments and exposure to VOCs is an important environmental health concern. The VOCs emissions that are unintentionally contained in household products are also of concern. In this study, the amount of selected VOCs (Toluene, Xylene, Benzene, Styrene, Ethylbenzene, Chloroform Methylene chloride, Tetrachloroethylene, Trichloroethylene, n-Hexane, Formaldehyde, Acetaldehyde) from the 9 cosmetic sprays such as hair spray, deospray and facial spray was analyzed. The estimated VOCs concentration was calculated based on the worst case scenario of consumer exposure and compared with indoor air quality guidelines by EU or WHO. The contents were collected by spraying the product in a tedlar bag and VOCs and aldehydes were analyzed using TD-GC/MS and High Performance Liquid Chromatography (HPLC). Formaldehyde and acetaldehyde were detected in hairspray. Styrene, formaldehyde and acetaldehyde were detected in deospray. But all other VOCs were below the detection limit and therefore not identified. The concentration of VOCs in the air was calculated on the assumption that cosmetic sprays were used in a 10m³ non-ventilated bathroom space. The estimated VOCs (styrene, formaldehyde and acetaldehyde) exposure concentrations are at 20-300 times below the established air guideline values. It was concluded that under normal conditions, cosmetic sprays (hair spray, deospray and facial spray) do not pose a safety concern to the consumer.

ABSTRACT NUMBER: 3505    Poster Board Number: P301
TITLE: Polyaromatic Hydrocarbons Measured in Dust Collected from Childcare Centers of Tampa Bay


KEYWORDS: Exposure Assessment; Exposure, Environmental; Persistent Organic Chemicals

ABSTRACT: Polycyclic Aromatic Hydrocarbons (PAHs) are a group of approximately 100 naturally occurring chemical compounds found in oil, gasoline, coal, crude oil and in their combustion byproducts. PAHs may also bind to other particles in the air. Human exposure to low-levels of PAHs is largely unquantified despite increased global interest in the potential effects of chronic low dose exposures. Little data are available for exposures in pediatric populations. The data gap in children presents unique challenges; few day care facilities or preschools welcome on site research that may expose low level exposure to chemicals of concern. This study determined concentrations of PAHs present in the dust found in day centers in the Tampa Bay area. Dust samples were collected from two childcare centers as a part of pilot study in the Tampa Bay area. The dust samples were collected from 8 classrooms in each child care center using a vacuum cleaner, into a pre-cleaned glass container. A total of 16 samples were collected. The samples were sieved through a 150 µm mesh and kept in amber glass jars at -4 °C before extraction. Samples were extracted twice with 10ml hexane in a sonication bath for 30 mins. The
extracts were combined and concentrated. Analysis was done using GCMS. There were fifteen different PAHs that were analyzed in our study. The preliminary results show that concentration of certain PAH’s like Acenaphthylene, Benzo[b]fluoranthene were found to be in higher concentration compared to other congeners. This may be due to individual chemical and physical properties of the congeners. It may also be due to the ratio of congeners present in materials responsible for shedding the dust. The presence of PAHs in child care environments has implications for higher exposures in children during critical windows of development. Further research is needed using a larger sample size. Long term cohort studies are also needed to draw reliable conclusions of the impact on PAH’s on children’s health.

ABSTRACT NUMBER: 3506    Poster Board Number: P302
TITLE: Dietary Nutrients Modulate the Toxicity of Heavy Metal Exposure in Algae and Daphnia

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: O. M. Awoyemi, K. N. Thompson, A. Velazquez, and G. D. Mayer. Texas Tech University, Lubbock, TX.

KEYWORDS: Exposure Assessment; Ecotoxicology; Biological Modeling

ABSTRACT: Accurately assessing the risk of contaminants requires more than an understanding of the effects of contaminants on individual organisms, but requires further understanding of complex ecological interactions, elemental cycling and the interactive effect of natural stressors such as resource limitations and contaminant stressors. The development of ecotoxicological models that incorporate such data would significantly contribute to interpreting how contaminants impact organisms and aquatic food webs in such a dynamic system. This study seeks to: (a) develop and analyze a series of empirically testable and robust mathematical models of population dynamics subject to stoichiometric and contaminant stressors and (b) integrate sufficient empirical data from existing and new experiments to parameterize, test and improve the model. Specific empirical measurements include: physiological traits (growth, survival, reproduction, respiration, heartrate), behavioral (distance moved, velocity), elemental (C, P, N), and toxicant (Cadmium-Cd, Arsenic-As, Copper-Cu) contents in Daphnia pulex and algae (Scenedesmus acutus) cultured in separate exposure media. These media include control (metal free COMBO media) and test media containing Cd, As, and Cu (25%, 50%, and 100% of daphnia-exposed LC50 values) with varying nutrient ratios of C:P (500:1, 200:1, 75:1) for acute (48 h) and chronic (7 d, 14 d and 21 d) durations. Preliminary results showed that As (NaAsO2) up to 10 mg/L was minimally toxic to the algae, while 6mg/L Cu (CuSO4) and 10mg/L Cd (CdCl2) were more toxic resulting in complete death of the algae in 96 h. In low phosphorus media (10% P of control media), As and Cu toxicities were minimally impacted after 96 h. while Cd toxicity was enhanced resulting in complete death at ~4mg/L. Furthermore, studies are currently underway to determine the effects of the stoichiometric modulation of the mineral nutrients on metal toxicity in D. pulex.

ABSTRACT NUMBER: 3507    Poster Board Number: P303
TITLE: Categorizing Exposure Reports

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: M. Osorio, and N. Pechacek. Ecolab, Eagan, MN.

ABSTRACT: Ecolab is a company that specializes in selling cleaning programs. Our cleaning programs typically include cleaning chemistries specific to the needs of the industry, training, equipment, and
technical support. Recognizing the need to provide a service to customers that would deliver information in emergency situations and enhance product stewardship, Ecolab contracts a third-party company providing 24-hour professional services in the areas of emergency health advice and treatment recommendations. Access to these professional services occurs via toll-free numbers on Ecolab product labels and safety data sheets. Historically, the data collected from these professional services has been periodically summarized and provided to Ecolab. To enhance our product stewardship, our goal is to define a process to further characterize and categorize unintentional exposures to our product chemistries as identified through our third-party emergency health provider and develop approaches to mitigate the potential for future exposure and lessen the health outcomes associated with these exposures. Over the past four years (2014-2017), there have been a total of 2,709 unintentional exposure reports recorded. To obtain a better understanding as to why and/or how unintentional exposures were occurring, the exposure reports were categorized based on information from call summaries across four primary exposure reasons (i.e., accidental, training, packaging, and equipment). Using a word frequency query tool within a qualitative analysis program and product use information, 28 key words were identified that assisted in categorizing the exposure reports. Overall, the “accidental” exposure reason was the largest category, containing approximately 87% of the exposure reports. Hard surface cleaners, biocides, and detergents for food contact items were the most frequently reported product categories with exposure reports from 2014-2017. By separating the reports by year, additional product categories emerged in prominence, such as various cleaners (e.g., equipment, multi-surface) and laundry treatments. Additionally, we can track exposure reports from varying temporal durations, reported routes of exposures, and the severity of self-reported human health outcomes. The enhanced post-market product surveillance using third-party emergency health data enables Ecolab to rapidly identify potential problem areas and develop effective solutions to improve our product formulation, design, and packaging, while also enhancing our customers’ experience.

ABSTRACT NUMBER: 3508   Poster Board Number: P304
TITLE: MicroRNAs as Contributor to Prostate Cancer Disparities between African American and European American


KEYWORDS: Metals; Pharmaceuticals

ABSTRACT: Prostate cancer (PCa) is a major public health burden, it has been the most commonly diagnosed cancer and the second leading cause of cancer deaths among American men. Notably, African-American (AA) men are 1.6 times higher incidence rate and 2.4 times higher mortality rate compared to European-American (EA) men. Multiple socioeconomic factors, and environmental differences have been major components contributing to the cancer health disparities between AA and EA PCa. Recurrence and mortality rates remained higher in AA population, even after adjustment of those socioeconomic status, suggesting that intrinsic genetic differences between AA and EA PCa may account for part of the observed disparities. MicroRNAs (miRNAs) are a class of small noncoding RNAs that regulate gene expression post-transcriptionally. In PCa, miRNA deregulation has been implicated in tumor initiation and progression through the regulation of the expression of target genes involved in multiple signaling pathways, including the ones contributing to tumor aggressiveness and metastasis development. From our initial analysis, 2 miRNAs were identified as AA PCa enriched and 8 miRNAs as
AA PCa depleted. We hypothesized that these 10 miRNAs may contribute to the differential activation of oncogenic signaling pathways in AA PCa and EA PCa. In this study, our main goal is to further validate the population-associated miRNAs and the corresponding mRNA targets that regulate the critical signaling pathways in prostate cancer cell lines and specimens derived from AA and EA patients, to evaluate whether miRNAs can serve as potential biomarkers of chemical-induced cell injury. Furthermore, RT-qPCR assays were used to validate expression profiles of candidate miRNA-mRNA pairing in PCa cell lines as well as clinical samples derived from AAs and EAs. This study particularly focused on two pathways, focal adhesion and insulin signaling pathways. Both pathways are known to promote the cell invasion, migration during cancer development/progression. Further identification of cadmium (Cd)-induced alteration in miRNA expressions may further elucidate the possible mechanism underlying PCa carcinogenesis. In summary, our preliminary data suggest that miRNA-mRNA may play a critical role in regulating oncogenic pathways that promote cell invasion, migration and/or inhibit apoptosis. The mis-regulation of miRNA-mRNA interaction, in turn, contributes to the PCa aggressiveness especially in AA patients.

ABSTRACT NUMBER: 3509    Poster Board Number: P305
TITLE: Race Specific Alterations in DNA Methylation Patterns among Middle Aged African Americans and Whites with Metabolic Syndrome
KEYWORDS: Metabolism; Bioinformatics; Epigenetics
ABSTRACT: Metabolic syndrome (MetS) is a cluster of interrelated cardiometabolic risk factors that may portend the development of age-related diseases. Recent studies suggest that MetS is characterized by epigenetic alterations in selective genes. Consequently, identification of epigenetic alterations associated with MetS in African Americans (AAs) and whites may provide further insights about the genes that influence the negative health outcomes in populations at risk. This study examines genome-wide DNA methylation (DNAm) signatures among AA and white adults with and without MetS. We analyzed race-specific DNA methylation differences and differential methylated interaction hotspots associated with MetS. We assessed age, race and poverty status associated DNAm among AA (n=225) and White (n=233) urban-dwelling adults aged 30-64 years from the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study. MetS was defined according to NCEP-ATPIII guidelines. DNA was extracted from peripheral blood mononuclear cells using standard methods followed by genome-wide DNAm measurement using Illumina Infinium Methylation EPIC BeadChip. Differential methylation analysis using dmpFinder was performed to identify differentially methylated positions (DMPs), bumphunter was performed to identify differentially methylated regions (DMRs). Significant hotspots of epigenetic deregulation were predicted using Functional Epigenetic Modules algorithm. There were significant DMPs associated with age, poverty status, and MetS in each race. Of these, HLA-C, GYPF, LDHC, MAD1L1, GSTT1 were identified as top five hypermethylated and CELF4, MIREP, FRMD4A, SNTG2, HLA-DQB1 as top hypomethylated genes among AAs with and without MetS. On the other hand, RBMXL2, AZ1, PPP1R13L, CDKN2D, NID2 were identified as top five hypermethylated and SCD, MICAL3, SMAD2, PPP4R2, PTPRN2 as top hypomethylated genes among whites with and without MetS. We identified seven interactome hot spots (ARRB2, F10, ITGB7, LDB1, NPTN, TRIM29, VPS41) of epigenetic deregulation among AAs and eight interactome hotspots (ATRIP, CREB1, EPAS1, NEDD4, TRIM29, VPS41) of epigenetic deregulation among AAs and...
ABSTRACT NUMBER: 3510    Poster Board Number: P306
TITLE: Systematic Review and Meta-Analysis of Epidemiological Literature Evaluating the Association between Exposure to Man-Made Vitreous Fibers and Respiratory System Cancers

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: N. S. Egnot1, S. M. Benson1, M. F. Vater1, R. Hazan2, O. Patel3, A. Bowman4, and G. M. Marsh1. 1Cardno ChemRisk, Pittsburgh, PA; 2Cardno ChemRisk, Brooklyn, NY; 3Cardno ChemRisk, San Francisco, CA; and 4Cardno ChemRisk, Chicago, IL. Sponsor: M. Kreider

KEYWORDS: Epidemiology; Lung; Pulmonary Or Respiratory System

ABSTRACT: The carcinogenic potential of man-made vitreous fibers (MMVF) has been extensively studied epidemiologically since the 1970’s, and in 2002, the International Agency for Research on Cancer (IARC) classified insulation glass wool, rock (stone) wool, and slag wool as not classifiable as to their carcinogenicity to humans (Group 3). However, since the release of the 2002 IARC Monograph, several studies evaluating the association between MMVF exposure and respiratory system cancers (RSC) have been published. We therefore conducted a systematic review of studies published since 2002, and performed a meta-analysis of all peer-reviewed epidemiological studies evaluating the association between MMVF exposure (specifically glass, rock, and slag wool) and RSCs (including cancers of the bronchus, trachea, larynx, and lung) published since the 1970’s. In the instance of multiple studies evaluating overlapping participants, only the most recently published cohort study was included in the analysis. We systematically conducted searches in MEDLINE/PubMed and Web of Science databases, and identified 10 case-control studies and 10 cohort studies appropriate for inclusion in our analysis. We utilized random effects models, and assessed several sources of between-study heterogeneity. The summary relative risk (RR) of RSC among those occupationally exposed to MMVF was 1.09 (95% CI=0.99-1.22). When limiting the analysis to include only effect estimates from studies that accounted for a priori risk factors for RSC (asbestos exposure and/or active smoking) the risk of RSC attenuated such that the asbestos-adjusted summary RR was 1.03 (95% CI=0.92-1.15), and the smoking-adjusted summary RR was 1.05 (95% CI=0.92-1.21). The summary RR was reduced to 1.01 (95% CI=0.91-1.13) among studies that adjusted for both asbestos and smoking. Notably, the results varied substantially by study design (RR=1.01, 95% CI=0.91-1.13, and RR=1.42, 95% CI=1.12-1.80 among cohort and case-control studies, respectively). This evaluation of the collective epidemiological literature provides no evidence of an association between occupational MMVF exposure and RSC, and indicates that the 2002 IARC Group 3 classification of insulation glass wool, rock wool, and slag wool remains valid.
ABSTRACT NUMBER: 3511   Poster Board Number: P307

TITLE: An Ecological Evaluation of Vinyl Chloride Exposure and Liver Cancer Incidence and Mortality in Texas

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KEYWORDS: Epidemiology; Liver

ABSTRACT: We performed an ecological study to evaluate the association between county-level ambient exposure to vinyl chloride (VC) and both liver cancer incidence and mortality in Texas. Ambient county-level VC exposure data were obtained from the National Air Toxics Assessment. Age-adjusted county-level liver cancer incidence rates were abstracted from the Texas Cancer Registry (TCR). Age-standardized county-level liver cancer mortality rates were obtained from the peer-reviewed literature and were based on an analysis of death records, population counts, and small area estimation models. County-level data on sex, race, income, smoking, heavy drinking, obesity, and hepatitis were obtained from the American Community Survey, Behavioral Risk Factor Surveillance System, Centers for Disease Control, Institute for Health Metrics and Evaluation, and peer-reviewed literature. Multivariable imputation was utilized to impute incidence rates in counties with less than 16 reported cases of liver cancer (suppressed by the TCR). Exploratory spatial data analyses were performed to evaluate associations among exposure, outcome, and potential confounders. Univariate and multivariable negative binomial and Poisson models were used to evaluate the association between VC exposure and liver cancer incidence and mortality rates, respectively (incidence/mortality rate ratios [IRRs]). Exposure data were log transformed and both exposure and confounder data were scored into tertiles. VC exposure was not associated with liver cancer incidence in the unadjusted model (IRR 1.05 [95% CI: 0.98-1.13]) or the multivariable model (IRR 0.99 [95% CI: 0.93-1.06]) adjusted for the statistically significant predictors of liver cancer risk (heavy drinking, hepatitis, income, and race). VC exposure was not associated with liver cancer mortality in the unadjusted model (IRR 0.98 [95% CI: 0.93-1.04]) or the multivariable model (IRR 0.94 [95% CI: 0.89-1.00]). In both the incidence and mortality models, hepatitis mortality rates and heavy drinking were associated with an increased rate of liver cancer, while income and race were associated with a decreased rate of liver cancer. Overall, our analysis provides no evidence that Texas county-level ambient VC exposure is associated with either county-level liver cancer incidence or mortality rates.

ABSTRACT NUMBER: 3512   Poster Board Number: P308

TITLE: Regarding the References for Reference Chemicals of Alternative Methods

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KEYWORDS: Alternatives to Animal Testing; Predictive Toxicology; Toxicity; Acute

ABSTRACT: The selection of reference and proficiency chemicals is an important component of method development (today that is mainly non-animal methods) and proficiency evaluations. Reference chemicals are a set of test substances used by a method developer to evaluate the reliability and
relevance of a new method, in comparison to reference data (usually in vivo data from regulatory tests that the non-animal test method is seeking to fully, or partially, replace). Proficiency chemicals, as defined in OECD Guidance Document on Good In Vitro Method Practices, are defined post validation as a subset of the reference chemicals that are used by naive laboratories to demonstrate technical competence with a validated test method. Proficiency chemicals should cover several chemical classes within the applicability domain of the method and yield negative and a range of positive responses, they shall be commercially available and have high quality reference data. If reference and subsequent proficiency chemicals are chosen without sufficient evidence for their inclusion, both test method evaluation and demonstration of technical proficiency can be hampered. In this report we present cases in which the selection of reference chemicals led to problems in the validation and/or demonstration of technical proficiency: The variability of results was not always taken into account in selection of several reference substances of the LLNA (OECD TG 429): Chlorobenzene (CAS 108-90-7), methyl salicylate (CAS 119-36-8), methyl metacrylate (CAS 80-62-6), nickel chloride (CAS 7718-54-9) and salicylic acid (CAS 69-72-7). Based on the available reference data the proficiency chemical tetraethylenepentamine (CAS 112-57-2) for the Corrositex skin corrosion test (OECD TG 435) should be replaced. Likewise, the expected in vitro result for the proficiency chemical dibenzyl-L-tartaric acid (CAS 2743-38-6) of the BCOP (OECD TG 437) was difficult to reproduce in several labs. Furthermore, it was not possible to obtain the proficiency chemical human chorionic gonadotropin gonadotropin (CAS 9002-61-3) for the Steroidogenesis Assay (OECD TG 456) at non-prohibitive costs at a reasonable purity. Based on these, we recommend changes of current proficiency chemicals lists with established OECD Test Guidelines and provide recommendations for developing future sets of reference chemicals.

