# ABSTRACTS

2025 OVSOT Annual Meeting

November 14, 2025

University of Louisville

Louisville, KY

# Platform Presentations

## Early-Stage Investigator Platform Presentation

**Idoia Meaza Isusi**, University of Louisville, “Mechanisms of hexavalent chromium-induced carcinogenesis: DNA double-strand breaks and DNA repair inhibition in a guinea pig model” (Abstract 001)

## PhD Platform Presentations

**Evangeline Schott**, Ohio State University, “Lipocalin 2 Production Mitigates Ozone Induced Lung Injury and Inflammation” (Abstract 002)

**Oluwanifemi Bolatimi**, University of Louisville, “Sex-Specific Hepatic Effects of an Environmentally Relevant Dioxin Mixture in a Humanized AHR Mouse Model” (Abstract 003)

**Wagner Tamagno**, Purdue University, “Lipid-Mediated Transgenerational Effects of Developmental Pb Exposure on Cognitive Impairments and Anxiety-Like Behavior in Zebrafish Larvae” (Abstract 004)

## Postdoctoral Platform Presentations

**Abigail Ekuban**, University of Louisville, “Sex-Dependent Hepatotoxicity of Trichloroethylene in Humanized PPARα Mice Fed a Western Diet” (Abstract 005)

**Ahmed Ismail**, University of Louisville, “FGF1ΔHBS prevents DICT in tumor-bearing young mice through inhibiting cardiac fatty acid oxidation andimproving vasculature integrity” (Abstract 006)

**Samuel Cochran**, Ohio State University, “CD163 mediated resolution of ozone-induced pulmonary injury and inflammation” (Abstract 007)

# Big Picture Science Presentations

**Samuel Vielee**, University of Louisville, “Pathways of Cr(VI) Toxicity Change with Concentration in a Caenorhabditis elegans Model: The Importance of Exposure Parameters in Toxicological Models” (Abstract 020)

**Sydnee Dressel**, University of Louisville, “Oxidative Stress and Apoptosis Induced by MDMA and Synthetic Cathinones in HepG2 Cells” (Abstract 021)

**Zianne Olverson**, Ohio State University, “Electronic Cigarette Exposure During Pregnancy Causes Uteroplacental and Fetal Impairments” (Abstract 022)

**Hamza Jamal**, University of Louisville, “Low Concentration Hexavalent Chromium Induced Neuroinflammation in Rats after a 90 Day Drinking WaterExposure” (Abstract 042)

**Aadil Umerani**, Wright State University, “Functional Inhibitors of Acid Sphingomyelinase Reduce UVB-Induced Erythema in Rosacea via Topical Imipramineand Amitriptyline: A Pilot Clinical Trial” (Abstract 058)

**Andrea Arroyo**, University of Louisville, “The RAD50 Zinc Hook Motif as a Vulnerable Target in Heavy Metal-Induced Genotoxicity” (Abstract 059)

**Kassidy Stewart**, University of Louisville, “Sex- and Diet-Specific Effects of a Low-Dose Dioxin Mixture on Adipose-Liver Axis Disruption” (Abstract 060)

**William Buchanan**, University of Louisville, “Hexavalent Chromium [Cr(VI)] Induces Blood-Brain Barrier Dysfunction and DNA Damage in Endothelial Cells: Using a Toxic Aging Coin to Assess Cr(VI) Neurotoxicity” (Abstract 061)

# Platform Presentation Abstracts

**Abstract 001 – Early-Stage Investigator**

## Mechanisms of hexavalent chromium-induced carcinogenesis: DNA double-strand breaks and DNA repair inhibition in a guinea pig model

**Idoia Meaza[1], Jamie Wise[2], Sandra Diven[1], Haiyan Lu[1], Aggie Brownell[1], Jack Easley[1], J. Calvin Kouokam[1], John Wise Jr.[3], Samuel Vielee[3], James Wise[4], Rachel Wise[1], John Pierce Wise Sr.[1]**

*[1]Wise Laboratory of Environmental and Genetic Toxicology, [2]Wise Laboratory of Liver and Environmental Health, University of Louisville; [3]NeuroWise Laboratory of Environmental Toxicology, University of Toledo; [4]Wise Laboratory of Nutritional Toxicology and Metabolism, University of Hawaii*

Hexavalent chromium [Cr(VI)] is an environmental and occupational health hazard, and a known lung carcinogen. Despite its exposure risks, it is unclear how Cr(VI) causes lung cancer. Prolonged Cr(VI) exposure induces DNA double strand breaks and inhibits their repair, leading to chromosome instability, a hallmark of lung cancer. Specifically, Cr(VI) inhibits RAD51, a key effector protein in the homologous recombination repair pathway. This mechanism has been well characterized in cell culture assays and was recently translated to a rat model. However, rats have different bronchial bifurcation patterns from humans, which is critical because Cr(VI) particles impact and form tumors at these sites in humans. In contrast, guinea pigs share these bronchial bifurcation patterns with humans, making them a more translatable model to study how Cr(VI) causes lung cancer. Our goal was to translate the mechanism of Cr(VI)-induced DNA double strand breaks and DNA repair inhibition to guinea pig lungs. We hypothesized acute Cr(VI) exposure would increase the levels of DNA double strand breaks and increase DNA repair, while subchronic exposure would increase DNA double strand breaks and inhibit their repair. Hartley guinea pigs were exposed to zinc chromate via oropharyngeal aspiration for acute (24 h) or subchronic (90-day) exposure. Zinc chromate was used as a representative particulate Cr(VI) compound, as epidemiological and toxicological data show it causes lung cancer in humans. At the end of each exposure, animals were euthanized and lungs were dissected. One piece of lung tissue was analyzed for metal contents by inductively coupled plasma mass spectrometry, which confirmed Cr reached the lungs. A second piece of lung tissue was used for immunofluorescence staining. DNA double strand breaks were measured using the gamma-H2AX marker, and homologous recombination repair was assessed using RAD51. Z-stack images were obtained using a confocal microscope, and nuclear gammaH2AX and RAD51 foci were quantified. We found acute Cr(VI) exposure induced DNA double strand breaks and DNA repair, whereas subchronic exposure also caused DNA double strand breaks but inhibited repair. This study translates the mechanism of Cr(VI)-induced DNA double strand break formation and repair inhibition to a human-relevant animal model, the guinea pig. Future studies will assess how Cr(VI) inhibits DNA repair pathways to drive lung carcinogenesis. This work was supported by the National Institute of Environmental Health Sciences grant R35ES032876 (JPWSr.).

## Abstract 002 – PhD Student

### Lipocalin 2 Production Mitigates Ozone Induced Lung Injury and Inflammation

**Evangeline Schott[1,2], Samuel J. Cochran[1], Grace Hutton[1], Anthony Lutton[3,4], Zachary Hurst[5], Aaron Vose[6], Zobeida Cruz-Monserrate[5], John Olesik[3,4], Robert M. Tighe[6], and Kymberly M. Gowdy[1]**

*[1] Department of Internal Medicine, Division of Pulmonary, Critical Care and Sleep Medicine, The Ohio State University Wexner Medical Center, [2]Department of Microbial Infection and Immunity, The Ohio State University, Columbus, OH [3]Trace Elements Research Laboratory, Columbus, OH [4]The Ohio State University School of Earth Sciences, Columbus, OH [5]The Ohio State University College of Medicine, Division of Gastroenterology, Hepatology and Nutrition, Columbus, OH [6]Department of Medicine, Duke University Medical Center, Durham, NC*

Air pollution exacerbates cardiopulmonary diseases resulting in increased morbidity and mortality. As global temperatures rise, the frequency and incidence of these exposures identify pollutants such as ozone($O_3$) as a critical health concern. $O_3$ has been shown to stimulate pulmonary inflammation through disruption of iron homeostasis. Previous work from our lab has shown elevations in airspace iron and iron associated proteins such as Lipocalin 2 (LCN2) are seen following an acute $O_3$ exposure. Although LCN2 is shown to be essential in the pulmonary host defense, its biological role following an $O_3$ exposure has not been explored. Therefore, we hypothesis that $O_3$-induced elevations in airspace iron stimulate production of LCN2, mediating $O_3$-induced lung injury and inflammation.

To test this hypothesis, male C57BL/6J (WT) mice or LCN2 deficient (*Lcn2*$^{-/-}$) mice were exposed to filtered air (FA) or 1ppm $O_3$ for 3h. Mice were euthanized 24h post-exposure and bronchoalveolar lavage fluid (BAL) and plasma were collected. Airspace iron concentrations, BAL total protein and immune cell recruitment were measured within the BAL, while airspace and systemic LCN2 production was quantified with both BAL and plasma. To better understand the cellular compartment responsible for LCN2 production following $O_3$ exposure, WT and *Lcn2*$^{-/-}$ mice underwent a bone marrow transplant (BMT) to generate bone marrow chimeras. Once fully engrafted, BMT mice were then exposed to FA or 1ppm $O_3$ for 3h and 24h following exposure BAL and plasma were collected.

24h following $O_3$-exposure, *Lcn2*$^{-/-}$ mice had a significant increase in airspace neutrophilia and total BAL protein compared to $O_3$-exposed WT mice. $O_3$ exposure increased airspace iron concentrations in WT mice when compared to FA exposed mice which was further augmented in *Lcn2*$^{-/-}$ mice. Following BMT, WT recipients given *Lcn2*$^{-/-}$ bone marrow had significantly increased airspace LCN2 when compared WT mice receiving WT bone marrow. Regardless of donor bone marrow genotype, *Lcn2*$^{-/-}$ recipients has elevated BAL total protein as a marker for lung injury, while no significant differences were seen in immune cell recruitment.

Taken together, these data suggest that LCN2 is protective against $O_3$-induced lung injury and inflammation perhaps through decreasing airspace iron concentrations. Furthermore, LCN2 production following $O_3$ exposure is structural cell dependent. Ongoing studies will identify which structural cells are responsible for LCN2 production following $O_3$ exposure and elucidate the mechanism by which iron is released.

**Abstract 003 – PhD Student**

## Sex-Specific Hepatic Effects of an Environmentally Relevant Dioxin Mixture in a Humanized AHR Mouse Model

**Oluwanifemi E. Bolatimi, Yuan Hua, Paxton Ames, Abigail Ekuban, Gavin Phillips, Frederick A. Ekuban, Walter H. Watson, Banrida Wahlang**

*University of Louisville, Louisville, KY, USA*

Legacy persistent organic pollutants like “dioxins” remain present in our environment in relatively low yet significant levels and are associated with metabolic disruptions that initiate or drive the progression of steatotic liver disease. Dioxins are potent aryl hydrocarbon receptor (AHR) activators and have been reported to induce hepatoxicity via this pathway. However, the majority of studies utilize murine models which have higher affinity and responsiveness to these pollutants compared to the human AHR (hAHR). Knowledge on how dioxins act through the hAHR to promote liver and metabolic dysregulation is still limited. Thus, this study aims to investigate the translational relevance of acute exposure to an environmentally relevant dioxin mixture: 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8 Pentachlorodibenzofuran (PeCDF) and Polychlorinated biphenyl 126 (PCB 126), focusing on liver outcomes and sex differences, using a humanized AHR mouse model. Male and female hAHR-C57BL/6 mice were fed a low-fat diet and administered a weekly dose of TCDD (10ng/kg) + PeCDF (80ng/kg) + PCB 126 (140ng/kg) or vehicle control for 2 weeks. At euthanasia, plasma and tissues were collected for downstream analyses. Females had higher fat mass, but lower lean mass compared to males, along with elevated levels of hepatic triglyceride and cholesterol. Plasma glucose levels and glucose intolerance were increased by dioxin exposure in males but decreased in females. Correspondingly, dioxin led to a reduction in insulin resistance and pancreatic insulin production in the male group, while exposed females displayed increased levels of these measurements. Furthermore, the dioxin mixture did not activate the hAHR as canonical target genes (*Cyp1a1* and *Cyp1a2*) were not induced with exposure in both sexes. Notably, transcription of the *hAhr* gene which had higher expression in females was reduced by dioxin exposure in this group alone. Additionally, hepatic gene expression of xenobiotic receptors *Car* and *Pxr*, as well as endobiotic receptors *Lxr* and Pparα were also selectively decreased in dioxin exposed females only. Overall, the dioxin mixture did not appear to activate the hAHR. However, phenotypic changes including lipid disruption patterns were consistent with our previous findings in the mAHR model, suggesting off-target effects. The current data also demonstrated sex-specific dioxin alterations on liver transcriptional machinery. Further evaluation of canonical and non-canonical mechanistic AHR pathways and longer-term studies in this human relevant model will provide further understanding on how dioxins impact sex-specific hepatotoxicity in humans.

**Abstract 004 – PhD Student**

## Lipid-Mediated Transgenerational Effects of Developmental Pb Exposure on Cognitive Impairments and Anxiety-Like Behavior in Zebrafish Larvae

**Wagner A. Tamagno[1], Hellen W. Mendes[1], Sydney Stradtman[2], Karthikeyan S. N. Chelladurai[1], Christina R. Ferreira[3], Jennifer L. Freeman[1]**

*[1] School of Health Sciences, Purdue University, West Lafayette, IN, United States of America. [2] Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR, United States of America. [3] Bindley Bioscience Center, Purdue University, West Lafayette, IN, United States of America.*

Lead (Pb) is a pervasive environmental neurotoxicant that poses significant risks to the developing nervous system. Developmental exposure to even trace amounts of Pb has been associated with persistent alterations in cognition, behavior, and cellular homeostasis. This study examined the multigenerational (F1) and transgenerational (F2) consequences of developmental Pb exposure at environmentally relevant concentrations (0.01, 0.1, and 1 ppb) in zebrafish (*Danio rerio*), with an integrated assessment of behavioral, physiological, and lipidomic endpoints. In previous work, zebrafish embryos were exposed to Pb from 1–72 hours post-fertilization (hpf). After exposure, fish were reared to adulthood (4 months) to generate the F1 generation, which was not directly exposed. F1 adults were subsequently bred to produce the F2 generation. Behavioral assays were conducted at early larval stages: shoaling test and visual motor response test at 120 hpf, and open field test at 168 hpf. F1 larvae exhibited clear neurobehavioral impairments, including hypolocomotion, disrupted anxiety-like responses, impaired social cohesion, and diminished decision-making ability. These outcomes were strongly associated with Pb-induced perturbations in sphingomyelins, phosphatidylcholines, and cholesterol esters, indicating compromised neuronal signaling and membrane stability. Conversely, F2 larvae showed negligible behavioral alterations, suggesting the absence of transgenerational inheritance under the tested conditions. Pathway enrichment analyses identified dysregulation of fatty acid transport and mitochondrial energy metabolism, with notable enrichment of carnitine–acylcarnitine translocase in F1 larvae exposed to 1 ppb Pb. Antioxidant system evaluation further revealed a metabolic shift favoring glutathione-mediated detoxification, characterized by enhanced activity of GSH-related enzymes and reduced catalase activity, consistent with oxidative imbalance. Previous evidence of Pb-induced epigenetic reprogramming of antioxidant genes supports the hypothesis that such molecular changes underline the observed phenotypes. Taken together, the findings demonstrate that early-life exposure to environmentally relevant Pb levels elicits multigenerational neurobehavioral toxicity linked to lipidomic and redox disturbances, while not producing fully transgenerational effects. The enrichment of pathways related to neuronal energy metabolism and membrane composition underscores the high vulnerability of developing organisms to Pb exposure.

**Abstract 005 – Postdoctoral Trainee**

## Sex-Dependent Hepatotoxicity of Trichloroethylene in Humanized PPARα Mice Fed a Western Diet

**Abigail Ekuban[1], Paxton Ames[1], Frederick A. Ekuban[1], Jianzhu Luo[1], Oluwanifemi E. Bolatami[1], Yuan Hua[1], Megana Iyer [1], Collette Gorga[1], Josiah Hardesty[1], Banrida Wahlang[1], Jennifer Schlezinger [2], Matthew Cave[1], Walter H. Watson[1]**

*[1]University of Louisville, Louisville, KY 40202, USA; [2]Boston University School of Public Health, Boston, MA, 02118, USA*

**Background**: Consumption of a high fat western-style diet is a major risk factor for metabolic dysfunction-associated steatotic liver disease (MASLD). Environmental exposures to toxic chemicals may contribute to the progression of this disease. Trichloroethylene (TCE) is a commonly used industrial solvent and environmental contaminant that has been linked to metabolic dysfunction and hepatic steatosis. Metabolites of TCE activate the transcription factor PPARα, a major regulator of lipid metabolism in the liver. Both diet and sex have been identified as important modifying factors in MASLD development. Therefore, we sought to test the hypothesis that TCE exposure exacerbates western diet-induced metabolic dysfunction and liver injury in a sex-dependent manner using a humanized PPARα mouse model.

