Ohio Valley Society of Toxicology
Annual Meeting Program

November 5, 2021

Virtual

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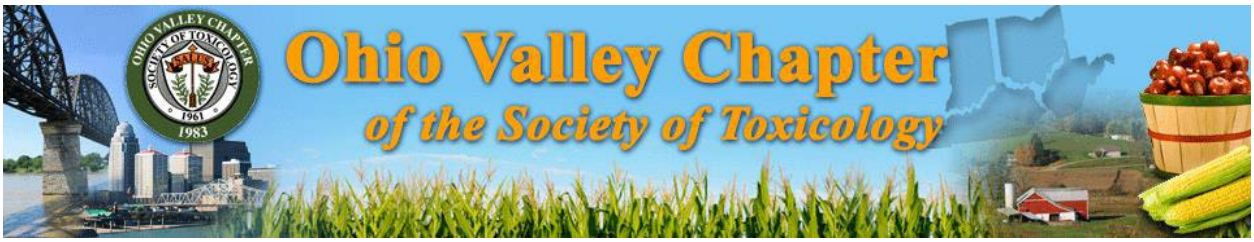

OVSOT Annual Meeting Agenda

Friday, November 5, 2021

8:30 – 9:00 am	Entry into Meeting *(SOT Moderator)* and Poster Viewing
9:00 - 9:15 am	**Welcoming Remarks**, Meeting Logistics & Acknowledgement of Sponsors - David Mattie, PhD, DABT, OVSOT President
9:15 - 10:15 am	**Postdoctoral Platform Presentations** and Judging Chair: Walter (Bert) Watson, PhD, OVSOT Vice President
10:15 - 11:00 pm	Poster viewing / Break
11:00 - 12:00 pm	**Poster Oral Presentations** and Judging Chair: Brandon Lewis, PhD, OVSOT Postdoc Rep
12:00 - 1:00 pm	Lunch Break / Q&A with Toxicology Experts in Government, Academia and Industry / Poster Viewing
1:00 - 2:00 pm	**Doctoral Student Platform Presentations** and Judging Chair: Jonathan Shannahan, PhD, Vice President-Elect
2:00 - 3:00 pm	**Tox-on-the-Clock** Chair: Eddie Sloter, OVSOT Education and Outreach Liaison
3:00 - 3:15 pm	Break / Poster Viewing
3:15 - 4:15 pm	**Keynote Speaker:** Environmental Risk Assessment: A Case Study of a Down-the-Drain UV-Filter in US Freshwater. **Kyle Roush**, Environmental Stewardship & Sustainability, Global Product Stewardship, The Procter & Gamble Company Chair: David Mattie, PhD

4:15 – 4:30 pm **Announcement of Results of the Judging** - Bert Watson, PhD

Close Meeting - Dave Mattie, PhD

KEYNOTE SPEAKER

Kyle Roush, Scientist
Environmental Stewardship & Sustainability
Global Product Stewardship
The Procter & Gamble Company

Presentation Title: Environmental Risk Assessment: A Case Study of a Down-the-Drain UV-Filter in US Freshwater.

Biography: Kyle Roush, Received a MSc in Biology from TCU with a focus in aquatic toxicology. Research background includes areas such animal alternatives, endocrine disruption, environmental effects method development, etc. Currently serving as an environmental steward for P&G Beauty Care supporting the Hair Care business. Specific responsibilities and expertise areas including environmental effects, regulatory toxicology, endocrine disruption, etc.

Abstract: Environmental risk assessment (ERA) is a robust process for evaluating the chance of harmful effects in an ecological system as a result of exposure to an environmental stressor. This process allows for assessing and ensuring the safety of chemicals in the environment. From the most basic viewpoint, ERA of a stressor (e.g., chemical) involves comparing the level of exposure to the level at which a hazard is present. The evaluation of exposure and hazard scales from screening level to high-tier methodologies dependent on the margin of safety and need for refinement. This process was utilized to assess the environmental risk of oxybenzone (BP-3), an organic ultraviolet filter used in cosmetic and personal care products. Specifically, the ERA was focused on safety in United States (US) freshwater as a result of down-the-drain release. Results indicate that oxybenzone is of low concern with a significant margin of safety in US freshwater.

Postdoctoral Platform Presenters

miR-186 Overexpression Suppresses BUB1 and CDC27 in Immortalized Human Keratinocytes

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Background: Chronic arsenic (As) exposure in drinking water is a global issue affecting >225 million people. Skin is a major target organ for As. Our preliminary data indicate miR-186 overexpression in As-induced squamous cell carcinoma relative to As-induced hyperkeratosis. Inhibition of DNA repair, miRNA dysregulation and chromosomal instability (CIN) have been proposed as mechanisms of As-induced carcinogenesis. DIANA-miRPath analysis indicates that miR-186 targets mRNA components of the cell cycle, such as mitotic checkpoint serine/threonine kinase B (BUB1) and cell division cycle 27 (CDC27). Hypothesis: miR-186 overexpression accelerates arsenic-induced transformation of keratinocytes by induction of CIN. Methods: Ker-CT cells were transduced with miR-186 overexpressing and control lentivirus. Stable clones were isolated after puromycin selection. Four clones with highest and four with lowest miR-186 expression were maintained with 0 or 100 nM As for 20 weeks. Cell lysates were collected and BUB1 and CDC27 levels were determined of exposure by western blot at 4 and 8 weeks. Epithelial to mesenchymal transition (EMT) was assessed at 20 weeks of exposure. Data were analyzed by 2-way ANOVA followed by Tukey's multiple comparison test; significant difference at $p<0.05$. Results: miR-186 overexpression significantly reduced CDC27 levels irrespective of arsenic exposure at 4 and 8 weeks of exposure. BUB1 levels were decreased with miR-186 overexpression at 8 weeks, but not at 4 weeks of exposure. EMT markers have not indicated cell transformation by 20 weeks. Discussion: BUB1 is a component of the spindle assembly checkpoint and CDC27, a component of the anaphase-promoting complex, the two major regulators of mitosis and chromosome segregation. Suppression of BUB1and CDC27 by elevated miR-186 correlates with incorrect segregation of chromosomes and lead to CIN. Dysregulation of cell cycle events leading to aneuploidy may be involved in arsenic-induced carcinogenesis. Funding: NIH grants R01ES027778, R21ES023627 and P30ES030283.

Reduced DNA Damage Response Activation in Keratinocytes Chronically Exposed to Toxicologically Relevant Concentrations of Sodium Arsenite

Alexandra Nichole Nail, Lakyn McCaffrey, Ana Paula Ferragut Cardoso, Mayukh Banerjee, J. Christopher States

Department of Pharmacology and Toxicology, University of Louisville

Background: An estimated > 225 million people, including > 2 million in the U.S., are exposed to high arsenic concentrations (>10 μg/L) in contaminated groundwater. Inorganic arsenic (iAs) is the major contaminant in drinking water. iAs does not form DNA adducts, but does induce chromosomal instability (CIN) which is a hallmark of cancer. The mechanism of iAs-induced CIN is not established and arsenic is a human class I carcinogen. Illumina RNA sequencing analysis from human keratinocytes chronically exposed to iAs suggested dysregulation of the DNA damage response (DDR). Based on these results, we investigated DDR signaling in keratinocytes chronically exposed to iAs. Hypothesis: iAs exposure results in CIN by perturbation of either upstream components required for DDR activation or downstream components required for DDR inactivation. Methods: Passage-matched, quadruplicate cultures of human keratinocytes (HaCaT or KerCT cells) were chronically exposed to media containing 0 or 100 nM iAs. Protein lysates collected at 7 (HaCaT) or 8 (KerCT) weeks were examined by immunoblotting for activated DNA damage response (DDR) proteins (i.e. MRN complex, ATM and downstream signaling proteins, ATR and downstream signaling proteins) and phosphatases. Statistical analyses of densitometric data was performed in GraphPad Prism software using two-tailed unpaired t-test; p-value <0.05 was considered significant. Results: Activated ATM is significantly reduced in HaCaT and KerCT cells exposed to toxicologically relevant iAs concentrations. Both phosphorylated and total protein levels within the Mre11-Rad50-Nbs1 (MRN) complex are significantly altered in KerCT and HaCaT cells chronically exposed to iAs whereas phosphatase expression was significantly reduced or unaffected. Conclusions: Perturbed MRN phosphorylation and diminished ATM pathway activation following chronic iAs treatment indicates impairment of the DDR which may contribute to CIN. Thus, reduced DDR activation may be a driving force for CIN and carcinogenesis in

keratinocytes chronically exposed to iAs.

Repeated Exposure to Eucalyptus Smoke Alters Pulmonary Gene and Metabolic Profiles in Male Long-Evans Rats

Katelyn Dunigan-Russell[1], Helen Nguyen[2], Janice A. Dye[3], Hongzu Ren[3], Mette C. Schladweiler[3], Dean P. Jones[4], Anna Fisher[3], Ian M. Gilmour[3], Kymberly M. Gowdy[1], Colette N. Miller[3], M. Ryan Smith[4]

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With the increasing prevalence and intensity of wildland fires in the U.S., there is significant concern for respiratory heath in occupational and public health settings. Wildfires increase toxins in the air including ultrafine particulate matter (PM2.5), noxious gases, and volatile organic chemicals, which are known to increase lung inflammation and injury. Therefore, we hypothesize that wildfire smoke inhalation induces pulmonary genetic and metabolic adaptations through inflammatory signaling. To test this hypothesis, adult male Long-Evans rats were exposed to filtered air or smoke from eucalyptus biomass burning under smoldering conditions at a particle (32nm – 10.57μm) concentration of 11 ± 1.89 mg/m3 (low) or 23.7 ± 0.77 mg/m3 (high) for 1 hr/day for 2 weeks. 24hrs following the last exposure, rats were euthanized and bronchoalveolar lavage (BAL) fluid and lung tissue were collected. BAL was used to measure differential cell counts, cyto/chemokine production in the airspace, as well as inflammatory gene expression in BAL cells. Lung tissues were assessed for changes in gene expression using RNA-seq paired with high-resolution metabolomics (HRM) (n=7-8/group). Differentially expressed genes (DEGs) were identified and analyzed by Ingenuity Pathway Analysis (IPA). BAL cell differentials revealed a dose dependent increase in macrophages, neutrophils and lymphocytes following smoke exposure. Interferon-gamma (IFN-γ), and anti-inflammatory cytokines interleukin-10, IL-5 were also increased in the airspace following low and high smoke inhalation. RNA-seq analysis revealed 1,712 upregulated DEGs and 1,413 downregulated DEGs in the high exposure smoke compared to filtered air. IPA analyses showed increased EIF2 and Rho Family GTPases with reductions in the eIF4, p7056K, and mTOR Signaling. HRM revealed perturbations to 23 metabolic pathways including nucleotide, amino and fatty acids, nitrogen and antioxidant pathways with smoke exposure. Integrative analysis of the transcriptome and metabolome data revealed significant changes in Wnt, NOS1, and Cox2 following smoke exposure. Our results suggest that acute exposures to wildfire smoke results in robust changes to the lung at both the genetic and metabolic levels. These results provide novel insight into the pulmonary response to wildfire smoke and support the association between wildland fire smoke exposure and adverse respiratory outcomes. This abstract does not reflect U.S. EPA policy.