ABSTRACT NUMBER: 3513  Poster Board Number: P309

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KEYWORDS: In Vitro and Alternatives; Alternatives to Animal Testing; Cell Culture

ABSTRACT: The recently approved OECD Guidance Document, Good In Vitro Method Practices (GIVIMP), coordinated by the European Commission Joint Research Centre's EU Reference Laboratory for alternatives to animal testing (EURL ECVAM), provides a framework of technical and quality practices to help ensure that the overall development and implementation of in vitro methods is of scientific integrity and of the highest quality possible. While the guidance is intended for all OECD member states and encompasses a wide range of audiences including method developers, validation bodies and end users, its greatest impact may be in regions where in vitro methods are just beginning to take root. Currently China is striving to adopt and implement non-animal, including in vitro, testing approaches for the safety assessment of cosmetics and ingredients. Collaborative efforts between industry and the Institute for In Vitro Sciences (IIVS, Gaithersburg, USA) have focused on the transfer of several OECD Test Guideline methods to government laboratories in China and have supported the creation of an in vitro toxicology testing laboratory within the Zhejiang Institute for Food and Drug Control (Hangzhou, China). Recently BASF SE (Ludwigshafen, Germany) and IIVS have partnered to introduce a cell based in
vitro skin sensitisation test, LuSens, into China using the principles of GIVIMP as a standard. This case study exemplifies the practical way in which the GIVIMP guidance can assist interested parties in the development, transfer and establishment of in vitro approaches.

ABSTRACT NUMBER: 3514  Poster Board Number: P310

TITLE: Defined Approach for Detection of Eye Irritants and Corrosives for Pesticide Formulations

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KEYWORDS: Alternatives to Animal Testing; Ocular Toxicity; Pesticides

ABSTRACT: In 2018, ICCVAM released a roadmap to expedite the development, use, and regulatory acceptance of new approach methodologies. Central to achieving these goals is the formation of public-private partnerships that allow cross-sector cooperation. These partnerships are being implemented to explore new approaches to assess eye irritation/corrosion potential for pesticide formulations. The PETA International Science Consortium Ltd., NICEATM, the U.S. Environmental Protection Agency, and CropLife America companies are collaborating to develop an in vitro defined approach for classification of eye irritation potential of pesticide formulations. A three-phase prospective evaluation is being employed to assess the applicability of seven in vitro eye irritation/corrosion methods to pesticide formulations and develop a defined testing approach for prediction of U.S. and international irritancy classifications. In Phase 1, six formulations were tested in seven eye irritation test methods: bovine corneal opacity and permeability, neutral red release, isolated chicken eye, porcine cornea reversibility, EpiOcular Eye Irritation Test, and two EpiOcular ET-50 approaches. Results showed that each test method misclassified at least one formulation, but none misclassified all tested formulations. All methods will be included in Phase 2, which will evaluate 10 formulations that represent a range of eye irritancy classifications. Based on these results, an expanded set of pesticide formulations may be tested in Phase 3 in one or more of the test methods. The outcomes of this analysis will suggest endpoints that can form the basis of a defined approach for pesticide formulations testing for eye irritation/corrosion potential. This was funded with US federal funds from NIEHS/NIH/HHS under Contract HHSN273201500010C.
ABSTRACT NUMBER: 3515    Poster Board Number: P311
TITLE: Application of Cryopreserved Human Intestinal Mucosa (CHIM) in the Evaluation of Enterotoxic Potential of Herbal Supplements

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KEYWORDS: Natural Products; Alternatives to Animal Testing; Gastrointestinal

ABSTRACT: Thirty commonly used herbal supplements (HS) were evaluated for their in vitro human enterotoxicity using a novel in vitro experimental system for the human intestine, cryopreserved human intestinal mucosa (CHIM). The HS evaluated were: aloe vera, black cohosh, black elderberry, cinnamon, cranberry, echinacea, evening primrose, fenugreek, flaxseed, garlic, ginger, ginkgo, ginseng, grapefruit juice, green tea, guarana, horehound, maca, milk thistle, oregano, red yeast rice, rhodiola, saw palmetto, soy isoflavone, spirulina, St. John’s wort, turmeric, valerian, wheat grass, and white kidney bean. Except for grapefruit juice, the HS were obtained commercially as pills intended to be administered orally. The HS pills were dissolved in protein free culture medium (HQM) followed by filtration to remove insoluble components and pH adjustment to 7.0-7.2. Grapefruit juice was pH adjusted without dilution. CHIM (1 mg protein per mL) was thawed and added directly in a volume of 50 uL per well to 96 well plates containing 50 uL of HS at 2x of the final concentration, following by incubation at 37 degrees. Cytotoxicity was quantified as relative viability as a ratio of the cellular ATP contents of the HS treated CHIM versus that of untreated control. An initial screen was performed with a 4-hour incubation to approximate the longest duration of enteric exposure upon oral administration. One hundred percent HS is defined as the recommended oral dosage dissolved in 200 mL of HQM (based on the commonly accepted enteric fluid volume of 214 mL). HS that yielded cytotoxicity less than 60% relative viability were evaluated in a second 4-hour experiment at final HS concentrations of 1.6, 3.15, 6.25, 12.5, 25, 50 and 100% of HS. Of the 30 HS evaluated, dose-response induction of cytotoxicity was consistently observed in the following, presented in descending order of cytotoxicity (relative viability at 100%): green tea (6.3%), grapefruit juice (27.9%), St. John’s wort (34.8%), soy isoflavone (43.6%), black elderberry (46.5%), echinacea (47.9%), and valerian root (55.3%). A third experiment was performed with five cytotoxic HS with an incubation of 0.5 hrs, which showed similar cytotoxicity. The results suggest that HS are associated with acute cytotoxicity towards intestinal mucosa. The physiological relevance of the observation is yet to be evaluated.

ABSTRACT NUMBER: 3516    Poster Board Number: P312
TITLE: Antioxidant Content and Fungal Presence on Mexican Honey Samples Compared to a New Zealand Manuka Honey

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KEYWORDS: Food Safety/Nutrition

ABSTRACT: Honey is a bee product with high antioxidant content and many therapeutic applications, including antimycotic functions. To prevent antioxidant degradation in honey, no chemical or heat treatments are used to preserve it, hence presence of unwanted natural substances or commensal
microorganisms is common. For this study, 20 honey samples were collected from different parts of Mexico and were compared to a Manuka honey obtained from New Zealand. Antioxidant properties of honey were analyzed using the Folin-Ciocalteu method to determine Total Phenolic Content (TPC) and the aluminum chloride (AlCl₃) colorimetric method was used to determine Total Flavonoid Content (TFC). Honey color was measured with a HANNA honey color portable photometer HI96785 to determine its relationship with antioxidant properties. The honey samples with the highest and lowest content of antioxidants (2) were used along with Manuka honey to determine presence of fungal contamination, antifungal activity, and total aflatoxin content. Honey samples were cultured in potato dextrose agar (PDA) at concentrations of 70%, 50%, 30%, and 10% w/v and fungal contamination was quantified after 3 days. To evaluate antifungal activity, honey samples (undiluted, 70% and 30% dilutions) were added to wells made on Aspergillus flavus cultures on petri dishes with PDA. Presence of total aflatoxins in honey was done using a VICAM fluorometer method. The TPC in honey varied from 120.43 to 1304.80 mg/kg while TFC ranged from 4.028 to 47.454 mg/100g. Higher TFC values were related to darker honey color based on a Pfund scale. Mycological examination revealed presence of fungi in all samples in various degrees, while antimycotic activity with A. flavus showed more fungal growth in wells with higher concentrations of honey. Regarding total aflatoxin presence, only one sample had detectable levels (0.17 ppm). In conclusion, antioxidant content in honey has wide variations and this is correlated with color; fungi seem to be a common contaminant in honey and this may pose potential risks that need further research.

ABSTRACT NUMBER: 3517        Poster Board Number: P313
TITLE: In Vitro Cytotoxicity Activity of Neutral Electrolyzed Water on Aspergillus spp. and Fusarium spp. Spores


KEYWORDS: Cytotoxicity

ABSTRACT: Neutral Electrolyzed Water (NEW) is a promising antiseptic solution for food applications. It is produced by electrolysis of a diluted NaCl solution passing through an electrolytic cell. NEW antimicrobial effects are mainly studied on bacteria and a few studies focus on its effects on fungi. Fungi such as Aspergillus and Fusarium species are common food contaminants and potential producers of mycotoxins, that need to be controlled. Therefore, we evaluated NEW cytotoxic activity against Aspergillus flavus, Aspergillus parasiticus, Fusarium oxysporum and Fusarium verticillioides spores in order to determine their minimal inhibitory concentration (MIC). Fungal strains were incubated on PDA media for 7 days in case of Aspergillus spp., and 14 days for Fusarium spp. in order to obtain spores. NEW 10, 40 and 60 mg/L of available chlorine content (ACC) were characterized by measuring their pH and oxidation reduction potential (ORP) and four working dilutions were made with potato dextrose broth (PDB) to achieve the concentrations of 50%, 25%, 12.5% and 6.25% (v/v). A working 2.5 x 10^5 spore/mL suspension was achieved with tween 20 (0.01%). Resazurin sodium salt was used to perform the cytotoxicity assay. A volume of 80 μL of spore suspension were reacted with resazurin on PDB media with control or NEW treatment. Triplicate measurements of each assay were done. The MIC was established as the concentration of NEW able to inhibit at least 50% of the cells ability to convert resazurin to resorufin. Images of NEW effects on spores were taken to document evident cell
morphological changes. Mean values and standard deviations of pH and ORP were as follows: NEW 10 had a pH of 5.15 ± 0.05 and an ORP of 506.33 ± 10.17, NEW 40 had a pH of 4.79 ± 0.08 and an ORP of 669.86 ± 05.44, and NEW 10 had a pH of 4.69 ± 0.02 and an ORP of 776.9 ± 6.44. Results of cytotoxicity assays showed higher percentage of cytotoxicity with increasing concentrations of NEW. MIC of NEW 10 was 5 mg/L for all fungal strains, while NEW 40 had MIC of 2.5 mg/L for A. flavus, 5 mg/L for A. parasiticus, and 10 mg/L for Fusarium strains. NEW 60 had MIC values of 3.75 mg/L for A. flavus, 7.5 mg/L for A. parasiticus, and 15 mg/L for Fusarium spp. Images of spores exposed to low concentrations of NEW revealed high germination rate and larger hyphal growth. NEW treatments are cytotoxic to Aspergillus and Fusarium, supporting its potential use as an antifungal alternative.

ABSTRACT NUMBER: 3518    Poster Board Number: P314
TITLE: The Association between Green Tea and Green Tea Supplements Consumption and Liver Biomarkers in US Adults

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KEYWORDS: Food Safety/Nutrition; Liver

ABSTRACT: There have been isolated case reports of liver toxicity in individuals consuming supplements containing green tea extracts, thereby questioning the safety of green tea and green tea supplements. The relationship between green tea and green tea supplement consumption and abnormal liver biomarkers in adults was evaluated using the 2009-2014 U.S. National Health and Nutrition Examination Survey (NHANES). Dietary intake data for Day 1 of the 2-day survey were available for 15,774 individuals aged 19 years or older, and liver biomarkers (i.e., bilirubin, gamma glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase) were available for 14,924 of these individuals. An individual was considered to have an abnormal liver biomarker if any one of the liver biomarkers exceeded the upper end of the age- and gender-specific normal reference ranges. Steeped green tea consumption was quantified 3 ways, as consumption versus no consumption, servings/doses per day, and amount (g) per day. Use of green tea supplements (containing green tea or an extract of green tea) was quantified as consumption versus no consumption and servings/doses per day. The associations between green tea and green tea supplement use and the odds of having abnormal liver function were assessed using multiple logistic regression models that included other confounding variables (i.e., gender, age, race, medication use, alcohol use, waist circumference, or liver disease). 23% of the individuals included in the analysis had at least one abnormal liver biomarker. 1.96% of the survey participants consumed steeped green tea, with an intake frequency of approximately 1.2 servings/doses per day, and a mean intake of 620 g/day. The consumption of green tea was associated with a significant reduction in the odds of having an abnormal liver biomarker (OR=0.48; 95% CI: 0.27, 0.84; p=0.01); however, neither the number of consumption occasions nor the amount of green tea consumed was associated with the odds of having an abnormal liver biomarker. The percentage of users of green tea supplements was 1.30%. Consumption of green tea supplements versus no consumption was not associated with abnormal liver biomarkers (OR=0.92; 95% CI: 0.51, 1.66; p=0.78). Consuming steeped green tea is associated with a significant reduction in the odds of having abnormal liver biomarkers.
ABSTRACT NUMBER: 3519     Poster Board Number: P315
TITLE: Distinct Carbon Sources Determine the Response of Induced Pluripotent Stem Cell-Derived Cardiomyocytes to Hypoxia

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: A. Bruening-Wright, and C. Obejero-Paz. Charles River Laboratories, Cleveland, OH. Sponsor: B. Roche

KEYWORDS: Safety Pharmacology; Toxicity; Acute; Cardiovascular System

ABSTRACT: Depending on the carbon substrate induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) are capable of generating ATP by glycolysis and/or oxidative phosphorylation. Here we investigated 1) whether changes in cellular ATP content are paralleled by electrophysiological and contractile changes measured using the xCELLigence RTCA CardioECR instrument and 2) the extent these properties are affected by hypoxia. Cardiomyocytes were exposed to culture mediums containing linoleic-oleic acid and either 2.75 mM galactose (galactose medium) to support the aerobic respiration or 2.75 mM glucose (glucose medium) to support both aerobic and anaerobic respiration. Hypoxic conditions were achieved using a hypoxic chamber from Billups-Rothenberg and ATP content/well was measured using a luminescence assay (Promega). The data indicates that: 1) Under normoxic conditions cardiomyocytes incubated in galactose medium showed an average 25% lower ATP content per well compared to glucose exposed wells. Changes in ATP were paralleled by a 9% significant reduction in the cell index suggesting cell shape rearrangement or decreased cell viability and a significant 9% shortening of the twitch duration. No significant differences in excitability (sodium spike amplitude, field potential duration and beat rate) were observed between glucose and galactose incubated cells. 2) Under hypoxic conditions (6 hours at 1% O2), wells containing galactose medium showed a 25% lower ATP content/well than wells containing glucose medium (Exp 1: 19%, n>15, p<0.05, Exp 2: 31%, n>10, p<0.05). Electrical and contractile activity was completely obliterated in galactose wells exposed to hypoxic conditions whereas cells in glucose medium showed robust activity. The data is consistent with results in isolated hearts where the presence of glucose reduces the deleterious effects of hypoxia. The evidence indicates that significant changes in cellular ATP content can be reached using defined culture conditions and that those changes are paralleled by a decrease in twitch duration. Notably, effects on electrical excitability were minimal. These experiments represent a basis for development of an iPSC-CM in vitro ischemia-reperfusion model.

ABSTRACT NUMBER: 3520     Poster Board Number: P316
TITLE: Toxicology Evaluation of Drug Administration via the Intra-Articular Route


ABSTRACT: The nonclinical toxicology studies to support safe use of drug products via the intra-articular (IA) route of administration must take into account this specific route. The US FDA guidance document titled Nonclinical Safety Evaluation of Reformulated Drug Products and Products Intended for Administration by an Alternative Route, does not specifically address the IA route of administration or how safety margins should be derived based on local toxicity findings to support human dosing. Due to limitations in the database for IA drug products, the Division of Anesthesia, Analgesia, and Addiction Product (DAAAP) has recommended that Applicants conduct short-term IA toxicology studies in two species. However, similar to intranasal and pulmonary drug products, a chronic IA toxicology study in a
single species may be justified based on the results of the short-term toxicity data. In general, DAAAP has provided the following nonclinical recommendations to support development of new drugs intended for IA administration: 1) Conduct short-term GLP nonclinical studies in two species (at least one nonrodent) that closely mimics the intended clinical use taking into consideration the drug concentration, the volume to be administered, and the size of the IA space of the animal. If short-term IA toxicity studies can clearly demonstrate that either there are no differences in the local toxicity profile or one species is clearly more sensitive to the drug product than another, a chronic IA toxicity study in a single species may be justified. However, for a new molecular entity (NME), systemic safety would also need to be studied in two species. 2) In the design of the toxicity study, the doses of drug product tested should be selected to identify a local and systemic NOAEL and to characterize the toxicity profile. The study must establish a safety margin for the concentration of the drug product in the synovial joint fluid. 3) The study should evaluate local tissues including joint fluid cytology and specialized tissue staining to assess the effects of the drug on the synovial tissues, such as toluidine blue or Safranin O staining for cartilage, unless adequately justified otherwise. As always, any Applicant intending to develop an IA drug should consult with appropriate FDA review division for detailed advice about any specific development program.

ABSTRACT NUMBER: 3521

TITLE: Generating Mechanistic Insight into On/Off-Target Mitotox Enables Design of Compound with Improved Safety Profile


ABSTRACT: AstraZeneca are developing novel inhibitors of Monocarboxylate transporter 4 (MCT4i) as potential ImmunoOncology treatments, aiming to reduce lactic acid mediated immunosuppression in the tumour microenvironment and disrupt tumour cellular energetics. Early MCT4i leads showed activity in a mitochondrial Glu/Gal screen (n=11/13). A high proportion of compounds (n=11/18) also produced a bell-shaped response in our primary efficacy assay (measuring lactate efflux in MCT4 expressing SKBR3 cells) with dose-dependent extracellular lactate reduction seen up to 1µM concentration of compound, the lactate levels rebounded at higher concentrations of test compound (>1µM). This phenotype was considered a potential safety risk with the hypothesis that it may be linked to undesirable changes in mitochondrial function (mitotox). To determine whether the hypothesized mitotox was on/off-target, we compared mitochondrial function in SKBR3s vs. low-level/null MCT4 cells (CoLo320) using the Seahorse XFe96 analyser, measuring Oxygen Consumption Rate (OCR) and Extra-Cellular Acidification Rate (ECAR). Dose-dependent effects were observed in SKBR3 cells with tool MCT4i. Similar IC50s and compound rank-order of potency were maintained in the CoLo320 cell line, indicating that the mitotox was off-target (not driven by MCT4 inhibition), significantly reducing the project safety risk. To elucidate the precise mechanism of mitotox, we evaluated the effect of MCT4i on respiration via individual mitochondrial respiratory complexes using the permeabilised cell approach in the XFe96. Mitotox was observed when permeabilised cells were treated with tool MCT4i, glutamate and maleate (Complex I (CI) substrates). Bypassing CI using succinate (CI substrate) plus rotenone (blocking CI) ablated the OCR effects, indicating CI inhibition as the mechanism of mitotox. Finally, we tested MCT4i effects in isolated bovine CI. Mitotox was observed, (indicating direct, not indirect inhibition) albeit at IC50s approx. 10-fold lower; potentially due to reduced potency at bovine CI vs. human. This mitochondrial safety cascade
resulted in the deprioritisation of our most potent MCT4i, redirection of chemistry towards an alternative compound showing no lactate rebound phenotype and a significantly improved mitochondrial profile. The new lead demonstrated good safety margins in preclinical toxicity studies, without evidence of mitochondrial toxicity.