**Methods**: Mice expressing human PPARα were used in this study because they are a better model for human responses to PPARα agonists. Male and female humanized PPARα C57BL/6J mice aged 9-12 weeks were placed on either a low-fat diet (LFD; 10% fat) or a western diet (WD; 42% fat) with concurrent exposure to 0.5 mg/mL of TCE or 1% ethoxylated castor oil (used to emulsify TCE) in drinking water for 12 weeks. Body weight and water consumption were measured each week. A glucose tolerance test was performed at week 10, and EchoMRI was performed at week 11. At week 12, blood and tissues were collected for measurement of plasma AST and ALT, tissue weights, and analysis of liver histopathology with H&E and Oil Red O. Statistical analyses were performed using two- and three-way ANOVA followed by Tukey's post hoc test, with significance set at $p \leq 0.05$.

**Results**: Male mice on WD, independent of TCE exposure, exhibited increased body weight, fat mass, white adipose tissue, and liver weight compared to their female counterparts. Interestingly, the female mice on WD and TCE exposed exhibited a significant decrease in body weight, white adipose tissue and fat mass relative to WD-fed females. TCE exposure relative to body weight was greater in females than in males. Glucose tolerance was impaired in male mice on a WD diet, independent of TCE exposure, whereas their female counterparts maintained glucose tolerance. Plasma AST and ALT levels were elevated in WD-fed males, independent of TCE exposure, but remained unchanged in females. Histological analysis revealed enhanced steatosis in WD-fed males, regardless of TCE exposure, as confirmed by H&E and Oil Red O staining, compared with their female counterparts.

**Conclusion**: This study revealed marked sexual dimorphism in diet- and TCE-induced hepatotoxicity in humanized PPARα mice. Male mice were more susceptible to liver injury and toxicity, whereas female mice showed alterations in systemic fat deposition.

**Abstract 006 – Postdoctoral Trainee**

## FGF1$^{\Delta HBS}$ prevents DICT in tumor-bearing young mice through inhibiting cardiac fatty acid oxidation and improving vasculature integrity

**Ahmed Abdelbaset-Ismail[1], Xiaodan Hui[1], Chunjie Gu[1], Jason Xu[1], Zhong-bin Deng[2], Ashok Raj[3], Lu Cai[1], Yi Tan[1]**

*[1]Department of Pediatrics, [2]Department of Surgery, University of Louisville, [3]Norton Children's Cancer Institute, Norton Children's Hospital, Louisville, KY 40202, USA.*

**BACKGROUND:** Doxorubicin (Dox)-induced cardiac toxicity (DICT) is increasingly recognized in children and adolescents, yet no effective preventive therapy is currently available for pediatric cancer survivors.

**Hypothesis/Objective:** We evaluated the therapeutic potential of a non-mitogenic human FGF1 variant (rhFGF1$^{\Delta HBS}$) against both early- and late-onset DICT in tumor-free and tumor-bearing young mice, modeling pediatric cancer survivors.

**METHODS:** Male EL4 lymphoma-bearing mice (3 weeks old) randomly received Dox alone or with rhFGF1$^{\Delta HBS}$. For late-onset DICT, male tumor-free mice were treated with rhFGF1$^{\Delta HBS}$ (62.5 μg/kg, i.p) for either a short-term or a long-term period. Cardiac-specific hFGF1$^{\Delta HBS}$ overexpression was induced in one-day-old mouse pups, which then received Dox at weaning. Hearts were collected and prepared for molecular and pathological assessment. Left ventricular (LV) systolic function was evaluated at defined time points. Bulk RNA-sequencing was performed to elucidate potential mechanisms underlying rhFGF1$^{\Delta HBS}$-mediated cardioprotection. The data were analyzed and assessed using one-way ANOVA, with significance set at $p < 0.05$.

**RESULTS:** rhFGF1$^{\Delta HBS}$ at 62.5 μg/kg improved heart weight compared to Dox alone ($p<0.05$). At both 62.5 and 125 μg/kg, rhFGF1$^{\Delta HBS}$ restored cardiac systolic function and mitigated Dox-induced cardiac pathologies without compromising Dox's tumor-suppressive effects. Short-term rhFGF1$^{\Delta HBS}$ treatment significantly increased survival and effectively prevented both early- and late-onset DICT. Consistently, cardiomyocyte-specific overexpression of hFGF1$^{\Delta HBS}$ preserved heart mass and enhanced LV systolic function compared with empty vectors plus Dox controls ($p < 0.05$). Bulk RNA-seq analysis revealed that hFGF1$^{\Delta HBS}$ overexpression upregulated signaling pathways related to endothelial cell migration, angiogenesis, and tissue remodeling, while downregulated signaling pathways related to fatty acid oxidation, steroid metabolism, and organic acid biosynthesis.

**CONCLUSION:** FGF1$^{\Delta HBS}$ prevents DICT in young mice, likely by maintaining cardiac lipid metabolic homeostasis and vasculature integrity. These findings support its therapeutic potential to prevent DICT in pediatric cancer survivors and provide a strong rationale for further translational studies.

**Grant Support:** The present study was supported, in part, by grants from the Kentucky Pediatric Cancer Research Trust Fund (PCRTF), R01 HL125877, R01 HL160927, and R01 HL174922.

**Abstract 007 – Postdoctoral Trainee**

## CD163 mediated resolution of ozone-induced pulmonary injury and inflammation

**Samuel J. Cochran[1], Brett Saunders[1], Grace M. Hutton[1], Katelyn Dunigan-Russell[1], Aaron Vose[3], Anastasiya Birukova[3], Cameron Rankin[3], Velayutham Murugesan[2], Salik Hussain[2], Tim McMahon[3], Robert Tighe[3], Kymberly M. Gowdy[1]**

*[1] Division of Pulmonary, Critical Care, and Sleep Medicine, OSU Wexner Medical Center, Columbus OH; [2] Department of Physiology and Pharmacology, West Virginia University, Morgantown, WVU; [3] Department of Medicine, Duke University, Durham NC*

CD163 is a scavenging receptor (SR) responsible for clearance of cytotoxic, cell free hemoglobin (CFH) that accumulates in the lungs during ozone ($O_3$)-induced injury. CD163 internalization and CFH metabolism also activate anti-inflammatory and antioxidant pathways. However, the relevance of CD163 in scavenging CFH and promoting anti-inflammatory responses in $O_3$-induced lung injury is not currently understood. We hypothesize that CD163-mediated CFH clearance is necessary for resolution of pulmonary inflammation caused by acute $O_3$ inhalation.

Female, wild type (WT) and CD163$^{tm1(KOMP)Vlcg}$ (CD163$^{-/-}$) knockout (KO) mice were exposed to either filtered air (FA) or 1 ppm $O_3$ for 3 hours, which models an environmentally relevant $O_3$ exposure hazardous to humans. A separate cohort of WT and KO mice were given a single oropharyngeal (op) dose of CFH (1 mg/ml, 100 ug/mouse) or vehicle control. Mice were necropsied 12 and 24 hours following all experiments to collect bronchoalveolar lavage (BAL) and lung tissue. Lung injury was measured BAL total protein and albumin concentrations. Lung inflammation was measured by airspace cell differential. CFH clearance was measured by BAL concentration of scavenging proteins, hemopexin (Hx) and haptoglobin (Hg). Airspace oxidative stress was measured by BAL electron paramagnetic resonance (EPR).

At 12 hours, $O_3$ significantly increased BAL neutrophilia, total cell numbers, albumin, and total airspace protein, augmented in CD163$^{-/-}$ mice. At 24 hours, $O_3$ significantly increased BAL neutrophilia and oxidative stress in CD163$^{-/-}$ mice compared to WT controls; however, there was no difference in markers of lung injury. Compared to vehicle controls, airspace total protein and immune cell numbers were significantly increased in both WT and CD163$^{-/-}$ mice 24 hours after CFH administration, with no difference between WT and CD163$^{-/-}$ mice. CD163$^{-/-}$ mice exposed to CFH had significantly greater airspace neutrophilia compared to WT mice exposed to CFH. CFH exposure led to significantly increased BAL Hp in CD163$^{-/-}$ mice compared to WT mice after 24 hours and significantly increased oxidative stress in CD163$^{-/-}$ mice only.

Cumulatively, these data indicate that CD163$^{-/-}$ mice experience increased pulmonary injury, inflammation, and oxidative stress in response to either $O_3$ or CFH. Additionally, our data suggests that there is impaired CFH clearance in CD163$^{-/-}$ mice, supporting a role for CFH scavenging by CD163 in resolution of $O^3$-induced pulmonary injury and inflammation. Further research will examine CD163 expression by different lung cell populations and the role of soluble CD163 (sCD163) in response to $O_3$ exposure.

# Big Picture Science & Poster Presentation Abstracts

**Abstract 008 – Postdoctoral Trainee**

**Human Keratinocytes Exhibit Poor Arsenic Methylation Capacity**

**Alexandra N. Nail[1], M. Banerjee[1], M. Xu[1], C.H. Reynolds[1], M. Stýblo[2], P.H. Cable[2], D.W. Wilkey[1], M.L. Merchant[1], A.P. Ferragut Cardoso[1], S. Thomas[1], and J. C. States[1]**

*[1]Center for Integrative Environmental Health Sciences, University of Louisville, [2]Department of Nutrition, University of North Carolina at Chapel Hill*

**Background:** Chronic inorganic arsenic (iAs) exposure is a worldwide environmental health problem and arsenical skin lesions are a hallmark of chronic arsenic exposure. Decreased arsenic methylation efficiency is associated with a higher risk of developing skin lesions. Enzymatic methylation is the major metabolic pathway for inorganic trivalent arsenic ($iAs^{III}$) in humans. Methylated arsenic metabolites generated primarily by Arsenic (+3 oxidation state) methyltransferase (AS3MT) exhibit distinct grades of toxicity governed by their ability to bind to C2H2-, C3H-, and C4-type zinc finger proteins whereas $iAs^{III}$ can bind to only C3H and C4type zinc finger proteins. Continuous $iAs^{III}$ exposure for 28 weeks malignantly transforms HaCaT human keratinocytes. This model is currently the gold standard in iAs-induced skin cancer research. However, it is unclear whether human keratinocytes have arsenic methylating capacity, and which proteins may be directly targeted by $iAs^{III}$ to drive HaCaT cell malignant transformation. Our central hypothesis is that malignant transformation of human keratinocytes is driven by $iAs^{III}$ rather than methylated arsenic species. **Methods:** Human keratinocytes (HaCaT, Ker-CT, and HEKn) cells and a liver cell line (HepG2) were exposed to sodium arsenite (iAsIII, 0 or 100 nM) for 24 h. iAs and methylated arsenic species within cells and media from 100 nM exposed cultures were determined by hydride-generation inductively coupled plasma mass spectrometry coupled with a cryotrap (HG-CT-ICP-MS). AS3MT mRNA expression was determined (RT-PCR and RT-qPCR). Proteomic analysis (Tandem-mass tagging) was used to determine relative changes in protein abundance in cells chronically exposed to iAsIII for 7 weeks (0 or 100 nM). Statistical analyses, including Chi Square test, student's t-test, or Fisher's exact test were performed with GraphPad Prism software, p £ 0.05 was considered significant. **Results:** Human keratinocytes exhibit poor arsenic methylation capacity. In addition, we discovered a novel alternatively spliced AS3MT mRNA isoform whose predicted protein does not contain arsenic methylating capacity. A total of 170 C3H- and C4-type zinc finger proteins expressed in HaCaT cells were identified as potential, direct targets for iAsIII that may drive malignant transformation. **Conclusions:** Direct targeting of C3H- and C4-type zinc finger proteins by iAsIII is likely necessary for malignant transformation of human keratinocytes.

**Grant support:** Jewish Heritage Foundation, American Cancer Society PF-23-1153675-01DMC, and NIH-NIEHS grants T35ES014559, R01ES027778, P30ES030283, and 1K99ES036286-01.

**Abstract 009 – Early-Stage Investigator**

## PPARα-Mediated Hepatic Responses to PFOS Exposure in a Humanized and Knockout Mouse Model of Alcohol-Associated Liver Disease

**Fredrick A. Ekuban[1]; Paxton M. Ames[1]; Abigal Ekuban[1]; Oluwanifemi E. Bolatimi[1]; Adrian Centers[1], Yuan Hua[1] Jianzhu Luo[1]; Josiah H. Hardesty[1]; Tyler C. Gripshover[1]; Collette Gorga[1]; Walter H. Watson[1]; Banrida Wahlang[1]; Dhimitter Bello[2]; Jennifer J. Schlezinger[3]; Craig J. McClain[1]; Matthew C. Cave[1]**

*[1]University of Louisville, Louisville, KY, USA; [2]University of Massachusetts Lowell, Lowell, MA, USA and [3]Boston University, Boston, MA USA.*

**Background**: Perfluorooctane sulfonate (PFOS) exposure is increasingly linked to MASLD (PMID: 35475652), yet its role in ALD remains understudied. Our recent wild-type studies showed that PFOS worsened ALD severity via PPARα (PMID: 40347464), yet the exact role of PPARα in mediating these effects remains elusive. Given that PFOS is a strong mouse but weak human PPARα agonist, the relevance of murine models to human health risk is uncertain. Here, we sought to investigate the species-specific hepatic responses to PFOS-ethanol co-exposure in humanized PPARα (hPPARα) and PPARα-knockout (KO) mice.

**Methods**: Male hPPARα and PPARα-KO mice (C57BL/6J) received modified Lieber-DeCarli diet (5% EtOH vs. control) for 10 days with concurrent PFOS gavage (1 mg/kg), followed by EtOH binge dose (5 g/kg) and euthanized 5-6h later. Assessments included body composition, biochemistry, liver histology (H&E), and high-content imaging of primary hepatocytes.

**Results**: EtOH decreased body weight with trends toward differential liver-to-body weight ratios (decrease in hPPARα, increase in PPARα-KO). Both genotypes showed ethanol-induced triglyceride and VLDL elevations, with PFOS augmenting VLDL levels. Notably, PFOS exposure attenuated EtOH-induced ALT/AST increases in both genotypes, contrasting with wild-type studies where PFOS exacerbated hepatotoxicity (PMID:40347464). Histological examination revealed marked genotype-dependent differences in hepatic architecture. While hPPARα mice maintained normal liver morphology in controls and developed progressive steatosis from mild (PFOS alone) to moderate (ethanol alone) to severe (PFOS+ethanol combination), PPARα-KO mice demonstrated significant baseline steatosis even in vehicle-treated controls, with persistent severe steatosis across all treatment groups. High-content imaging confirmed striking cellular differences: hPPARα hepatocytes showed 4-fold increased mitochondrial function with PFOS-ethanol, while PPARα-KO hepatocytes exhibited heightened oxidative stress and 5-fold increased lipid peroxidation, indicating compromised homeostasis.

**Conclusions**: These findings reveal species-specific PPARα-mediated responses to PFOS-EtOH co-exposure. Baseline steatosis in PPARα-KO controls demonstrates PPARα's fundamental role in hepatic lipid homeostasis. Attenuation of EtOH-induced liver injury suggests varying PPARα activity levels modulate injury mechanisms differently than wild-type mice. More data are needed on species-specific nuclear receptor signaling that may vary with environmental pollutant exposure, particularly in alcohol consumers.