Doctoral Platform Presenters

Excessive Copper Impairs Adult Neurogenesis in Brain Subventricular Zone *In Vitro*

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Copper (Cu), an essential metal for human health, exists in nearly all brain regions. The subventricular zone (SVZ) is the largest geminal region in the adult brain which harbors neurogenesis and supplies newborn neurons for normal brain function. Recent data from this group has established that the SVZ has the highest Cu concentration within brain. However, the role of Cu in the SVZ with regards to the adult neurogenesis remains elusive. This study, by using adult mouse SVZ-derived neurospheres to model the SVZ neurogenesis in vitro, was designed to explore the hypothesis that an excessive Cu impaired the adult neurogenesis in SVZ-derived neurospheres, which may contribute to certain Cu-dyshomeostasis-associated neurodegenerative disorders. When neurospheres were incubated with 0, 1.0, 10.0, and 100.0 μM Cu in culture media for 7 days, the presence of high Cu caused a dose-dependent inhibition of neural cell migration, proliferation, differentiation, and maturation in the neurospheres as observed in diminished migration area, reduced numbers of BrdU(+) proliferating progenitor cells, decreased DCX(+) neuroblasts, and fewer NeuN(+)

mature neurons, respectively. By contrast, incubation of neurospheres with a therapeutic Cu chelator D-penicillamine (D-Pen) at 20, 50, and 100 μM for 7 days exhibited a significant, dose-dependent stimulatory effect on adult neurogenesis. Co-treatment of neurospheres with 20 μM D-Pen and 10 μM Cu demonstrated that the presence of D-Pen significantly rescued the Cu-induced neurogenesis impairments. Assays by Phen Green Cu sensor further showed a significantly reduced cellular Cu level by D-Pen as compared to controls. Furthermore, qPCR analysis revealed that several key genes regulating the Cu transport, such as Atp7b and Mt2, appeared to be upregulated in a compensatory response to Cu overload; treatment with D-Pen also reversed Cu-caused impairment in the expression of genes that regulate the neurogenesis pathway, such as Shh and Slit1. Taken together, these in vitro observations suggest that excessive Cu exposure impairs the SVZ adult neurogenesis possibly by depleting GFAP(+) neural progenitor pool; this may partly explain manganese exposure-induced Parkinsonian disorder where an increased Cu accumulation in the SVZ as well as in the choroid plexus has been observed. Supported by NIEHS R01 ES028078.

Particulate Hexavalent Chromium Causes DNA Repair Inhibition Leading to Increased Chromosome Instability in Human Bronchial Epithelial Cells

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Particulate hexavalent chromium [Cr(VI)] is a well-established human lung carcinogen with environmental and occupational exposure risks. Epidemiology, experimental animal and cell culture studies all indicate Cr(VI) targets chromosomes inducing chromosome instability (CIN) and CIN is the proposed driver of Cr(VI)-carcinogenesis. RAD51, a key protein in the homologous recombination (HR) repair pathway, is inhibited after prolonged exposure to Cr(VI) and accumulates in the cytoplasm inhibiting its function. HR repair is a major DNA repair pathway that prevents development of CIN by repairing DNA double strand breaks with high fidelity. These effects on RAD51 and HR repair were observed in human lung fibroblasts, but Cr(VI)-induced tumors originate from epithelial cells. Therefore, the aim of this study is to translate DNA repair deficiency and CIN from human bronchial fibroblasts to epithelial cells (BEP2D). First, we characterized the toxicological effects of zinc chromate, a particulate Cr(VI) compound, in epithelial cells, by measuring cytotoxicity with clonogenic assay and intracellular Cr levels with atomic absorption spectrometry. Cr(VI)-induced DNA double strand breaks were measured by neutral comet assay and CIN was evaluated with chromosome aberration assay. Effects on RAD51 were measured as: 1) immunofluorescent foci formation and 2) cytoplasmic accumulation using confocal microscopy and ROI analyses. Results show Cr(VI) is cytotoxic and intracellular Cr ion levels increase with concentration and time. We found Cr(VI) induced DNA double strand breaks. However, prolonged Cr(VI) exposure inhibited RAD51 foci formation and increased inappropriate cytoplasmic accumulation, indicating DNA break repair was compromised, leading to an increase in CIN after prolonged Cr(VI) exposure. Comparison of these results with previous data on fibroblast cells showed 1) fibroblast cells have higher levels of intracellular Cr and 2) epithelial cells develop more CIN for the same amount of intracellular Cr levels. These results match epidemiological data showing fibroblast cells accumulate Cr, whereas epithelial cells originate tumors and are characterized by CIN. In conclusion, Cr(VI) induces DNA double strand breaks and targets RAD51 to inhibit HR repair leading to an increase in CIN, successfully translating the outcomes in human bronchial fibroblasts to human bronchial epithelial cells.

Heterocyclic amines induce changes in glucose production and insulin signaling in human hepatocytes

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Humans are exposed to heterocyclic amine (HCA) mutagens produced in meats cooked at high temperatures or until well-done. A recent epidemiological study documented associations of high HCA intake with increased prevalence of insulin resistance, which is one of the hallmarks of type II diabetes mellitus and metabolic syndrome. However, it is unknown if HCAs directly induce insulin resistance. To investigate the effects of HCAs on insulin sensitivity, we treated HepG2 (hepatocellular carcinoma) cells and cryopreserved human hepatocytes with 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline (MeIQx), or 2-amino-1-methyl-6-phenylimidazo(4,5-b) pyridine

(PhIP), which are 3 common HCAs found in cooked meats, and measured changes in gluconeogenic gene expression, insulin signaling, and glucose production. HepG2 cells and cryopreserved hepatocytes were treated with varying concentrations of HCAs (0-100 μM), then treated with insulin and analyzed for changes in (1) mRNA transcript levels of genes involved in gluconeogenesis via qRT-PCR, (2) insulin signaling via p-AKT induction on Western blot, and (3) extracellular glucose production via glucose oxidase assay. Statistical significance was determined by one-way ANOVA (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). HCA treatment significantly upregulated the transcripts of genes involved in gluconeogenesis (e.g., G6PC and PCK1) in both HepG2 and cryopreserved human hepatocytes. MeIQ treatment decreased insulin-induced phosphorylated AKT (ser473; p-AKT) levels in cryopreserved human hepatocytes. The decrease in insulin-induced p-AKT levels following HCA treatment suggest that hepatocytes become insulin resistant in the presence of HCAs. Additionally, MeIQ and MeIQx treatment increased glucose production in cryopreserved human hepatocytes, compared to vehicle-treated control cells. The increase in extracellular glucose production following HCA treatment indicate that HCA exposure potentially cause a sustained increase in glucose levels and may lead to hyperglycemia which often accompanies insulin resistance and type II diabetes mellitus. The current study provides an evidence, for the first time, that exposure to HCAs via consumption of cooked meat may contribute to development of insulin resistance and hyperglycemia. Support for these studies was provided by the following: T32- ES011564, P20-GM113226, P42-ES023716, P30-ES030283, and pilot project funding from UofL School of Medicine.

Poster Oral Presenters

Titanium dioxide and zinc oxide nanoparticles in sunscreens: impact on cytokine expression in human skin post-UVB exposure

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Nanoparticles (NP) have recently been widely used in sunscreen products to prevent UVBmediated skin damage. Research has shown that Zn and TiO2 NP effectively scatter, reflect, and absorb light in the UV range. However, there is little research on the impact of UVB exposure with or without NP therapy related to inflammatory cytokine expression. This study investigates the influence of ZnO and TiO2 NP on the expression of pro- and anti-inflammatory cytokines in the setting of UVB radiation on in-vivo human skin samples. Three human skin explants obtained post-surgically were treated as follows: UVB alone (control), ZnO or TiO2 NP with or without UVB, and ZnO/TiO2 combination therapy with UVB exposure. Samples were analyzed with BioRad Bio-Plex Pro Human Cytokine 27-plex Assay to determine cytokine expression of: IL-1β, IL-1RA, IL-6, IL-8, IL-15, FGF basic, Eotaxin, GM-CSF, IFN-γ, MCP-1, MIP-1β, RANTES, TNF-α, and VEGF under various experimental conditions. Our preliminary analysis of results confirms previous research suggesting a baseline increase in pro-inflammatory cytokines when skin is exposed to UVB, Zn or TiO2 NP. We also found that baseline inflammation with NP-only treated samples is generally higher than UVB exposure alone. Interestingly, some inflammatory cytokines were synergistically increased with either NP application before or after UVB exposure. These results suggest a potential for NP sunscreen and UVB exposure to induce inflammation and supports further studies to evaluate the safety and efficacy of using NP sunscreens.