ABSTRACT NUMBER: 3522     Poster Board Number: P318
TITLE: Range Finding Toxicity Study of AXER-204 Administered Intrathecal in Non-Human Primates
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KEYWORDS: Safety Evaluation; Nervous System; Pharmaceuticals

ABSTRACT: AXER-204, a Nogo Receptor Decoy (NgR-Fc Decoy), is a stimulator of axonal fiber re-growth that is being developed for patients with spinal cord injuries. We evaluated the toxicity of AXER-204, when administered for up to 60 minutes as a single dose and repeat dose for 14 consecutive days by intrathecal infusion via catheter implanted in the lumbar region to non-human primates (NHP), followed by a 7-day recovery period. Naive one animal/sex/group was administered vehicle, 1.92, 15, or 30 mg/day AXER-204 formulated at 10 mg/mL. AXER-204 was slowly released into the serum following intrathecal administration. Exposure, as assessed by AXER-204 mean Cmax and AUC0-24 values, increased with the increase in dose level. The increases in AXER-204 mean Cmax and AUC0-24 values were generally dose proportional and the sex differences were less than 2-fold. Accumulation of AXER-204 was observed after multiple doses in NHP. On Day 22, the incidence of anti-drug antibody (ADA) induction against AXER-204 was 50% at 1.92 mg/day, and 100% at 15 and 30 mg/day. On Day 15, the ADA induction was 50% and only detected at 30 mg/day. The presence of ADA did not appear to impact exposure during the dosing phase. No mortalities or AXER-204-related clinical observations occurred during the study. AXER-204-related clinical pathology effects were minor and reversible, and included increases in fibrinogen for females at all dose levels and within 2-fold increases in alanine aminotransferase for the female administered 30 mg/day. These findings indicate that AXER-204 was well tolerated up to 30 mg/day dose with corresponding Day 14 average blood Cmax and AUC0-24 values of 12.900 μg/mL and 241 μg·hr/mL, respectively, and average CSF concentration of 288 μg/mL at 1 h post-dose and 2.990 μg/mL at 24 h post-dose.

ABSTRACT NUMBER: 3523     Poster Board Number: P319
TITLE: Weighting of Translational Agreement between Human and Animal Adverse Events among Key Target Organ Systems
AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: P. Huie¹, and J. A. Phillips². ¹Johns Hopkins University, Baltimore, MD; and ²Vertex Pharmaceuticals, Boston, MA.

KEYWORDS: Computational Toxicology; Predictive Toxicology; Undergraduate Student

ABSTRACT: Preclinical safety studies in animals are critical for assuring safe administration of new molecular entities to people. To understand the strength and consistency of risk predictors derived from preclinical studies, a systematic analysis of animal and human adverse events was conducted. Traceable
concordance and discordance of adverse events observed in humans and animals exposed to the same drug was established. Using a commercially available database of documented adverse events from toxicology studies and clinical trials, a statistical analysis was performed to weigh the categorical risk of a toxicology finding by organ system. The dataset included over 100,000 individual findings across 6 organ systems and the 5 most commonly used preclinical species. Confidence metrics are also furnished to characterize the predictivity of adverse events among organ classes, and species. Our results indicate gastrointestinal adverse events as the strongest predictors of adverse findings in clinical studies. Skin disorders were found to have the least concordant findings among organ systems evaluated. This study also confirmed dogs as the strongest translational model for cardiac and GI disorders. Results from this study reveal mathematical drawbacks from relying on animal models to uniformly predict human risk across target organs. From this work, options to strengthen data collection during animal testing can be considered to allow for stronger statistical relationships and improve future decisions based on historical concordance classification. This study also provides a historical concordance for rationalizing species based on anticipated target organ.

ABSTRACT NUMBER: 3524          Poster Board Number: P320
TITLE: Safety Assessment in the Development of CAR-T Cells Projects


KEYWORDS: Safety Evaluation; Cell Lines, Transfected

ABSTRACT: The development of gene therapies to activate the immune cell response targeting specific antigens is currently one of the most promising and innovative approaches for the treatment of hematological malignancies. Particularly, ex-vivo gene modified autologous or allogeneic cells obtained and manufactured from human Peripheral Blood Mononuclear Cells (PBMC) demonstrate significant response in the treatment of different leukemias. In this perspective, the preclinical safety programs should be properly designed to identify the bio-distribution of the therapeutic cells, their persistency, and any adverse effects of cell administration, while discriminating the consequence of the administration to a different species and their relevance and predictivity in the clinical settings. In addition, one of the major clinical complication (and limitation) of human cells administration in cancer patients is Graft versus Host Disease (GvHD). The preclinical program should therefore take into careful consideration the different claims to properly evaluate the effects observed in preclinical species. The most appropriate study design and the analysis planned should therefore take into account the endpoint of the program and the technical limitations of the animal models. Particularly, the effect of lymphoid cells, presenting different modifications or obtained through different culture programs, to the receiving organism need to be investigated. The study plans used with different CAR-T cells projects and the effects observed with unmodified or mock cultured cells will be presented, including biodistribution and persistence evaluated through immunohistochemistry, flow cytometry and PCR technologies. Animals receiving the unmodified cells showed clear signs of GvHD with some differences in their onset and findings related to the different cells preparations. Findings observed included body weight decrease, and deterioration of general conditions leading to preterm euthanasia of the involved subjects. At histological examination, changes characterized mainly by perivascular inflammatory cell infiltration/inflammation, perivascular fibrosis in the spleen and liver, bile duct hyperplasia in the liver, epidermal hyperplasia and adnexal atrophy in the skin were present in these animals, comparable to
GvHD histological changes reported as indicative of GvHD in both humans and animal models of the disease.

**ABSTRACT NUMBER:** 3525         **Poster Board Number:** P321

**TITLE:** What To Do with Unwanted Inflammation: Dealing with the Impact of Delayed Downstream Immunomodulation Induced by Test Article Administration

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** G. N. Wilson, and B. M. Roche, Charles River Laboratories, Ashland, OH.

**KEYWORDS:** Inflammation; Cytokine, Signalling; Mechanisms

**ABSTRACT:** The field of nonclinical safety assessment is tasked with characterizing physiological effects of new drugs and identifying potential risks to target populations. A most troublesome and unpredictable physiological response that can occur downstream of test article administration is immune activation. While certain drugs take advantage of immune modulation, **unintended** off-target deregulation of the immune system is dangerous. Under the worst cases, a cytokine ‘storm’ may ensue due to the unrestrained, overactivation of the immune system. The purpose of this work was to provide a review of downstream molecular cascades initiated by common classes of compounds that may induce delayed or unintended inflammatory responses, demonstrate mechanisms of how these cascades interact with pre-existing conditions, and provide mitigation strategies in effort to attenuate drug attrition rates and improve tolerability in the clinic. Classes of compounds with potential immunomodulatory properties include oligonucleotides, mitogen activated protein kinase activators, receptor TKIs, ACE inhibitors, opioid agonists/antagonists. Downstream effects include direct release of pro-inflammatory cytokines via activation of toll-like receptor pathways as with siRNAs, increased cytokine synthesis as with some MAPKs, or a shift in immune effector versus immune regulatory cell balance to favor immunostimulatory environments via proliferation, infiltration or activation of various immune cells as with some TKIs. These effects are complicated by pre-existing conditions associated with chronic immunostimulation such as metabolic disorders, asthma, neurodegeneration, or cardiac conditions; e.g. ACE inhibitors can lead to worsening of symptoms in asthmatics due to bronchial hyper-reactivity. Consideration of these conditions within intended populations may encourage **in vitro** prescreening assays (e.g. cytokine release assays), thereby providing important biomarkers for clinical monitoring and mitigation of serious immune responses. For **in vivo** tests, revision or extension of data collection timelines may be sufficient to detect unanticipated responses, but other compounds may warrant prescreening or inclusion of immunotoxicological endpoints (e.g. blood/tissue sampling for multiplexed cytokine analyses) in effort to improve the likelihood of candidate molecules successfully passing through clinical development.
ABSTRACT NUMBER: 3526    Poster Board Number: P322
TITLE: Intrathecal Administration by Direct Lumbar Injection in the Rat and Nonhuman Primate

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: C. Voyer, J. Douville, L. Chouinard, F. Émond, C. Foucault, and M. Halle. Charles River Laboratories, Senneville, QC, Canada. Sponsor: M. Vezina

ABSTRACT: Neurological diseases represent a large portion of the rare and orphan diseases which remain a largely unmet medical need. Over the past few years there has been a shift towards the development of large molecules to treat various disorders, however this type of molecule has poor penetration across the blood brain barrier making targets located in central nervous system difficult to reach when using conventional routes of administration. To circumvent these limitations, our laboratory has developed and refined procedures to allow for intrathecal administration by direct injection (single or repeated dose) under anesthesia in both rats and nonhuman primates (NHPs). This approach has the additional benefit of closely mimicking the clinical approach for intrathecal administration. The same approach can be used to collect cerebrospinal fluid (CSF) samples for bioanalysis or biomarker evaluation purposes. In addition, we have adapted the techniques to allow use for juvenile nonhuman primate populations. Briefly, animals receive a standard protocol of antibiotics and analgesics prior to and following the procedures, and are anesthetized with isoflurane. Rats are placed in ventral recumbency, the lumbar site is incised at the L4/L5 level. A spinal needle is slowly inserted until the vertebral body is attained; tail flick and CSF flow indicate proper placement of the needle. For NHPs, animals are placed in lateral recumbency and similar procedures are followed, but the skin is not incised; proper placement of the needle is confirmed by CSF flow in the hub of the needle, at which point the dosing syringe is connected and the dose is slowly injected, followed by a flush. Herein, we present historical data showing that the experimental procedures caused no abnormal clinical observations, no changes in body weight, food consumption, electrocardiography, respiratory rate, hematology, coagulation or clinical biochemistry parameters, no effects on cytokines or complement activation, and only minimal background changes in histopathology. There were also no abnormal findings in more specialized endpoints such as neurological evaluations or functional observation battery and CSF chemistry and cell count, when compared to age-matched animals dosed via more conventional routes over a similar period. These results demonstrate that the intrathecal route of administration using direct injection under anesthesia can be safely used on toxicology studies.

ABSTRACT NUMBER: 3527    Poster Board Number: P333
TITLE: Successful Application of Automated Liquid Handlers in Bioanalysis to Meet the Increasing Demand of Faster Timelines in Support for Toxicology Studies


ABSTRACT: Development of bioanalytical assays to support IND enabling nonclinical studies using specific and selective reagents is becoming increasingly difficult to manage with respect to program timelines. This is often due to the increase in speed from receipt of test article to initiation of the toxicology study. The procurement of high quality reagents such as anti-id antibodies or recombinant target proteins is nearly impossible in many of the proposed timelines for IND submission. The assay development and validation, as well as sample testing can quickly become the rate-limiting step to filing
an IND, as the exposure data is critical to interpretation of the toxicology data. Bioanalytical testing is often a laborious manual process that requires strong technical expertise as well as specific reagents. In this study, we provide a strategy to expedite bioanalytical sample testing from these IND enabling toxicity studies. We have developed and automated generic human IgG method that is performed 100% using the Hamilton® Microlab STARlet liquid handling platform. This platform can be optimized and used for any humanized IgG with a minimal amount of time for method optimization and validation. Due to the use of generic reagents, the need for article specific reagents is eliminated. The instrumentation equipped with a plate washer and a reader was programmed to run a fully automated ELISA process. Once the plates, bar-coded samples, and reagents were placed on the deck, the instrument ran the pre-programmed experimental procedure. This includes washing, dilution of samples, performing the minimum required dilution in assay buffer, management of incubation times, and reading the assay plate on the integrated spectrophotometer. This process was tested for intra- and inter-assay reproducibility for assay performance and sample dilution. The results have demonstrated precision of <20% and sample dilution accuracy within ±20%. The automation workflow sample throughput has increased to >270 samples/day per instrument compared to <90 samples/day with a manual process. This resulted in an increase of sample analysis throughput by at least 3-fold. By using Hamilton®, we are able to significantly improve efficiencies in method optimization, validation and sample testing while reducing the opportunity for human error. The total time from receipt of test article to sample results can support rapid IND filing with high quality bioanalytical data.

**ABSTRACT NUMBER:** 3528  
**Poster Board Number:** P324

**ABSTRACT:** Extractables and leachables program (E&L) evaluate safety of the future drug product (DP) from toxicological prospective of any potential leachable from all contact surfaces during its entire manufacturing, handling, shipment and long term storage history. Biotherapeutics manufacturer is responsible to prove that the DP container closure systems, process equipment and packaging are toxicologically safe. Simulated extraction studies are currently an emerging approach, that are designed to be representative, albeit harsher than the real time conditions of the DP exposure to a studied system. The present study investigated how simulation extraction studies with single organic co-solvent can be used and applicable to various drug programs with different aqueous formulations. These studies evaluate an existing profile of extractable components, obtained via controlled extraction studies, to identify potential leachables, such as antioxidants, plasticizers, dyes and metals. It is critical that E&L studies are designed specifically for each DP, dose regimen and the container closure materials so that the risks associated with leachable impurities can be assessed.
ABSTRACT NUMBER: 3529   Poster Board Number: P325
TITLE: Toxicologic Evaluation of Nicotinamide Riboside Chloride

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KEYWORDS: Natural Products; Safety Evaluation

ABSTRACT: Nicotinamide riboside is a naturally occurring and efficient vitamin B3 precursor of nicotinamide adenine dinucleotide (NAD+), a coenzyme found in all living cells and play a key role in many biological functions. In animal studies, Nicotinamide riboside has shown significant value in maintaining robust health and preventing age-related health problems. Hereby, the safety of Nicotinamide riboside chloride was investigated using a bacterial reverse mutation test (Ames assay), a 28-day bone marrow micronucleus assay in rats, and a 90-day oral toxicity study in rats. No bacterial mutagenicity was observed in the Ames assay. The 28-day bone marrow micronucleus assay was negative for clastogenic activity and/or disruption of the mitotic apparatus. In the 90-day oral toxicity study with dose levels of 0, 300, 500, and 1200 mg/kg/day of Nicotinamide riboside chloride, there were no mortality or clinical observations attributable to the test substance administration. Decreases in mean weekly body weights, daily body weight gain, daily food consumption, and food efficiency were observed in the 1200 mg/kg/day-treated male rats, but not in female rats. There were no test substance-related changes in clinical pathology parameters, with the exception of decreases in selected serum chemistry parameters in all dose groups that were within PSL historical control range, had no correlated histopathological or clinical observations, and were interpreted to be non-adverse. There were no test substance-related macroscopic or microscopic findings. Increases in several organ-to-body weights in 1200 mg/kg/day-treated males were interpreted to be due to the decreased body weights. Increases in liver-to-body weights in 1200 mg/kg/day-treated females were without significant change in absolute liver weight, correlating hepatic microscopic or serum chemistry finding, and were interpreted to have little toxicological significance and no adversity. Under the conditions of the study, the NOAEL for systemic toxicity of Nicotinamide riboside chloride in Sprague-Dawley rats was determined to be 500 mg/kg/day for males and 1200 mg/kg/day for females.

ABSTRACT NUMBER: 3530   Poster Board Number: P326
TITLE: New TTC Database Compilation to Support Thresholds of Toxicological Concern in the Risk Assessment of Antimicrobials beyond Cramer Classes

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ABSTRACT: Threshold of Toxicological Concern (TTC) is an alternative method applied in the risk/safety assessment for substances whose exposure is very low and when appropriate data are not available. The aim of this work was to expand the original Munro TTC dataset through integration of existing public data sources to extend the TTC approach to antimicrobials. A global antimicrobial inventory was defined based on records from US EPA (319), EFSA (170), and ECHA (240) spanning the chemical types of disinfectants, antimicrobial, biocides, and preservatives. The expanded database includes over 1600 chemicals and data from several well-established datasets, e.g., COSMOS TTC, MUNRO, EFSA, EPA IRIS and ToxRefDB. Strict study inclusion criteria (e.g., study type/duration, route of exposure, species,
number of doses) have been applied. Although the TTC for Cramer Class III is 90 mg/day and approximately 85% of the AM inventory is Cramer Class III, applying this threshold for most antimicrobials (AMs) is overly simplistic. Instead of using the Cramer Decision Tree, an AM category concept was developed to bin the compounds structurally, and further delineate to sub-categories according to their potency. This large database increases the robustness of the chemical domains already covered by the Munro dataset and enables performing chemoinformatics analysis to go beyond the Cramer decision tree. In this study, a set of AM chemotypes based on ToxPrint chemotypes is identified to develop categories, taking into account the physical and biological properties that are related more directly to toxicity. Potency categories of antimicrobial chemotypes are then developed by correlation with NO(A)EL values. The possibility of grouping chemicals into potency categories using chemotypes is then validated against the full dataset. Using these AM categories, several use cases are presented to establish a framework for potential thresholds. This new method intends to reduce the need for chronic animal testing of active antimicrobial ingredients in premarket reviews. The TTC database is equipped with tools supporting the study evaluation in terms of reliability and applicability to derive safe human exposure thresholds applicable to antimicrobial chemicals.