**Abstract 010 – Postdoctoral Trainee**

## Long-read isoform sequencing reveals Aroclor1260-induced isoform expression in mouse liver

**Belinda J. Petri[1,2,3], Kellianne M. Piell[1], Banrida Wahlang[4,6,7,8] Julia H. Chariker[2,3], Eric C. Rouchka[1,2], Matthew C. Cave[4-8], and Carolyn M. Klinge[1,8]**

*[1]Dept. of Biochemistry and Molecular Genetics, UofL School of Medicine, [2] KYINBRE Data Science Core, UofL School of Medicine, [3]Dept. of Neuroscience Training, UofL School of Medicine, [4]UofLHepatobiology and Toxicology Center, [5]UofL Alcohol Research Center, [6]The UofL Superfund Research Center, [7]Division of Gastroenterology, Hepatology & Nutrition, Dept. of Medicine, UofL School of Medicine, [8]UofL Louisville CIEHS*

Polychlorinated biphenyls (PCBs) are legacy environmental contaminants that act as metabolic disrupting chemicals (MDCs) and are implicated in the pathogenesis of human metabolic dysfunction-associated steatotic liver disease (MASLD). While the effects of PCBs on whole-gene expression are well-documented, the more subtle regulatory role of alternative splicing and differential transcript usage (DTU) is less understood. This study investigated the transcriptomic effects of PCB exposure, focusing on DTU as a key mechanism of gene regulation. Normal diet-fed C57BL/6J male mice were exposed to a single oral gavage of the PCB mixture Aroclor1260. After 34 weeks, RNA was isolated from the livers and then analyzed using PacBio long read IsoSeq. Differential transcript usage was identified with SQANTI3 and DRIMseq. DTU was determined by comparing the IsoSeq data from Aroclor1260-exposed to control (DMSO) mice. Network analysis using STRINGdb was performed on the proteins encoded by genes with significant DTU to identify enriched biological pathways and protein-protein interactions. Our IsoSeq analysis identified four genes with significant DTU in the Aroclor1260-exposed livers: *Adpgk*, *Blvra*, *Mup2*, and *Ndufaf6*. Network analysis of the corresponding proteins revealed a strong association with pathways relevant to MASLD including lipid metabolism, glycolysis, and oxidative stress. These findings suggest that PCB exposure alters the transcript isoform landscape of key metabolic genes, leading to changes in the protein interaction network. Our findings demonstrate that PCB exposure orchestrates a profound and subtle regulatory shift in gene function through differential transcript usage, which in turn perturbs the protein interaction network. This study highlights the utility of IsoSeq in identifying novel mechanisms of metabolic disruption and provides a foundation for future research into the therapeutic potential of targeting these pathways.

**Abstract 011 – Postdoctoral Trainee**

## Particulate Cr(VI) Compromises Genomic Stability via Disruption of Mismatch Repair in Human Lung Cells

**Haiyan Lu[1], James T. F. Wise[2], Idoia Meaza[1], Aishwarya Prakash[3], John B. Vincent[4] and John Pierce Wise, Sr[1]**

*[1]Wise Laboratory of Environmental and Genetic Toxicology, University of Louisville. [2]University of Hawaii, [3]University of South Alabama; [4]University of Alabama*

Lung cancer remains a significant public health concern due to its high mortality rate, with many cases linked to environmental carcinogens beyond tobacco exposure. Particulate hexavalent chromium [Cr(VI)] is a well-established human lung carcinogen, yet its underlying mechanisms of carcinogenicity remain poorly defined. Previous studies have shown that Cr(VI) induces microsatellite instability and inhibits DNA double-strand break repair in exposed workers. The DNA mismatch repair pathway is essential for maintaining genomic stability by correcting replication-associated errors. While a few reports have implicated mismatch repair in Cr(VI)-induced genotoxicity by treating repair-deficient cells, direct impacts of Cr(VI) on mismatch repair in human lung cells are lacking. In this study, we tested the hypothesis that particulate Cr(VI) disrupts the mismatch repair pathway in human lung cells. Transcriptomic analysis revealed that Cr(VI) exposure downregulated key mismatch repair genes, including MLH1, MSH2, MSH3, and MSH6, with more pronounced suppression following prolonged exposure. These transcriptomic changes were confirmed by RT-qPCR. Functionally, Cr(VI) reduced MSH2 foci while increasing MLH1 foci, indicating differential targeting of mismatch repair components. The Cr(VI)-induced decrease in MSH2, accompanied by elevated gamma-H2A.X levels and increased co-localization with MLH1, suggests that impaired mismatch repair contributes to DNA double-strand break accumulation. Moreover, Cr(VI) exposure increased microsatellite instability in a concentration- and time-dependent manner. Collectively, these findings demonstrate that prolonged exposure to particulate Cr(VI) disrupts the mismatch repair pathway and promotes microsatellite instability in human lung cells, implicating mismatch repair dysfunction in Cr(VI)-induced genomic instability and carcinogenesis. This work was supported by the National Institute of Environmental Health Sciences [R35ES032876 and R01ES016893 to JPW; and R15ES033800 to JBV and JPW].

**Abstract 012 – Postdoctoral Trainee**

## Hydralazine N-acetylation and genotoxicity are N-acetyltransferase 2 (NAT2) allele dependent

**Mariam R. Habil, Makayla A. Stephens, Alexandra A. Cass, David W. Hein**

*Department of Pharmacology and Toxicology, School of Medicine, University of Louisville, KY, USA*

Background/Introduction: Arylamine N-acetyltransferases (NATs) are xenobiotic metabolizing enzymes that work by adding an acetyl group from acetyl coenzyme A to arylamines or arylhydrazines. Two NAT genes (NAT1 and NAT2) have been annotated in humans. NAT2 exhibits interindividual differences in both efficacy and toxicity following treatment with common hydrazine drugs such as hydralazine. Hydralazine is a potent vasodilator widely used to treat hypertension during pregnancy and has been shown to have an epigenetic role in cancer treatment. Some previous studies reported that hydralazine is mutagenic in bacteria and mammalian cells, but their reported effects are inconsistent. Studies using human metabolic machinery are needed to investigate the effect of NAT2 polymorphism on hydralazine N-acetylation and genotoxicity.

Hypothesis/Objective This study was done to investigate the association between NAT2 allelic variants and genotoxicity using mammalian cells transfected with recombinant human CYP1A2 and either *NAT2*4* (reference) or *NAT2*5B* (variant).

Methods: N-acetyltransferase assays containing Chinese Hamster ovary (CHO) cell lysates, hydralazine (10 – 1000 µM) and acetyl coenzyme A (300 – 1000 µM) were incubated to measure in vitro acetylation rate of hydralazine using HPLC. Cytotoxic effect of hydralazine following 24 hours exposure was assessed using resazurin (Alamar Blue) cell viability assay. DNA damage response was measured using γH2AX in-cell western assay. Reactive oxygen species (ROS) level was measured using 2',7'-dichlorofluorescin diacetate (DCFDA) fluorescence assay.

Results: CHO cells with *NAT2*4* showed higher acetylation rate than those with *NAT2*5B* ($p < 0.001$) using acetyl CoA 300 or 1000 µM. Cell viability levels did not differ significantly between alleles following hydralazine treatment ($p > 0.05$). Hydralazine treatment caused a concentration dependent increase in DNA damage response with more genotoxicity in *NAT2*4* versus *NAT2*5B* cells ($p< 0.0001$). Hydralazine treatment reduced ROS levels in CHO cells with *NAT2*4* and *NAT2*5B*.

Conclusions: Hydralazine is genotoxic in mammalian cells. Hydralazine N-acetylation and genotoxicity in mammalian cells are modified by NAT2 genetic polymorphism.

**Abstract 013 – Postdoctoral Trainee**

## Obesogenic Effects of Bisphenol A and Its Analogues on Feeding Behavior and Appetite Gene Expression in Zebrafish Larvae as a New Approach Method

**Rachna Mirchandani*, Silvia Karim, Maria Bondesson.**

* *Indiana University*

Introduction: Obesity is a complex disease linked to multiple comorbidities, influenced in part by environmental endocrine disruptors known as obesogens. Bisphenol A (BPA) and its analogues disrupt metabolism and adipocyte function via hormonal receptor pathways. This study employs zebrafish larvae, an emerging and translational New Approach Method (NAM) in toxicology, to investigate obesogenic effects of BPA and five substitute analogues (BPAF, BPE, BPC, BPC-Cl, BPS) on feeding behavior and appetite gene regulation.

Methods: Zebrafish larvae were exposed to BPA and analogues at multiple concentrations. Feeding was assessed by quantifying gut intake of stained egg yolk. RT-qPCR measured mRNA expression of key hunger-satiety regulatory genes (pck1, hcrt, leptin, ghrelin). Lowest effect levels (LELs) and proportion of larvae with increased feeding were determined for each compound.

Results: BPAF and BPC-Cl induced increased feeding at lower concentrations (0.01 µM) than BPA, BPE, and BPC (0.1 µM), with BPS effects at higher doses (1 µM). The highest percentage of larvae exhibiting overeating was observed with BPE (94%), BPAF (85%), and BPC-Cl (84%). BPA, BPC-Cl, and BPAF significantly upregulated pck1 and hcrt expression, while leptin and ghrelin levels remained unchanged. BPA produced the largest fold change in gene expression, followed by BPC-Cl and BPAF.

Conclusion: Zebrafish larvae, as a recognized NAM bridging *in vitro* and mammalian toxicology, effectively revealed differential obesogenic potencies and appetite gene modulation by BPA and analogues. These findings highlight disrupted hunger-satiety feedback as a mechanism for obesogenic effects and advance understanding of endocrine disruptor impacts on metabolic health and obesity risk.

**Abstract 014 – Postdoctoral Trainee**

## Mitochondrial Bioenergetics Drive PFAS-Induced Dopaminergic Vulnerability in Zebrafish

**Umamaheswari Sathisaran[1], Jennifer L. Freeman[1]**

*[1]School of Health Sciences, Purdue University, West Lafayette, IN, USA*

Per- and polyfluoroalkyl substances (PFAS) are environmentally persistent chemicals with growing concern for neurotoxicity. While long-chain PFAS such as perfluorooctanoic acid (PFOA- C8) are well studied, the toxicity mechanisms of shorter-chain alternatives, including hexafluoropropylene oxide dimer acid (GenX- C6) and perfluorobutanoic acid (PFBA- C4), remain poorly understood. This study investigated how developmental exposure to PFOA, GenX, and PFBA impairs mitochondrial bioenergetics and dopaminergic signaling in zebrafish (*Danio rerio*), an integrative vertebrate model of early neurodevelopment. Embryos were exposed to 0, 0.04, 0.4, 4, or 40 ppb (µg/L) of each PFAS from 1–72 hpf, and endpoints were analyzed at 120 hpf. Fluorescence-based quantification of intracellular ATP demonstrated marked reductions in cellular energy levels, with significant declines at 0.4, 4, and 40 ppb in PFOA and GenX, and at 4 and 40 ppb in PFBA, indicating impaired oxidative phosphorylation and reduced neuronal energy capacity. Monoamine oxidase activity increased at 4 and 40 ppb in PFOA and GenX, and at 0.4–40 ppb in PFBA, suggesting enhanced dopamine catabolism. Correspondingly, ELISA-based analyses demonstrated reduced dopamine levels alongside elevated DOPAC, confirming disruption of dopaminergic metabolism. Tyrosine hydroxylase (TH) immunofluorescence revealed a loss of TH-positive neurons, and confocal-based morphometric analysis identified decreased brain size in PFAS-exposed larvae, consistent with impaired neurodevelopment. Cell death across the larval head was detected using a membrane integrity assay, consistent with mitochondrial dysfunction and ATP depletion, with significant increases at 40 ppb in PFOA and PFBA. MitoTracker imaging further confirmed disrupted mitochondrial distribution, providing a mechanistic link between PFAS exposure, energy failure, and neuronal vulnerability. These findings reveal that both legacy and emerging PFAS compromise mitochondrial bioenergetics, accelerate dopamine catabolism, and reduce dopaminergic neuron populations during early brain development. By integrating mitochondrial dysfunction with neurotransmitter imbalance, neuronal loss, and structural brain deficits, this study identified dopaminergic circuits as a critical target of PFAS neurotoxicity. The mechanistic link between bioenergetic failure and dopaminergic vulnerability underscores the potential role of PFAS in the pathogenesis of neurodevelopmental and neurodegenerative disorders, highlighting the urgent need for regulatory reassessment of shorter-chain PFAS.

**Abstract 015 – Postdoctoral Trainee**

## Dual CXCR4/CXCR7 Modulation: TC14012 as a Novel Therapeutic Strategy Against Doxorubicin-Induced Cardiotoxicity

**Xiaodan Hui[1], Chunjie Gu[1], Ahmed Abdelbaset-Ismail[1], Jason Xu[1], Ashok Raj[2], Zhongbin Deng[3], Wuqiang Zhu[4], Lu Cai[1], Yi Tan[1]**

*[1]Department of Pediatrics, University of Louisville, Louisville, KY, United States.[2]Norton Children's Cancer Institute, Norton Children's Hospital, Louisville, KY, United States.[3]Department of Surgery and Brown Cancer Center, University of Louisville, Louisville, KY, United States.[4]Department of Cardiovascular Medicine, Physiology and Biomedical Engineering, Mayo Clinic, Scottsdale, AZ, United States.*

Background: Doxorubicin (DOX) is an effective chemotherapy but limited by severe cardiotoxicity. Chemokine receptors CXCR4 and CXCR7 are involved in regulating cardiac survival, inflammation, and fibrosis, yet their specific roles in DOX-induced cardiotoxicity (DICT) remain unclear.

Objective: We investigated the cardioprotective effects of TC14012, a dual-target CXCR4 antagonist and CXCR7 agonist, against DICT.

Methods: Male C57BL/6J mice received chronic DOX administration with twice-weekly TC14012 treatment. Cardiac function was evaluated by echocardiography. Markers of cardiac fibrosis and inflammation were assessed. RNA-seq analysis was performed to explore associated molecular pathways. Key pathways were validated in cardiac cells and bone marrow-derived mast cells. The data were analyzed and assessed using one-way ANOVA, with significance set at $p<0.05$.

Results: TC14012 significantly improved cardiac function, indicated by preservation of ejection fraction and fractional shortening, and increased survival rates optimally at 5 mg/kg, which were accompanied by reductions in cardiac collagen deposition and infiltration of macrophages and neutrophils. RNA-seq revealed that TC14012 protecting against DICT was associated with a remarkable stimulation of signals involving in cardiac contraction and energy reserve metabolism processes, particularly glycogen and glucan metabolism that are crucial for meeting the cardiac energy demand in response to DOX induction. These signals could be further enriched into AMPK signaling pathway and validated by enhancement of cardiac AMPK phosphorylation both in vivo and in vitro. Meanwhile, TC14012's cardioprotective effects were accompanied by a reduction in signals involving in cardiac mast cell activation and degranulation, which could be enriched into Rap1 signaling pathway and validated by TC14012 inhibiting mast cell activation and Rap1-GTP activity in vitro. Importantly, TC14012 did not compromise DOX's antitumor efficacy in EL-4 lymphoma-bearing mice.

Conclusions: TC14012 may prevent DICT by activating CXCR7-AMPK signaling to reprogram cardiomyocyte metabolism and antagonizing CXCR4-Rap1 signaling to blunt mast cell-mediated sentinel effects without compromising the anticancer efficacy DOX.

**Abstract 016 – Postdoctoral Trainee**

## Sirtuin pathway as a target to developmental exposure to methylmercury in hiPSC-derived cortical neurons

**Isabella Bramatti[1], Anke Tukker[1], Ribhav Mishra[1], Aaron B. Bowman[1]**

*[1] School of Health Sciences, Purdue University, West Lafayette, IN, USA*

Methylmercury (MeHg) is a well-known environmental neurotoxicant with human exposure primarily through dietary sources such as fish and rice. MeHg exposure is strongly linked to oxidative, disruption of redox homeostasis, and mitochondrial dysfunction. Developmental exposure to MeHg is of particular concern, as it can lead to long-lasting neurotoxic effects that persist in adulthood. Sirtuin 1 (SIRT1), a NAD+-dependent protein deacetylase, plays a critical role in cellular responses to oxidative stress, cell proliferation viability and cell death. Given its involvement in neurodevelopment and mitochondrial biogenesis, dysregulation of SIRT1 signaling has been implicated in various neurodegenerative disorders, including Alzheimer's and Huntington's disease. Studies suggest that sirtuin pathway may also mediate the toxic effects of environmental pollutants, including mercury. Previous scRNA-seq and proteomics analysis from our lab demonstrated that the Sirtuin pathway is significantly altered after developmental exposure to MeHg. Therefore, we hypothesized that MeHg disrupts SIRT1 signaling, leading to persistent neurotoxic effects, and that pharmacological modulation of this pathway could either mitigate or exacerbate MeHg-induced phenotypes. To investigate this, we exposed human induced pluripotent stem cells (hiPSCs) to low doses of MeHg (1, 10, and 100 nM) during a developmental window of neuronal differentiation (D7–D13) and are assessing SIRT1 pathway expression and SIRT1 downstream targets (e.g., NF-kB). Additionally, we are modulating SIRT1 activity using the selective inhibitor EX-527 (Selisistat) and the activator Resveratrol (RSV). Our previous results demonstrate that SIRT1 protein expression and its nuclear localization are not significantly altered following developmental MeHg exposure. However, our scRNA-seq analysis revealed significant enrichment of the sirtuin signaling pathway, with most of the differentially expressed genes linked to mitochondrial function. This suggests that while SIRT1 expression and localization may remain unchanged, downstream signaling or potential redistribution between the nucleus and cytoplasm could still be affected. To address this, we are investigating whether pharmacological modulation of SIRT1 influences these outcomes. Together, these studies will help clarify the extent to which SIRT1 contributes to the long-term effects of MeHg-induced neurotoxicity.