Potential Physiological role of AhR in Antibody Production

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The aryl hydrocarbon receptor (AhR) mediates the immunosuppressive effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in murine B cells. The effects of AhR activation on the regulation of expression of human immunoglobulin (Ig) isotypes (μ, γ1-4, α1-2 and ε) and Ig secretion is unclear. Our previous results using the CL-01 cell-line originating from a Burkitt's lymphoma patient, demonstrated an inhibitory effect of TCDD on IgG expression but a surprising and marked loss of IgG secretion when the AhR was knocked out by siRNA or

CRISPR/Cas9 gene editing. To determine if the AhR is a critical mediator of IgG expression, the current study is focused on characterizing IgG expression in another human B-cell line (SKW 6.4 or SKW WT) originating from a different, non-related Burkitt's lymphoma. We confirmed that SKW WT cells do not have endogenous expression of AhR using PCR analysis and Western blotting. We also demonstrated that SKW 6.4 cells can be stimulated in-vitro using CD40L and IL-4 to produce more IgM antibodies as detected by ELISA assays. Further, we demonstrate that total IgG secretion induced by CD40L and IL-4 stimulation is severely impaired in SKW WT cells. Conversely, the qRT-PCR studies show that the expression of ε, γ2-4 transcripts that code for IgE and IgG2-4 respectively is significantly increased with stimulation as compared to un-stimulated SKW cell lines. The expression of γ1 was low in naïve as well as stimulated SKW WT cells. The α1-2 transcripts coding for IgA1-2 respectively are not expressed at all in SKW cells regardless of stimulation. To further investigate, we used CL-01 AhRTA cells that express AhR with functional transactivation domain (TAD), to compare the expression of different isotypes. We found that the expression of γ1-4 and ε transcripts was significantly higher in AhR expressing CL-01 AhRTA cells as compared to the AhR-deficient SKW WT cells. Our observations imply that the AhR plays a critical role in the expression of the Ig heavy chain (IGH) gene.

Investigating the Interaction of PCB 126 and Ethanol in a Rodent Alcohol Model

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Fatty liver disease prevalence in the United States is on the rise and its subtypes differ in etiology but have similar pathology. Alcohol-associated Liver Disease (ALD) is characterized by triglyceride accumulation and impaired energy metabolism with possible inflammation of the liver. Pathologically, exposure to environmental toxicants have shown to induce similar outcomes but characterized as "Toxicant-associated Fatty Liver Disease". The objective of this study is to examine hepatic injury and disrupted metabolism in mice that were exposed to Polychlorinated Biphenyl 126 (PCB 126) while consuming an ethanol diet. It is hypothesized that exposure to PCB 126 followed by ethanol consumption will interact to disrupt lipid metabolism endpoints as both compounds are known to target the liver. Male C57BL/6 mice were exposed via oral gavage to 0.2 mg/kg PCB 126 or corn oil vehicle. Mice were then fed a 5% ethanol or pair fed (0% ethanol) diet for ten days followed by 31.5% ethanol binge. At euthanasia, tissues were collected for downstream analysis. Steatosis was indicated in the ethanol fed group and to a greater severity in the ethanol+PCB 126 exposed group by increased liver-to-body weight ratio, elevated hepatic triglycerides and lipid droplet formation as shown by Hematoxylin and Eosin staining. White adipose-to-body weight ratio was decreased due to PCB 126 in both pair fed and ethanol fed groups. Hepatic cholesterol was increased in pair fed mice while decreased in ethanol fed mice due to PCB 126 exposure. Plasma glucose, LDL, HDL and cholesterol were decreased due to ethanol and/or PCB 126 exposure. Plasma triglycerides and VLDL were increased due to ethanol consumption but were decreased in the ethanol+PCB 126 group, indicating an interaction effect. Luminex analysis indicated increased plasma insulin and PAI-1 due to ethanol consumption; however, insulin levels were not as high in the ethanol+PCB 126 group. qPCR displayed activation of AhR and CAR nuclear receptors by increased target gene expression due to PCB 126 or ethanol. There are millions of individuals who consume alcohol while also exposed to environmental toxicants daily and this interaction is largely unknown. Here, PCB 126 and ethanol interacted to enhance fatty liver disease endpoints by altering lipid metabolism and storage. Our investigation will continue to elucidate metabolic disruption and injury for how hepatic steatosis develops in this model.

Flavored E-cigarette Aerosols Induce Cardiopulmonary Toxicity in Mice

Cory Kucera[1,2], Anand Ramalingam[2], Dawson Stephens[1], Upasana Ghosh[2], Andrew Hodges[1,2], Aruni Bhatnagar[3], Alex P. Carll[1,2,3]

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Introduction: JUUL is a pod-based electronic cigarette (e-cigarette) with a compact design, youth-friendly flavors, and a high nicotine content. A majority of high school and middle school students who currently use e-cigarettes recently reported JUUL as their preferred e-cigarette

brand. This is alarming because nicotine can harm the developing brain, and youth who use e-cigarettes are more likely to smoke cigarettes later in life. Since JUUL owns 60% and 53% of the menthol- and tobacco-flavored markets, respectively, a thorough examination of the potential health effects of these two flavors is critical. Objective: Assess the role of flavors in the cardiopulmonary effects of JUUL using a murine model. Methods: Male C57BL/6 mice were implanted with electrocardiogram (ECG) radiotransmitters and exposed to air or JUUL aerosols from PG:VG ± nicotine (5%), JUUL Virginia Tobacco (5% nicotine), or JUUL Menthol (5% nicotine). Acute exposures (270 puffs/4.5 hours/day in ≥4-day intervals; n=4) and subacute exposures (360 puffs/6 hours/day over 4 days; n=8/group) were conducted in separate mice. Ventricular premature beats (VPBs) were quantified from continuous ECG signals throughout exposures. Pulmonary glutathione in its reduced (GSH) and oxidized (GSSG) states, and urinary 8-isoprostane, were assayed with commercial kits. Correlation analysis and one-way ANOVA were performed using GraphPad Prism, with $p < 0.05$ considered significant. Results: Only acute exposure to JUUL Menthol increased incidence of VPBs (vs. air, PG:VG, and Virginia Tobacco). Urinary 8-isoprostane levels were elevated with exposures to JUUL Virginia Tobacco and Menthol but did not reach statistical significance. Subacute exposure to JUUL Menthol decreased GSH:GSSG ratio (vs. PG:VG) concurrent with a significant decline in GSH, indicating oxidative stress. Conclusions: Menthol-flavored e-liquids may pose greater cardiopulmonary risks than other e-liquids. Human studies should be conducted to determine if menthol alters cardiac conduction and rhythmicity more than alternate flavors. If validated by human studies, our findings suggest that the FDA could implement regulatory policies targeting individual flavors to reduce toxicity from e-cigarettes.

Neurotoxic potential of titanium dioxide nanotubes in the zebrafish model system

Athira Sairanthry Suku, Parayanthala Valappil Mohanan, and Jennifer L. Freeman

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One dimensional (1D) nanomaterials comprise the most prevalent category of nanostructures with a long array of application potentials owing to their structural and functional properties. Among the large numbers of 1D nanostructures, nanotubes denote one of the most studied materials in nanoscience research; especially nanotubes formed from metal oxides possessing desirable unique features (e.g., excellent stability, tunability, surface properties, and biocompatibility). TiO2 nanotubes (TNTs) comprise one among the leading members of this category with an innumerable number of application potentials in various medical as well as non-medical scenarios. The question on the toxicity outcomes of nanomaterials in the central nervous system (CNS) has been one of the most heard among the scientific community. TNTs are applied widely for different therapeutic approaches targeting the CNS including as high-throughput electrodes for neurochemical detection (e.g., for dopamine). In view of this, one must know the toxicity limit of TNTs in the brain before being applied to any application procedures. The present study addressed the neurotoxicity of TNTs using the zebrafish model (Danio rerio). TNTs were first sonified in fish water for 40 min at room temperature and nanotubular morphology confirmed using Scanning Electron Microscope (SEM). Zebrafish were exposed to a range of TNT concentrations (0, 10, 50, 100, 500, or 1000 ppb (μg/L)) within 1 hour post fertilization (hpf) through 120 hpf. The dosing solution was renewed every 24 h after initial exposure. After the exposure period, a visual motor response test, morphological parameters, and dopamine concentrations using an ELISA were assessed. Statistical differences were determined with a repeated measures analysis of variance (ANOVA) for the behavior assay and an ANOVA for morphological and dopamine measurements (α=0.05). A noticeable alteration in behavioral outcomes were observed with significant hyperactivity for higher concentrations ($p<0.05$). Morphological features, such as head length, head width, body length, and brain length, were analyzed with comparable treatments showing significant differences ($p<0.05$). A decreased dopamine concentration was also observed at higher exposure concentrations (500 and 1000 ppb; $p<0.05$). Overall, the study is denoting neurotoxicity outcomes of TNTs, which requires further research into the toxicity mechanisms.