ABSTRACT NUMBER: 3531   Poster Board Number: P327
TITLE: Toxicological Evaluation of Carboplatin Using an In Vivo Gene Mutation Assay (Pig-a) in Rodents
KEYWORDS: Genetic Toxicology; Mutation
ABSTRACT: Carboplatin [cis-Diammine(1,1,-cyclobutanedicarboxylato)platinum(II)] is a second generation platinum anticancer drug used in the treatment of various cancers, including testicular, ovarian, lung, head and neck. Platinum drugs such as carboplatin are mutagenic, carcinogenic, and teratogenic. They damage DNA by forming monofunctional DNA adducts or and DNA-protein crosslinks. Although carboplatin has the same mechanism of action as its parent compound, cisplatin, it is known to be a less toxic. Carboplatin has shown positive results in a number of in vitro and in vivo genotoxicity tests, such as the Ames assay, in vitro HPRT and sister chromatid exchange assay in Chinese hamster V79 cell line, in vivo transplacental micronucleus assay, comet assay in primary ovarian carcinoma cells. To assess the genotoxic potential, carboplatin was tested in the rat Pig-a and micronucleus assays. A dose range finding (DRF) study was conducted to determine the maximum tolerated dose (MTD) for the three days dosing regimen. Doses for the main study were selected based on the DRF results. Groups of six male Sprague-Dawley rats were administered vehicle (0.9% saline), 7.5, 15 or 30 mg/kg/day of carboplatin for three consecutive days. No adverse clinical observations were noted during or after the dosing phase. Blood was collected for the Pig-a assay on Days -1, 15 and 29 and for the micronucleus assay on Day 4 (First Day of dosing was considered as Day 1). Analysis of micronucleated reticulocytes (MN-RETs) by flow cytometry resulted in a statistically significant increase compared to the concurrent vehicle control at the highest tested dose (30 mg/kg/day). Statistically significant increases in both the mutant reticulocyte (RET\textsuperscript{cos\#}) and mutant erythrocyte (RBC\textsuperscript{cos\#}) frequencies were observed in the Pig-a assay at all three carboplatin dose groups on Days 15 and 29. Pig-a mutant frequencies in the vehicle control group were similar on Days 15 and 29 and were consistent with the pre-dose (Day -1) analysis. In conclusion, carboplatin was detected positive in inducing increase in the micronucleus and the Pig-a
mutant frequencies in the Sprague-Dawley rats when treated up to 30 mg/kg/day for three consecutive
day.

**ABSTRACT NUMBER:** 3532  **Poster Board Number:** P328  
**TITLE:** Generation and Characterization of Inhalation Atmosphere for Conducting Whole-Body Inhalation Exposures Using Cigarette Smoke and E-Cigarettes  
**KEYWORDS:** Inhalation Toxicology  
**ABSTRACT:** The generation and characterization of aerosol atmosphere for whole-body inhalation exposures pose significant technical challenges as compared to nose-only setups: e. g., due to large volume of whole-body chambers, additional time is required to achieve stable aerosol concentration within the chamber; higher aerosol concentration is required at the output of aerosol generator to achieve target concentration; and a higher deposition surface area which includes chamber walls and presence of animals within the chambers creates a potential for enhanced losses of nicotine on the chamber walls and animal fur during the exposures. During this study, we have developed methods for generation and characterization of inhalation atmosphere in whole-body Hazelton H-1000 chambers for a 6-hour exposure duration. For combustible 3R4F cigarettes, cigarette smoke was produced using CIR regimen and three rotary cigarette smoking machines (CH Technologies, JB2096). The outputs from three machines were combined in a custom-designed manifold and delivered to the whole-body chamber. A high WTPM target concentration of 1000 µg/L and a low target concentration of 100 µg/L were achieved in the chamber. The temporal (measured using real time aerosol monitor) and spatial uniformity (measured using gravimetric filters) of aerosol in the chamber were in the acceptable range. Aerosol particle size in the chamber was in respirable range with a MMAD of 0.9 µm and GSD of 1.6. Mean cigarette butt-length ranged from 43.0 to 45.5 mm (based on 8 puffs per cigarette). The CO to WTPM ratio across the range of concentration ranged from 1.27 to 1.31. Nicotine to WTPM ratio ranged from 6.7 to 8.5%. For e-cigarettes (Joyetech Unimax 25), aerosol was produced using two linear smoking machines (Borgwaldt, LM24E). The e-cigarettes were button activated and held at 45 degrees to the horizontal. The outputs of two machines were combined and delivered to the whole-body chamber. Current efforts are focused on achieving a high and stable WTPM concentration of 1000 µg/L in the chamber.

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**ABSTRACT NUMBER:** 3533  **Poster Board Number:** P329  
**TITLE:** Key Steps to Validate “In House” a Reconstructed Human Epidermis for Skin Irritation Potential Testing and In Vitro Efficacy Evaluation of New Chemicals or Plant Extracts  
**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** E. Bauza1, I. Garcia1, M. Arcioni1, M. Brulas1, C. Plaza1, C. Meyrignac1, S. Kim2, C. Capallere1, and J. Botto1. 1Ashland LLC, Sophia Antipolis, France; and 2Ashland Inc., Dublin, OH.  
**KEYWORDS:** In Vitro and Alternatives; Alternatives to Animal Testing; Cutaneous Or Skin Toxicity  
**ABSTRACT:** Nowadays reconstructed human epidermis is one of the main methods used as a screening tool to evaluate both skin irritation and in vitro efficacy of new compounds or plant extracts. This is
fast and predictive method. We first developed “in house” a bioengineered model of reconstructed human epidermis (RHE) based on the cultivation of human keratinocytes in a specific culture insert and in a chemically defined medium. We first demonstrated that our model shows similarities to native human skin in terms of morphology and marker expression with a very well-differentiated epidermis. To demonstrate this, several immunodetection including β1-integrin, keratin-10, claudin-1, e-cadherin, filaggrin and loricrin were performed 17 days post-reconstruction as well as a hematoxylin & eosin staining (H&E) to determine the tissue morphology. The next step was to evaluate our epidermal model for acute irritation according to OECD testing guidelines TG 439 and the Skin Irritation Test 42bis. TG 439 guidelines require the examination of several specific criteria including tissue morphology (H&E), cell viability of negative control (optical density), the tissue integrity and the barrier function (ET50). After confirming that our model presented all quality control parameters required for skin irritation testing, we evaluated whether our model was useful to discriminate between irritant and non-irritant materials. For that the performance study was performed with the completion of three independent runs including 20 reference chemicals (each run was performed in triplicate). The skin irritation test method applied on our developed RHE model appears to be relevant and reliable, with a specificity of 70%, a sensitivity of 100%, an accuracy of 85% and a within-laboratory reproducibility of 100%. In conclusion, we developed a proprietary epidermal model suitable for skin irritation testing “in house” and for use in the prediction of the irritant potential of chemical compounds. Furthermore, this model is also of a great interest to screen in vitro efficacy of a large set of dermo-cosmetics ingredients. Finally, these data summarize the key steps to validate "in house" a reconstructed human epidermis and shows that this approach is feasible and accessible to reduce costs during the initial development of new ingredients.

ABSTRACT NUMBER: 3534       Poster Board Number: P330
TITLE: Neurobehavioral and Respiratory Effects of Acute Trimethylsilanol Inhalation in Rats
AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: F. Golich¹,², A. Keebaugh¹,³, S. McInturf¹, D. Holtzapple¹,³, M. Goodwin¹,³, R. James¹,³, and K. Mumy¹. ¹NAMRU-Dayton, Wright-Patterson AFB, OH; ²Oak Ridge Institute for Science and Education, Oak Ridge, TN; and ³Henry Jackson Foundation, Bethesda, MD.
KEYWORDS: Exposure, Environmental; Inhalation Toxicology; Volatile Organic Compound
ABSTRACT: Trimethylsilanol (TMS) has recently been identified as a contaminant in some breathing gas systems. It is thought to be a break-down product or degradant of certain silicone rubber-containing components. Acute TMS exposure is believed to impact the central nervous system (CNS) as a CNS depressant in a similar manner to the structurally similar alcohol, tert-butanol. Currently, the only acute inhalation toxicity data for TMS is a 50% lethal concentration (LC₅₀) study. Animals were exposed via oral or intravenous routes during all other existing acute toxicity studies. Therefore, a study was undertaken to understand the impact of acute TMS inhalation exposure on neurobehavioral measures of motor activity and coordination in rats to determine what level of exposure could put individuals at risk of impairment while performing complex tasks. Separate cohorts of Sprague Dawley rats were exposed to TMS concentrations of 0 ppm, 100 ppm, 500 ppm, or 1000 ppm for two hours while inside of whole body plethysmographs. Acute changes in animal behavior were assessed by functional observational battery and open-field activity immediately following exposure. Changes in respiratory rate and tidal volume were monitored with the plethysmographs during the exposure. TMS exposures resulted in a concentration-dependent decrease in ventilation with increasing exposure concentration. Additionally,
there was a concentration-dependent reduction in motor activity with increasing TMS exposure in the post-exposure open-field testing. The findings of this study will support a refinement of safe TMS exposure levels that protect against acute CNS effects, particularly for occupations requiring extreme focus and attention.

**ABSTRACT NUMBER:** 3535  **Poster Board Number:** P331
**TITLE:** Nutritional Composition and Subchronic Toxicity of Genetically Modified Rice with Lysine Rich Gene

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** Y. Hu, and L. He. *National Institute for Nutrition and Health, China CDC, Beijing, China.* Sponsor: T. Wang

**KEYWORDS:** Food Safety/Nutrition; Toxicity; Chronic; Transgenic Models

**ABSTRACT:** Objective: To compare the difference of nutritional components between genetically modified rice with lysine-rich gene (lysine rice) and its parental rice, and to observe whether the lysine rice had sub-chronic toxicity. The main components, minerals and vitamins in rice were determined and compared. The weaning Wistar rats were randomly divided into three groups, 10 males and 10 female rats for each group. The feed for lysine rice group (71.85% rice added), parental rice group (72.80% rice added) and AIN-93G control group were formulated by maximum protein incorporation method. The body weight, food intake, blood routine, blood biochemistry, organ coefficient and bone density of rats were observed, and the organs of rats were examined by pathology. There was no significant difference in terms of total nutritional components, minerals and vitamins between lysine rice and its parental rice. The ratio of lysine to protein in lysine rice was increased by 15.6% compared with the parental rice. There were no significant differences in food intake and bodyweight between the lysine rice group and the other groups. There were significant differences were found in some indicators such as blood routine (MCH, PLT, RBC, LYM), blood biochemistry (GLU, LDLC), organ coefficient (spleen/bodyweight), and bone density between the three groups. Although there are differences in some indicators between groups, they were still within the normal range, and no toxic and side effects of lysine rice on health were found.

**ABSTRACT NUMBER:** 3536  **Poster Board Number:** P332
**TITLE:** Suppressive Effects of Platycodic Acid A, Platycodi Radix-derived Saponin, on TGF-beta1-induced Hepatic Stellate Cells Activation via Blocking Smad and Activating PPARgamma Signaling Pathway

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** Y. Chung1, J. Choi2, G. Lee2, S. Jin2, and H. Jeong2. 1International University of Korea, Jinju, Korea, Republic of; and 2Chungnam National University, Daejeon, Korea, Republic of.

**ABSTRACT:** Platycodi radix, the root of *Platycodon grandiflorum*, is mainly found in northeast Asia, and has been used as a food resource in the Asian countries. Aqueous extract and saponin derived from Platycodi radix have been shown a variety of suppressive effects including atopic dermatitis-like skin diseases, airway inflammation, osteoporosis, tumor metastasis, liver injuries. Nevertheless, the suppressive effects of platyconic acid A (PA), the active component of Platycodi radix-derived saponin, on the anti-fibrotic activity involving the SMAD and PPARy pathway remains unknown. This study investigated the anti-fibrogenic activity of PA and reveal the possible molecular mechanisms in HSC-T6.
cells. PA suppressed the TGF-β1-stimulated cell proliferation in a concentration-dependent manner. Furthermore, PA strongly inhibited TGF-β1-induced α-SMA and collagen Iα1 mRNA and protein expression. PA suppressed TGF-β1-induced smad2/3 phosphorylation, smad binding elements 4 (SBE4) luciferase activity, and type 1 TGF-β receptor (TβRI) and type 2 TGF-β receptor (TβRII) mRNA expression. Reversely, PA restored TGF-β1-reduced expression of smad7 and peroxisome proliferator-activated receptor (PPAR)y. Interestingly, PA significantly increased the expression of smad7 and PPARγ in time- and concentration-dependent manner. Taken together, these findings proved novel perspectives that suppressive effect of PA on HSCs occurs via blocking of SMAD and promoting of PPARγ pathways, leading to suppression of α-SMA and collagen Iα1 expression. Therefore, PA, the active component of Platycodi radix-derived saponin, represents a potential candidate for the development of novel chemotherapeutic agents that may contribute to prevention the liver fibrosis.

ABSTRACT NUMBER: 3537  Poster Board Number: P333
TITLE: Protective Effects of Rutaecarpine on Acetaminophen-induced Hepatotoxicity via Promotion of Antioxidants Enzymes and Autophagy in Mice

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ABSTRACT: Rutaecarpine, a pentacyclic indolopyridoquinazolinone alkaloid found in Evodia rutaecarpa, have been used to treat gastrointestinal disorders, headache, amenorrhea, and postpartum hemorrhage in traditional oriental medicine. Although rutaecarpine has been used for traditional oriental medicine, the protective effect of rutaecarpine on acetaminophen-induced hepatotoxicity remains unclear. This study investigated the protective effects of rutaecarpine on acetaminophen-induced acute liver injury in mice model. Rutaecarpine treatment significantly attenuated the acetaminophen-induced serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities and hepatic malondialdehyde (MDA) content. Also, cytochrome P450 2E1 (CYP2E1) expression was decreased by rutaecarpine treatment in a dose-dependent manner. Rutaecarpine treatment inhibited the acetaminophen-induced inflammatory cytokines production via inhibition of NF-κB activation. Furthermore, rutaecarpine promoted activation of nuclear erythroid 2-related factor 2 (Nrf2)-mediated antioxidant enzymes, including in glutamate-cysteine ligase catalytic (GCLC), heme oxygenase-1 (HO-1), and NAD(P)H quinone oxidoreductase 1 (NQO1). Rutaecarpine prevented APAP-induced kelch-like ECH-associated protein-1 (Keap1) expression. Interestingly, rutaecarpine treatment increased the APAP-induced the conversion of LC3 I to LC3 II and the degradation of p62. Taken together, these findings proved novel perspectives that protective effect of rutaecarpine on acetaminophen-induced hepatotoxicity via activation of antioxidant enzymes and autophagy. Therefore, rutaecarpine could be a useful candidate for the discovery of new chemotherapeutic agents that may contribute to protecting the liver injury by hepatotoxicants.
TITLE: The Effect of Sesamin in Regulating CYP1A1 and CYP1B1 Expression in Breast Cancer Cells

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: T. Pham, S. Jin, and H. Jeong. Chungnam National University, Daejeon, Korea, Republic of.

ABSTRACT: The majority of breast cancer tumors constitutively express CYP1A1 and CYP1B1. Both CYP1A1 and CYP1B1 are induced by environmental xenobiotic chemicals or endogenous ligands through the activation of the aryl hydrocarbon receptor (AhR). Sesamin, a lipid-soluble lignan from sesame (Sesamum indicum), displays anticancer activities through an unclear mechanism. Here, the effects of the sesamin on expression of CYP1A1 and CYP1B1 in human estrogen receptor positive (MCF-7) and negative (MDA-MB-231) breast cancer cell lines were investigated. Sesamin decreases the expression of CYP1A1 and CYP1B1 in both MCF-7 and MDA-MB-231 breast cancer cells follow concentration- and time-dependent manner. Besides, sesamin also inhibits the expression of CYP1A1 and CYP1B1 in breast cancer cells under TCDD-induced condition. Finally, sesamin blocks XRE luciferase activity and AhR nuclear translocation. These results indicate that sesamin reduces CYP1A1 and CYP1B1 expression in breast cancer cells. Sesamin would be able to act as a potential chemo-preventive agent against CYP1A1 and CYP1B1-mediated carcinogenesis and development of cancer.

TITLE: The Effect of Sesamin in Regulating Hepatic Lipid Metabolism in Hepatocytes

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: T. Thai, T. Pham, S. Jin, and H. Jeong. Chungnam National University, Daejeon, Korea, Republic of.

ABSTRACT: Sesamin, the major lignan extracted from sesame seed and oil, has been found to exert various pharmaceutical functions, including anti-hypertensive, hypo-cholesterolemic, anti-fibrotic, anti-oxidative, and anti-inflammatory actions, but the effect on hepatic lipid metabolism remains largely uncharacterized. The aim of this study is to provide new data on the molecular mechanism underlying the role of sesamin in the prevention of palmitate-induced lipid accumulation in HepG2 cells. Sesamin suppresses palmitate-induced lipid-droplet formation. Besides, sesamin also inhibits sterol regulatory element binding protein (SREBP)-1c and fatty acid synthase (FASN) expression in a concentration-dependent manner. Moreover, use of the compound C, pharmacological AMPK inhibitor, reveals that AMPK is essential for suppressing SREBP-1c expression in sesamin-treated cells. Finally, sesamin promotes calcium/calmodulin-dependent protein kinase kinase (CaMKK) activity and increases intracellular calcium concentration. These results indicate that sesamin prevents lipid accumulation by blocking the expression of SREBP-1c and FASN through CaMKK/AMPK activation. This finding suggests that sesamin is a novel AMPK activator with a role in the prevention and treatment of obesity.
ABSTRACT NUMBER: 3540    Poster Board Number: P336

TITLE: *Cola acuminata* Contains Potent Antimicrobial Activity against Selective Multidrug Resistant Bacteria


KEYWORDS: Natural Products

ABSTRACT: The rapid emergence of multidrug resistant pathogens has created an urgent need for discovering novel antibiotics with natural plant products as potential antimicrobial agents. *Cola acuminata* (Bizzy Nut), possesses a variety of bioactive compounds which exhibit important activities against the growth of certain mammalian cell lines, bacteria and fungi. However, the specific chemicals responsible for the antimicrobial bioactivity has not been identified. Therefore, we investigated a bioactive extract from *Cola acuminata* for the presence of antimicrobial activity using two multidrug resistant pathogenic bacterial species, *Staphylococcus aureus* and *Klebsiella pneumoniae*. The specific bioactive compounds present in *C. acuminata* was partially purified using sequential extraction with solvents of increasing polarity, followed by SPE purification. The antimicrobial activity associated with each extraction was evaluated using the agar diffusion and MIC assay screened with *Staphylococcus aureus* as a model multidrug resistant pathogenic organism. An enriched antimicrobial activity (Biz-3w) obtained from the acetone extract (Biz-3) and SPE purification was effective against both Gram-positive and Gram-negative bacteria. We observed a dose and time dependent inhibition of *Staphylococcus aureus*, *Bacillus subtilis* and *Enterococcus faecalis* with an effective dose (ED50) of 4 ug/ml, 3 ug/mL, and 3.2ug/ml respectively. The order of potency of Biz-3w was *Bacillus subtilis* ≥ *Staphylococcus aureus* > *Enterococcus faecalis*. The results obtained in this study provide preliminary evidence of the presence of secondary metabolites in *C. acuminata* capable of inhibiting multidrug resistant bacteria.