**Abstract 017 – Postdoctoral Trainee**

## Embryonic Atrazine Exposure in Zebrafish: Neurotoxic Impacts on Amyloid Pathway and Behavior Independent of Oxidative Stress

**Karthikeyan S. Chelladurai, Jennifer L. Freeman**

*School of Health Sciences, Purdue University, USA*

Atrazine is a widely used herbicide, primarily in agriculture to control broadleaf and grassy weeds. Despite its extensive use, concerns have emerged regarding its potential neurotoxic effects in humans. Environmental exposure to atrazine and other neurotoxicants has been increasingly linked to neurodegenerative diseases, particularly Alzheimer's (AD) and Parkinson's disease (PD); both characterized by neuronal dysfunction, oxidative stress, mitochondrial impairment, and disruption of pathways including antioxidant enzyme regulation. These features contribute to gradual central nervous system degeneration, leading to cognitive and motor deficits. Environmental toxicants like atrazine may exacerbate these conditions by inducing oxidative damage, disrupting mitochondria, and interfering with pathways such as glutamate signaling. This study examined the neurotoxic effects of atrazine on antioxidant enzyme activity, glutamate signaling, and expression of genes associated with AD in a zebrafish (*Danio rerio*) model. Briefly, wild-type embryos (n=50, <2 hpf) were exposed to atrazine (0, 0.3, 3, or 30 ppb, µg/L) until 72 hpf. Embryos were washed, pooled (45/plate), and experiments replicated 6 times. Antioxidant enzyme activity, glutathione/glutamate levels, and amyloid-beta peptide concentrations were quantified using colorimetric assays. AD risk gene expression was analyzed by qPCR, and behavior assessed with a Visual Motor Response (VMR) assay. Data were evaluated using ANOVA with LSD post hoc tests ($\alpha=0.05$). Atrazine exposure had no significant effects on CuZnSOD, MnSOD, catalase, glutathione Stransferase activity, lipid peroxidation, or glutathione/glutamate levels ($p>0.05$). Interestingly, amyloidosis pathway genes were selectively downregulated with *appa* decreased at 0.3 ppb, and *bace1* decreased at 0.3 and 30 ppb ($p<0.05$), while amyloid-beta peptide and other AD risk genes were unchanged. Behavioral analysis revealed significant hypoactivity in the 30 ppb group ($p<0.05$). These findings suggest atrazine at these concentrations does not induce oxidative stress or alter glutamate signaling but selectively affects behavior and downregulates amyloidogenic pathway genes, likely via mechanisms independent of classical oxidative stress. Further research is necessary to explore the long-term implications of these changes and their potential contribution to neurodegenerative disease mechanisms.

**Abstract 018 – Postdoctoral Trainee**

## Persistent neurotoxic effects of developmental methylmercury (MeHg) exposure causes alterations in proteostasis pathway of autophagy

**R.[1], Tukker A.M. [1], Kim H. [1], Arayl U.[2], Bowman A.B. [1]**

[1] School of Health Sciences, Purdue University, West Lafayette, IN, USA; [2] Bindley Bioscience Centre, Purdue University, West Lafayette, IN, USA.

Neurotoxic effects of methylmercury (MeHg) in the human brain are often linked with its chronic or acute exposure to the human population. Interestingly, we observed that neurotoxic effects of MeHg persist to later timepoints even when the exposure of MeHg is limited to early neurodevelopmental stage of cells. To explore these persistent effects at the protein level, we used neurotypical human induced pluripotent stem (hiPSC) cells and differentiated them to a cortical neuron lineage using dual SMAD inhibition. The differentiated hiPSCs were exposed during developmental phase Days 7-12 (D7-12) to an environmentally relevant MeHg (100nM) concentration. We let these cells differentiate and divide until we observed a high number of immature neurons in the culture by D43 when cells were collected to quantify protein alterations using global proteomics. Analysis of significantly altered protein shows a significant enrichment of proteosasis mechanism of autophagy, and its linked pathways e.g. mitophagy, protein ubiquitination /deubiquitination, and mTOR. Hence, we hypothesize that disturbance in autophagy is the major proteostasis mechanism which contributes to the neurotoxic effects of MeHg. To examine molecular level changes in autophagy following developmental MeHg exposure (D7-12), we performed immunofluorescence (IF) image analysis for critical autophagy marker proteins (p62, LC3 and Ub) on hiPSC cortical cells at D43 (31 days after cessation of exposure). Our findings suggest upregulation of p62 and LC3 expression in the hiPSC-derived cortical cells indicating that autophagy-based degradation is compromised as p62 itself is degraded by autophagy and a simultaneous LC3 increase suggests a decreased autophagosome turnover. We further confirmed changes in autophagosome turnover with IF images by detecting alterations in autophagic flux at D43 with the use of the autophagy inhibitor bafilomycin. In IF images, we observed a significant accumulation of autophagy marker proteins (p62, LC3) in forms of puncta in MeHg-exposed cells suggesting that autophagosomes are formed but not degraded at the same rate. Overall, our analysis indicate that developmental MeHg exposure makes cells prone to autophagic dysfunctions and hence can cause them to become more susceptible to developing autophagy-associated neurodegenerative disorders in later life-phase.

**Abstract 019 – Postdoctoral Trainee**

## Investigating the Effects of Chronic Arsenic Exposure in Human Keratinocytes Overexpressing hsa-miR-362

**J. L. Scott, G. Rodriguez, T. B. Emran, S. Thomas, L. Abozaid, and J. C. States**

*Center for Integrative Environmental Health Science, Department of Pharmacology and Toxicology, University of Louisville*

Background: Arsenic is a Group I human carcinogen, a known clastogen that does not directly interact with DNA, but dysregulates DNA repair, chromosomal stability, mRNA alternative splicing, and miRNA (miR) expression. miRs are small non-coding RNAs that can suppress mRNA translation. hsa-miR-362 (miR-362) expression was elevated in arsenic-induced human skin tumors, suggesting a role in arsenic-induced carcinogenesis.

Hypothesis: miR-362 overexpression will suppress proteins involved in biological pathways known to be dysregulated by arsenic.

Methods and Results: Ker-CT cells were transduced with lentiviral particles overexpressing either miR-362 or a scrambled miR control (SC-miR). miR-362-5p expression was measured with TaqMan RT-qPCR with hsa-miR-191-5p as control. Four miR-362 or SC-miR clones with similar expression were selected, cultured with +/- 100 nM NaAsO2, and passaged for several weeks. Predicted miR-362-5p targets were ranked by their combined score from three miRNA – mRNA binding databases: DIANA-MicroT, TargetScan, and mirDIP. Expression of target proteins with roles in DNA repair, chromosomal stability, alternative splicing, and their mRNAs were assessed by immunoblot and RT-qPCR. Their downstream protein interactions were determined with STRING. Clonal Ker-CT cell lines transduced with miR-362 and SC-miR lentiviruses were produced. miR-362-5p overexpression was confirmed and ranged between 250- and 2000-fold over basal expression. The 54 miR-362-5p target hits that were found in all three databases were categorized by biological pathway and ranked by combined score. The top results in DNA repair (RMI1), alternative splicing (LUC7L3), and chromosomal stability (RMI1, LUC7L3, and CENPK) were confirmed to be translationally suppressed in miR-362 overexpressing cells. STRING interactions revealed that CENPK primarily interacts with other centromere proteins, RMI1 interacts with members of the Bloom and Fanconi Anemia complexes, and LUC7L3 interacts with several splice factors. Chronic culture assays demonstrated that both miR-362 overexpression and arsenic exposure inhibited cell growth compared to unexposed SC-miR. Statistical analyses were completed using 2 and 3-way ANOVAs.

Conclusions: Overexpression of miR-362 was shown to suppress the expression of proteins involved in DNA repair, maintenance of chromosomal stability, and alternative splicing. The resulting dysregulation of these pathways could contribute to arsenic-induced transformation and skin carcinogenesis, but further studies are needed.

Grant support: R01ES027778, P30ES030283, T35ES014559

**Abstract 020 – PhD Student**

## Pathways of Cr(VI) Toxicity Change with Concentration in a *Caenorhabditis elegans* Model: The Importance of Exposure Parameters in Toxicological Models

**Samuel T. Vielee[1], Haiyan Lu[1], Natalie Besaw[2], Omar Prudencio[2], John P. Wise, Jr.[2]**

*[1]Department of Pharmacology & Toxicology, University of Louisville School of Medicine*
*[2]Department of Pharmacology & Experimental Therapeutics, University of Toledo*

Hexavalent chromium [Cr(VI)] is a naturally occurring metal and potent toxicant. It is currently ranked as the 17th greatest potential chemical threat to human health and one study recently estimated 94% of the U.S. population is exposed to Cr(VI) in drinking water above health reference levels. Cr(VI) is best recognized for its role as a clastogen and genotoxicant, but Cr(VI) also induces mitochondrial toxicity, cellular senescence, oxidative stress, and alters post-translational protein modifications. Here, we use a *Caenorhabditis elegans* model to consider how pathways of toxicity change following exposure to a wide range of Cr(VI) concentrations. Briefly, we synchronized worms to collect a synchronized L4 population and exposed worms to sodium chromate (0, 5, 50, 500, and 1000 mg/L) in 85 mmol sodium chloride + 0.001% Tween-20 for 24 h. These exposures result in internal Cr concentrations of approximately 0, 0.04, 0.05, 0.09, and 0.1 ng Cr/worm, respectively. Following exposure, we collected, washed, and freezecracked worms in TRIzol to extract total RNA and protein. We performed untargeted proteomics with the University of Louisville Proteomics Technology Core to investigate changes in protein abundance, relative to controls. Cr(VI) affected pathways distinctly across exposure groups. We observed: 1) exposure to 5 mg/L down-regulated proteins related to cholinergic signaling and RNA processing, 2) exposure to 50 mg/L up-regulated expression of histone proteins, suggesting chromatin remodeling, 3) exposure to 500 mg/L yielded the most changes in protein abundance, including up-regulation of stress response pathways alongside downregulation of proteins related to metabolism, cytoskeletal function, and stress defense, and 4) exposure to 1000 mg/L induced similar effects to those observed in worms exposed to 500 mg/L. In all, these data demonstrate how pathways of chemical toxicity may shift across exposure concentrations. Exposure to lower Cr(VI) concentrations (5, 50 mg/L) primarily altered pathways related to cellular signaling, neurotransmission, and DNA damage; whereas exposure to higher Cr(VI) concentrations (500, 1000 mg/L) primarily altered pathways related to mitochondrial function and oxidative stress. These data emphasize the importance of relevant exposure concentrations in toxicological assessments and emphasize distinct pathways for Cr(VI) toxicity in a novel *C. elegans* model. Transcriptomic data are pending. Future investigations will assess distinct neurotoxic pathways in *C. elegans*. Support from SOT GIFT (STV), T32-ES011564 (STV), LRC Voucher Program (Q3-FY2025), and R21-ES033327 (JPW).

**Abstract 021 – PhD Student**

## Oxidative Stress and Apoptosis Induced by MDMA and Synthetic Cathinones in HepG2 Cells

**Sydnee P. Dressel[1], Faith E. Latherow[2], Alexander S. Wisner[2,3], Frederick E. Williams[1], Isaac T. Schiefer[2,3], F. Scott Hall[1]**

*[1]Department of Pharmacology and Experimental Therapeutics, [2]Department of Medicinal and Biological Chemistry, [3]Center for Drug Design and Development, The University of Toledo, Toledo, OH, USA*

**Introduction:** Synthetic psychoactive cathinones (SPCs) such as methylone and pentylone are emerging MDMA-like substances. While MDMA is known to trigger oxidative stress–mediated liver injury, the mechanisms underlying SPC toxicity remain unclear. This study compared reactive oxygen species (ROS) production, glutathione status, apoptosis, and necrosis in HepG2 hepatocytes treated with MDMA, methylone, and pentylone.

**Methods:** HepG2 cells were exposed to varying concentrations of MDMA, methylone, and pentylone. ROS generation was quantified using real-time CellROX live-cell imaging and endpoint ROS-Glo assays. Intracellular redox balance was assessed by quantifying reduced glutathione (GSH) and the GSH/GSSG ratio. Apoptosis and necrosis were monitored via live-cell imaging with Apopxin Green/7-AAD.

**Results:** MDMA induced the strongest ROS production, accompanied by significant GSH depletion and increased apoptosis. Pentylone also triggered robust ROS, with sustained elevation detected by real-time imaging. Pentylone uniquely increased total GSH while decreasing the GSH/GSSG ratio, suggesting compensatory redox imbalance. These oxidative changes corresponded with pronounced apoptosis at 24 hr. and necrotic cell death at high concentrations. Methylone generated moderate ROS, reduced GSH, and lowered the GSH/GSSG ratio, but produced minimal apoptosis.

**Conclusions:** HepG2 studies reveal compound-specific oxidative stress responses among MDMA-like SPCs. Both MDMA and pentylone promoted ROS-driven apoptosis, with pentylone also eliciting necrosis at high doses. Methylone caused redox disruption without significant apoptotic activation, suggesting weaker hepatotoxic potential. These findings highlight oxidative stress and redox imbalance as key mechanisms driving MDMA-like cathinone hepatotoxicity.

**Abstract 022 – PhD Student**

## Electronic Cigarette Exposure During Pregnancy Causes Uteroplacental and Fetal Impairments

**Zianne A. Olverson, Sierra Saldaña, Roy A. Miller, Matthew W. Gorr**

*Dorthy M. Davis Heart and Lung Research Institute, Division of Cardiac Surgery, Department of Surgery, Biomedical Research Tower, The Ohio State University, Columbus, OH*

**Background and Purpose:** Electronic cigarettes (e-cigs) use has surpassed traditional cigarette use, with 7.2 million adults in the U.S. reporting e-cig usage, including 1-15% of pregnant women. Although e-cigs are marketed as safer alternatives to tobacco and as a nicotine cessation device, their effects during pregnancy remain poorly defined.

**Methods:** Our study investigated the impacts of e-cig aerosol exposure during pregnancy. Pregnant FVB mice were exposed to filtered air (FA) as a control, e-cig aerosol vehicle (50:50 propylene glycol and vegetable glycerin, PV) or vehicle with nicotine (PV + Nic; 2% Nic). Utilizing high-frequency ultrasound and echocardiography, maternal cardiac and vascular physiology was assessed at embryonic day 16.5 (E16.5). At E17.5, embryos and placental tissues were collected via c-section for gravimetric assessment and molecular and histological analyses via hematoxylin and eosin (H&E) staining, immunohistochemistry (IHC), and qPCR. **Results:** We found that e-cig aerosol exposure in pregnant mice, PV and PV+ Nic, significantly impaired cardiac diastolic function (E/A and E/E') ($P$<0.05, $P$<0.005 compared to filtered air (FA) group, via ANOVA). Concurrently, uterine and umbilical artery blood flow was significantly reduced ($P$<0.05 compared to FA group, via ANOVA) and fetal heart rates were reduced ($P$<0.001 and $P$<0.00001 compared to FA group, via ANOVA). At E17.5, pups assessed from e-cig aerosol-exposed dams had reduced weight and length; placental efficiency was also reduced ($P$<0.05, $P$<0.005, $P$<0.0002, and $P$<0.0001, compared to FA group, via ANOVA). Uterine artery H&E analysis demonstrated reduced lumen area in both exposure groups, and histological analysis of placentas showed a reduced placental labyrinth zone area in only the PV group, including reductions trophoblast giant cell (TGC) density in the junctional zone ($P$<0.05 compared to FA group, via ANOVA). Lastly, qPCR analysis of trophoblast giant cell markers (Tpbpa, Ctsq, Prl3d1) showed decreased gene expression in the placental decidua/junctional zone ($P$<0.07, $P$=0.0567, and $P$=0.0961 respectively compared to FA group, via ANOVA).

**Conclusions:** Taken together, these results indicate that exposure to e-cig aerosol during pregnancy, even without nicotine, reduces maternal cardiovascular function, uteroplacental blood flow, lumen area, and placental development, ultimately restricting fetal growth. Although the mechanisms are not fully uncovered, we hypothesize that *in utero* e-cig exposure to either PV or PV + Nic causes separate mechanisms of uteroplacental impairment, such as disrupted trophoblast-mediated decidual spiral artery remodeling or oxidative stress, respectively. This challenges the perception that e-cigs are a safe alternative to tobacco use in pregnancy.