Comparative Toxicity Assessment of Legacy and Emerging Perfluoroalkyl Substances Using Zebrafish Model

Ola Wasel, Hanna King, Kathryn Thompson, and Jennifer L. Freeman

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Perfluoroalkyl substances (PFAS) are synthetic compounds that are composed of a fluorinated carbon chain. PFAS are persistent in environment and bioaccumulate in organisms. Exposure to perfluorooctanoic acid (PFOA, C8) and perfluorooctane sulfonate (PFOS, C8) has been linked with multiple adverse health effects. Shorter chain chemicals (such as perfluorobutyrate (PFBA, C4) and perfluorobutane sulfonate (PFBS, C4)) and compounds with chemical modifications (such as GenX, C6) were used as a replacement to the long chain PFAS in order to increase their degradation potential. PFAS were detected in breast milk and cord blood, therefore, there is potential developmental exposure to PFAS. PFAS exposure induced developmental neurotoxicity in animal studies, but majority of these studies focused on PFOA and PFOS. There is limited information on the developmental neurotoxicity of the emerging PFAS. In this study, we compared toxicity of five PFAS in order to assess the role of chain length, functional group and chemical structure in their toxicity. We compared the toxicity of PFOS, PFOA, PFBS, PFBA and GenX using zebrafish (Danio rerio). To determine LC50 of each chemical, zebrafish embryos were exposed to a range of concentrations of each chemical within 1-hour post fertilization (hpf) through 120 hpf. The toxicity of these compounds was assessed by monitoring the survivability every 24 hours through 120 hpf. 120hpf-LC50 were determined using GraphPad 8.0 software. In addition, behavioral analysis using a visual motor response test was performed. For behavioral analysis, we used concentrations of 0, 4, 40, 400, and 4000 part per billion (ppb). The exposure was terminated at 72 hpf and the test was done at 120 hpf. Results of 120hpf-LC50s that toxicity ranking was PFOS> PFOA> PFBS> GenX> PFBA. Results showed that toxicity increases with increasing the chain length. Also, presence of sulfonate group increased toxicity for PFAS of a given chain length. Behavioral analysis showed that embryonic exposure to PFOS, PFBS, PFBA or GenX induced changes in the locomotor activities in larvae, while PFOA didn't cause any changes. Future work will focus on identifying the mechanism behind the observed behavioral changes.

Tox on the Clock Presenters

Combining ethanol + thermal burn injury results in elevated microvesicle particle production via dramatic production of the lipid mediator Platelet-activating factor

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Ethanol, in combination with thermal burn injury, is a clinically significant problem resulting in an increase in morbidity and mortality due to acute multi-organ toxicity from bacterial translocation permeating from the gut. This intoxicated thermal burn affects close to 50% of admitted burn patients and currently has no standard treatment. However, using in vitro cellular and murine models, our group has provided data implicating the augmented production of the lipid mediator Platelet-activating factor (PAF) in keratinocytes in response to intoxicated thermal burn injury. Our group has also demonstrated that activation of the keratinocyte PAF receptor (PAFR) results in the generation of subcellular microvesicle particles (MVP) which carry and thus serve to protect this labile lipid mediator PAF. The goal of the current studies is to define the exact mechanisms by which EtOH + burn injury generates augmented PAF production, and the role of MVP in this process. Using the human keratinocyte-derived cell line HaCaT, we demonstrate that EtOH and burn injuries alone can generate MVP and synergize MVP release when in combination. To study the role of PAF signaling, we used siRNA knock-down approaches of the PAFR in HaCaT cells in conjunction with EtOH + burn studies, to indicate that burn injury-induced MVP release is PAFR-dependent. Moreover, use of the cytosolic phospholipase A2 (cPLA2) inhibitor pyrrophenone, which blocks enzymatic PAF synthesis, also hinders the exaggerated MVP formation in response to EtOH + thermal burn injury. These studies fit with a novel model whereby EtOH + thermal burn injury generates increased PAF via cPLA2 activation, which then generates high levels of PAF-laden MVP which are in part responsible for the subsequent pathology of intoxicated thermal burn injury.

Assessing Adult Learning and Memory in Three Genotypes of Mice Exposed to Benzo[a]Pyrene During Early Brain Development

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Benzo[a]pyrene (BaP) is a polycyclic aromatic hydrocarbon most commonly found in traffic-related air pollution. BaP is linked to learning deficits and neurodevelopmental delays in human and animal studies. We are using a mouse model to determine if genetic differences increase susceptibility to BaP exposure during early brain development. We are specifically interested in the the aryl hydrocarbon receptor which is a ligand-activated transcription factor that moves to the nucleus to upregulate transcription of the CYP1 genes. Mice with variations in the aryl hydrocarbon receptor, lacking the CYP1A2 metabolic enzyme, mice lacking the CYP1A1 metabolic enzyme, and wild type control mice were exposed to 10mg/kg/day BaP from gestational day 10 (GD10) through weaning at postnatal day 25 (P25). One male and one female per litter were randomly selected for neurobehavioral testing. A battery of cognitive and motor function tests were performed when the mice reached early adulthood (P60). We used Novel Object Recognition and three increasingly difficult phases (smaller platforms in new locations) in Morris Water Maze to assess hippocampal dependent non-spatial and spatial learning and memory. There was a trend for significance in the Novel Object Recognition test with BaP exposed mice spending less time observing the novel object. In the Acquisition Phase of Morris Water Maze, there was a significant gene x treatment interaction on two days of testing and a trend toward significance on two other days with BaP-treated *AhrbCyp1a2(-/-)* knockouts having longer path lengths compared to corn oil controls. In the Reverse phase, there was a significant main effect of treatment on four days of testing with BaP-treated mice having longer average distances to the escape platform and a significant sex x treatment interaction on two days of testing with BaP-treated males having longer path lengths compared to corn oil-treated control males. The opposite trend was seen in females. In the Probe trials, BaP-treated mice had significantly longer average distances to the platform in the Acquisition and Reverse phases ($P < 0.05$) and a trend for significance in the Shift-reduced phase ($P = 0.68$). Our indicate that all genotypes are affected by developmental BaP exposure with *AhrbCyp1a2(-/-)* knockouts appeared most susceptible.

Neurotransmitter Differences Following Developmental Benzo[a]pyrene Exposure in *Cyp1a1* Knockout and Wild Type Mice

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Benzo[a]pyrene, a polycyclic aromatic hydrocarbon (PAH) commonly used to model traffic-related air pollution (TRAP), has neurotoxic effects that are particularly harmful during early brain development. Human and animal studies have linked prenatal TRAP exposure with neurobehavioral and neurochemical changes persisting into childhood and adolescence. Our previous studies indicate that genetic differences in the aryl hydrocarbon receptor (AHR) pathway affect susceptibility. The AHR regulates enzymes involved in BaP metabolism, including genes in the cytochrome P450 (CYP1) family. Our behavioral studies found that *Cyp1a1(-/-)* knockout mice were more susceptible to developmental BaP exposure compared with wild type *Cyp1a1(+/+)* mice. In this study, we measured neurotransmitter levels in multiple brain regions of adult offspring following behavioral testing. Pregnant dams were treated with 10mg/kg/day BaP in corn oil-soaked cereal or the corn oil vehicle from gestational day 10 to postnatal day 25. One male and one female per litter were randomly selected for behavioral testing. Around postnatal day 120, we collected striatum, hippocampus, prefrontal cortex, and hypothalamus. Dopamine (DA), serotonin (5HT) and their metabolites DOPAC and 5HIAA were measured using High-Performance Liquid Chromatography with Electrochemical Detection. While multiple main effects of genotype, treatment, and sex were found across all brain regions, the most compelling results came from the hippocampus and hypothalamus. In the hippocampus, there was a significant main effect of genotype with knockout mice having higher DOPAC levels ($P < 0.001$) and dopamine turnover levels ($P < 0.05$) compared to controls. Knockout mice had significantly lower 5HIAA levels ($P < 0.001$) and a trend for lower 5HT levels ($P = 0.055$). In the hypothalamus, there was a significant main effect of sex with females having higher levels of DA, DOPAC, 5HT, and 5HIAA compared to males ($P < 0.05$). There was also a significant gene x treatment x sex interaction for DOPAC levels ($P < 0.05$)

and a trend for a gene x treatment x sex interaction for 5HIAA levels (P = 0.07). These results suggest that genotype and sex may have the greatest influence on neurotransmitter difference and that both genotype and sex affect susceptibility to developmental BaP exposure.

N-acetyltransferase 1 (NAT1) allele *NAT1*14B* phenotype is substrate-dependent

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N-acetyltransferase 1 (NAT1) is phase II metabolizing enzyme that plays an important role in acetylation of many drugs and carcinogens. NAT1 has many genetic polymorphisms which predict acetylator phenotype. Previous studies reported that some adverse drug reactions and cancer susceptibilities are associated with NAT1 genetic polymorphisms. However, variation in activity among the different allelic variants has not been fully examined. In our study, we investigated the role of NAT1 genetic polymorphism on the N- acetylation of p-aminobenzoic acid (PABA), benzidine an aromatic amine carcinogen and 3,4-dimethyllaniline (3,4-DMA) an alkylaniline carcinogen. We used Chinese hamster ovary (CHO) cells that were stably transfected with either human *NAT1*4* (the reference allele) or *NAT1*14B* (the variant allele). In vitro *N*-acetylation rates of PABA, benzidine and 3,4-DMA were carried out at a range of substrate concentrations (31.3 to 500 μM) using acetyl CoA (AcCoA) 300 μM as a cofactor. PABA, benzidine, 3,4-DMA and their *N*-acetyl products were separated and measured by high performance liquid chromatography (HPLC). Statistical analysis was done using two-way ANOVA and student t-test. Michaelis-Menten model was used for determination of kinetic constants. Our results revealed that *NAT1*4* had higher PABA and 3,4-DMA N-acetylation rates compared to *NAT1*14B* ($p < 0.001$). On the other hand, *NAT1*14B* showed higher N-acetylation rate compared to *NAT1*4* towards benzidine ($p < 0.01$) although it is identified as a slow acetylator allele. Significant differences between *NAT1*4* and *NAT1*14B* towards PABA or benzidine apparent Km were not observed. On the other hand, *NAT1*14B* had greater affinity towards 3,4-DMA compared to *NAT1*4* reflected by lower apparent K_m value ($p < 0.05$). V_{max}/K_m ratio that reflects the intrinsic clearance showed different patterns for both carcinogens. *NAT1*4* had lower V_{max}/K_m for benzidine ($p < 0.05$) but higher V_{max}/K_m for 3,4-DMA ($p < 0.01$) compared to *NAT1*14B*. These results suggest that *NAT1*14B* phenotype is substrate dependent which should be incorporated in cancer risk studies for precise determination of the individual risk. Further studies will be done to determine the effect of NAT1 allelic variants on benzidine and 3,4-DMA induced genotoxicity. Research was supported by NIH grants P20-GM113226, P42-ES023716 and P30-ES030283.