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ABSTRACT NUMBER: 3541    Poster Board Number: P337

TITLE: A Toxicological Assessment of Methylliberine

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: T. S. Murbach¹, R. Glávits², J. R. Endres¹, A. E. Clewell¹, G. Hirka², A. Vértisi², E. Béres², and I. P. Szakonyiné³. ¹AIBMR Life Sciences, Inc., Seattle, WA; ²Toxi-Coop Zrt., Budapest, Hungary; and ³Toxi-Coop Zrt., Budapest, Hungary.

ABSTRACT: Methylliberine (CAS 51168-26-4) is a methoxiuric acid found at low levels in various *Coffea* plants (and likely other caffeine containing genera) as a metabolite of caffeine, theacrine, and liberine. No toxicological investigations on this compound were found in the literature. We studied the toxicological potential of a pure form of this compound (supplied by Compound Solutions, Inc.) according to internationally accepted guidelines in (1) a bacterial reverse mutation test, (2) an *in vitro* mammalian chromosomal aberration test, (3) an *in vivo* mammalian micronucleus test, and (4) a 14-day repeated-dose oral toxicity study in rats. The *in vitro* studies revealed no mutagenic or clastogenic activity of the test article both in the absence and presence of metabolic activation and up to the maximum OECD recommended test concentrations. There was also no genotoxicity noted in the mammalian micronucleus study up to the highest dose tested of 700 mg/kg bw (given twice at a 24-hour interval). No mortality or effects that were considered toxicologically relevant were observed in...
Hsd.Han:WIST rats in the 14-day gavage study at doses of 55, 110, and 220 mg/kg bw/day, and the NOAEL was determined to be the highest dose level tested in both male and female rats.

**ABSTRACT NUMBER:** 3542  **Poster Board Number:** P338  
**TITLE:** A Toxicological Assessment of Bis (2-carboxyethylgermanium sesquioxide) (Organic Germanium)  
**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** A. Clewell. AIBMR Life Sciences, Inc., Seattle, WA.  
**ABSTRACT:** Bis (2-carboxyethylgermanium sesquioxide) is an organic compound containing the trace element germanium. While inorganic germanium (e.g. germanium dioxide) has been shown to cause renal toxicity and accumulation in the body, the toxicity and accumulation of this organic form of germanium appears to be low. Of concern has been contamination/adulteration of this organic germanium product with inorganic germanium (such as germanium dioxide). We investigated the toxicological potential of a pure form of this compound (bis (2-carboxyethylgermanium sesquioxide), manufactured by Designed Nutritional Products, Inc. in the U.S.) according to internationally accepted guidelines, in (1) a bacterial reverse mutation test, (2) an *in vitro* mammalian chromosomal aberration test, (3) an *in vivo* mammalian micronucleus test, and (4) a 90-day repeated-dose oral toxicity study in rats. The *in vitro* studies revealed no mutagenic or clastogenic activity of the test article, both in the absence and presence of metabolic activation and up to the maximum OECD recommended test concentrations. There was also no genotoxicity noted in the mouse micronucleus study up to the highest dose tested of 2000 mg/kg bw (given twice at a 24-hour interval). No mortality, general toxicity, or toxic effects on organs or tissues was observed in Han:WIST rats in the 90-day gavage study at doses of 500, 1000 and 2000 mg/kg bw/day, and the NOAEL was considered the highest dose level tested in both male and female rats.

**ABSTRACT NUMBER:** 3543  **Poster Board Number:** P339  
**TITLE:** Effects of Fumonisin B1 on the Promoter Methylation of Tumor Suppressor Genes and Their Relation with Histone Modifications in HK-2 Kidney Cells  
**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** E. Karaman, S. Omerustaoglu-Bayoglu, and S. Özden. Istanbul University, Faculty of Pharmacy, Istanbul, Turkey.  
**KEYWORDS:** Non-Genotoxic; Epigenetics; Mechanisms  
**ABSTRACT:** Mycotoxins are secondary metabolites of *Aspergillus*, *Penicillium*, *Fusarium*, *Claviceps*, and *Alternaria* molds and fungi. Fumonisins are the most studied Fusarium mycotoxins which contaminate cereal grains, animal feeds and human food products, and pose a threat to animal and human health. Fumonisin B1 (FB1) has a toxic effect by causing accumulation of sphinganine and impairment of sphingolipid biosynthesis which of these may play an important role in apoptotic modulation and cell proliferation pathway associated with cancer development. FB1 is classified by the IARC as a ‘possible human carcinogen’ (Group 2B). However, little is known about early molecular changes associated with FB1 carcinogenicity. It has been shown that in kidney and liver cells FB1 disrupts DNA methylation and histone modifications which are key in the expression profile of many tumor suppressor genes in tumor cells and neoplasia development. In this study, the effects of FB1 on promoter methylation of tumor suppressor genes and their relation with histone modifications in HK-2 kidney cells were aimed to
investigated. Cytotoxicity was evaluated by MTT and Neutral Red tests and IC₅₀ determined to be greater than 200 μM. 5-methylcytosine (5-mC) was assayed with Elisa kits and methyl array panel was used for gene specific methylation levels or tumor suppressor genes (totally 84 genes) in HK-2 cells exposed to FB1 (0, 10, 50, 100 µM) for 24 h. FB1 significantly increased the global 5-mC% levels (2.23 fold) at the high dose FB1 exposure. Interestingly, mRNA levels of DNMT1 and MGMT decreased while DNMT3b showed increase after FB1 exposure compared to control. FB1 caused gene specific DNA methylation of some important tumor suppressor genes such as CDKN1A, CDKN1B, CDKN2A, CDKN2B, PTEN and JUN by methyl array panels in HK-2 cells. Thus, gene specific histone modifications of p16 (CDKN2B) gene is currently analysed by chromatin immunoprecipitation (ChIP) analysis. The global occurrence of many mycotoxins with the hazardous toxicities makes important for risk assessment and public health. Consequently, this study will be contribute to the risk assessments of FB1 through well-defined mechanisms and pathways. This work was supported by Scientific Research Projects Coordination Unit of Istanbul University (Project numbers: TDP-2017-23293 and BYP-2018-28846).

ABSTRACT NUMBER: 3544        Poster Board Number: P340

TITLE: Developmental Nicotine Exposure Attenuates Female Sex Behavior

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: R. Joglekar, M. Cauley, H. White, E. Levin, and S. Murphy. Duke University, Durham, NC.

KEYWORDS: Mating Behavior; Neurotoxicology; Developmental Toxicity; Prenatal

ABSTRACT: Mammalian brain sexualization is developmentally mediated well after sex determination via gonadal hormones, and ultimately results in sexually dimorphic brain regions, both in structure and function. One such region, the preoptic area (POA), is responsible for adult sexual behavior and copulatory preference. Estradiol-mediated masculinization of the POA was found to involve the inhibition of DNA methylation and activation of methylation-dependent masculinizing genes (MDMGs) during postnatal days 0-4 in male rats. Although the epigenetic mechanisms of POA sexualization are understood, little is known about how exposure to developmental toxicants may alter these pathways. Tobacco use during pregnancy is common, and developmental nicotine exposure is known to impact DNA methylation. Further, a relationship between developmental nicotine exposure and altered sexual preference in exposed human females has been reported. Therefore, we hypothesized that developmental nicotine exposure would alter the methylation-dependent sexualization of the POA in rats. We used a rat model of gestational nicotine exposure via osmotic minipump (2mg/kg/day nicotine) from prematuring through postnatal day 4 (PND4). PND2 POA was analyzed for MDMG expression using real-time PCR. Male sexual behavior (#mounts, #intromissions, #ejaculations, #anogenital sniffs) and female sexual behavior (#darts, #hops, #lordoses, #anogenital sniffs) were assessed at PND80. Using repeated measures analysis, we found an attenuation of female sexual behavior, including number of lordosis and darts/hops, in exposed vs control females (p=0.0074), but not in exposed or control males. We also found increased MDMG expression (p=0.0398) in nicotine-exposed male and female POA. Our results suggest that developmental nicotine exposure is capable of triggering the epigenetic masculinization of the rat POA.
ABSTRACT NUMBER: 3545    Poster Board Number: P341
TITLE: Cytotoxic Effect of Extracted Bioactive Materials of Dioscorea Alata Cv. White Yam Tuber on Androgen Insensitive Prostate Cancer Cells

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KEYWORDS: Cytotoxicity

ABSTRACT: Dioscorea alata cv. White Yam is a widely produced yam in the Caribbean and Latin America and is an important staple amongst many individuals in these regions. However, the nutritional and phytochemical composition of the Dioscorea alata tuber may vary with respect to its origin. The objective of this study was to identify the presences of bioactive anticancer compounds in Dioscorea alata c.v White yam from Columbia (DaC) and Jamaica (DaJA). Solid-liquid soxhlet extraction was carried out on Dioscorea alata tuber using solvents of increasing polarity (hexane, diether, acetone and ethanol) and the resulting extract screened for anti-prostatic activity. In vitro cytotoxicity of the crude extracts was assessed using MTT assay on androgen-insensitive prostate cancer DU145 cells. The screening revealed that the anticancer bioactive compounds were present in both DaC and DaJA yam tuber with the inhibition properties present in the DaC’s hexane extract and DaJA acetone extract. The greatest inhibition was seen in DaC’s hexane extract at an IC50 of 23.76, CI95%(16.79- 30.72ppm) and DaJA acetone extract at an IC50 of 46.29 CI95%(21.21 -71.36ppm). The solid-liquid extraction screen signified that the phytochemical composition and bioactivity of D. alata c.v White Yam varied with location which may be attributed to a difference in the various environmental conditions under which both yam samples were cultivated.

ABSTRACT NUMBER: 3546    Poster Board Number: P343
TITLE: Plasticizer Di-(2-Ethylhexyl) Phthalate Inhibits Myogenesis and Activates Lipogenesis in Differentiating Myoblasts via the AMP-Activated Protein Kinase-Regulated Pathways

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KEYWORDS: Phthalates; Signal Transduction; Muscle Toxicity

ABSTRACT: Several epidemiological studies have shown that plasticizer Di(2-ethylhexyl) phthalate (DEHP) is associated with low birth weight in newborns. Low birth weight may be related to immature muscle differentiation. Here, we investigated the effects and possible mechanisms of DEHP and its metabolite mono(2-ethylhexy) phthalate (MEHP) on myogenic differentiation in cultured mouse skeletal myoblasts C2C12. Myoblasts were cultured in a differentiation medium for 4 days with or without DEHP or MEHP. Both DEHP and MEHP at non-cytotoxic concentrations (10-100 μM) significantly inhibited the multi-nucleated myotube formation and the protein expressions of muscle differentiation markers myogenin and myosin heavy chain (MHC) in a dose-dependent manner. Both DEHP and MEHP could also increase the phosphorylation of AMP-activated protein kinase (AMPK) and decrease the phosphorylation of Akt in myoblasts during differentiation in a dose-dependent manner. Compound C, an AMPK inhibitor, significantly reversed the inhibitory effects of DEHP or MEHP on myogenin and MHC.
protein expressions, Akt phosphorylation, and myotube formation. Unexpectedly, both DEHP and MEHP were capable of enhancing lipid accumulation and increasing the PPARγ protein expression in differentiating myoblasts in a dose-dependent manner. Compound C could significantly reverse the increased effects of DEHP on lipid accumulation and PPARγ protein expression. Moreover, the protein expressions of AMPK and PPARγ were increased in the soleus muscles isolated from mice treated with DEHP (10 and 100 mg/kg) for 4 weeks. Taken together, DEHP and its metabolite MEHP are capable of activating AMPK signaling to inhibit Akt-regulated myogenesis and to increase PPARγ-related lipogenesis in myoblasts during differentiation.

**ABSTRACT NUMBER:** 3547  
**Poster Board Number:** P344  
**TITLE:** Prediction of Transcription Factor Binding Sites and Methylation Patterns in LCR Sequences of HPV-16 Variants

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** E. C. Veranes-Font. ONAT, Marianao, La Habana, Cuba.

**ABSTRACT:** High risk human papillomavirus (HPV) is the primary risk factor for cervical cancer (CC). The HPV genome (~8.0 Kb) is functionally divide into two coding regions (early and late) and one regulatory region or LCR. The LCR region (~850 bp) contains the origin of replication (ori) and multiple transcription binding sites, thus controlling the expression of HPV gene. HPV gene expression is mainly regulated at the transcriptional and post-transcriptional levels. DNA methylation is thought to occur early in malignant transformation. HPV 16 is the most isolated virus type and has a high GC content with part of non-traditional CpG islands, particularly in LCR region. Based on whole-genome sequence data, HPV16 can be subdivided into subtypes and variants. A difference of between 2-10% constitutes a subtype, and less than a 2% difference defines a variant. Variants that match host ethnicity, in turn, have been associated with increased risk of persistence and more recently with increased risk of CIN3+. Aims: To evaluate if changes in LCR region modify the prediction patterns of transcription binding sites and methylation for HPV-16 variants. Six representative LCR sequences of each variant (E (prototype sequence), As, AA, Af1, and NA) were obtained from Nucleotide and HPV sequences (Los Alamos National laboratory). First, we searched for putative transcription binding sites and methylation patterns associated with position with divergent nucleotides among different variants. Second, it was analyzed which differences were exclusives for E and AA variants. Finally, we tried to find similarities with sites described for regulatory sequences of other viruses. All nucleotide substitutions, except C/T transition at nt. 7689, modified the pattern of transcription factor binding site prediction respect to reference sequence. In addition, A/C change at nt. 7729 (NA, AA) was related with transcription factor binding sites for retinoic acid receptor alpha and thyroid hormone receptor described for herpes simplex virus type 1. T/G transition at nt. 7743 (AA) were associated with NF-1 and C/EBP alpha sites described for hepatitis B virus S gene promoter and murine sarcoma virus, respectively. The E sequence had the highest number of methylation site prediction. These results suggest that variation in regulatory sequences may modify gene expression, by different mechanisms and it could be the basis for carcinogenesis mechanism common to several viruses.
A Gain of Function Allele Encoding Theragulator Ortholog Overstimulates Torc1 in Nutrient-Starved Conditions

S. Carlson. West Virginia University, Morgantown, WV. Sponsor: J. Gallagher

Undergraduate Student; Cell Proliferation; Metabolism

Both nutrient availability and sensing are fundamental to cellular growth and division. In the absence of nutrients, genetic variation in regulatory pathways can bypass the tight regulation of growth through hyper-stimulation of intracellular growth pathways. The widely conserved Target of Rapamycin Complex 1 (TORC1) is activated in response to cellular nitrogen sources and induces proliferation through kinase cascades that induce ribosome biogenesis, protein synthesis, and inhibition of autophagy. High cytosolic amino acid concentrations stimulate and yet limit TORC1 activity through several vacuolar-localized signaling cascades. Unregulated cell growth is a hallmark of cancer and overlapping pathways mitigate negative effects of excess cell growth. The TORC1 Ragulator complex (EGO complex) is required for growth recovery after either TORC1 inhibition via rapamycin treatment or amino acid starvation. There is genetic variation in the EGO complex proteins in different individuals of the same species. In order to investigate the integration of amino acid availability signals into growth cues, we analyzed TORC1 activity in the absence of aromatic amino acid synthesis with two different, but naturally occurring variants of Ego3 in the model organism, Saccharomyces cerevisiae. Phosphorylation of Sch9, the yeast ortholog of mammalian S6 kinase and a target of active TORC1, was different in genetically identical yeast but contained different alleles of Ego3. In slow recovery strains, lacking the aromatic amino acid biosynthesis enzyme (Aro1), we show that Ego3 allelic variation differentially rescued growth recovery via TORC1 signaling. Signaling from the hyper-stimulatory Ego3 allele in respect to TORC1 is sufficient to prevent sporulation, the process by which diploid yeasts meiotically divide into stress-resistant haploid gametes. Sporulation is the canonical yeast response to nitrogen starvation conditions and is partially initiated through the inhibition of TORC1. This finding further propagated the idea that this particular Ego3 allele may drive uncontrolled cell growth when recovery from starvation would otherwise be slow. Interestingly, this growth response to starvation conditions was found to require Vam6, the GEF (GTP exchange factor) which activates Gtr1 in order to stimulate TORC1. Given the implications of mammalian TORC1 dysregulation in tumorigenesis, further understanding in its upstream activators leads to more possibilities for cancer treatment.

Whole Transcriptome Profiling of Focal Areas of FFPE


Histopathology; Gene Expression/Regulation; Biomarkers

Formalin fixed paraffin embedded (FFPE) tissues offer many benefits for histology, since it retains morphology and can be stored for extended periods of time. Unfortunately, measurement of gene expression from FFPE has been problematic, requiring large amounts of tissue which is heterogeneous (different cell types/histologic regions), and requiring extraction and reverse-
transcription of RNA, which often fails due to low quality/yield of RNA. The utility of the large archives of FFPE has thus been limited. We demonstrate that the commercial TempO-Seq® assay of FFPE can be used to profile focal, histologically discrete areas of H&E stained FFPE as small as 1 mm². The assay of these small focal areas of FFPE is possible because TempO-Seq does not require extraction or reverse transcription of RNA, nor does H&E staining interfere. The scraped tissue is simply lysed and then assayed directly with no further RNA manipulation. We demonstrate high correlation of gene expression measured from FFPE to fresh samples, and high correlation of FFPE lysates profiled by TempO-Seq to RNAseq analysis of RNA extracted from those same samples, demonstrating that the TempO-Seq FFPE assay produces reliable data. We found that using H&E stained FFPE we were able to precisely visualize and select subareas of interest, differentiated by histology, from within a single section. As an example, we were able to profile cancer tissue areas and directly adjacent normal colon tissue from a single section of resected patient colorectal tissue. This provided a true “100%” pure cancer whole transcriptome profile, whereas typically, using whole sections, profiling data is obtained from cancer areas that may contain as much as 20% normal tissue. What was striking is that a significant number of key molecular pathway genes associated with normal tissue are not expressed at all in cancer, and vice versa. In contrast, the historical methods used to build the TGCA database (microarray or RNAseq assay of frozen tissue, without the ability to profile areas of discrete histology) do not identify the discrete differences focal TempO-Seq assay did, rather, there is lower but not absent expression. We suggest this is an artifact due to being performed on heterogeneous sample, compared to the result when focal “histological homogeneous” areas of FFPE are selectively profiled. The result is that important genes and pathways can easily be missed unless histologically discrete areas of tissue are profiled.