**Abstract 023 – PhD Student**

## Synergistic Toxicity of E-Cigarette Flavoring Aldehydes and Nicotine in Lung Epithelial Cells

**Benjamin Dierolf, Thivanka Muthumalage**

*School of Health Sciences, Purdue University, West Lafayette, IN.*

**Background:** Flavored ENDS aerosols contain aldehydes, ketones, and esters, as well as nicotine. The mixture toxicity of these ENDS-derived chemicals on the lungs is not well understood. We hypothesized that mixtures of flavoring aldehydes, or aldehydes with nicotine, would exert synergistic toxicity in lung epithelial cells compared to individual components. **Methods:** Bronchial epithelial cells (BEAS-2B) were treated with individual aldehydes–benzaldehyde (B), furfural (F), decanal (D), salicylaldehyde (S), citral (C), p-tolualdehyde (T), 2,5-dimethylbenzaldehyde (DB), benzaldehyde(B), cinnamaldehyde (C), and veratraldehyde(V), at 10 μM – 2.5 mM, and in combination of two aldehydes at varying ratios (1:1- 1:49), to assess their individual and combined toxicological effects on lung epithelial cells. Effects of nicotine were determined by combining aldehyde with *S*-nicotine (N). Twenty-four hours post-treatment cytotoxicity was measured with MTT assays. Elicited inflammatory response was determined by a Luminex 17-plex cytokine panel. Individual chemical treatment-induced dose-response and synergism of combined chemicals were determined using CompuSyn analysis. **Results:** B:F combination showed no cytotoxicity; however, IL-6 and IL-8 levels were significantly decreased. B:D caused significant cytotoxicity, decreased IFN-γ, MCP-1, IL-5, IL-6, and IL-8, but increased GM-CSF, IFN-γ, and MCP-1. B:S showed significant cytotoxicity and a significant decrease in MCP-1, IL-6, and IL-8. B:C caused significant cytotoxicity, a significant decrease in MCP-1, IL-6, and IL-8. B:T caused significant cytotoxicity and decreased MCP-1, IL-6, IL-7, and IL-8. B:DB caused significant cytotoxicity, increased IL-6, while decreasing TNF-α and IL-7. Combining nicotine with aldehydes, such as N:C caused significant cytotoxicity proportional to the increased cinnamaldehyde ratio in the mixture. N:C caused a decrease in MCP-1 but elevated levels of MIP-1β, IL-6, and IL-8 with increased nicotine concentration. **Conclusion:** Binary mixtures of ENDS flavoring aldehydes, as well as aldehyde–nicotine combinations, caused synergistic and ratio-dependent cytotoxicity with differential inflammatory responses. These findings highlight the importance of mixture toxicity analysis for ENDS toxicity assessment and suggest that even low-level chemical combinations can exacerbate pulmonary toxicity. This study was funded by the National Institutes of Health R00ES033835.

**Abstract 024 – PhD Student**

## Vasoactive Effects of Spree Bar E-Liquid and its Nicotine Analog, 6-Methylnicotine, in Murine Aorta

**Brandon Capps II, Lexiao Jin, Daniel J. Conklin**

*Dept. of Physiology and the Center for Cardiometabolic Science, School of Medicine, 580 S. Preston St., Delia Baxter Building, University of Louisville, Louisville KY 40202*

Spree Bar, a new electronic cigarette (E-cig), contains 6-methyl nicotine (6MN; Metatine), an unregulated nicotine analog. The toxicity of 6MN is largely unknown. Because 6MN is structurally similar to nicotine, and nicotine is vasoactive, this study evaluated the direct toxic effects of 6MN on isolated murine aorta using isometric myography. We compared these effects with those elicited by nicotine and Spree Bar E-liquid. We tested 6MN effects both before and after precontraction. For pretreatment, aortas were treated with either E-liquid vehicle (PG: VG), 6MN, nicotine (up to 1 mM), or Spree Bar E-liquid (≈30–330 µM 6MN). After 5 or 60 min incubation, aortas then were contracted with phenylephrine (PE, 10 µM), and then acetylcholine (ACh, 0.1 nM-300 µM) to assess endothelium-dependent relaxation. 6MN produced concentration-dependent endothelial dysfunction (ED; n=4). For example, ACh [300 µM] relaxed PE-contracted aorta by -85±4% in the control group (n=4), -82±3% in the nicotine group, yet only -65±5% (n=4; $P<0.05$) in the 6MN group – significant ED. Aorta were also pretreated with Spree Bar (Blue Razz Ice, BRI; up to 44 µL) and then challenged with one of three contractile agonists: HiK, U4, and PE. Spree Bar BRI significantly inhibited contractions of all three agonists. HiK contraction was reduced by -71.2±3.1%, (n=3); U4 contraction by -60.9±13.0%, (n=3); and, PE contraction reduced by -90.4±10.0%, (n=3) compared with controls. In post-treatment, aortas were contracted with either PE or HiK; Spree Bar was added up to 44 µL (approx. 330 µM of 6MN). The three Spree Bar flavors (BRI, Sweet Spearmint [SS], Blood Orange Peach [BOP]) induced similar volume-dependent loss of contraction. In PE-contracted aorta, BRI reversed tension by -41.8 ±15.9% (n=3), SS relaxed tension by -69.7±9.1% (n=3), and BOP reversed tension by -44.9±12.2% (n=3). Similarly, with HiK: BRI reduced tension by -44.1±8.6% (n=3), SS relaxation was -83.5±7.3% (n=3), and BOP relaxed tension by -37.5±7.8% (n=3). These data suggest that neither 6MN nor PG:VG recapitulated contractile inhibition of Spree Bar. Although 6MN induced ED, it did not inhibit agonist-induced contraction as with Spree Bar, and thus, another toxic constituent is present in Spree Bar. That E-cigs are on the market delivering an unregulated nicotine analog and additional toxic compounds should cause concern amongst E-cig users and regulatory authorities alike.

**Abstract 025 – PhD Student**

## The Interactions between Chromosomal and Gonadal Sex Affect Lung Function After Sub-Chronic Ozone Exposure

**Dustin Rousselle, Patricia Silveyra**

*Department of Environmental and Occupational Health, Indiana University - School of Public Health Bloomington*

Epidemiological studies have shown that when exposed to ambient ozone, women typically have worse lung health outcomes such as higher mortality and decreased lung function compared to men. Animal models of acute ozone exposure have recapitulated sex differences in pulmonary responses; however, data remain conflictive. Previously, our lab found that hormone status modulates lung responses to acute ozone exposure. The contributions of sex hormones and the sex-specific responses to chronic or sub-chronic exposures remain understudied. To address this gap, we are using the Four-Core Genotype (FCG) mice, a mouse model where gonadal sex and chromosomal sex are independent of each other, to understand how same-sex and opposite-sex hormones alter the response to sub-chronic ozone.

We exposed adult FCG mice to 1.5 ppm of ozone for 2 hours, twice a week, for 6 weeks (4-7 mice per experimental group). At 24 hours after the last exposure, mice were anesthetized, administered a paralytic, and attached to a rodent ventilator (flexiVent, Scireq). Baseline lung function data was recorded, including PV loops, inspiratory capacity, airway resistance, elastance, and compliance. Three-way ANOVAs with Tukey tests were used for statistical analyses with significance at $p\text{-value} < 0.05$.

Our preliminary results indicate that PV loops for the FCG mouse model varied after exposure to ozone depending on their combined chromosome and gonad composition. Mice with the XY sex-chromosome complement had no significant response to the ozone exposure, however mice with the XX sex-chromosome complement had changes to their PV loops. XX mice with testes had a reduction in volume for the given pressures, and XX mice with ovaries had an increase in volume for the given pressures. The XX mice with ovaries also showed a significant decrease in elastance, a significant increase in inspiratory capacity, and a significant decrease in compliance.

After exposure to ozone, the increase in volumes for given pressures on the PV loop for the XX mice with ovaries suggests a shift towards emphysema, while the decrease in volumes for given pressures in the XX mice with testes suggests a shift towards fibrosis. Furthermore, the emphysema phenotype for the XX mice with ovaries is further evident by the significant increase in inspiratory capacity and decrease in elastance. In contrast, the XX mice with testes did not display the typical increase in elastance expected of a fibrosis phenotype. In conclusion, our data show that the combination of gonads and hormones affect lung function after sub-chronic exposure to ozone. Future histological analysis will confirm these lung disease progressions in the response to ozone.

**Abstract 026 – PhD Student**

## Investigating Lipid and Energy Dyshomeostasis Induced by Per- and polyfluoroalkyl Substances (PFAS) Congeners in Mouse Model Using Systems Biology Approaches

**Esraa Gabal and Priyanka Baloni**

*School of Health Science, Institute for a Sustainable Future, Institute for Integrative Neuroscience, Purdue University, West Lafayette, Indiana.*

Exposure to per- and polyfluoroalkyl substances (PFAS), including 7H-Perfluoro-4-methyl-3,6dioxaoctanesulfonic acid (PFESA-BP2), perfluorooctanoic acid (PFOA), and hexafluoropropylene oxide (GenX), has been associated with liver dysfunction. While previous research has characterized PFAS-induced hepatic lipid alterations, their downstream effects on energy metabolism remains unclear. This study investigates metabolic alterations in liver following PFAS exposure to identify mechanisms leading to hepatoxicity. **Methods:** We analyzed RNA sequencing datasets of mouse liver tissues exposed to PFAS to identify metabolic pathways influenced by the chemical toxicant. We integrated the transcriptome data with mouse genome-scale metabolic model to perform *in silico* flux analysis and investigated reactions and genes associated with lipid and energy metabolism. **Results:** PFESA-BP2 exposure caused dose- and sex-dependent changes, including upregulation of fatty acid metabolism, βoxidation, and cholesterol biosynthesis. On the contrary, triglycerides, sphingolipids, and glycerophospholipids metabolism were suppressed. Simulations from the integrated genome-scale metabolic models confirmed increased flux for mevalonate and lanosterol metabolism, supporting potential cholesterol accumulation. GenX and PFOA triggered strong PPARα-dependent responses, especially in β-oxidation and lipolysis, which were attenuated in PPARα$^{-}$/$^{-}$ mice. Mitochondrial fatty acid transport and acylcarnitine turnover were also disrupted, suggesting impaired mitochondrial dysfunction. Additional PFAS effects included perturbations in the tricarboxylic acid (TCA) cycle, oxidative phosphorylation, and blood-brain barrier (BBB) function, pointing to broader systemic toxicity. **Conclusion:** Our findings highlight key metabolic signatures and suggest PFAS-mediated disruption of hepatic and possibly neurological functions. This study underscores the utility of genomescale metabolic modeling as powerful tools to interpret transcriptomic data and predict systemic metabolic outcomes of toxicant exposure.

**Abstract 027 – PhD Student**

## Combined Toxicity of E-Cigarette Flavoring Chemicals and Metals in Human Lung Epithelial Cells

**Ehsanul Kabir, Thivanka Muthumalage**

*School of Health Sciences, Purdue University, West Lafayette, IN.*

**Background:** Menthol- and tobacco-flavored electronic cigarettes (e-cigarettes) are widely marketed as safer alternatives to combustible cigarettes. E-cigarette aerosols contain both flavoring chemicals, such as aldehydes, and metals, including nickel. The combined toxicity of these chemical–metal mixtures remain poorly understood. We hypothesized that e-cigarette-derived chemicals and metals exert synergistic toxicity on lung epithelial cells. **Methods:** Human bronchial epithelial cells (BEAS-2B) were exposed to common flavoring chemicals (vanillin, ethyl vanillin, p-anisaldehyde, and WS23) at 10 µM-1000 µM to assess dose-responses, individually and in mixtures (1:1-1:49), with or without nickel oxide (10 – 50 ug/cm$^2$). Twenty-four hours post-treatment, cytotoxicity was determined by MTT assay and AO/PI assays, and the elicited inflammatory response was assessed by quantifying IL-6 and IL-8 cytokine levels using ELISA in conditioned media. **Results:** Aldehydes induced differential toxicity thresholds (LOAELs: vanillin, 200 µM; ethyl vanillin, 300 µM; p-anisaldehyde, 100 µM; WS23, 50 µM). Mixture toxicity of vanillin and WS23 induced synergistic cytotoxicity, whereas ethyl vanillin and p-anisaldehyde showed antagonism. Ethyl vanillin and nickel oxide induced significant cytotoxicity and augmented IL-6 and IL-8 responses compared to either component alone. **Conclusion:** E-cigarette flavoring aldehydes interact with each other and with metal constituents to drive augmented epithelial injury and inflammation. Our data underscore the importance of mixture toxicity analysis in evaluating e-cigarette safety and suggest that regulatory policies should account for combined chemical–metal exposures rather than single-agent effects. This study was supported by the National Institutes of Health R00ES033835.

**Abstract 028 – PhD Student**

## Cr(VI) in Drinking Water Induces Sex Dependent Essential Metal Dyshomeostasis in Guinea Pig Liver, Brain, and Blood

**M.E. Delnicki[1], S.T. Vielee[1,2], G. Griffin[1], I. Meaza[1,3], O. Ogunsuyi[1,3], F. Gueye[1,3], A.W. Brownell[1,3], N. Butzke-Souza[1,3], J. Easley[1,3], H. Lu[1,3], S.S. Diven[1,3], J.C. Kouokam[1], J. Wise, Jr.[4], J.P. Wise Sr.[1,3], J.L. Wise[1]**

*[1]Department of Pharmacology and Toxicology, [2]Department of Pediatrics,[3] Wise Laboratory of Environmental & Genetic Toxicology, University of Louisville; [4]NeuroWise Laboratory, University of Toledo*

Hexavalent chromium [Cr(VI)] is a toxicant that enters the environment due to natural and man-made processes. Drinking water is at an elevated risk of Cr(VI) contamination due to industrial run-off settling in downstream drinking water basins. The liver is the main target organ of oral Cr(VI) exposure through contaminated drinking water, which has been found to induce hepatic inflammation and increase the incidence of liver disease and primary liver cancers in exposed individuals. Recent studies have also identified the liver as a major organ involved in the clearance of plasma amyloid beta (Aβ) from circulation, posing significant ramifications for the brain. Aβ is a protein that, if not properly cleared, accumulates and aggregates to form Aβ plaques largely involved in the pathogenesis of Alzheimer's disease (AD). Therefore, it is crucial to understand the role Cr(VI) plays in disrupting crosstalk along the liver-brain axis. Hartley guinea pigs were chosen as an *in vivo* model due to their substantial organ homology to humans, allowing for enhanced translation of data. However, while Hartley guinea pigs have a rich history in research, they have never been used as a model for studying oral Cr(VI) toxicity. To begin investigating the impact of Cr(VI) on the liver-brain axis, we first aimed to investigate essential metal dyshomeostasis that occurs in response to sub-chronic Cr(VI) exposure in the liver, brain, and blood. Hartley guinea pigs were given either 0 or 5 mg Cr(VI)/L for 90 days. Water volume was measured twice daily, and body mass recorded weekly. Liver, brain, and blood samples were digested in 70% nitric acid to quantify Cr and essential metal levels using inductively coupled plasma mass spectrometry. Statistical significance was determined using either the Student's-t test or the Mann-Whitney-U test depending on the distribution of data ($p < 0.1$). After 90 days, essential metal dyshomeostasis was observed in all three organs, with exposed females displaying altered essential metal distribution compared to exposed males and controls. This is the first study to use guinea pigs as an oral Cr(VI) exposure model. Exposed female guinea pigs appear to be more sensitive to oral Cr(VI) exposure than males, marking a notable sex difference. Future studies will consider how pro-inflammatory cytokine production is impacted by oral Cr(VI) exposure, and how this drives disease severity and progression in the liver and brain. This work was supported in part by the Jewish Heritage Fund for Excellence Faculty Recruitment Grant Program (JLW), NIEHS R35ES032876 (JPWSr) and NIEHS T32-ES011564 (MD).

**Abstract 029 – PhD Student**

## *In utero* exposure to E-cigarette vapor alters macrophage response to LPS challenge in offspring

**Rachel M. Rosenzweig, Zianne A. Olverson, Roy A. Miller, Matthew W. Gorr**

*Immunology & Immunotherapeutics Graduate Program, College of Medicine, Department of Surgery, Biomedical Research Tower, The Ohio State University, Columbus, OH*

**Rationale:** Exposure to E-cigarettes (ECs) *in utero* can have deleterious effects on offspring. Up to 7% of women use ECs during pregnancy, but the effects of exposure to EC aerosol *in utero* have yet to be fully elucidated, particularly as it pertains to the function of the immune systems of the offspring.