Using the zebrafish to elucidate developmental neurotoxicity after a binary arsenic and lead mixture exposure

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Arsenic (As) and lead (Pb) are environmental pollutants found in common sites and linked to adverse health effects. This study evaluated As and Pb to determine if developmental toxicity significantly changes at lethal and sub-lethal mixture concentrations using the zebrafish model. Joint action models were applied to survival data to determine the type of interaction. Metal exposures were from 1 -120 hours post fertilization (hpf). As concentrations were 0–10E6 μM. Pb concentrations were 0–480 μM. The LC_{25}, LC_{50}, and LC_{75} values at 120 hpf from single metal exposures were used to select mixture concentrations for modeling. The survival data indicated an additive effect occurred at lethal concentrations. The impact of the mixture on behavior, morphology, and gene expression were evaluated at sub-lethal concentrations of 10 and 100 ppb As (0.133, 1.33 μM) and Pb (0.048, 0.48 μM) individually or in mixtures. Data was analyzed with a repeated measures ANOVA (behavior) or an ANOVA (morphology and qPCR) with the least significant difference test (α=0.05). Zebrafish larvae exposed to 10 ppb As exhibited hyperactivity in all dark phases for the distance moved, time moving, and velocity, while those exposed to 10 ppb Pb only showed an increase in distance moved and velocity in the first dark phase. The 10 ppb mixture was found to have an intermediate impact with increased time moving in all dark phases and increased distance moved and velocity only in the first dark phase. In contrast, hyperactivity was observed only in the 100 ppb mixture in the last two dark phases for time moving and in the last dark phase for the distance moved. No significant behavioral alterations occurred in the single 100 ppb treatments. A decrease in mean brain length and brain length ratio to the total length in the 10 ppb mixture was observed with no significant morphology changes observed for head length, head width, or total length. Four genes related to the development of cerebral blood

vessels were assessed and included: *vegfaa*, *cldn5a*, *cldn5b*, and *wnt7aa*. The 100 ppb As and 100 ppb mixture treatment groups showed a significant decrease in *vegfaa* expression and significantly increased *cldn5b* expression. Expression of both claudins (*cldn5a* and *cldn5b*) significantly increased in the 10 ppb Pb group. *wnt7aa* expression significantly decreased in the 10 ppb Pb group. Overall, the 10 ppb Pb treatment group showed significant and unique alterations in gene expression. Overall, there are many significant changes at lower concentrations in single and mixture treatments compared to higher concentrations indicating a nonlinear exposure-response.

Particulate Hexavalent Chromium Inhibits Homologous Recombination Repair in Rat Lung

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Particulate hexavalent chromium [Cr(VI)] is a well-established human lung carcinogen, but the mechanism for Cr(VI)-induced cancer is uncertain. Chromosome instability (CIN) is a hallmark of lung cancer and is considered a driving event in Cr(VI)-induced lung cancer. Studies in cultured human lung cells show Cr(VI) induces DNA double strand breaks that occur in late S and G2 phases in the cell cycle when homologous recombination repair (HR) is the dominant repair pathway, and, indeed, data show HR repair is essential to prevent Cr(VI)-induced CIN. In addition, showing Cr(VI) induces DNA double strand breaks, lung cell culture studies also show Cr(VI) simultaneously inhibits the repair of those breaks by targeting RAD51 in the HR repair pathway resulting in CIN. However, the observations that Cr(VI) induces DNA double strand breaks and targets RAD51 to inhibit HR repair have only been reported in cell culture. No studies have evaluated these outcomes in lung tissue. We decided to translate these outcomes to rats as this species has been shown to be susceptible to developing Cr(VI)-induced lung tumors. Accordingly, we exposed 12-week old male and female Wistar rats to zinc chromate particles in a saline solution or saline alone by oropharyngeal aspiration. We conducted the exposures once a week for 90 days. We measured Cr levels in the lung tissue by inductively coupled plasma mass spectrometry (ICP-MS) and decided to focus our efforts on the middle lobe of the right lung. We found these outcomes do indeed translate from human lung cells as we found zinc chromate induced DNA double strand breaks (measured as gamma-H2AX foci formation) and targeted RAD51 to inhibit HR repair (measured as RAD51 foci formation). Notably, these effects were distinct in the bronchioles of the lung and more muted in the alveolar region, consistent with observations of Cr(VI)-induced lung tumors in humans. This work was supported by the National Institute of Environmental Health Sciences [R01ES016893 and R35ES032876 to JPWSr.].

Securin Disruption and Chromosome Instability Persist After Chronic Hexavalent Chromium Exposure

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Hexavalent chromium [Cr(VI)] is a potent lung carcinogen with widespread environmental and occupational exposure risks, however, its carcinogenic mechanisms are not fully understood. A key effect of Cr(VI) exposure is chromosome instability (CIN) including changes in chromosome number, which may be explained by its ability to induce centrosome amplification. Numerical CIN and centrosome amplification are characteristics of lung tumors and phenotypes induced in human lung cells by prolonged (>24 h) exposure to Cr(VI). However, little is known about how chronic Cr(VI) exposure leads to carcinogenesis. Using clonal cell lines derived from 180-day Cr(VI) exposure we determined effects that persist long after exposure has ended. Our hypothesis is Cr(VI) disrupts securin, a key centrosome regulator, leading to centrosome amplification, which drives numerical CIN. Human lung cells were exposed to acute (24 h) and prolonged (120 h) Cr(VI) concentrations of 0.1, 0.2, and 0.3 ug/cm2. After 120 h exposure, particulate Cr(VI) induced numerical CIN and supernumerary centrosomes. Securin protein and mRNA levels were measured by western blot and RT-qPCR, respectively. Cr(VI) caused loss of both securin protein and mRNA after 120 h exposure at all concentrations. To test the persistence of effects after chronic exposure, human lung cells were

exposed to Cr(VI) continuously for 180 days at concentrations of 0.0125, 0.025, and 0.05 ug/cm2. Untreated, passage-matched cells served as controls. Treatment was removed and cells were seeded at colony-forming density. Colonies were isolated and expanded, without any further treatment. Cell lines were characterized for securin protein levels, mRNA levels and karyotypes. In cell lines exposed to 180 days Cr(VI), disruption of securin protein and mRNA persisted and these cell lines displayed striking numerical chromosome abnormalities. These data support the conclusion that Cr(VI)-induced centrosome amplification may be due to disruption of securin expression, which leads to centrosome amplification. Ongoing studies will quantify securin levels after in vivo Cr(VI) exposure in rats and in human lung tumors. This work was supported by NIEHS grant R01ES016893 and R35ES032876 (JPW) and T32ES011564 (JHT & JPW).

Modulation of Pulmonary Toxicity in Metabolic Syndrome (MetS) due to Variations in Nanoparticle-Biocorona Composition

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Following introduction into a biological environment, nanoparticles (NPs) interact with biomolecules forming a biocorona (BC) which alters cell interactions and toxicity. Metabolic syndrome (MetS) is a prevalent condition and enhances susceptibility to inhaled exposures. We hypothesize distinct NP-biomolecular interactions occur in the lungs due to MetS forming unique NP-BCs mediating enhanced toxicity. Bronchoalveolar lavage fluid (BALF) was collected from C57BL/6J mice receiving either a healthy diet or a high-fat western diet for 16 weeks. BCs were formed by incubating 20 nm iron oxide (Fe_3O_4) NPs in collected BALF for 8h at 4 °C. Fe_3O_4 NPs without or with BCs were characterized for hydrodynamic size and zeta potential. Protein and lipid components of the BCs were evaluated via a proteomic/lipidomic approach. This assessment demonstrated the association of unique biomolecules and differential abundance of shared components. Specifically, 35 common proteins bound to Fe_3O_4 NPs in healthy and MetS BALF. There were 11 and 93 unique proteins associated with the healthy-BC and MetS-BC, respectively. Proteins including serine protease inhibitor A3N, vitronectin, clusterin, complement C3, gelsolin, apolipoprotein A, and others were increased in the MetS-BC compared to the healthy BC. Increased abundance of lipids such as PC(32:1), DG16:0_16:1, PG(32:1), DG 16:1_16:0, and others was also determined in the MetS BC. A mouse macrophage cell line was utilized to examine differential toxicity due to BCs. Exposures to 6.25, 12.5, 25, and 50 µg/ml of Fe_3O_4 NPs with BCs for 1 or 24h did not demonstrate overt cytotoxicity. Darkfield microscopy and X-ray fluorescence (XRF) analyzer determined enhanced Fe_3O_4 NP internalization due to the MetS BC compared to healthy. Additionally, 1h or 24h exposure to Fe_3O_4 NPs with a MetS-BC at a concentration of 25 µg/mL enhanced gene expression of inflammatory markers: *CCL2*, *IL6*, and *IL-1β* compared to Fe_3O_4 NPs with a healthy BC. Inflammatory pathways were examined by western blots to determine activation of specific proteins within the MAP kinase, Jak/Stat, and NF- κB signaling pathways. Jak/Stat pathway was determined to be the most upregulated inflammatory pathway due to the MetS-BC. In conclusion, our assessment suggests that the formation of distinct NP-BCs occurs following inhalation of particles in MetS, which contributes to exacerbated inflammatory effects and susceptibility.