**ABSTRACT NUMBER:** 3550  
**Poster Board Number:** P347  
**TITLE:** Mapping of Damage and Repair across the Nuclear and Mitochondrial DNA Adductomes  
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¹Florida International University, Miami, FL; ²Prince Sattam bin Abdulaziz University, Alkharkh, Saudi Arabia; and ³University of Leicester, Leicester, United Kingdom.  
**ABSTRACT:** The DNA adductome can represent either the genome-wide distribution of specific forms of DNA damage, mapped to discrete locations, or the comprehensive characterization of all the DNA adducts present in the genome. To address the former, we have developed a method which comprises a combination of Damaged DNA Immunoprecipitation and next generation sequencing (DDIP-Seq), and have applied it to study the induction and repair of two representative DNA lesions: cyclobutane thymine dimers (T<>T), and 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG). DDIP-seq produced a high-resolution map of T<>T location and intensity, across both the nuclear and mitochondrial genomes, following exposure to solar simulated radiation. For nuclear DNA, the distribution of damage and repair was heterogeneous, at both the sequence and chromosome level. Levels of T<>T were much higher in the mitochondrial DNA, compared to nuclear DNA, heterogeneously distributed, and decreased with time, despite no reported mechanisms for their repair. The loss of T<>T was confirmed using short-range qPCR. To date, little attention has been paid to mapping damage and repair across the mitochondrial genome - we predict that access to techniques such as DDIP-seq will change this. These data indicate the existence of regions of sensitivity and resistance to damage formation, together with regions that are fully repaired, and those for which >90% of damage remains, after 24 h. Initial experiments confirm the
potential to apply DDIP-seq to study the 8-oxodG adductome. This methodology offers a more detailed approach to studying cellular DNA damage and repair, compared to the targeted analysis of global genome adduct levels, which will further aid our understanding of the link between DNA damage and disease.

**ABSTRACT NUMBER:** 3551  **Poster Board Number:** P348

**TITLE:** Development of a High-Throughput Cell-based Genotoxicity Testing to Address 21st-Century Genetic Toxicology Needs

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** H. Li¹, R. Chen¹, J. Aubrecht², and A. J. Fornace¹. ¹Georgetown University, Washington, DC; and ²Takeda Pharmaceutical Company Ltd, Boston, MA.

**KEYWORDS:** Genotoxicity; In Vitro and Alternatives; Toxicogenomics

**ABSTRACT:** Standard *in vitro* genotoxicity assays are not high-throughput and frequently generate positive results that are subsequently found to be irrelevant *in vivo*. New approaches enabling more efficient and accurate hazard and risk assessment for genotoxicity are required. The transcriptomic biomarker, TGx-DDI (previously called TGx-28.65), is capable of differentiating compounds with irrelevant false positive findings in chromosome damage assays from true DNA damaging agents. TGx-DDI is the first transcriptomic biomarker that has been shown to perform robustly and consistently on different assay platforms and is under a formal biomarker qualification review at the FDA. In this study, we develop the application for high-throughput cell-based genotoxicity testing using the nCounter Elements technology (Plexset assay). The high throughput capability allows the adaption of a highly-automated workflow requiring minimal hands-on time for large-scale multi-condition screening by directly using cell lysates, which can accurately and simultaneously quantify the abundance of up to 800 transcripts. Using the Plexset design we can run up to 96 samples simultaneously. To optimize the performance of nCounter Elements technology, a titration assay was performed to determine the optimal sample input for the Plexset assay. Results showed high dynamic range of sample inputs, e.g. the detection efficiency of target genes was highly concordant when cell number ranged from 2,000 to 20,000. To further validate the Plexset assay, we treated cells with radiation or bleomycin (positive controls) and caffeine (negative control) in replicates and multiplexed these sample to run on one nCounter cartridge. The analysis of high-throughput Plexset assay data show consistency with results from microarray and the nCounter assay using single sample probe-set. In order to evaluate the extended utility of TGx-DDI nCounter Plexset assay for highly automated high-throughput genotoxicity screening, we also tested agents from ToxCast Chemical Library, and compared with other assay results available in the Tox21 database, which should help in the delineation of biologically relevant pathway responses. Overall, our goal is to use this biomarker in an automated, inexpensive, and high-throughput manner that can be readily integrated into the genetic safety evaluation of chemicals.
ABSTRACT NUMBER: 3552  
Poster Board Number: P349

TITLE: Risk Assessment of Novel Tobacco Vapor Product Using ToxTracker and High-Content Screening Systems In Vitro


KEYWORDS: Genotoxicity; Cytotoxicity; Risk Assessment

ABSTRACT: A Novel Tobacco Vapor product (NTV) reportedly shows lower genotoxic and cytotoxic potential in the in vitro bacterial reverse mutation assay, micronucleus assay and neutral red uptake assay compared with a combustible tobacco product. This study aims to further evaluate and compare the risk assessment of an aerosol from the NTV with that of 3R4F cigarette smoke. One system we employed was the ToxTracker, where six different mouse embryonic stem reporter cell lines were designed to exhibit fluorescence upon induction of various pathways relevant to (geno)toxicity and cancer. In the other system we used, human bronchial epithelial cells (BEAS-2B) were analyzed for double DNA strand break (γ-H2AX immunostaining), oxidative stress (H2-DCFDA staining), apoptosis (Caspase-3 immunostaining) and autophagy (LC3B immunostaining). In the ToxTracker, DNA damage, oxidative stress, p53 activation or unfolded protein response was significantly induced in each the reporter cell line treated with 3R4F, but no significant induction was observed in any of the reporter cell lines treated with NTV. In addition, 3R4F cigarette smoke resulted in marked γ-H2AX, H2-DCFDA, Caspase-3 and LC3B staining in BEAS-2B cells, whereas NTV aerosol produced no significant staining. We conclude that NTV aerosol has a very different risk assessment profile from that of combustible cigarette smoke under the study conditions.

ABSTRACT NUMBER: 3553  
Poster Board Number: P350

TITLE: Genotoxic Assessment of Reference Tobacco Cigarette Smoke Condensate Using Human Lung Epithelial A549 Cells in an OECD Protocol for the In Vitro Micronucleus Assay

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ABSTRACT: The in vitro micronuclei (MN) assay using human A549 cells is commonly used to evaluate the potential genotoxicity of inhaled nanomaterials but surprisingly not electronic nicotine delivery systems (ENDS). Since the harm reduction assessment of ENDS may require a comparison with the effect of reference tobacco cigarette smoke condensate (CSC), we investigated the effect of CSC exposure on the presence of MN in cultured A549 cells. The study plan was based on OECD Guidelines TG487. Positive controls (PCs; colchicine, cyclophosphamide), vehicle controls (VCs, DMSO) and DMSO-extracted CSC (3R4F) were tested with and without metabolic activation (S9 fraction) and with short- and long-term exposures for cytotoxicity (trypan blue) and genotoxicity (MN) using submerged culture conditions. Manual counting of MN with fluorescence microscopy were conducted in fixed cells stained with acridine orange. A549 cell cultures incubated in the presence and absence of S9 fractions demonstrated an expected dose-dependent increase in % MN formation (3.5 to 18.0x mean fold increase vs. VC, n=6) when exposed to PCs after short- and long-term exposures. Likewise, A549 cells exposed to CSC showed a clear dose-dependent increase in the % MN formed vs. VC (n=6). Without metabolic activation, short term CSC exposure (175 to 350 µg/ml) resulted in a mean fold MN increase of 2.4 to 13.0x and long-term CSC exposure (40 to 125 µg/ml) resulted in a mean fold MN increase of 2.9
to 9.4x. With metabolic activation, short term CSC exposure (150 to 330 µg/ml) resulted in a mean fold MN increase of 3.3 to 15.2x. The study results also indicated that A549 cells exposed to the PCs, cyclophosphamide or benzo(a)pyrene, in the absence of S9 produced significantly fewer MN than that observed in cells incubated with S9. In conclusion, we have demonstrated using OECD guidelines for the in vitro MN assay that human lung A549 cells appear to be a promising lung cell system to evaluate the possible harm reduction potential of ENDS as compared with the genotoxic effects of reference cigarette smoke condensates.

**ABSTRACT NUMBER:** 3554  
**Poster Board Number:** P351  
**TITLE:** Re-evaluation of Discordant Results in Related OECD TG471 Tester Strains  
**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** C. S. Farabaugh, B. Lallier, C. Tydrick, R. Baba, S. Tincher, J. Callupe, V. Y. Kwok, and L. F. Stankowski. Charles River Laboratories, Skokie, IL.  
**KEYWORDS:** Genetic Toxicology; Safety Evaluation; Regulatory/Policy  
**ABSTRACT:** The International Workshops on Genotoxicity Testing met in 2017 with one group assessing the sensitivity/selectivity of tester strains used for the Bacterial Reverse Mutation (Ames) Test performed under OECD TG471. Results from large (>10,000 compound) databases were analyzed to assess relative responses between related tester strains: TA100/TA1535; TA97/TA1537; and TA102/WP2uvrA/WP2uvrA (pKM101). Comparisons between TA100/TA1535 were straightforward, since both are required by OECD TG471 and comparison data usually were generated concurrently. However, only one strain from the other pairs/triplets is required, and comparison data generally were from different labs. Discordant results were often noted, especially when using a “2- (or 3-) fold” criteria. During this past year, ten chemicals producing discordant results in different labs were re-evaluated concurrently (some in multiple trials): altertoxin I, chlorambucil, CI basic red, retorsine and 2-methylpropanenitrile in TA97/TA1537; and N,N-diethylnitrosamine, folpet, acrylonitrile, NiCl\(_2\) and p-toluene sulfonfyl hydrazide in TA102/WP2uvrA/WP2uvrA (pKM101). Our OECD TG471- and GLP-compliant studies did not confirm the previous discrepancies. For example, altertoxin I was reported to induce 8.7- to 10-fold increases in revertant frequencies in TA1537, but only 1.5- to 2.0-fold increases in TA97. In our testing, altertoxin I produced 4.5- to 11-fold increases in TA1537 and 2.5-fold increases in TA97 (due to differences in spontaneous revertant frequencies); however, the response was ~4-fold higher in TA97 based upon revertants/µg-plate\(^{-1}\). Similarly, folpet previously gave conflicting results in TA102/WP2uvrA with and/or without S9, but was uniformly positive here in TA102/WP2uvrA/WP2uvrA (pKM101) ±S9. Chlorambucil, previously reported to be TA1537-positive and TA97-negative (+S9 only), was uniformly negative here in both tester strains. These results and the database analyses suggest: strain TA1535 adds little value to a set containing TA100; TA97 is more sensitive than TA1537; and WP2uvrA (pKM101) is more sensitive than TA102 or WP2uvrA. Our testing did not confirm previously reported differences in response between related strains. The inability to confirm previous responses is worrisome. Possible explanations include technical error, poor test article characterization, impurities, differences in exposure methods, and genetic drift.
Comet Assay in 3D HepaRG Spheroid Model: A Promising Tool to Evaluate DNA Strand Breaks Using Human Liver Cells

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KEYWORDS: Genetic Toxicology; Clastogen; Genotoxicity

ABSTRACT: Genetic toxicity information is critical for the safety assessment of all xenobiotics, however, the current in vitro and in vivo genotoxicity assays have some limitations since they are mostly performed using rodent metabolism to predict human response. HepaRG are cells derived from human hepatocellular carcinoma and retain a metabolic capacity similar to primary human hepatocytes. Liver spheroid models have been shown to maintain their viability and liver functions better than 2D or suspension cultures. The objective of this study was to demonstrate the viability of 3D HepaRG spheroids in Comet assay evaluation. Cryopreserved HepaRG were seeded in 96-well ultra-low adhesion plates at 1,000 cells/well. Spheroids were maintained in culture for 10 days, followed by a 24 hour treatment with the direct mutagen ethyl methanesulfonate (EMS) at 1 and 2 µL/mL and negative control. Ten spheroids per condition, in duplicate cultures, were collected for comet assay analysis. Spheroids were dissociated into a single cell suspension and analysed for viability, hedgehogs and % tail intensity. The negative control group presented high cell viability, low hedgehog counts and low % tail intensity. This shows that the cultured spheroids were healthy with no necrotic core and that the cell dissociation method did not compromise cell integrity. Spheroids treated with EMS presented high viability, low hedgehog counts and a significant increase in DNA damage when compared to the negative control. These results suggest that the detection of DNA breaks by Comet assay is due to a genotoxic mechanism rather than a cytotoxic one. In conclusion, the data demonstrates the viability of performing the Comet assay on HepaRG spheroids. Future investigations will determine if the Comet assay using this culture system is sensitive enough to detect pro-mutagens that require metabolism to elicit their genotoxicity.

Application of a 3D Model of Human Liver Cells Co-Cultured with Human Lymphoblast in the Evaluation of Comet and Micronucleus

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KEYWORDS: Genetic Toxicology; Hepatocytes; Alternatives to Animal Testing

ABSTRACT: The liver metabolic capacity evaluated in the current pre-clinical genetic toxicology test battery does not reflect the specific and complex metabolism of the human liver as this parameter is assessed by addition of a metabolic activation system (induced rat S9 mix) in the in vitro assays or by evaluating rodent liver in the in vivo assays. The objective of this study was to demonstrate a proof of concept for the creation of a metabolic competent human co-culture system that can be applied to genotoxicity assessment. The culture system is comprised of human lymphoblastic TK6 cells co-cultured with a 3D liver sandwich model. The system allows for communication between two compartments and the performance of the Comet assay in liver and the Micronucleus assay in TK6 cells. The liver model
(extracellular matrix, endothelial cells, HepaRG cells, and matrigel) was maintained in a 96-well-plate and the TK6 cells were cultured on top of the plate using transwells. Both compartments were treated for 24 hours with either negative control, direct mutagen methanesulfonate (MMS) or indirect mutagen benzo[a]pyrene (BaP). Liver cells were collected for the comet assay and the TK6 cells were collected for the Micronucleus assay. The results demonstrate a low level of DNA damage in the negative control treated cultures and an increase in micronuclei incidence and % tail intensity in the Comet assay in cultures treated with MMS and BaP. The results indicate that the human liver model can metabolize BaP in vitro and that the DNA reactive metabolites were dispersed across the two compartments eliciting genotoxicity in both the lymphoblastic and liver cells. The culture system has shown promising results to combine an integrated co-culture metabolically competent human 3D liver model with TK6 cells, a system able to detect different genotoxic parameters, i.e. induction of chromosomal damage or damage to the mitotic apparatus (micronucleus test) and DNA strand breakage (comet assay), within a single model. Additional studies are ongoing to further characterize the metabolic activation and sensitivity to genotoxicants.

**ABSTRACT NUMBER:** 3557  
**Poster Board Number:** P354  
**TITLE:** Comparison of TK6 Micronucleus Data Generated by Flow Cytometry and Microscopy  
**KEYWORDS:** Genotoxicity; Safety Evaluation; Clastogenesis  
**ABSTRACT:** Good product stewardship requires assessment of potential genotoxicity using a battery of tests. The most recent addition to the genetic toxicity core battery of in vitro assays is the mammalian cell micronucleus assay. Historically, micronuclei (MN) are scored following chemical exposure by microscopy. However, flow cytometry also may be used to enhance the precision of the assay by increasing the number of cells scored per culture (e.g., 10,000 vs. 2,000 cells). Here we report the direct comparison of test results obtained from the same cultures using each method. Utilizing eight known genotoxic chemicals, including some promutagens requiring metabolic activation (S9), TK6 cells were exposed for 4 hours ±S9 and for 27 hours -S9 and dose-response curves were generated by both scoring methods. Cultures were harvested at the appropriate time and processed for each scoring method. Pooled data across the assays (N=198 cultures) indicated a good association between the scoring platforms (r² = 0.67). However, residuals indicated a bias towards higher MN frequencies in the flow cytometric data, which could be explained by the larger dynamic range of the assay. This inherent methodological difference did not adversely impact study outcome, as each chemical was either positive or negative across all exposure conditions and both scoring methods. The bias of flow cytometry to yield higher MN frequencies than microscopy was largely driven by results for the 27-hour -S9 treatment, as 5 of the 6 chemicals were positive in this treatment design at lower concentrations. This assessment was limited by the exclusion of weak genotoxicants, which would allow a more in-depth analysis of the sensitivity between the two methods. Cytotoxicity data also will be compared, as relative-nuclei counts are a built-in metric that may be appropriate regardless of the different generation times across exposure conditions. Overall, these data support the use of flow cytometry to score MN in TK6 cells.
**ABSTRACT NUMBER:** 3558  
**Poster Board Number:** P355  
**TITLE:** Identification and Quantification of PARP1 in Human Tissues and Cultured Cells by Liquid Chromatography/Isotope-Dilution Tandem Mass Spectrometry

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**ABSTRACT:** Poly(ADP ribose) polymerase 1 (PARP1) is a multifunctional DNA repair protein of the base excision repair pathway and plays a major role in the repair of DNA strand breaks and in replication and transcriptional regulation among other functions. Mounting evidence points to the predictive and prognostic value of PARP1 expression in human cancers. Thus, PARP1 has become an important target in cancer therapy, leading to the development of inhibitors as anticancer drugs. In the past, PARP1 expression levels in tissue samples have generally been estimated by indirect and semi-quantitative immunohistochemical methods. Accurate measurement of PARP1 in normal tissues and malignant tumors of patients will be essential for evaluating PARP1 as a predictive and prognostic biomarker in cancer and other diseases, and for the development and use of its inhibitors in cancer therapy. In this work, we present an approach involving liquid chromatography–isotope-dilution tandem mass spectrometry to positively identify and accurately quantify PARP1 in human tissues and cultured cells. We identified and quantified PARP1 in human normal ovarian tissues and malignant ovarian tumors, and in three pairs of human cell lines, each pair consisting of a normal cell line and its cancerous counterpart. Significantly greater expression of PARP1 was observed in malignant ovarian tissues than in normal ovarian tissues. In the case of one pair of cell lines, the cancerous cell line also exhibited greater expression of PARP1 than in normal cell line. We also show the simultaneous measurement of PARP1 and apurinic/apyrimidinic endonuclease 1 (APE1) in a given protein extract. The approach presented in this work is expected to contribute to the accurate quantitative assessment of PARP1 levels in basic research and clinical studies.