**Methods:** Pregnant mice were exposed to filtered air (FA), EC aerosol alone (50:50 propylene glycol:vegetable glycerin, PV), or EC aerosol with 2% nicotine (PVN), 4 hrs/day, 5 days/week for the length of gestation. We isolated bone marrow from adult male and female offspring (F1) mice and cultured the cells in DMEM with M-CSF for 7 days to differentiate the cells into bone-marrow derived macrophages (BMDMs). F1 BMDMs were exposed to a single dose of LPS (1 ng/mL to 100 ng/mL) for 24 hours. Cell culture supernatant was then collected to determine cytokine concentration via ELISA. RNA was extracted from cell lysates, and cDNA was generated to perform qPCR to determine gene expression levels of cytokines associated with macrophage-mediated pro- and anti-inflammatory immune response, and angiogenic factors. ELISA was performed on cell culture supernatant to determine cytokine expression levels. Data were analyzed via ANOVA.

**Results:** After exposure to 10 ng/mL LPS, TNFα concentration was increased in culture supernatant of male BMDMs from PV and PVN offspring ($p = 0.01$ and $p = 0.08$, respectively), as compared to FA offspring. This effect was not seen at higher LPS concentrations. Following 100 ng/mL LPS exposure, *Tnfa* gene expression in male BMDMs was increased in FA and PV offspring ($p < 0.03$), but not PVN offspring, while in female BMDMs *Tnfa* expression was increased in all groups ($p < 0.0004$). *Il-10* expression was increased in female BMDMs from FA and PV offspring ($p < 0.009$), but not PVN offspring, while no effect was seen in male BMDMs. *Il1b* expression was increased in female BMDMs from PV and PVN offspring ($p < 0.002$), but not FA offspring, but this effect was not evident in male BMDMs. *Vegfa* expression decreased in male BMDMs from FA offspring in a dosedependent manner following LPS exposure ($p < 0.008$), but this effect was abrogated in BMDMs from PV and PVN offspring. In female BMDMS, there was no change in *Vegfa* expression in any group. *Hif1a* expression was increased in female BMDMs from FA and PVN offspring ($p \leq 0.0001$) but not PV offspring, while this effect was not seen in male BMDMs.

**Conclusion:** *In utero* exposure to EC aerosol caused changes in expression of pro- and antiinflammatory factors at gene and protein levels following exposure of offspring BMDMs to LPS. These changes, associated with macrophage immune response to LPS, demonstrate implications for the effects of EC aerosol exposure on immune health of offspring.

**Abstract 030 – PhD Student**

## Neurotoxicity of glyphosate and dicamba exposures on zebrafish (Danio rerio) embryo-larval model and adult systems

**Vasisht Varsh Sridhar and Jennifer. L. Freeman**

*School of Health Sciences, Purdue University, Indiana*

Glyphosate is the most widely used agricultural herbicide in the US. Over the past 20 years use and production has tripled. Glyphosate moves into drinking water sources and is regulated at 700 parts per billion (ppb, µg/L) by the US EPA. In contrast, glyphosate-based herbicides are banned in many countries over carcinogenicity risk (IARC Group 2A, probably carcinogenic to humans). Dicamba, a herbicide not widely used at high rates historically, is gaining in popularity due to an increase in glyphosate-resistant weeds and introduction of Roundup Ready Xtend products (containing glyphosate and dicamba). Currently, there are limited toxicity studies with dicamba but those that exist report genotoxicity and biochemical toxicity. This study was designed to test the hypothesis that binary mixtures of these herbicides are more neurotoxic than singular exposures and exposure to either of these herbicides will result in developmental and adult neurotoxicity. The zebrafish (Danio rerio) was used to test this hypothesis. Exposure was initiated in embryos at 1-2 hours post fertilization (hpf) to varying concentrations of glyphosate (0.7 – 7000 ppb), dicamba (0.01 - 1000 ppb), or two binary mixtures (700 ppb glyphosate/100 ppb dicamba, 7000 ppb glyphosate/100 ppb dicamba). Fish were exposed through 72 or 120 hpf and split into two groups for early life (120 hpf) and adult stage (9-12 months) analyses. Neurotoxicity (behavior with visual motor response assay in larvae and memory related behavior in adults) and molecular alterations (expression of genes involved in the BDNF and APP pathways) were assessed. Larval results indicated glyphosate and the two binary mixtures caused significantly impaired movement concomitant with downregulation in genes involved in the BDNF and APP pathways with the binary mixtures causing more severe impairments and alterations. In the adult behavior assessments, glyphosate, dicamba and the binary exposure groups all exhibited impaired learning and memory responses in the novel object recognition test. Similarly, all exposure scenarios altered social behavior and increased anxiety-like behavior in the novel tank diving test. Along with these observations, gene expression analysis of the adult brain showed upregulation of genes in the BDNF and APP pathways. Moreover, fish with the developmental binary mixture exposure exhibited a greater impact than the single herbicide exposure groups when compared with those in the negative control treatment. Overall, neurotoxicity was observed in the glyphosate, dicamba, and mixture treatment groups with the binary mixtures exhibiting more severe impacts compared to the single herbicide exposures.

**Abstract 031 – PhD Student**

## Pulmonary mTOR disruption mediates metabolic syndrome associated susceptibility to nanoparticle exposure by regulating lipid signaling, autophagy and insulin sensitivity

**Akshada Shinde[1], Arjun Pitchai[1], Jenna Swihart[1], Kiley Robison[1], Christina Ferreira[2], Jonathan Shannahan[1]**

*[1] School of Health Sciences, Purdue University, West Lafayette, IN, USA; [2] Purdue Metabolite Profiling Facility, Purdue University, West Lafayette, IN, USA*

Individuals with metabolic syndrome (MetS) exhibit exacerbated inflammatory responses following inhalation of particulates. MetS and other metabolic diseases cause disruption in mTOR signaling which regulates lipogenesis, inhibits autophagy, and causes insulin resistance. Nanoparticles (NP) exposure may exacerbate inflammatory responses and toxicity in MetS, via further mTOR dysregulation, yet the role of mTOR in MetS-related susceptibility remains unclear. To investigate the impact of NP exposure on mTOR mediated lipid signaling and its susceptibility to inflammation, we utilized C57BL6/J mice with myeloid-derived cells (macrophages) lacking the mTORC1 specific component, Raptor, by breeding $Raptor^{fl}$ mice with $LysM^{cre}$ mice, and examine the mTORC1-dependent disruption of lipogenesis, autophagy, and insulin signaling. At 6 weeks, mice were fed either a control or high-fat western diet for 14 weeks, generating four groups: healthy wildtype, MetS wildtype, healthy Raptor KO, and MetS Raptor KO. Mice were exposed to silver nanoparticles (AgNP) via oropharyngeal aspiration or water (control). Altered mTORC1 signaling, autophagy, inflammation and insulin resistance endpoints were evaluated 24 hours after post exposure. Genotyping assessment confirmed Raptor KO myeloid-derived cells in animals of 4-5 weeks old, with reduced Raptor expressions in macrophages of KO mice compared to WT mice, validating the targeted KO model. Moreover, Raptor expression was relatively reduced in isolated macrophages compared to whole lung tissue in KO mice. We performed exploratory study in female cohorts to generate preliminary insights of study. Gene expression of inflammatory markers chemokine ligand-1 (CXCL1), macrophage inflammatory protein-2 (CXCL2), interleukin-6 (IL-6), interleukin-1 beta (IL-1$\beta$), monocyte chemoattractant protein-1 (CCL2) were upregulated in KO mice exposed to AgNP compared to KO control mice. Moreover, the inflammatory gene expressions were enhanced in WT exposed mice compared to KO mice following AgNP exposure, demonstrating the role of mTORC1 in exacerbating inflammatory response. Pulmonary lipids will be evaluated utilizing an MRM profiling approach demonstrating the effect of mTOR mediated lipid dysregulation and induction of pro-inflammatory lipid mediators following AgNP exposure. Specifically, arachidonic acid (AA), prostaglandin-E2 (PGE2), 12-hydroxyeicosaterraenoic acid (12-HETE), leukotriene-B4 (LTB4) and others will be determined. Overall, MetS in ongoing male cohort is expected to result in inhibited autophagy, increased inflammation, and elevated insulin resistance following exposure to NP. However, these responses are likely to be less pronounced in Raptor KO mice, highlighting the importance of mTORC1 in NP induced toxicity. Together, this work will provide mechanistic insights into mTORC1-dependent pathways and their contribution to MetS-related susceptibility to particulate exposures.

**Abstract 032 – PhD Student**

## The Effect of Chronic Cadmium Exposure in Pulmonary Arterial Hypertension Pathogenesis

**Dakotah Cathey , Yi Tan, Jun Cai, Lu Cai, and Jiapeng Huang**

*University of Louisville School of Medicine Department of Pharmacology and Toxicology 500S Preston Street Louisville, KY 40202*

Pulmonary arterial hypertension (PAH) is a disease wherein chronic high pulmonary arterial pressure causes right ventricular (RV) dysfunction, resulting in global heart failure, and eventually patient death. Our previous clinical studies demonstrated that PAH patients had increased blood and urine cadmium (Cd) concentrations compared to non-PAH controls. Therefore, we hypothesize that environmental factors, specifically chronic heavy metal exposure such as Cd, could directly or indirectly contribute to PAH pathogenesis. Thus, we tested whether Cd could directly cause PAH pathogenesis or worsen PAH outcomes with a SU5416 and hypoxia (SuHx) PAH mouse model.

Forty male C57/6J mice were initially split in 2 groups: control and 5ppm Cd in drinking water (n=20/each) for 8 weeks (Wks). Diastolic and systolic function was evaluated with echocardiography (echo), then, 10 mice per group were subject to PAH induction, netting 4 subgroups: CTRL, Cd, PAH, and Cd+PAH. Echo was performed 2, 3, & 4wks post-PAH induction, followed by 3 mice per group having RV systolic pressure (RVSP) measured. The remaining 7 mice per group were euthanized, where heart and lung tissue were collected for pathology and biochemical analyses: focusing on fibrosis, hypertrophy, and vascular remodeling, respectively. GraphPad Prism 10 software was used regarding statistics. After normality was established, the parametric or nonparametric equivalence of the ONE-WAY or TWO-WAY ANOVA was used, with a $p\text{-value}<0.05$ being considered significant.

Results show 12wks' Cd exposure alone significantly increased: (1) RV systolic function indicating systolic stimulation, (2) Cd accumulation within heart and lung tissue, (3) collagen content in both ventricles, and (4) LV cardiomyocyte size. This indicates pulmonary vascular and ventricle remodeling when compared to CTRL. Furthermore, western blot and RT-qPCR analyses determined that 12wks' Cd exposure alone significantly upregulated the concentration of proteins and genes involved in pulmonary arterial remodeling including alpha smooth muscle actin and elastin in the lungs, further confirming our histological results. Thus, we conclude that chronic exposure to 5ppm presents a PAH-like phenotype, with further mechanistic experiments currently being conducted to further our hypothesis.

**Abstract 033 – PhD Student**

## $PM_{2.5}$ exposure promotes premature aging and leads to endothelial dysfunction

**Daniel C. Gomes[1,2], Jingjing Zhao[2], Marina Malovichko[2], Daniel C, Conklin[2], Timothy E. O'Toole[2]**

*[1] Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY; [2] Christina Lee Brown Envirome Institute, Division of Environmental Medicine, University of Louisville, Louisville, KY*

**Introduction:** While exposure to fine air-borne particulate matter ($PM_{2.5}$) is associated with a wide range of health disorders, a complete understanding of the mechanisms for these effects is lacking. Exposure to $PM_{2.5}$ can induce oxidative stress, DNA damage, and inflammation leading to larger tissue dysfunction. While we and others have identified leukocyte telomere attrition as one outcome of $PM_{2.5}$ exposure, it is unclear if this apparent in multiple cell types, if it also induces a senescent phenotype, and if these outcomes together can impact endothelial function.

**Hypothesis:** We hypothesize that exposure to $PM_{2.5}$ accelerates the aging and senescence of multiple cell types resulting in endothelial activation and promoting susceptibility to cardiovascular disease (CVD).

**Methods:** To test this, we exposed mice to $PM_{2.5}$ or filtered air for 6h per day for 9d using Core facilities at UofL. To assess endothelial activation *in vivo*, exposed mice leukocytes and blood vessels were stained to visualize leukocytes and vascular structures and the number of leukocytes rolling on and adhering to the endothelium visualized and quantified using a multiphoton microscope. At euthanasia, harvested peripheral blood mononuclear cells (PBMNCs) and endothelial progenitor cells (EPCs) were used to assess telomere lengths by RT-PCR and levels of senescence by measuring β-galactosidase activity. During some exposures, some mice received the anti-oxidant, carnosine in drinking water, while others received the senolytics Dasatinib and Quercetin (DQ).

**Results:** $PM_{2.5}$ exposure promoted telomere attrition, increased β-Galactosidase activity and the expression of senescence-related genes in both PBMNCs and EPCs. Telomere shortening appeared to be a result of the impaired activity of telomerase reverse transcriptase. Telomere attrition and increased senescence were reversed in mice exposed to $PM_{2.5}$ and receiving carnosine or senolytics. Furthermore, $PM_{2.5}$-exposed mice had increased numbers of leukocytes rolling and adhering to the vascular endothelium and this outcome was reversed in mice receiving senolytic compounds. All statistical analysis was done using a student's t-test or One way ANOVA as needed.

**Conclusion:** $PM_{2.5}$ exposure promotes the aging and senescence of multiple cell types leading to endothelial dysfunction. These outcomes may be a common mechanism for the diverse pathological outcomes of $PM_{2.5}$ exposure.

**Abstract 034 – PhD Student**

## A Time-Based Analysis of Ventricular Dysfunction Progression in FVB Akita Mice

**Genell Jasper, Dakotah Cathey, Yi Tan, Lu Cai, Jiapeng Huang**

*Pediatrics, Anesthesia and Perioperative Medicine, University of Louisville, Kentucky*

Based on a 2021 American Diabetes Association report, 11.6% of the United States population had diabetes, and 95% of the diabetic population had type 2 diabetes (T2D). Diabetes-associated cardiovascular complications are well-documented but are often studied in the context of T2D. In preclinical research, Akita mice are used to study T1D cardiovascular diseases, but the focus is often on left ventricle (LV) function using the C57BL/6 background. We have demonstrated that FVB background OVE26 T1D mice develop diabetic complications faster than C57BL/6 background OVE26 mice. The objectives of the present study are to determine (1) whether Akita T1D mice develop right ventricular (RV) dysfunction in addition to LV dysfunction and (2) whether FVB Akita mice will develop diabetic cardiac dysfunction faster than C57BL/6 Akita mice.

A total of 15 males and 10 females were used to observe diabetic and cardiac complications. Blood pressure and non-fasting (NF) blood glucose were measured bi-weekly starting at 3.5 months of age. Mice showing signs of ascites underwent transthoracic echocardiography (echo) to assess their LV and RV function. Statistical analysis was performed using GraphPad Prism 10 software, with a combination of both descriptive statistics and normality tests being used to determine whether the parametric or nonparametric equivalence of the one-way ANOVA or student T-test was to be used. A p-value $<0.05$ was considered significant.

NF blood glucose levels for control mice were 121.1 mg/dL on average. Akita T1D mice developed diabetes and showed NF blood glucose levels above 400 mg/dL by 4 months of age. Male T1D mice lost body weight age-dependently, but not female T1D mice. Echo analysis observed significant RV failure by 5 months in Akita males as indicated by pronounced ascites. The tricuspid annulus plane systolic excursion (TAPSE) in T1D mice significantly decreased to 0.883±0.049 mm, which is low compared to recently published reference values of nondiabetic mice. Symptoms of heart failure were less severe in T1D female mice, with variable blood glucose levels and no display of ascites at 5 months of age.

These preliminary outcomes showed the FVB Akita model to be an efficient and promising model for the evaluation of diabetic effects on right heart functions. Continuing evaluation will compare cardiac dysfunction development in T1D mice between the FVB and C57BL/6 backgrounds.