Poster Gallery Presenters

Parentally Exposed Zebrafish Larvae Have Altered Craniofacial Measurements

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Atrazine is a herbicide used throughout the Midwest US to prevent broadleaf weeds in crops. The US EPA has set the maximum contaminant level at 3 ppb (µg/L) in drinking water. Atrazine (ATZ) is an endocrine disrupter interfering with the function of hormones and disrupting normal physiology and homeostasis throughout development and the life course of an organism. The zebrafish model was used to test the hypothesis that an embryonic parental ATZ exposure will cause modifications in morphology in developing offspring. AB adult zebrafish were bred. Their embryos were collected and exposed to ATZ concentrations of 0 ppb, 0.3 ppb, 3 ppb, or 30 ppb from 1-72 hours post fertilization (hpf; the end of embryogenesis). ATZ exposure was ceased at 72 hpf and larvae were grown into adulthood in aquaria water (ATZ F0). ATZ F0 adult zebrafish were then bred within their treatment group, their embryos were

collected, and placed in petri dishes in aquaria water until 120 hpf. At 120 hpf, larvae were collected for morphological analysis including general morphology measurements and co-staining with alcian blue and alizarin red for cartilage and skeletal assessments. Head length and ratio of head length to total length was significantly increased in the F1 of 0.3 and 30 ppb ATZ groups ($p<0.05$). Additional craniofacial morphology was completed with a decreased distance for cartilaginous structures, decreased surface area and distance between saccular otoliths, and a more posteriorly positioned notochord ($p< 0.05$). The posteriorly positioned notochord indicates delayed ossification and skeletal growth. These findings signify that a single embryonic parental exposure leads to changes in craniofacial development in their offspring.

Genome Wide Characterization of a CRISPR/Cas9-Edited B Lymphocyte Cell Line

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CRISPR/Cas9 gene editing has allowed for precise genomic editing in cell lines, tissues, and animals. CRISPR/Cas9 editing has high fidelity and precision when inducing double strand breaks in the genome. However, it is always important to fully characterize the edits that were induced. Off target editing is also a potential risk when using guidance RNA-mediated CRISPR/Cas9 editing due to abundance of protospacer adjacent motifs (PAMs) in non-bacterial genomes. Detection of potential off targeting should be considered as a best practice when generating genome edited models. We previously generated a cell line variant with a monoallelic edit in the transactivation domain of the aryl hydrocarbon receptor (AhR) gene. To fully understand the edit induced and potential off-target edits, we first characterized the parental cell line with high throughput whole genome sequencing (WGS) via whole genome Illumina NextSeq paired end sequencing (HudsonAlpha). We obtained a 32x coverage of the genome. The reads were preprocessed and then analyzed with the Broad Institutes GATK best practices guidelines and custom scripts developed by our lab. Genomic variants, such as insertions/deletions (INDELs) and single nucleotide variations (SNPs), were called as compared to the GRCh38 human reference and annotated using the ensembl variant effect predictor (VEP). Similarly, our AhR edited line was also sequenced using the NextSeq platform and achieved 31x coverage. In parallel with the parental cell line analysis, the AhR-edited variant was called and annotated. Genomic variations were compared by location and cross compared with predicted off-target editing. Here we show a relatively low variation between parental cell line and the AhR-edited variant, especially in the context of predicted off target. These results demonstrate the precision and accuracy of CRISPR/Cas9 editing within our cell line. The results also provided a high quality reference for our cell lines and the exact targeted edits induced. The above pipeline also provides a robust and reproducible framework for WGS analysis and CRISPR/Cas9 off target detection.

Campus wastewater surveillance enables early detection of coronaviruses before an outbreak

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Coronavirus infects the gastrointestinal epithelia in humans, leading to virus shedding in feces. This allows for the detection of coronavirus genome fragments in wastewater prior to an outbreak. In this study, we tracked SARS-CoV-2 genome in dormitory wastewater at five locations within Wright State University from January 2021, as an early indicator of COVID-19 spread on our campuses. Using a portable autosampler, wastewater was collected twice weekly at each location. RNA was then extracted from the samples, followed by the detection of SARS-CoV-2 genome using RT-qPCR. Our data showed a correlation between the detection of coronavirus genomes in wastewater samples and the positive cases of COVID-19 reported in the dormitory. Additionally, there was an increase in SARS-CoV-2 positive wastewater samples when students returned to campus. We shall continue to monitor wastewater at both Dayton and Lake campuses for the next year to inform student COVID-19 testing and University response efforts.

Analysis of Metabolic Profiles to Detect Changes in the Immune Function of B cells

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The aryl hydrocarbon receptor (AhR) is a transcription factor that is activated by ligands such as TCDD, and other compounds such as aromatic hydrocarbons, indoles, and

6-Formylindolo[3,2-b]carbazole (FICZ). AhR is responsible for regulating the metabolism of xenobiotic substances and is expressed by cells responsible for immunity. TCDD is minimally metabolized by AhR and is a sensitive target of antibody production. AhR can bind many chemicals, however, it is uncertain if the antibody response would be inhibited by nonpersistent AhR ligands. The objective of this study is to determine the metabolic profile of cellular changes when AhR ligands are introduced are ligand specific. Literature searches of metabolic studies have demonstrated that a decrease in B-cell antigen receptors in Burkitt's Lymphoma resulted in a decrease in mitochondrial function with weakened metabolic flexibility. In a separate study, a 24 hour treatment exposure of TCDD to egg embryos demonstrated glycolytic changes in the thymus and the liver of the embryo. The Agilent Seahorse can measure the oxygen consumption rate and extracellular acidification rate in the mitochondria to determine cellular metabolism.. This capability is significant, because real time analysis will provide a metabolic profile of the interaction of AhR ligands and B cells or future consideration in alternative treatment possibilities.

Exploiting the Efficacy of Metformin as a Drug Reposing for Non-Small Cell Lung Cancer Treatment

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Lung cancer is one of the most commonly occurring malignancies in both men and women, and is responsible for 25% cancer-related deaths. Of major types, non-small cell lung cancer (NSCLC) is the highly aggressive and the most prevalent subtype with a poor prognosis. Despite having FDA approved treatment options, the survival rates remained relatively low, therefore new approaches have been explored to achieve improved therapeutic responses. To that end, repurposed drugs such as metformin has been evaluated against many cancer types, including NSCLC. Metformin has been widely used for the treatment of type-2 diabetes and also found to exhibit anti-cancer properties due to its ability to target several oncogenic signalling pathways, including repression of miR-381-YAP-Snail axis activity, AMP-activated protein kinase (AMPK-dependant and AMPK-Independent) and inhibition of mammalian target of rapamycin (mTOR). Metformin has also been tested with several anti-cancer agents and found to synergize their effectiveness resulting in increased therapeutic outcomes. Here, we highlight the anti-cancer mechanisms of metformin in various in vitro and in vivo cancer models as well as its translational relevance for the treatment of NSCLC.

Exposing Mice to Benzo[a]Pyrene During Early Brain Development to Analyze Depressive-like Behavior and Stress Hormones

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Benzo[a]pyrene (BaP) is a carcinogenic pollutant found in cigarette smoke, grilled food, and vehicle exhaust. It belongs to a group of pollutants known as polycyclic aromatic hydrocarbons. In human and animal studies, BaP exposure is associated with impaired learning, memory, and motor skills. Interestingly, BaP is also associated with changes in serotonin and anxiety. We used a mouse model to determine if genetic differences in the enzymes required to metabolize BaP would affect the response to developmental BaP exposure. Mice were exposed to BaP starting from gestational day 10 until postnatal day 25- equivalent to the second and third trimesters in humans when brain development is at its peak. We used a forced swim test to analyze their depressive-like behavior and measured their stress hormone levels at baseline and immediately after the swim test to determine if genetic makeup affected their response to a stressful stimulus. BaP exposure showed no effect, but genotype had a significant difference, with the AhrdCyp1a2(-/-) mice having a longer float time compared to the AhrbCyp1a2(+/+) and AhrbCyp1a1(-/-) mice. Analyzing the stress hormone levels indicated that the AhrbCyp1a2(+/+) had higher cortisol levels but this was not statistically significant. The data appears to indicate that the low affinity mice were not able to properly metabolize the BaP; consequently, causing them to show more depressive signs and lower stress levels.

Melatonin as a Drug Repurposing in the Treatment of Melanoma

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Melanoma is the most aggressive form of skin cancer with high metastatic potential and an increased prevalence and mortality rates. This cancer type has been shown to be resistant to currently ongoing treatment options. Among new approaches, drug repurposing has been explored as a

potential therapeutic option. To that end, recent studies have suggested that melatonin produced by the pineal gland exerts anti-proliferative and oncostatic effects in various in vitro and in vivo models of melanoma. Mechanistically, melatonin-induced anticarcinogenic activity is due to its ability to target various oncogenic signaling pathways, including heat shock proteins (HSPs), P13/Akt/mtor and inflammatory pathways such as tumor necrosis factor α (TNF α), Cyclooxygenase related factors resulting in the induction of apoptosis. Additionally, preclinical studies show melatonin in combination with chemotherapeutic agents has synergistic outcomes in melanoma models. Our goal is to highlight mechanistic insights of melatonin as a combinational therapy or monotherapy for melanoma treatment.