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**ABSTRACT NUMBER:** 3559  
**Poster Board Number:** P356  
**TITLE:** How Low Can You Go? An Analysis of Lowest Effective Dose in the Ames Test

**AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS:** G. Kocks¹, P. J. Rawlinson², M. J. Tate², and R. V. Williams¹. ¹Lhasa Limited, Leeds, United Kingdom; and ²Gentronix Limited, Alderley Edge, United Kingdom.

**KEYWORDS:** Dose-Response; Regulatory/Policy; Genetic Toxicology

**ABSTRACT:** Non-intentionally added substances (NIAS) are a challenge for the food contact materials’ industry. European framework regulation (EU 1935/2004, Article 3) states suppliers must assess the safety of the final product. However, the risk assessment of NIAS has proven difficult due partly to questions regarding the sensitivity of test methods, potency and the small amount of migrate produced for toxicological testing. Thus the question is whether conventional genotoxicity assays are sufficiently sensitive to detect toxicity at very low doses? Publicly available Ames test dose response data for more than 1,200 substances have been collated from the Vitic Nexus database. Data analytics have been applied to determine the lowest dose and the fold increase at which a mutagenic response could be...
detected. This was found to be 0.001 µg/plate for this data set, although in some cases that also equated to the lowest tested dose.

ABSTRACT NUMBER: 3560    Poster Board Number: P357
TITLE: Synthetic Hydrogels with Different Chemical Compositions and Stiffnesses Affect Neural Organoid Folding


KEYWORDS: Cell Culture; Neurotoxicology

ABSTRACT: Cost effective, high-throughput in vitro models that recapitulate human disease pathology and developmental events are needed to enable improvements in drug testing and toxicity screening. Previous studies have demonstrated that chemically defined synthetic hydrogels enable induced pluripotent stem cell (iPSC)-derived neural cell populations to reproducibly self-assemble into aggregates. However, it is unknown how the mechanical properties of synthetic hydrogels influence the self-assembly, or folding, of iPSC-derived neural organoids. To address this gap in knowledge, we hypothesized that varying hydrogel properties would affect the rate of folding of neural organoids. Synthetic hydrogels were made by reconstituting poly(ethylene glycol) (PEG) and synthetic peptides, including metalloproteinase (MMP)-degradable peptide crosslinkers and cyclic RGDS cell adhesion peptides. Different amounts of PEG (40, 50, 60 mg/mL) and varying crosslinking percentage (40, 50, or 60%) were used in combination to alter hydrogel mechanical properties. Hydrogel formulations were characterized using rheometry, swelling ratio, and Ellman’s assay. PEG hydrogel precursor solutions were polymerized in a 96-well plate 24 hours prior to seeding iPSC-derived endothelial cells (EC), neural progenitor cells (NPC), pericytes (PC), and microglia (MG). After seeding, the surface area of the organoids was measured on day 7, day 14, day 21, and day 28 to assess folding. Organoids were characterized using endothelial (CD31+), neuronal (βIII Tubulin+), and microglial (Iba1+) markers with confocal microscopy. Results showed that organoids generated on hydrogels with a higher amount of PEG (50 mg/mL or 60 mg/mL) folded significantly faster than those on hydrogels with lower PEG (40 mg/mL) at Day 14. However, organoids cultured on hydrogels with PEG 40 mg/mL with 50% or 60% crosslinking folded significantly slower than organoids on hydrogels with PEG 40 mg/mL with 40% crosslinking. Altered mechanical properties of synthetic hydrogels influenced the rate of folding. The findings are significant, as hydrogel properties can be manipulated for specific applications, allowing adaptation of the neural organoids to specific developmental timepoints or better recapitulation of disease pathology.
TITLE: The NSAID Diclofenac Causes Epithelial Cytotoxicity and Increased Barrier Permeability in an In Vitro Model of Primary Human Small Intestinal Epithelium

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: A. P. Bhatt1, D. B. Gunasekara1, J. Speer1, M. I. Reed1, A. N. Peña1, B. R. Midkiff2, B. Zwarycz2, S. Magness2,1, S. Bultman1, N. Allbritton2,1, and M. R. Redinbo1. 1University of North Carolina at Chapel Hill, Chapel Hill, NC; and 2Altis Biosystems, Chapel Hill, NC. Sponsor: N. Allbritton, American Association for the Advancement of Science

KEYWORDS: Alternatives to Animal Testing; Gastrointestinal; Cell Culture

ABSTRACT: Intestinal toxicity resulting from non-steroidal anti-inflammatory drug (NSAID) use has been documented for decades; however, NSAIDs are the most commonly prescribed medication for pain and inflammation with over 30 million people taking NSAIDs each day. In the small intestine, NSAIDs can cause intestinal inflammation resulting in erosions or ulcers ultimately lead to life-threatening intestinal bleeding, strictures, and perforations. Most investigation into NSAID toxicity utilizes mouse models for in vivo studies or Caco-2 cells, an immortalized human colon cancer cell line, for in vitro studies; however, these models fail to recapitulate major physiologic attributes of the human small intestine. To understand NSAID toxicity in the most physiologically-relevant platform available, we utilized a newly developed in vitro human primary small intestinal cell monolayer system, RepliGut Planar. RepliGut Planar is a confluent, differentiated monolayer of primary human intestinal epithelial cells that recapitulates key aspects of the human intestinal epithelium in vivo and is grown on a transwell, which allows for access to the apical and basal sides of the monolayer. We used primary human jejunal epithelium cultured on RepliGut Planar to pinpoint the consequences of the NSAID diclofenac on the small intestinal epithelium. Physiologically relevant doses of diclofenac were found to reduce epithelial cell proliferation and induce epithelial cytotoxicity by reducing mitochondrial membrane potential and inducing reactive oxygen species, which were directly observed using florescent Mitotracker dyes. Diclofenac also impaired barrier integrity by disrupting tight junctions, measured by a decrease in tight junction immunostaining, a decrease in transepithelial resistance measurements, and an increase in permeability of Lucifer Yellow. This epithelial cytotoxicity was increased and permeability decreased in a dose-dependent manner, similar to symptoms in patients who use NSAIDS long-term. These results suggest that the RepliGut platform recreates critical features of the human intestinal epithelium and represents a physiologically relevant system to study toxicity and permeability in vitro across different human populations.
heterogeneity observed in iHep differentiation, we took advantage of a genetically defined mouse population, the Collaborative Cross (CC) mouse resource composed of 8 genetically extant founder lines that have been intercrossed for multiple generations to derive recombinant inbred (CCRI) mouse lines that breed true. CCRI lines are useful for mapping toxicant susceptibility/resistance genes. CCRI iPSCs provide the opportunity to conduct such studies in vitro, saving both animals and costs associated with in vivo studies. In this study, 3 independent iPSC lines were selected for differentiation into hepatocyte-like cells. Morphology, uptake of low-density lipoprotein (LDL), and mRNA-based biomarkers of differentiation (using RT-PCR and RNAseq) were determined, including mRNAs characteristic of undifferentiated iPSCs (Oct4 and Nanog), definitive endoderm (Sox17), hepatoblasts (Afp, Sox17, Hnf3b); and hepatocyte-like cells (Ttr, HNF4α, Alb, Cyp3a11, Cyp3a13, Cyp1a1, Cyp2e1). Cell culture mRNA expression was compared to whole mouse liver. Evidence for hepatocyte-like cell differentiation occurring was indicated by LDL uptake and mRNA expression of Alb, HNF4α, Ttr and Afp. Phase contrast microscopy and LDL uptake suggested significant cell-type heterogeneity present in the cultures, including the persistence of stem-like cells as indicated by continued expression of Oct4 and Nanog. The numbers of hepatocyte-like cells appear to be relatively low and significant heterogeneity in morphology and hepatocyte-like differentiation markers is apparent among the 3 CCRI iPSC lines. These differences will be important for characterizing the extent to which genetics controls differentiation of iHeps, and for mapping genes and developmental pathways important for hepatocyte differentiation and their susceptibility to toxicant exposures. Supported by NIH Grants P30ES007033 and P30ES023512; and EPA Grants R83573801 and R83580201.

ABSTRACT NUMBER: 3563  Poster Board Number: P360
TITLE: Developing a Human Embryonic Stem Cells Based High-Throughput Platform to Screen for Developmental Toxicants

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ABSTRACT: Every year, millions of infants worldwide are born with a serious birth defect, which not only raises the risk for lifelong disabilities to those who survive but also increases the economic burden to their families and society. Besides genetic or hereditary factors, many of these defects can be caused by environmental chemical exposure, such as alcohol, smoking, and drugs. While there are over 80,000 chemicals registered for use in the United States, many of them have undergone little safety testing. Therefore, a rapid and accurate method for predicting developmental toxicants in the environment to humans and understanding their toxic mechanisms is strongly desired. Pluripotent human embryonic stem cells (hESCs) possess the capacity to differentiate into any cell type which makes them an ideal in vitro model to investigate developmental toxicity. In this study, we aim to develop a cost-effective transcriptomic-based high-throughput platform using hESCs to screen for environmental chemicals and pharmaceutical compounds with embryotoxic potential. In general, three-dimensional embryoid bodies (EBs), which recapitulate many developmental processes of early embryogenesis, were formed from hESCs. 30 chemicals with known or suspected teratogenicity (i.e., thalidomide, sodium arsenate, and tretinoin) were administered to EBs for seven days at concentrations causing a minimal cell viability loss (i.e. LC10). Pluripotency and embryonic differentiation of EBs were assessed by measuring the expression of 16 hallmark genes of these processes. The impacts of tested chemicals on key signaling pathways (Wnt, Notch, Sonic hedgehog, and TGF-β) required for the early embryogenesis were investigated as
Hierarchical clustering analysis of our preliminary data allowed us to separate embryonic toxicants apart from the negative controls. Furthermore, consistent with previous reports, our results also indicate that tretinoin, benomyl, and perfluorooctanoic acid possess neural developmental toxicity since they drastically decrease the expression of genes associated with ectoderm formation (≥2-fold). Together, these results indicate that our screening platform could be successfully applied for identifying developmental toxicants and understanding their etiology.

ABSTRACT NUMBER: 3564    Poster Board Number: P361
TITLE: Functional Expression of GLC-3, a Glutamate-Gated Chloride Channel from the Parasitic Nematode *Brugia malayi*

AUTHORS (FIRST INITIAL, LAST NAME) AND INSTITUTIONS: M. Abongwa, B. Akanji, S. Kashyap, S. Verma, M. McHugh, G. R. Mair, R. J. Martin, and A. P. Robertson. *Iowa State University, Ames, IA.*

ABSTRACT: Glutamate-gated chloride channels (GluCls) are targets of the macrocyclic lactone (ML) class of anthelmintics which include ivermectin. These channels are unique to invertebrates, making them an attractive target for antiparasitic drugs. Molecular and genetic studies have revealed the presence of multiple GluCl subunit genes and isoforms in *Caenorhabditis elegans* and parasitic nematodes. GluCl subunit combinations and receptor pharmacology also vary across species. We have cloned a GluCl subunit gene, GLC-3 from *Brugia malayi* (*Bma*-GLC-3), the causative agent of lymphatic filariasis. In the *Xenopus laevis* oocyte heterologous expression system, functional homomeric channels were formed when oocytes were injected with *Bma*-glc-3 cRNA. Two-electrode voltage-clamp electrophysiological recordings showed that channels formed by *Bma*-GLC-3 responded to L-glutamate in a concentration-dependent manner with an EC50 value of 64.8 ± 4.0 µM. The responses to L-glutamate were rapid in onset and were completely reversible. Current-voltage (I-V) relationships for the glutamate-gated currents produced a reversal potential (Erev) of -35.3 ± 3.2 mV demonstrating the receptors formed by *Bma*-GLC-3 are selectively permeable to chloride ions. No responses were detected with application of 1 mM L-aspartate, glycine, γ-aminobutyric acid (GABA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA). Remarkably, *Bma*-GLC-3 was insensitive to the chloride channel antagonist, fipronil, whereas picrotoxin was a weak antagonist with an IC50 of 165.3 µM. More importantly, ivermectin exhibited an unusual and complex mode of action on *Bma*-GLC-3 involving direct activation of the channel (EC50 = 4.1 nM) and inhibition of the responses to L-glutamate (IC50 = 95.1 pM). Our results show *Bma*-GLC-3 is pharmacologically different from *C. elegans* GLC-3 and other parasitic nematode GluCls. Further investigation of *Bma*-GLC-3 and the in vivo subunit composition and functions of the different GluCls present in *B. malayi* will provide insights to ivermectin’s limited activity on adult filaria.
ABSTRACT NUMBER: 3565  Poster Board Number: P362

TITLE: Identification of Mechanism of Toxicity and Disease Implication of Microplastics Using Caenorhabditis elegans Transcription Factors RNAi Library Screening and Comparative Toxicogenomics Database (CTD) Analysis


KEYWORDS: Transcription Factors

ABSTRACT: With recent reports of the detection of microplastics in seafood and bottled water, concern about the effects of microplastics on human health has been growing. To gain insight into the human health implications of microplastics, in this study, we investigated the possible mechanisms affecting the toxicity of microplastics using Caenorhabditis elegans RNAi screening and a bioinformatics-based unbiased approach. C. elegans transcription factors (TFs) RNAi library screening was conducted to explore the toxicity-related pathways. For this, age-synchronized RNAi-fed worms were exposed to various microplastics, and the toxicity to the RNAi-fed worms was subsequently investigated. Combined bioinformatics analysis was conducted using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and REACTOME pathway databases to identify the pathways involved in toxicity. The results of polyethylene microplastics showed that the nucleotide excision repair (NER) and transforming growth factor-beta (TGF-β) signaling pathways were significantly associated with microplastics exposure. The expression of components of NER and TGF-β signaling pathway genes was confirmed individually. Finally, gene-disease interaction analysis using the Comparative Toxicogenomics Database (CTD) revealed the possible human health implications of microplastics. Concomitantly, label-free Raman mapping was also conducted to investigate whether or not C. elegans could uptake microplastics. Further experiments using mammalian in vitro and in vivo models with realistic exposure scenarios are required to confirm the human health implications of microplastics. Keywords: Microplastics, Caenorhabditis elegans transcription factors RNAi library screening, Comparative Toxicogenomics Database. Acknowledgments: This study was also supported by a research project titled, “Environmental Risk Assessment of Microplastics in the Marine Environment” from the Ministry of Ocean and Fisheries, Korea for Y.L., S.L., and I.C.

ABSTRACT NUMBER: 3566  Poster Board Number: P363

TITLE: Linking Environmental Chemicals to Health Outcomes Using a Computational Systems Toxicology Approach


ABSTRACT: Integrative computational approaches combining systems biology and toxicology can help in deciphering the mechanisms of action of environmental chemical substances, thereby speeding up the identification of their putative health effects and accelerating their association with adverse outcome pathways (AOPs). The concept of AOP summarizes key information across various biological levels to connect biological perturbations at the molecular or cellular levels to adverse outcomes (AOs). We have developed a computational tool called AOPHelpFinder to link chemicals to biological key events, and applied it to bisphenol S (BPS), a substituent of bisphenol A, which is suspected to be an endocrine disruptor and is considered to be of high concern. The developed methodology is based on text mining of large-scale existing texts (in vitro, in vivo, and in silico data) which have been published in peer-review
journals. The text mining was used to find associations between environmental chemicals and AOPs. We combined graph theory to text mining to generate various scoring functions in order to keep only relevant information for the development of AOP network for BPS. Using such an approach, it is possible to identify linkages between a molecular initiating event (MIE) which is the initial target of a stressor (here the chemical) in a biological system, and an adverse outcome that impacts health of an individual or a population. Using the AOPHelpFinder tool and integrative systems biology, we were able to link BPS to various pathways leading to putative adverse effects, including obesity and reproductive disorders. Such computational approaches have the advantage of exploring in a systematic manner large amounts of available toxicological information, which is difficult by manual curation. Therefore, such approaches open new possibilities for accelerating the development of new AOPs and for establishing associations between AOPs and putatively toxic compounds. When considering substitution of available chemicals, such methods could prove to be useful for decision makers.

ABSTRACT NUMBER: 3567    Poster Board Number: P364
TITLE: Regional Differences of Acetaminophen and Naproxen Enterotoxicity in Human Small Intestine

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KEYWORDS: Gastrointestinal; In Vitro and Alternatives; Methods/Mechanism

ABSTRACT: We have recently developed a novel in vitro model of the human intestine, namely, cryopreserved human intestinal mucosal epithelium (CHIM). CHIM was isolated via collagenase digestion of human small intestine lumen and were consisted of multicellular fragments which retained the cell types (mainly enterocytes) and cell-cell junctions of the intestinal mucosal epithelium in vivo. Upon thawing, CHIM was found to retain all key drug metabolizing enzyme activities of the small intestine (Li et al., 2018, Drug Metab Disp). In this study, we evaluated the in vitro enterotoxicity of acetaminophen and naproxen, two NSAIDS known to have clinical enterotoxicity as evidence by the manifestation of intestinal bleeding. Clinical findings have established that naproxen has a higher enterotoxic potential than acetaminophen. In this study, we also evaluated potential regional differences in the enterotoxicity of the two NSAIDS in the small intestine. Small intestine from a human donor (49 year old female Caucasian) was dissected into 10 12-inch segments starting at the pyloric valve (segments A to J, with A being the first and J the last segment) and with CHIM prepared from each segment. For the enterotoxicity study, CHIM from the 10 segments (A to J) were thawed, with the suspensions adjusted to 1 mg protein/mL. Volumes of 50 uL of the CHIM suspension were plated in a 96 well plate preloaded with acetaminophen and naproxen at 2X of the final concentrations of 0 (medium control), 1.56, 3.125, 6.25, 12.5, 25 and 50 mg/mL. After an incubation duration of 4 hrs, cellular ATP was determined. Results are expressed as relative viability calculated as the ratio of ATP contents in treated samples versus that for medium control. Dose-dependent cytotoxicity was observed for both drugs in all the segments. The IC50 values (mg/mL) for the two drugs at the various regions, expressed as region: Acetaminophen IC50/Naproxen IC50, are as follows: A: 0.92/0.76; B: 1.21/0.53; C: 1.28/0.31; D:0.51/0.31; E: 0.71/0.29; F: 0.62/0.15; G: 0.99/0.38; H: 1.22/0.45; I: 0.48/0.19; and J: 0.19/0.15. The results showed that naproxen consistently had a high cytotoxicity than acetaminophen, an observation consistent with that observed in humans in vivo. There is also apparent regional differences in sensitivity, with the highest cytotoxicity observed for both drugs in segments I and J. Our results suggest
that CHIM represents a physiologically relevant in vitro experimental system the evaluation of enterotoxicity.