**Abstract 035 – PhD Student**

## Dose and Time-Dependent Changes of Pulmonary and Systemic Lipid Composition and Spatial Distribution

**Jenna Swihart[1], Eduardo Rossini[1], Christina Ferreira[2], Arjun Pitchai[1], Jonathan Shannahan[1]**

*[1] School of Health Sciences, Purdue University, West Lafayette, IN, USA; [2] Purdue Metabolite Profiling Facility, Purdue University, West Lafayette, IN, United States*

Inhaled exposures, including ambient particulate matter and engineered nanomaterials, have been shown to disrupt lipid metabolism both locally and systemically. Despite these observations, comprehensive assessments of lipid dysregulation following inhalation exposures remain limited, and lipids are often overlooked in studies of systemic toxicity and disease. Among these exposures, silica nanoparticles (SiNPs), naturally occurring and frequently generated through human activities such as mining, construction, and manufacturing, are of particular concern. Respirable crystalline silica is a well-established cause of silicosis, chronic obstructive pulmonary disease, and lung cancer. Respiratory SiNP exposure has also been associated with extrapulmonary effects, contributing to chronic kidney disease, cardiovascular disease, and autoimmunity. Traditional lipidomic analyses rely on homogenized tissue preparations, an approach that may obscure localized effects since nanoparticles deposit heterogeneously across the lung. Desorption electrospray ionization mass spectrometry (DESI-MS) imaging overcomes this limitation by providing spatially resolved lipidomic data, thereby enhancing sensitivity and revealing site-specific lipid alterations. We hypothesized SiNP exposure induces dose- and time-dependent lipid dysregulation in both pulmonary and systemic tissues. To evaluate this, mice were exposed to 0.25, 0.5, or 1 mg of SiNPs by oropharyngeal aspiration, with controls receiving water (n=5/group). At 24 hours or 7 days post-exposure, bronchoalveolar lavage fluid and lung, liver, and kidney tissues were collected for quantitative real-time PCR of inflammatory and lipid metabolism genes. DESI-MS imaging was performed on tissues to evaluate SiNP-induced lipid alterations. Gene expression analyses demonstrated significant upregulation of the proinflammatory mediators *CCL2*, *CXCL-1*, *CXCL-2*, *IL-6*, and *IL-1β* in the lung 24 hours postexposure, with return to control levels by 7 days. While systemic tissues exhibited limited inflammatory changes, several lipid metabolism genes were altered compared to controls. Notably, *COX-2* was induced across the lung, liver, and kidney at 24 hours after exposure. DESI-MS imaging revealed dose-dependent SiNP-induced lipid dysregulation, most prominently within the lung, with histological overlays suggesting lipid alterations localized to terminal bronchioles. MS imaging of systemic tissues demonstrated lipid alterations that may mediate extrapulmonary responses to inhalation exposures. Collectively, these findings demonstrate that spatial lipidomics provide unique insights into SiNP-induce toxicity, uncovering lipid disruptions that may underlie exposure consequences. This approach offers a more holistic framework for understanding and ultimately mitigating the health risks of inhaled exposures.

**Abstract 036 – PhD Student**

## Effects of MC-100093, a GLT-1 modulator, on neuroinflammation in the prefrontal cortex and nucleus accumbens of female P rats exposed to ethanol

**Khokon Kanti Bhowmik[1], Ahmed Alotaibi[1], Magid Abou-Gharbia[2], Wayne Childers[2], Youssef Sari[1]**

*[1] Department of Pharmacology and Experimental Therapeutics, The University of Toledo, Toledo, OH 43614, USA. [2] Department of Pharmaceutical Sciences, Temple University School of Pharmacy, Philadelphia, PA 19140, USA.*

Chronic ethanol intake can elevate extracellular glutamate concentrations in key reward-related brain regions, including the prefrontal cortex (PFC) and nucleus accumbens (NAc), ultimately leading to oxidative stress and inflammation. There is potential involvement of several pro-inflammatory cytokines associated with glutamate neurotoxicity. HMGβ-1 is produced in response to elevated oxidative stress and acts as an indicator of inflammation by activating various receptors, including RAGE. Interaction between RAGE and its ligands, such as HMGβ-1, activates several cellular processes, including neuroinflammation. TNF-α, a proinflammatory cytokine implicated in numerous chronic inflammatory and neurodegenerative disorders and serves as a principal mediator of neuroinflammation. Recent studies from our laboratory demonstrated that MC-100093, a novel synthetic beta-lactam, lacking antibacterial properties and functioning as a modulator of one of the major glutamate transporters such as glutamate transporter 1 (GLT-1). This latter drug was found to decrease ethanol consumption, and this reduction in ethanol consumption is associated with the normalization of GLT-1 in the NAc. In this current study, we examined the impact of the GLT-1 modulator, MC-100093, on chronic ethanol consumption and neuroinflammation in specific subregions of the NAc (core and shell) and PFC (Prelimbic, PL; and Infralimbic, IL) of female P rats in a dose-dependent manner. MC-100093 treatment decreased ethanol consumption at both doses (100 and 150 mg/kg, i.p.) following a five-week drinking paradigm. MC-100093 reduced ethanol-induced elevation of the pro-inflammatory cytokines HMGβ-1 and TNF-α across all investigated mesocorticolimbic brain regions. Moreover, MC-100093 treatment attenuated the ethanol-induced elevation of RAGE in these brain regions at both doses. MC-100093 treatment reduced ethanol consumption, and this behavioral outcome correlated with a decrease in elevated pro-inflammatory markers, suggesting that MC-100093 may serve as a potential therapeutic agent for mitigating the effects of chronic ethanol exposure.

**Abstract 037 – PhD Student**

## Physicochemical Influences on Metal Interactions and Toxicity in Exposure to Real-World Micro- and Nanoplastics

**Kiley Robison[1], Arjun Pitchai[1], Somenath Mitra[2], Jonathan Shannahan[1]**

*[1]School of Health Sciences, Purdue University, West Lafayette, IN, USA; [2]Department of Chemistry and Environmental Science, New Jersey Institute of Technology, Newark, NJ, USA*

Micro- and nanoplastics (MNPs) are environmentally abundant as plastic production has increased. Degradation of plastic waste generates MNPs, which are commonly found in air, water and soil, where interactions can occur with environmental pollutants. Due to their environmental abundance and potential to interact with other pollutants, MNPs may pose a significant risk to human health. Epidemiology has reported the presence of MNPs in blood, feces, sputum and placenta. Exposure to MNPs has been linked to respiratory diseases such as pulmonary fibrosis and intestinal diseases such as Crohn's Disease. Inhalation or ingestion of MNPs can initiate systemic inflammation, oxidative stress and cytotoxicity, promoting cardiovascular diseases, reproductive disorders and carcinogenesis. Traditional studies use engineered polystyrene nanomaterials (E-PS NMs) with uniform shape and size, however, environmental MNPs have a wide size distribution and irregular shapes. These physicochemical differences can impact interactions with environmental pollutants and toxicity. We hypothesize MNPs made from real-world plastic waste will differentially interact with environmental contaminants based on their physicochemical properties, resulting in altered toxicity compared to pristine MNPs and those without environmental contaminants. Mouse macrophages were exposed to 0, 0.01, 0.1, 1, 10, 25, 50, or 100 ug/ml of representative E-PS NMs, MNPs generated from salt milling (PET, PS, PP), MNPs with adsorbed Pb, MNPs with an adsorbed metal mixture (Mn, Co, Zn, Cd, Pb), or to metal concentrations consistent with the amount adsorbed on MNP surfaces. Endpoints of toxicity, inflammation, and oxidative stress were evaluated 24 hours post-exposure. Assessments of viability identified MNP-specific alterations where MNPs with adsorbed Pb or metal mixtures were more cytotoxic than Pb or metal mixtures alone, respectively. Gene expression analyses revealed significant upregulation of key inflammatory mediators following exposure. At 50 and 100 ug/ml all MNPs without metal induced expression of IL-6 and IL-1b without modifying CXCL2. Additionally, MNPs with heavy metal associated on their surface induced IL-6 and IL-1b expression that was less than MNP or metals alone. Examination of HO-1 expression as a marker of oxidative stress demonstrated no induction at 50 ug/ml or 100 ug/ml. Overall, exposure to MNPs with or without surface adherence of heavy metals induce concentration-dependent cytotoxic responses and activate inflammatory pathways. This suggests investigating real-world MNPs with co-occurring exposures may enhance translatability of toxicity evaluations.

**Abstract 038 – PhD Student**

## Oxidative Stress as a Mediator of Long-Chain PFAS-Induced Mitochondrial Impairment

**Moriom Khatun, Umamaheswari Sathisaran, Jennifer L Freeman**

*School of Health Sciences, Purdue University, West Lafayette, IN 47907, USA*

Per- and polyfluoroalkyl substances (PFAS) are a large group of man-made chemicals that are made of a chain of carbon (C) atoms with multiple fluorine (F) atoms. Among all the PFAS, long-chain PFAS (C>6) are more bioaccumulative, persistent, and toxic. As such, long-chain PFAS such as perfluorooctane sulfonate (PFOS, C-8), perfluorodecanoic acid (PFDA, C-10), and perfluoroundecanoic acid (PFUnDA, C-11) are of concern because they persist in the environment and bioaccumulate in fish tissues, which are consumed by humans and pose a serious threat to public health. PFAS exposure is associated with developmental toxicity, developmental neurotoxicity, carcinogenicity, endocrine disruption, and immunotoxicity. This study utilized zebrafish (*Danio rerio*) embryos to investigate the developmental neurotoxicity of these compounds, with a focus on oxidative stress markers and mitochondrial distributions. Zebrafish embryos were exposed to PFOS, PFDA, or PFUnDA at concentrations of 0, 0.004, 0.04, 0.4, 4, or 40 ppb (µg/L) from 1 to 72 hours postfertilization (hpf). Oxidative stress markers such as reactive oxygen species (ROS), thiobarbituric acid reactive substances (TBARS) as an indicator of lipid peroxidation, antioxidative activity such as catalase, and the relative mitochondrial content were assessed at 120 hpf. All the three PFAS showed increased levels of ROS in all concentrations, with the exception of 0.004 ppb, where PFDA and PFUnDA did not show significant changes. For all the PFAS compounds, TBARS levels also increased in almost all the concentrations compared to 0 ppb group. The results revealed a general decline in antioxidative enzyme activity across PFAS treatments compared to the 0 ppb group. The activities following PFDA exposure caused reductions at all tested concentrations, whereas the other PFAS showed a different response. Finally, relative mitochondrial content showed changes when compared to the 0 ppb group. These findings suggest that PFAS exposure increases oxidative stress through the induction of ROS generation resulting in lipid peroxidation and disruption of the oxidative balance, leading to mitochondrial impairment. However, future studies are needed to further investigate the molecular mechanisms underlying long-chain PFAS-induced oxidative stress and their role in mitochondrial impairment.

**Abstract 039 – PhD Student**

## Strain-Dependent Effects of Trichloroethylene Exposure and Western Diet in Wild Type and Humanized PPARα Mice

**Paxton Brizendine-Ames; Abigail Ekuban; Matthew Cave; Walter Watson**

*University of Louisville; 505 S Hancock St, Louisville, KY 40202-1617*

Metabolic dysfunction-associated steatotic liver disease (MASLD) affects nearly one-third of US adults. MASLD diagnosis requires the presence of excessive hepatic fat and at least one component of metabolic syndrome of which consumption of a western diet (WD) is a major risk factor. Environmental toxicants can also cause liver injury, as seen in toxicant-associated steatohepatitis (TASH). Our research combines the concepts of MASLD and TASH focusing on an environmentally relevant, hepatotoxic chemical: trichloroethylene (TCE). TCE was the main contaminant in the water at U.S.M.C. Base Camp Lejune, North Carolina, where as many as 1,000,000 individuals were exposed to contaminated drinking water. The TCE metabolites trichloroacetic acid (TCA) and dichloroacetic acid (DCA) are agonists of peroxisome proliferator-activated receptor α (PPARα), a nuclear receptor with a critical role in maintaining lipid homeostasis and thus steatosic development and MASLD progression. PPARα displays notable species differences; therefore, mice may not be good models to study human health effects of PPARα agonists. We hypothesize that TCE metabolites alter the expression of PPARα dependent genes and predispose the liver to fat accumulation in a strain dependent manner. To elucidate potential species differences, we subjected male and female WT and transgenic humanized PPARα mice to a 12-week exposure to TCE (0.5 mg/mL), WD, or their combination. We used two-way ANOVA with the Tukey's post hoc test. Regardless of strain, male mice displayed little response to TCE exposure but were susceptible to WD-induced liver injury as evidenced by elevated serum enzymes, increased liver weight, and decreased glucose tolerance. Both WT and hPPARα female mice responded similarly to WD but displayed differential response to TCE exposure; TCE exposure did not affect WT female mice but protected against the WD-induced injury in the female hPPARα mice as evidenced by a return to control values of white adipose tissue weight, relative fat mass, true lean mass, and percentage body weight change. This demonstrates a strain specific response to TCE exposure in which TCE protected against the injury of WD only in the female hPPARα mice. This effect may involve differential PPARα activation thereby altering the hepatic response to WD and inducing protection from WD-induced injury in a strain and sex specific manner. These results highlight the importance of considering sex and strain specific factors when evaluating environmental exposures alongside dietary factors.

**Abstract 040 – PhD Student**

## Developing a high-throughput assay for analyzing damaged Cell-free DNA

**Vijayalakshmi Jogi, Michael G. Kemp**

*Wright State University, 3640 Colonel Glenn Highway, Dayton, OH 45435*

Cell-free DNA in biological fluids is widely used for noninvasive molecular testing and can be used for diagnosis and treatment monitoring of various disease conditions, yet whether cell-free DNA carries these damaged DNA adducts has not been well studied. DNA damaging agents like UV radiation, anti-cancer drug Cisplatin and Benzo(a)pyrene diol epoxide (BPDE) from cigarette smoke causes formation of bulky DNA adducts in the genomic DNA like Cyclobutane pyrimidine dimers (CPDs), Cisplatin DNA adducts and BPDE DNA adducts respectively. We developed a high-throughput, 96-well plate-based assay to detect these bulky DNA adducts in the cell-free fractions. To study the regulation of adduct release we used both pharmacological and targeted genetic approaches of DNA-repair and cell death pathways. This assay could potentially be used to detect DNA adducts in cell-free DNA from research and clinical samples and for studying cell-free DNA release and therapeutic modulation of treatments in variety of disease states.

**Abstract 041 – Medical Student**

## Validating Predicted miR-362-5p Targets in Ker-CT Human Keratinocytes

**Leen S. Abozaid, Jared L. Scott, J. C. States**

*Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, KY, USA*

Inorganic trivalent arsenic is a Group 1 human carcinogen that contaminates drinking water affecting over 200 million people globally. Chronic exposure is linked to cancers of the lung, bladder, and especially skin, including cutaneous squamous cell carcinoma, an aggressive form of skin cancer. Arsenic promotes carcinogenesis through disrupted DNA repair mechanisms, mitotic disruption, and chromosomal instability. Epigenetic changes, particularly microRNA (miR) dysregulation, have been increasingly recognized as drivers of cancer progression. miRs are post-transcriptional regulators that bind to specific mRNA sequences and alter their expression. hsa-miR-362 upregulation has been found in arsenic-induced skin tumors and several other types of cancer, but its role in skin carcinogenesis remains unclear. Mature miR-362-5p is predicted to target hundreds of genes, including several genes involved in genomic stability. RMI1, CENPK, and LUC7L3 are predicted targets essential for DNA repair via homologous recombination, chromosomal segregation and mitotic progression, and R-loop resolution and mRNA alternative splicing, respectively. We hypothesized that miR-362-5p overexpression in Ker-CT cells will significantly inhibit expression of the proteins encoded by predicted mRNA targets. Ker-CT human keratinocytes were transduced with lentiviral constructs expressing miR-362 or a scrambled miR control (SC-miR). Transduced cells were plated in sextuplet, three plates for RNA and three for protein. RT-qPCR was utilized to confirm miR-362-5p overexpression and to measure target mRNA expression. Western blots were performed to assess relative protein expression and quantified using ImageJ. Statistical analysis: two-tailed, unpaired t-tests; $\alpha = 0.05$. miR-362-5p was overexpressed an estimated 700-fold in miR-362 transductants relative to SC-miR. RMI1, CENPK, and LUC7L3 mRNA expression was not significantly altered, however, Western blot analysis revealed significantly reduced protein expression for all three targets. Predicted miR-362-5p mRNA target expression remained unchanged, but Western blot analysis revealed significantly reduced protein expression, suggesting post-transcriptional repression consistent with miR inhibition. This work helps to validate these predicted miR targets and could help to explain some of the mechanisms of arsenic-induced skin cancer.