Cigarette Smoke-induced Platelet Activation: Acute Effect of Crotonaldehyde

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Conventional cigarette smoking remains the largest risk factor for cardiovascular disease. Research shows exposure to mainstream cigarette smoke (MCS) induces platelet-leukocyte aggregation in vivo, a biomarker of platelet activation and thrombosis, via damaging blood vessels, and thus, increasing the risk of blood clots that lead to myocardial infarctions and/or stroke. Our lab has published that acute exposure of mice to crotonaldehyde (CR), an α,β-unsaturated aldehyde and a harmful or potentially harmful constituent (HPHC) in MCS, causes changes in platelet-leukocyte aggregate (PLA) formation, indicating CR may contribute to MCS effects. Yet, the mechanism by which CR affects PLAs is not well understood. The purpose of this research was to compare MCS- and CR-induced PLA formation by examining PLA composition as including platelet-granulocyte aggregates (PGAs) and platelet-lymphocyte:monocyte aggregates (PLyMAs) via flow cytometry. Adult male C57BL/6 mice were exposed (6h/d, 4d) to filtered air (control), MCS (3R4F; 12 cigs/d, 4d), or CR (1 & 3 ppm, 6h/d,4d). After final exposure, mice were euthanized and PLA levels were measured in peripheral blood by flow cytometry as the number of double positive aggregates ($CD41^+$, platelet marker, and $CD45^+$, pan-leukocyte marker). IgG isotype negative controls were used to guide gating. Exposures induced significant changes in PLA levels in mice following MCS (2.0±0.8% vs. 3.7±1.0%), 1 ppm CR (6.0±1.0% vs. 3.0±0.0%), and 3 ppm CR (6.0±1.0% vs.12.0±2.0%) exposures compared with their respective air controls. In the MCS study, the PGAs on average (65.8%±11.0% of PLAs) made up a greater % of PLAs than the PLyMAs (34.2%±11.0% of PLAs). In the 1 ppm CR study, the PGAs on average (70.4%±13.5% of PLAs) made up a greater % of PLAs than the PLyMAs (29.6%±13.5% of PLAs). In the 3 ppm CR study, the PGAs on average made up a greater % of PLAs (62.9%±11.4% of PLAs) than the PLyMAs (37.1%±11.4% of PLAs). In each study, the proportions of PGAs and PLyMAs were unchanged by toxicant exposures. With PLAs increasing in both MCS and 3 ppm CR exposures, these findings suggest that high levels of CR, as present in MCS, can increase thrombotic risk and that tobacco-derived aerosols influence thrombotic activation. More studies are needed in order to determine their influence on sensitizing granulocyte, lymphocyte, and monocyte activation. This research will be of use to the FDA in regulating the levels of HPHCs in tobacco product aerosols, as the levels of CR should be less than 3 ppm. Our research will also aid in understanding the potential mechanisms of MCS- and CR-induced thrombogenesis by highlighting the sensitivity of leukocyte activation.

Metabolism and genotoxicity of new psychoactive substances (NPS) and 4,4'-oxydianiline (ODA) is modified by N-acetyltransferase 2 genetic polymorphism

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The use of new psychoactive substances (NPS) as drugs of abuse is common and increasingly popular, particularly among youth and neglected communities. Similarly, hookah smoking is increasingly popular; where 4,4'-oxydianiline (ODA), a carcinogenic aromatic amine, is one of the main components in the smoke. Recent studies have reported toxic effects from these chemicals; however, their long-term toxicity is unknown. It is likely that genetic differences between individuals affect the toxicity risk. Arylamine N-acetyltransferase capacity differs among individuals due to genetic inheritance. Our goal is to investigate the gene-environment interaction between NAT2 polymorphism and toxicity after exposure to these chemicals. We compared N-acetylation by

human NAT1 and NAT2, found that N-acetylation of NPS and ODA is carried out exclusively by NAT2. Differences in N-acetylation between NAT2*4 (reference allele) and NAT2*5B (common variant allele) were highly significant ($p<0.0001$). Using genetically engineered CHO cells, expressing CYP1A2 and either NAT2*4 or NAT2*5B, we tested the induction of DNA double-strand breaks (γH2Ax); cells were exposed to increasing concentrations of NPS including 3,4-methylenedioxymethamphetamine (MDMA), dimethocaine (DMC), mescaline (TMPEA), metaphedrone (3MMC), 2,5-Dimethoxy-4-ethylthiophenethylamine (2C-T2) or ODA. The induction of γH2Ax showed a NAT2 allele dependent response, being higher in the NAT2*4 vs NAT2*5B alleles ($p<0.05$). Finally, induction of oxidative stress (ROS/RNS) was evaluated; we observed NAT2 allele dependent response for all compounds in concentrations as low as 10 μM, where NAT2*4 showed increased ROS/RNS vs NAT2*5B ($p<0.05$). In summary, these results provide evidence that ODA and NPS are N-acetylated by NAT2, and such metabolic reaction is dependent on NAT2 genotype. Furthermore, our results provide evidence that exposure to psychoactive chemicals results in NAT2 genotype dependent genotoxic and oxidative damage. Research reported in this publication/presentation was supported by the National Institute of Environmental Health Sciences of the National Institutes of Health under Award Number P30ES030283. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Exploring the effectiveness of aspirin as drug repurposing against pancreatic cancer

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Pancreatic cancer currently represent the seventh leading cause of cancer related deaths worldwide. It is often diagnosed at advanced stages, and associated with poor prognosis and low 5-year survival rates of approximately 10% for all the stages combined. Despite currently available treatment options, pancreatic cancer remained difficult to treat malignancy and is often resistant to various chemotherapeutic drugs. Therefore, there is an urgent need of new treatment approaches such as adjuvant therapy that can improve the efficacy of therapeutic agents. Aspirin (also known as acetylsalicylic acid) has traditionally been used as one of the anti-inflammatory drugs which exerts its therapeutic effect by inhibiting cyclooxygenase (COX) and thromboxane dependent inflammatory pathways. Since many tumour types, including pancreatic cancer exhibit increased COX-2 expression, treatment with aspirin has been shown to reduce tumor burden via its ability to target various oncogenic signalling pathways, including nuclear factor-kappa B (NF-kB). Importantly, aspirin has also been evaluated in combination with several chemotherapeutic agents and radiation therapy, and found to increase their therapeutic efficacy against pancreatic cancer. This article highlights the mechanistic insights of aspirin as drug repurposing as well as its synergy with other therapeutic agents in various in vitro and in vivo models and clinical studies.

Developmental toxicity testing of acidic environmental chemical pollutants in aquatic systems: Are reported findings based on direct chemical toxicity or indirect changes in pH?

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With the vast commercial production of various chemicals, there is a need to investigate and identify the toxicity profile for each of these chemicals, especially those with a high risk for environmental contamination, to identify the potential adverse ecological and human health risks. The basis for this project was to determine the direct and indirect effects of an acidic pesticide, glyphosate, which is an active ingredient in the herbicide, Roundup. Previous studies have reported various physiological and genotypic deviations from normalcy, but the effect observed has not been verified as a direct contribution of the chemical in question or because of environmental alterations caused by the chemical, causing indirect effects. Glyphosate, being an acidic environmental pollutant, at high concentrations can alter the pH of a solution to very acidic levels depending on buffering capacity. Hence, toxicity observed in aquatic studies could be because of the chemical or the acidic pH. While it is assumed that most toxicity studies would adjust the solution to a neutral pH, when reviewing the zebrafish glyphosate toxicity studies, it was identified that most papers in the last decade have not mentioned pH or neutralizing of the test solution, leading to the question,

are the observed effects due to the acidic pH or the chemical. In this study, we conducted a pH toxicity curve for developing zebrafish embryos through 120 hours post fertilization (hpf) and compared the toxicity curves for unadjusted pH and adjusted pH for technical grade glyphosate to the pH curve. These results were then compared with recent glyphosate toxicity studies using the zebrafish to address the research question. Results showed that at concentrations greater than 10 ppm (mg/L) glyphosate, the pH of the fish water used in this study decreased to 5.5. As the glyphosate concentration increased, the pH continued to decrease as low as 2.98. To further check the influence of pH on glyphosate toxicity, LC50 curves were completed for unneutralized and neutralized solutions for zebrafish exposed from 1 through 120 hpf. The LC50 for 120 hpf zebrafish larvae without neutralization was close to 50 ppm, while the neutralized solutions for the same duration was greater than 1000 ppm. These findings were then compared to other zebrafish glyphosate toxicity studies to check if the pH of the testing solution was mentioned and only a handful of studies was found to mention this detail. Next, concentrations that were used and findings of these published studies were compared to the current study to determine if toxicity was due to glyphosate or acidic pH of the test solution. A large number of the studies were found to report toxicity based on acidic pH of solution only and not glyphosate toxicity. Overall, this study implies the need for proper quality control and reporting of neutralized solutions in toxicity assessments of acidic chemicals using aquatic models to accurately reflect direct effects of the chemical in question.

Analysis of Neuroendocrine Molecular Targets Following an Embryonic Atrazine Exposure in Zebrafish

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Atrazine is an herbicide used to control broadleaf and grassy weeds on agricultural fields in the US but this herbicide has been banned from use in the European Union since 2003, based mainly on risk of contamination of surface and groundwater. Atrazine is categorized as an endocrine disrupting chemical (EDC), altering release of luteinizing hormone from the pituitary through gonadotropin-releasing hormone in the hypothalamus. The specific mechanism that leads to this disruption is not yet clearly defined. In this study, molecular targets within the neuroendocrine system are being explored to assist in elucidating a mechanism that coincides with the observed adverse health outcomes along the endocrine axes. Using the zebrafish model, gene expression of multiple hypothalamic and pituitary targets were examined to determine if an embryonic atrazine exposure perturbed neuroendocrine development. Gene targets were chosen based on association with the neuroendocrine hormones that have been reported to be adversely affected following atrazine exposure in many models at various atrazine exposures. Adult wild type zebrafish were bred to attain embryos. Embryos were collected at 1 hour post fertilization (1 hpf) and randomly assigned to 0, 0.3, 3, or 30 ppb (µg/L) atrazine treatment to represent chemical treatment concentrations around the current US EPA regulatory level in drinking water of 3 ppb. Exposure was ceased at the end of embryogenesis (72 hpf), RNA isolated from pools of 40 eleuthero-embryos, cDNA synthesized, and qPCR completed on neuroendocrine-related genes including 12 hypothalamic targets and 6 pituitary targets. A total of six biological replicates were statistically compared using an analysis of variance (ANOVA, α=0.05). Overall, these findings have determined the specificity of gene expression alterations observed at the end of embryogenesis between the hypothalamic and pituitary gene targets.