ABSTRACT NUMBER: 3568   Poster Board Number: P365
TITLE: A Novel Tool for Structural Toxicity Testing with In Vitro Models

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KEYWORDS: Predictive Toxicology; Hepatocytes

ABSTRACT: There is increasing interest in using in vitro cell models, such as human induced pluripotent stem cells (iPSCs), in early drug development as they have shown physiologically relevant drug responses that may predict clinical drug effects. The development of accurate phenotypic assays for these cell models plays a critical role in their successful utilization. Cell-based phenotypic assays are currently used in early screens to predict adverse drug side effects related to cell death (e.g. mitochondrial deficiency or nuclear count), or functional defects. Here, we propose a novel method, PhenoTox, which uses artificial intelligence (deep neural nets) to capture drug-induced morphological changes in cell cultures that relate to toxic drug effects but have no noticeable cellular damage or death. The input to PhenoTox is a collection of microscopy images captured and grouped at multiple doses and time-points for the drug of interest and a control set for each time point where no drug is applied. PhenoTox performs a series of 2-class neural-net trainings comparing controls to the test conditions and generates a classification accuracy for each training. The final output is a heatmap of the z-factors across all test conditions, depicting the doses and time-points at which morphological changes have happened and how strongly they differ from controls. PhenoTox was applied in preliminary evaluation studies to characterize the effect of tamoxifen, a drug known to cause liver toxicity, on both primary and iPSC-derived hepatocytes. The goal was to demonstrate that PhenoTox can capture subtle morphological changes that correlate with the known toxicity profiles for both in vitro models. The cells were cultured, fixed and stained with phalloidin-conjugated fluorescent dyes. Both bright-field and fluorescence images were collected on multiple wells and multiple locations per well using an inverted microscope with fluorescence capabilities. PhenoTox detected differences that related with loss of Cytochrome P450 3A4 activity. In future work, we will compare morphological changes induced by tamoxifen and compounds that are not hepatotoxic, such as aspirin and caffeine. The presented experiment are part of large ongoing research study to perform structural toxicity testing for a library of drugs using PhenoTox and correlating the results with functional toxicity assays.

ABSTRACT NUMBER: 3569   Poster Board Number: P366
TITLE: Ambulatory Intravenous Infusion in the Beagle Dog


KEYWORDS: Toxicity; Chronic

ABSTRACT: An emphasis on improving animal welfare has led to the refinement of certain practices. Historically, for infusion studies using a surgically implanted catheter, jacket tether systems were
generally used in light of their reliability but the approach does require single housing conditions. In order to better comply with recent changes in European requirements as well as to ensure the highest standard in terms of animal welfare, our laboratory established use of an ambulatory model for longer term infusion studies in dogs. Four Beagle dogs were surgically implanted in the femoral vein as per our laboratory standard procedures, with a modification to the catheter length (shortened). The catheter was exteriorized into a jacket with a customized backpack, which also held the pump and the infusion bag. Animals were group housed and allowed post-operative recovery of at least 7 days during which time a saline intravenous infusion was maintained at a rate of 2 mL/hour. The rate was then increased to 2.0 mL/kg/hour, in order to mimic a rate commonly used for continuous infusion toxicology studies, and animals were administered saline continuously (24 hours/day) for 55 days. The infusion regimen was then modified to assess intermittent administrations. Animals underwent 4 weeks of once weekly 2-hour intermittent infusion at 7 mL/kg/hour. Following successful establishment of these procedures, an additional group of 10 dogs were implanted and monitored over 28 days of continuous intravenous infusion at 1 mL/kg/hour. Further to that we were also able to confirm that infusion line patency could be successfully maintained by a daily flush of saline during the prestudy period and in between intermittent dose occasions. Clinical condition was monitored daily and body weight once weekly. Accuracy of the pumps was measured at each occasion of dosing. There were no abnormal clinical signs noted, and no significant changes in body weights. Group housing of the animals was generally successful for the duration of the project, and no damage to the jackets or pumps was observed in group housed dogs with one exception which was attributed to incompatibility within the animal trio. Incidents requiring surgical repair of the catheter were limited, which suggests this model is suitable. Overall, these results show that the ambulatory system can be successfully used for both continuous and intermittent intravenous infusion studies for a duration of up to 8 weeks in the dog while improving the welfare of the animals.
across platforms and assay content, certain compounds were outliers in all 3 data sets. Norethindrone segregated from beta-estradiol and ethinylestradiol, consistent with its action as a progesterone agonist but weak estrogen agonist. Miconazole segregated from fluconazole, clotrimazole, and econazole, consistent with its reported dual MOAs. In contrast, leflunomide (LEF) segregated from 3-methylcholanthrene and beta-naphthoflavone although all three share an AhR MOA. This was also the case for N-nitrosodimethylamine (NIT), which segregated from ifosamide and aflatoxin B1, although all three are DNA alkylating agents. Interestingly, LEF and NIT clustered near each other across the three data sets. Upon examination in MSigDB, LEF and NIT share an enriched gene set that was not found in the other members of the AhR and DNA damage compounds. These results suggest not only that WT or focused expression profiling can distinguish compounds with multiple MOAs but can potentially reveal additional MOAs by co-clustering with each other. As such, a targeted TempO-Seq assay offers sufficient sensitivity for MOA definition while optimizing sequencing space utilization, lowering barriers to high throughput transcriptomics in compound screening.

**ABSTRACT NUMBER:** 3571  **Poster Board Number:** P368

**TITLE:** Assessing the Efficiency of a Cytosine-to-Thymine Base Editor for Inducing On-target LacZ Mutations in E. Coli

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**ABSTRACT:** Gene editing is being widely used for eliciting specific DNA modifications in various organisms. It is traditionally performed by CRISPR-Cas9 systems and user-defined guide RNAs (gRNAs) to induce DNA double-strand breaks (DSBs) at target sites. Desired mutations result from defective DNA repair at such sites. While these CRISPR-Cas9 systems are effective, inducing and repairing DSBs is stressful for most cells and can lead to off-target mutations and other undesired effects. To avoid these issues, less disruptive systems called “base editors” have been recently developed. Base editors are composed of a nuclease-deficient Cas9 (dCas9) fused to a DNA-modifying enzyme, a combination that can potentially achieve specific point mutagenesis at target sites with less DNA damage than traditional CRISPR-Cas9 systems. In this study, we used a temperature-sensitive cl repressor to control the expression of a cytidine deaminase-dCas9 base editor and assessed its ability for creating nonsense mutations in three sites of the native LacZ gene of E. coli MG1655. Colonies bearing nonsense cytosine-to-thymine on-target mutations were quantified by blue/white selection. We observed that greater induction of the system, either by higher temperatures or longer induction times, increased on-target mutations in all three target sites tested. In addition, we observed marked differences in mutation efficiencies from site to site, ranging from ~10% to ~90%. These results suggest, that like other gene editing systems, the efficiency of this cytidine deaminase-dCas9 base editor is largely dependent on its interactions with gRNAs and target sites. Non-targeting gRNAs and controls lacking cytidine deaminase-dCas9 base editors generated no white colonies (0% mutation efficiency). Overall, we found that this inducible cytidine deaminase-dCas9 base editor system can be used to elicit cytosine-to-thymine mutations, but that its efficiency is largely dependent on the sites being targeted and the corresponding gRNAs.
ABSTRACT NUMBER: 3572    Poster Board Number: P369
TITLE: Identifying Microbial Markers of Xenobiotic Exposures in Zebrafish

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ABSTRACT: Growing evidence indicates that microbiota modify the toxicokinetics and/or toxicodynamics of xenobiotic exposures. Current risk assessment strategies do not consider interactions between host-associated microbes and chemical toxicity. We previously showed that developmental exposure of colonized zebrafish to bisphenol A or bisphenol F selected for several genus-level taxa, including Rheinheimera. To identify microbial markers of xenobiotic exposure, CC, AX, and AX zebrafish colonized on day 1 (AC1) were exposed to 0.1-0.3 µM triclosan or 0.1% DMSO on days 1, 6, 7, 8, and 9 and microbiota community structure was assessed on day 10. Putative functional analysis revealed that triclosan-resistant microbes exhibit multiple xenobiotic resistance strategies. Relative to control zebrafish, triclosan exposure significantly selected for Rheinheimera in both CC and AC1 cohorts. Specifically, in control zebrafish, Rheinheimera accounted for <5% of sequence reads whereas in triclosan-exposed zebrafish, Rheinheimera accounted for 66-70% of all reads. We obtained Rheinheimera makueensis as a representative species. Utilizing marine broth media, we determined a growth rate constant (µ) of 0.16 hr⁻¹. Monocolonization of AX zebrafish with 100 cells/ml of R. makueensis was not sufficient to block locomotor hyperactivity at 10 dpf, a phenotype we previously showed to be associated with axenic status. These data indicate that this microbe may not colonize the zebrafish intestinal tract. Together, these data suggest that triclosan-resistant microbes like Rheinheimera may serve as a marker of xenobiotic-mediated microbiota disruption and that more work is needed to identify strains of Rheinheimera that colonize the host organism. This abstract does not necessarily reflect US EPA policy.

ABSTRACT NUMBER: 3573    Poster Board Number: P370
TITLE: Establishing a Link between Microbiota and AHR in Zebrafish Neurobehavioral Development

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KEYWORDS: Receptor; Aryl Hydrocarbon; Neurotoxicity; Developmental; Behavioral

ABSTRACT: Host-associated microbiota is a dynamic microbial system that plays an important role in nervous system development. We have previously shown that axenic (microbe-free) larval zebrafish exhibit behavioral hyperactivity, in comparison to conventionally colonized zebrafish. To understand the underlying mechanism by which microbes stimulate neurobehavioral development, unbiased RNA sequencing was performed in brain-enriched tissue isolated from axenic (AX), axenic colonized on day 1 (AC1), or conventionally colonized (CC) zebrafish larvae at 10 days post fertilization (dpf). No significantly differentially expressed genes were identified when comparing the two colonized cohorts (i.e. AC1 vs. CC). In stark contrast, there were 567 genes (>2-fold differentially expressed; 0.5 FDR) that were differentially expressed with the same directionality when comparing either colonized group to the axenic microbe-free cohort. Bioinformatics analysis revealed that Aryl Hydrocarbon Receptor (AHR) was a predicted regulator of the observed transcriptomic changes indicating that microbial colonization
might affect neurobehavioral development via AHR-dependent signaling pathways. There are three AHRs in zebrafish: AHR1a, AHR1b, and AHR2. To date, the AHR has not been systematically assessed as a mediator of neurobehavioral development. To elucidate AHR essentiality in the context of microbiota-gut-brain communication, CRISPR/Cas9 gene editing was used to create AHR1a, AHR1b, and AHR2 F0 chimeric zebrafish larvae. The effectiveness of guideRNA design was confirmed using the T7E1 endonuclease assay. Locomotor activity, used here as a functional readout of nervous system development, was assessed at 6 dpf. We report that AHR2 F0 chimeric larvae were significantly hyperactive in comparison to larvae injected with a scrambled guide. In comparison, AHR1a chimeric mutants presented with control-like behavioral activity. These data illuminate the importance of host-associated microbes in neurodevelopment and raise the question of whether xenobiotic and microbial-mediated signaling can converge via AHR2 signaling to affect neurobehavioral development in zebrafish. This abstract does not necessarily reflect US EPA policy.

ABSTRACT NUMBER: 3574       Poster Board Number: P371
TITLE: Dynamics of Microglial Morphology as an Acute Biomarker of Ketamine Neurotoxicity

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ABSTRACT: Neurotoxicity studies provided to the US FDA by industry for regulatory review of medical products currently focus on animal behavior and cellular abnormalities visible within fixed tissue. While essential, data obtained with these methods may lack sensitivity. A live imaging-based assay could potentially increase sensitivity by tracking the dynamics of neural responses to neurotoxicants at the cellular level. Our research focused on evaluating whether the dynamics of the resident immune cell, microglia, in the mouse cortex could serve as a biomarker of neurotoxicity. In vivo two-photon microscopy (TPM) was used to track the morphological changes in microglia acutely following local ketamine (600µM in saline) application through a thinned skull in both adolescent and adult Cx3Cr1 GFP+/− mice in which microglia express green fluorescent protein. Single high dose or chronic low dose ketamine exposure has been shown to induce changes to neuronal architecture and trigger neuronal cell death. In our study, 3D morphology of microglia in each animal was repeatedly imaged every 5 minutes from 1 hour pre to 2 hours post continuous ketamine application. Control animals received only saline application for 3 hours. In FIJI, the Simple Neurite Tracer and Sholl Analysis plugins were used to quantify the ramification and coverage of microglial processes over time. Preliminary results suggest that within 1 hour of ketamine exposure, microglial process spread and branching complexity both slightly decrease, morphological changes that are indicative of immune reactivity. These changes are not observed in control animals. Further studies are planned to determine the progression of microglial responses through 24 hours. Dynamic changes in microglial morphology shortly following neurotoxicant exposure may provide a promising avenue for rapidly detecting early neurotoxic events.
ABSTRACT NUMBER: 3575   Poster Board Number: P372
TITLE: Circulating Biomarkers of Neurotoxicity: Identifying Fluidic Endpoints Correlating with Central Nervous System Toxicity in a Rodent Model of Neurotoxicity

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ABSTRACT: Neurotoxicity has been linked to exposure to a number of common drugs and chemicals, yet efficient, predictive, and minimally-invasive methods to detect it are lacking. Fluid-based biomarkers such as those found in serum, plasma, urine, and cerebrospinal fluid (CSF) have great potential due to the relative ease of sampling, but at present, data on their expression and translation are lacking or inconsistent. Here, we present data on biomolecules that have some promise for detection and characterization of neurotoxicity induced by a single intraperitoneal injection of the known neurotoxic agent, trimethyltin (TMT). A single dose of TMT led to significant alterations in total oxidative stress markers, changes in lipid homeostasis, circulating interleukins and related factors, and markers of neuroinflammation. These findings provide an opportunity to explore the correlation of these fluid biomarkers with traditional neuropathology and magnetic resonance imaging (MRI) that serve to define TMT-induced neurotoxicity. Our data demonstrate a comprehensive correlation of TMT-induced neuropathology with several potential neurotoxicity biomarkers and MRI-based endpoints, findings suggestive of an involvement of specific pathways that can be assessed using peripheral fluids.

ABSTRACT NUMBER: 3576   Poster Board Number: P373
TITLE: An Evaluation of In Silico Predicted Neurotoxicity in Embryonic Rat Dorsal Root Ganglion (DRG) Cultures: Effects on Cytotoxicity, Neurite Length, and Neurophysiology


KEYWORDS: Neurotoxicology; Toxicity; Acute; Predictive Toxicology

ABSTRACT: Numerous chemicals are proposed to produce non-receptor/ion-channel mediated neurotoxic responses through adverse interactions with intracellular proteins and macromolecules. These molecular initiating events may induce an adverse outcome pathway (AOP) that can hinder synaptic nerve terminal protein function and result in peripheral neuropathy. To test the Hard-Soft Acid and Base (HSAB) hypothesis which is based on the potential chemical bio-reactivity (electrophilicity) to cause a neuropathic effect, a test set of chemicals from the USEPA ToxCast database was evaluated in vitro in DRG cultures. Chemicals predicted to be neurotoxic were tested acutely, using a 3-tiered assessment of: 1. Cytotoxicity (% lactate dehydrogenase (%LDH) release); 2. Structural alteration (total neurite length per neuron via high content microscopy) and 3. DRG neurophysiology, as measured by mean firing rate (MFR), recorded on microelectrode arrays. DRG primary cultures were generated from
embryonic day 16 Long-Evans rats and treated with cytosine arabinoside (500 nM) on day 3 in vitro (DIV 3) to inhibit glial overgrowth. DRGs were dosed acutely on DIV 7 with 1, 5, 10, 50 or 100 µM of a chemical and LDH release was measured at 24 and 48 hrs after exposure. To assess for neurite length, DRGs where fixed and antibody (PGP9.5) stained 48 hrs after dosing (DIV 7). Of the 10 chemicals tested, only 4-cyclohexylhexanone (4C) increased %LDH release at 50µM (22%) and 100 µM (49%) after 48 hrs. Two chemicals, 4C and Phenyacetylaldehyde (PAA) decreased mean neurite length per neuron at 48 hrs; 4C at 50 µM (28%) and 100 µM (60%) and PAA at 100 µM (46%). Separately, mature DRG cultures were dosed with a single concentration (10, 50 or 100 µM) of one of 13 chemicals on DIV 14 and changes in MFR were recorded for 1 hr. Octanal and 2,5 hexanedione had no effect on MFR. However, 11 compounds altered MFR in a dose-dependent manner, the most potent being PAA (-67%), 4C (-82%), and 4-Tertbutyl cyclohexanone (-92%). With the exception of 4C, the observed changes in MFR or neurite length occurred in the absence of cytotoxicity. These results provide support that HSAB physical properties may be used to predict chemical-induced neurotoxicity. *This abstract does not necessarily reflect US EPA policy.*

**ABSTRACT NUMBER:** 3577  **Poster Board Number:** P374

**TITLE:** Analysis of Media Effects on Human iPSC-CMs Responses to CiPA Compounds

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**KEYWORDS:** In Vitro and Alternatives; Safety Pharmacology; Cardiovascular System

**ABSTRACT:** Human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) have been widely used for drug-induced proarrhythmia detection. It has been noticed that the responses of iPSC-CMs can be different in serum-free (SFM) and serum-containing normal media (NM), presumably because of the difference in drug solubility and changes of cardiomyocyte electrophysiology. In this study, the responses of human iPSC-CMs (iCells² from CDI) to 10 CiPA compounds were compared side-by-side. We also measured the free fractions of 10 CiPA drugs in NM and SFM. Surprisingly, there were significant non-specific bindings of drugs in SFM, which reduced the concentrations of free drugs, particularly those high protein-binding drugs. Moreover, the compound preparation methods for in vitro studies can significantly affect the availability of drugs in media. The responses of human iPSC-CMs tested in NM and SFM were likely determined by the availability of unbound drugs. More comprehensive analysis of free drugs in testing media and the responses of cultured cells are needed for better data extrapolation from in vitro to in vivo studies.