AhR-mediated transcriptional regulation of the human immunoglobulin hs1.2 enhancer

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The 3'IGHRR, an ~ 17kb transcriptional regulatory region within the human immunoglobulin heavy chain gene (IGH), is thought to be responsible for the transcription of the IGH locus, which is essential for antibody production. The 3'IGHRR contains the hs1.2 enhancer which is polymorphic in humans and consists of a 53 bp invariant sequence containing transcription factor binding sites, including a potential dioxin response element (DRE), that can be duplicated one to four times. Previous experiments have shown that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) can induce transcriptional activity of the human hs1.2 enhancer alleles via the aryl hydrocarbon receptor (AhR) signaling pathway. The objective of this study is to assess the role of the AhR transactivation domain (TAD) in TCDD-induced hs1.2 enhancer activity and the transcriptional

impact on an increased number of invariant sequences. Luciferase reporter plasmids containing one of the four human polymorphic hs1.2 enhancers (α1A, α1B, α1C, or α1D corresponding to one, two, three, or four invariant sequence repeats, respectively) were transfected via electroporation into a human B-cell line (CL-01) expressing an AhR with either a functional or nonfunctional TAD as determined by the ability to induce a reporter plasmid regulated by 6 DREs. In B cells with a nonfunctional AhR TAD, TCDD activated the hs1.2 enhancer in a concentration-dependent manner but the number of invariant sequences did not impact TCDD-induced activation suggesting a TAD-independent activation of the hs1.2 enhancer by AhR ligands. Ongoing studies are evaluating the impact of TCDD on the hs1.2 enhancer alleles in B cells expressing a functional AhR TAD to determine if a functional TAD will increase the sensitivity of hs1.2 enhancer alleles with a greater number of invariant sequences to TCDD-induced activation. Since the polymorphic hs1.2 enhancer has been associated with altered antibody levels and a number of hypersensitivity and autoimmune diseases, these results will provide greater insight in assessing risk by exposure to environmental, dietary, and endogenous ligands of the AhR.

Particulate Hexavalent Chromium Induces Loss of the BCDX2 Complex Leading to Loss of Homologous Recombination Repair

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Lung cancer mortality rate remains on the rise with many cases attributable to causes other than tobacco; however, alternative causes of lung cancer are often overlooked as important. Metals cause human lung cancer by inducing chromosome instability (CIN) as a part of their carcinogenic mechanism. Metals are poor base mutagens, but potently damage chromosomes indicating CIN as a major factor in their carcinogenic mechanism. We focused on hexavalent chromium [Cr(VI)] as a representative metal, as it is a lung carcinogen and an environmental toxicant. Cr(VI) induces DNA double strand breaks while simultaneously inhibiting the repair of those breaks which results in CIN. The homologous recombination (HR) repair pathway plays a major role in preventing CIN by repairing DNA double strand breaks. Cr(VI) targets RAD51, a key effector protein within the HR repair pathway, and prevents its loading onto a nucleoprotein filament. Five classical RAD51 paralogs: RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3, form two distinct multi-protein complexes: BCDX2 and CX3, which are integral to RAD51 nucleofilament formation and function. It is unknown if Cr(VI) impacts these paralogs and their complexes. We hypothesized that Cr(VI) inhibits HR repair in human lung cells by targeting the complexes responsible for RAD51 nucleoprotein filament formation. RAD51D and XRCC3 were investigated as representatives of the BCDX2 and CX3 complexes, respectively. Using immunofluorescence to measure DNA repair function, Western blot for protein levels, and qPCR for mRNA levels, we showed acute and prolonged Cr(VI) exposure greatly inhibited RAD51D foci formation, protein levels, and gene expression. In contrast, Cr(VI) had a minimal effect on XRCC3 foci formation suggesting the CX3 complex was not affected. These data suggest RAD51D as a part of the BCDX2 complex may be a key initial target in Cr(VI)-induced loss of RAD51 function and HR repair. Future work will investigate the remaining members of the complex and connect outcomes to loss of filament formation and HR repair. *This work was supported by NIEHS grants R01ES016893 and R35 ES032876 (J.P.W.) and T32-ES011564 (A.W. and J.P.W.).*

What Happens to the Metabolism of Aromatic Amines During Incubation with Hexavalent Chromium in Human Lung Cells?

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Humans are exposed to carcinogenic chemicals via occupational and environmental exposures. Common chemicals of concern that can occur in exposures together are aromatic amines (*e.g.,* 4-aminobiphenyl [4-ABP] and β-naphthylamine [BNA]) and hexavalent chromium. Arylamine *N*-acetyltransferases 1 and 2 (NAT1/2) are key to the metabolism of aromatic amines and their genotoxicity. The implication of Cr(VI) on the metabolism of aromatic amines by NATs remains unknown as well as how it may affect its ensuing toxicity. The research presented here is to determine if Cr(VI) will

increase the *N*-acetylation of aromatic amines and increase their toxicity in immortalized human lung epithelial cells (BEP2D and HBEC2-KT) expressing NAT1 and NAT2. We measured the effect of Cr(VI) on NAT activities (*N*-acetylation levels determined using HPLC) and will measure genotoxicity in the future. Our results show that exposure to Cr(VI) for 48 h increased NAT1 activity as measured by *N*-acetylation of para-aminobenzoic acid (PABA) in BEP2D cells but not NAT2 *N*-acetylation of sulfamethazine, which are the prototypic NAT1 and NAT2 substrates respectively. For BEP2D cells NAT1 *N*-acetylation of PABA was 0.048 ± 0.003, 0.054 ± 0.004, 0.098 ± 0.009 ($P<0.001$), and 0.126 ± 0.01 ($p<0.0001$) nmol/1x10^6 cells/h for 0, 1, 2.5, and 3 μM Cr(VI) respectively. For our results for Cr(VI) using 4-ABP as a substrate, we see that Cr(VI) increases the *N*-acetylation levels of 4-ABP and BNA. Specifically, in BEP2D cells the *N*-acetylation of 4-ABP (1 μM) was 0.95 ± 0.06, 0.93 ± 0.03, 1.37 ± 0.11, and 1.7 ± 0.29 nmol/1x10^6 cells/h for co-exposure with 0, 1, 2.5, and 3 μM Cr(VI) respectively. We also tested 2 and 3 μM 4-ABP with Cr(VI) and saw comparable trends. We conclude that treatment of cells grown in culture with Cr(VI) causes the activity of NAT1 in human lung cell lines to be increased.. On-going work is to determine if Cr(VI)-mediated increase NAT1 *N*-acetylation activity will alter the genotoxicity of aromatic amine (4-ABP and BNA) carcinogens. This work is supported by USPHS grant T32-ES011564 and P42-ES023716 & P30-ES030283.

A Toxic Aging Coin: Cr(VI) Neurotoxicity and Gerontogenicity

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Within 9 years 20% of the U.S. population will be over the age 65. Millions of them will reach their 100th birthday. Consequently, understanding how aging and toxicology interact is an area of research with an urgent need for expansion. We consider this interaction from two perspectives: 1) how age affects susceptibility to a chemical's toxic effect, and 2) how chemicals accelerate biological aging processes in our organs (i.e. how chemicals are gerontogens). Genomic instability is one of the leading contributors to aging, but the mechanisms behind genomic instability and aging remain to be elucidated. We propose chromosome instability, a type of genomic instability often seen in aging paradigms, arises from accumulation of unrepaired DNA damage and promotes aging by contributing to the induction of cellular senescence. We also propose metals as a class of gerontogens that induce aging by inducing DNA damage and chromosome instability. A couple recent papers highlighted the importance of creating a new animal model to investigate this mechanism, which we are developing using a novel Cr(VI) exposure study. We are testing these hypotheses using hexavalent chromium [Cr(VI)], a widespread environmental contaminant, as a representative metal. We used extant brain tissues from a study exposing rats to zinc chromate via oropharyngeal aspiration to assess Cr(VI) neurotoxicity. Our results show widespread neurodegeneration, oxidative damage, and DNA double strand breaks. We further investigated a mechanism of chromosome instability for biological aging using M059K and M059J human brain cells. M059J has dysfunctional DNA-PK, an essential protein for DNA double strand break repair, while M059K exhibits proficient break repair. We show M059J cells are more susceptible to Cr(VI)-induced cytotoxicity than M059K cells. Intriguingly, M059J cells can escape Cr(VI) induced growth arrest, while M059K cells cannot, suggesting DNA damage repair signaling must be intact for Cr(VI)-induced growth arrest, and perhaps cellular senescence. Mechanistically these differences are under investigation now. Future studies will evaluate cellular senescence and the senescence-associated secretory phenotype. This work was supported by NIEHS grants R01ES016893 and R35ES032876 to J.P.W.,Sr.

Implications for Zinc Treatment in Non-Alcoholic Fatty Liver Disease

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Chronic liver disease is a top ten cause of death worldwide, accounting for over two million deaths each year. The prevalence nonalcoholic fatty liver disease (NAFLD), which has recently surpassed alcohol liver disease (ALD) as the most prominent chronic liver disease, has more than doubled over the past two decades and continues to rise. Zinc deficiency is common in both

obese and NAFLD patients. Clinically, zinc (Zn) supplementation has been used for the treatment of ALD; however, its therapeutical role in NAFLD is unknown. We have previously shown that male mice fed a 60% high fat diet (HFD) for 24 weeks developed fatty livers and when we supplemented the HFD with Zn we were able to eliminate HFD-induced hepatic steatosis. Although exciting, we realized that in a clinical setting, a NAFLD patient would already have a fatty liver and supplementation would occur after prognosis. Therefore, in our upcoming study we will feed male mice a normal diet (ND) or a 60% HFD for 12 weeks to induce steatosis. Body weight and food consumption will be recorded weekly. At 12 weeks Echo MRI will be used to assess body composition and intraperitoneal glucose tolerance tests (IPGTT) will be performed. Half of the mice will continue their diet regime for 4 more weeks while the other half of the mice will have Zn (90 mg zinc/4057) added to their respective diets. Mice will then undergo a second round of Echo MRI and IPGTT tests at the end of the 4 weeks followed by euthanasia. Blood samples will be collected, and liver tissue will be snap-frozen, fixed for histopathological analyses or used for metals analysis. Liver to body weight ratios, plasma aminotransferase, and hepatic triglycerides and total cholesterol will be determined. Results from this study will elucidate a potential role for zinc supplementation in the treatment of NAFLD and possibly other diet-induced metabolic disorders.

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