**Abstract 042 – Master Student**

## Low concentration hexavalent chromium induced neuroinflammation in rats after a 90 day drinking water exposure

**Hamza Jamal[1], Spencer H. Roof[1], Samuel T. Vielee[1], William J. Buchanan[1], Haiyan Lu[1], Sandra S. Diven[1], Idoia Meaza[1], Jamie L. Wise[1], John P. Wise, Jr [2]**

*[1] Department of Pharmacology & Toxicology, University of Louisville, School of Medicine;*
*[2] Department of Pharmacology & Experimental Therapeutic, University of Toledo*

We are living in an era of unprecedented aging and extended lifespan. The global geriatric population (65+) is expected to reach 20% by 2050 and at the same time, the centenarian population (100+) will increase 7-fold. These dramatic demographic shifts are set to bring a host of new challenges, in particular the health impacts of environmental pollution on an aging population. Hexavalent chromium [Cr(VI)] is a ubiquitous environmental pollutant and highly potent toxicant – recently identified as the #1 chemical hazard in U.S. public drinking water. However, few studies have considered the interactions between Cr(VI) and brain aging. Reports suggest Cr(VI) induces neuroinflammation, a complex immunological reaction involving activation of microglia and astrocytes. Astrocytes are crucial for brain health as they maintain neurotransmitter homeostasis, provide structural support, help form the blood-brain barrier, among many other roles, while microglia act as resident immune cells. We investigated the intersection of toxicology and aging with the Toxic Aging Coin approach; the heads side considers how different ages are impacted by chemical toxicity, while the tails side focuses on how chemicals accelerate biological aging. We investigated Cr(VI) neurotoxicity by exposing young (3-m.o.), middle-aged (7-m.o), and geriatric (18-m.o.) male Sprague-Dawley rats to Cr(VI) in drinking water consistent with the World Health Organization (WHO) and United States Environmental Protection Agency (U.S. EPA) maximum contaminant levels (0.05 and 0.1 mg/L, respectively). Effects of Cr(VI) on astrocyte (GFAP) and microglia (IBA-1) activation in the dorsal and ventral hippocampus were assessed as markers of neuroinflammation, using immunohistochemistry and Sholl analysis. We hypothesized that Cr(VI) induces neuroinflammation. Initial results from the ventral hippocampus show microglial activation with increasing Cr(VI) concentration seen by decreases in both cell processes length and distance from cell body. Astrocyte activation was also noted with higher average intersection maxima and shifts in intersections further from the nucleus with increasing concentration. Our data begins to characterize a mechanism for which Cr(VI) exposure through drinking water induces neurotoxicity. Future work will investigate mechanisms driving microglial and astrocyte activation and better define sex-, age-, and brain region-specific differences. These data will be critical for determining how low concentrations of Cr(VI) in drinking water contribute to neurological diseases. Funding provided by T32-ES011564 (STV) and R21-ES033327 (JPW).

**Abstract 043 – Master Student**

## Impact of the hs1.2 Enhancer Alleles on TCDD-Induced Modulation of Human *Antibody* Production

**Maitreyee Chavan, Mili Bhakta-Yadav, Courtney Sulentic**

*Department of Pharmacology and Toxicology, Boonshoft School of Medicine at Wright State University, Dayton, OH 45435.*

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), a high-affinity ligand of the aryl hydrocarbon receptor (AhR), is a well-established immunotoxicant that alters antibody production in B cells. Previous studies in our laboratory using the CL-01 human Burkitt lymphoma B-cell line showed that TCDD inhibits stimulation-induced IgG secretion, leaves IgM largely resistant, and exerts a stimulation-dependent inhibition on IgA. However, how genetic variation in the immunoglobulin heavy chain (IgH), which encodes each antibody isotype (i.e. IgM, IgG, IgA), influences these effects remain unknown. The hs1.2 enhancer within a large regulatory region at the 3′ end of the *IgH* gene (3′*IgH*RR) exhibits allelic variations that have been linked to altered antibody expression and immune-related disorders.

This project proposes to investigate if genetic variations in the hs1.2 enhancer modulate the response of human B cells to TCDD. Using a panel of cell lines and CRISPR/Cas9-edited clones exhibiting different hs1.2 alleles, the effects of TCDD on IgM, IgG, and IgA secretion will be evaluated. Antibody secretion will be measured by ELISA, and corresponding *IgH* constant region transcripts will be quantified by RT-qPCR.

The outcomes of this work are expected to clarify whether hs1.2 enhancer variants result in differential sensitivity of antibody isotype production to environmental AhR ligands. Establishing this connection could inform how genetic variation contributes to differential immune response and may help assess individual susceptibility to immunotoxic exposures.

**Abstract 044 – Master Student**

## Evaluating the effects of perfluorooctanoic acid (PFOA) on antibody production and gene expression in a human B-cell line

**Syaron Ghising, Poorna Pranavi Komatineni, Sailaja Rachakonda, Courtney Sulentic**

*Microbiology and Immunology, College of Science and Mathematics, Wright State University, 3640 Colonel Glenn Highway, Dayton, OH 45435, USA*

Perfluorooctanoic Acid (PFOA) (C8) is a human-made contaminant that is very resistant to degradation and can bioaccumulate in the body despite not being lipophilic, causing potential harm to human health. Despite it being widespread, the effects of PFOA on human antibody production and related gene expression are still uncertain. Utilizing a human B-cell line (CL-01) that can be induced to express different antibody isotypes (i.e. IgM, IgG, IgA), we will evaluate the concentration-dependent effects of PFOA on antibody secretion and immunoglobulin (Ig) gene transcription. We hypothesize that exposure to PFOA will inhibit antibody production through inhibition of Ig gene transcription. If we see the reduction, we will explore the underlying molecular mechanisms and genetic variations that influence the Ig gene expression.

**Abstract 045 – Master Student**

## Influence of the Aryl Hydrocarbon Receptor on Human Antibody Production

**Tejasvini Anil Bajad, Uma Maheswary Nedumaran, Shakti Pallikaranai Venkatesa Prasath, Clayton Allex-Buckner, Nasser Alhamdan, Bassam Kashgari, Courtney Sulentic**

*Boonshoft School of Medicine. Pharmacology and Toxicology Department*

The aryl hydrocarbon receptor (AhR) is a protein that responds to environmental chemicals and can affect the immune system, including the ability of B cells to make immunoglobulins (Ig) or antibodies. In mouse studies, AhR activation reduced antibody production by inhibiting a transcriptional regulatory region within the Ig heavy chain (*IgH*) gene (i.e. 3′*IgH*RR). The 3′*IgH*RR controls *IgH* gene expression and the ability to switch from making IgM to other antibody isotypes (i.e. IgG, IgA, IgE). However, the role of the AhR protein in human B cells remain unclear, because of genetic differences between the human and mouse *IgH* gene. We generated cells with a transient AhR knockdown and results suggested that reduced AhR expression may differentially impair antibody production. The objective of the current study is to fully and stably knockout AhR expression in the CL-01 human B cell line using CRISPER-Cas9 gene editing. These cells can be induced to express and secrete antibody and switch to making different antibody isotypes. These studies will determine the role of the AhR in antibody production.

**Abstract 046 – Master Student**

## Endothelial Dependent Effects of 6-Methylnicotine, a Nicotine-derivative Present in SPREE BAR, a Novel E-cigarette, on Murine Superior Mesenteric Artery: Implications for Cardiovascular Risk

**Alyssa Nguyen, Lexiao Jin, Brandon Capps, Daniel J. Conklin**

*Center for Cardiometabolic Sciences, Dept. of Physiology, School of Medicine, 580 S Preston St., Delia Baxter, University of Louisville, Louisville KY 40202*

Electronic nicotine delivery systems (ENDS), including E-cigarettes, were introduced in China in 2003 and have since gained global popularity. These devices heat and aerosolize an E-liquid typically containing nicotine, flavorings, propylene glycol, vegetable glycerin, and other additives. In the United States, the Food and Drug Administration (FDA) has sought to regulate ENDS under the Family Smoking Prevention and Tobacco Control Act, primarily targeting nicotine containing products. However, the emergence of synthetic nicotine analogs, such as 6-methylnicotine (6MN), also known as Metatine, has challenged regulatory action. Present in SPREE BAR E-cigs, Metatine is not a tobacco product, and thus, currently falls outside the scope of FDA's regulatory framework.

Although the vascular effects of nicotine are well known, the vascular effects of 6MN are little studied. As both 6MN and nicotine are structurally similar and agonists of the α7 nicotinic acetylcholine receptor (α7nAChR), we hypothesized they would have similar vasoactivity in isolated murine superior mesenteric artery (SMA). To test this, we evaluated SMA contractility and relaxation to both 6MN and nicotine using wire micrography. Both compounds induced concentration-dependent relaxation in phenylephrine (PE) precontracted SMA. 6MN was 3x more potent than nicotine. The relaxation of either 6MN or nicotine was significantly attenuated by SMA treatment with the nitric oxide synthase (NOS) inhibitor, L-NAME. Moreover, pretreatment with the α7nAChR antagonist, mecamylamine, also blocked the relaxation of 6MN and nicotine.

Thus, 6MN compared with nicotine more potently yet similarly activated an α7nAChR to promote a nitric oxide (NO)-dependent vasorelaxation that is likely endothelium-dependent in SMA. This signaling cascade is a well-known target of nicotine and it plays a vital role in maintenance of endothelial function, promotes angiogenesis, and regulates blood pressure and blood flow. Because of 6MN's greater vasoactive potency than nicotine, concern over potential vascular complications with its abuse is warranted.

**Abstract 047 – Master Student**

## Effect of plastic leachates on the reproduction and survival of *Ceriodaphnia dubia*, a model zooplanktonic organism found in Lake Erie

**Ashlynn Malloy, Gillian Champoir, Joseph Basden, Zach Morris, Kevin E. McCluney, Christopher Ward, James S. Metcalf**

*Bowling Green State University. 1001 E Wooster St, Bowling Green, OH 43403*

Plastic pollution has been ranked the second largest global threat to environmental and human health by the United Nations. Within the Great Lakes, especially Lake Erie, the most documented plastic pollution is from pre-production pellets (PPPs) that are major contaminants of shorelines. The main concern of such pollution is the release of microplastics and leached chemicals from plastic debris that spread into the environment. Out of the seven common plastic types used in consumer goods, five were obtained for leachate experiments and toxicity testing in *C. dubia* bioassays: polyethylene terephthalate (PET, Type 1), high-density polyethylene (HDPE, Type 2), polyvinyl chloride (PVC, Type 3), polypropylene (PP, Type 5), and polystyrene (PS, Type 6). Following the recommended EPA method 1002.0 for conducting bioassays, acute toxicity was not observed in any of the five plastic types. Further evaluation of adverse effects on reproduction and survival of *C. dubia* are underway using chronic toxicity bioassays, measuring heart rate, reproduction, and mortality. It is expected that the results will show indicators of increased stress in *C. dubia* through reduced reproductive capacity, masculinization, and changes in heart rate. With the essential ecosystem and economic services that Lake Erie provides, investigating potential toxicities of plastic pollution, specifically PPP leachates to important components of the food web, will provide insights regarding the consequences of plastic accumulation and their leachates in freshwater ecosystems.

**Abstract 048 – Master Student**

## Effect of Electron-withdrawing Groups on 1,4-Dichlorobenzene Nephrotoxicity In Vitro

**Dianne K. Anestis, Mika McCormick, Cade Cole, Mia Jarrell, Jana Sherif, Jude Sherif, Linh Tran, Gary O. Rankin**

*Department of Biomedical Sciences, Marshall University, Huntington, WV*

Para-dichlorobenzene (1,4-DCB) has been identified as the most nephrotoxic dichlorobenzene amongst the three dichlorobenzene isomers in previous studies.( Rankin et.al.) 1,4-DCB is a common chemical widely used in daily life, such as in mothballs and as a fumigant for mold. It is a building block for pesticides in agriculture, which can have a direct effect on human health. Additionally, its use as a pesticide can have a significant environmental impact. 1.4-DCB is toxic to fish and other aquatic organisms. The purpose of this study was to examine the effects of various electron-withdrawing groups added to dichlorobenzene on 1,4-DCB nephrotoxicity. Compounds tested include 1,4-dichloro-2-nitrobenzene, 2,5-dichlorobenzoic acid, and 2,5-dichloro-acetophenone. Isolated kidney cells (IKCs) from male Fischer 344 rats were incubated with these chemicals (0.25-1.0 mM) or dimethyl sulfoxide (DMSO, vehicle) for 30 or 60 minutes at 37 °C in a 95% oxygen/ 5% carbon dioxide atmosphere. The release of lactate dehydrogenase (LDH) was utilized to determine cytotoxicity. The nitro group is the strongest electron-withdrawing group, and the acetyl group is the weakest electron-drawing group. All the examined electron-withdrawing groups reduced the level of 1,4-dichlorobenzene toxicity. 1,4-Dichloro-2-nitrobenzene caused the least damage to isolated kidney cells, while 2,5-dichloroacetophenone induced the highest toxicity of the tested compounds. These results indicated that the stronger the electron- withdrawing group, the greater the reduction in 1,4-dichlorobenzene nephrotoxicity. These results also support previous findings that 1,4-dichlorobenzene must be oxidized to form a toxic metabolite(s), as oxidation rates would be reduced by the addition of an electron-withdrawing group.

**Abstract 049 – Master Student**

## Synthetic Nucleoside labels for studying cell-free DNA

**Dibya Guragai, M. Alexandra Carpenter, Michael G. Kemp**

*Wright State University, 3640 Colonel Glenn Hwy, Dayton, OH 45435*

Cell-free DNA (cfDNA) originates from dying cells or can be actively secreted by living cells, and it exists unbound, in extracellular vesicles, or associated with macromolecular complexes. This cfDNA plays roles in intercellular communication and immune responses and is clinically used for diagnostics through PCR and DNA sequencing. In our lab, lesions in cfDNA were previously observed after treatment with DNA-damaging agents such as UVB and cisplatin. Building on these findings, we sought a mechanism of labeling DNA with a synthetic nucleoside to enable robust tracing using fluorescent labels and sensitive detection of low-abundance cfDNA.

We evaluated three nucleoside analogs—BrdU (bromodeoxyuridine), EdU (5-ethynyl-2'-deoxyuridine), and F-ara-EdU (2-deoxy-2-fluoro-5-ethynyluridine)—for their ability to incorporate into DNA and serve as tracers in the cell-free space. We monitored labeling efficiency, cell viability, and the involvement of apoptotic pathways, including the use of the pan-caspase inhibitor zVAD-FMK to block apoptosis.

BrdU was found to be poor nucleoside label. EdU generated strong labeling signals even in the absence of UVB treatment; however, it reduced cell viability and induced caspase-dependent apoptosis. Blocking apoptosis with zVAD-FMK did not reduce the release of EdU-labeled cfDNA, indicating that cfDNA can also be released via caspase-independent mechanisms such as active secretion. In contrast, F-ara-EdU produced robust labeling after UVB exposure without reducing cell viability.

Our findings demonstrate that while EdU is effective for cfDNA labeling, its cytotoxicity and induction of apoptosis limit its use in applications where cell viability must be preserved. F-ara-EdU, by contrast, provides strong labeling following UVB exposure with minimal toxicity, making it a safer and more reliable for studies where minimizing toxicity is important. Overall, these results underscore the importance of choosing nucleoside analogs for tracking cfDNA.

**Abstract 050 – Master Student**

## Platelet-activating factor-receptor pathway mediates solar radiation-induced extracellular vesicle release in human keratinocytes

**Divyanshu Aggarwal[1], Anita Thyagarajan[1], Ravi P. Sahu[1]**

*[1]Department of Pharmacology and Toxicology, Boonshoft School of Medicine Wright State University, Dayton, OH 45435*

Solar ultraviolet (UV) radiation is a major environmental toxicant that contributes to inflammation, immunosuppression, and carcinogenesis. Previous studies suggest that UVBinduced oxidative stress generates oxidized lipids with platelet-activating factor (PAF) agonistic activity, which act through the PAF receptor (PAFR) to activate downstream signaling responses. Extracellular vesicles (EV), including microvesicle particles (MVPs) and exosomes, are increasingly recognized as mediators of intercellular communication during environmental stress. This study examined the role and mechanisms of the PAFR signaling in solar radiationinduced EV release in human keratinocytes using various biochemical and molecular biology methodologies, including cell viability assays and nanoparticle tracking analysis. Our studies demonstrated that exposure to solar radiation reduces cell viability in a dose- and timedependent manner. Solar radiation also induced a significant dose-dependent increase in MVP and exosome release at various timepoints. Importantly, inhibition of PAFR significantly attenuated the solar radiation-induced EV release, supporting a mechanistic role for PAFR in regulating the cellular response. Our studies support that solar radiation alters keratinocyte responses by inducing EV release via the PAFR pathway. This mechanistic insight links environmental radiation to toxic intercellular signaling processes and highlights PAFR as a potential target for mitigating phototoxicity and related skin pathologies.

**Abstract 051 – Master Student**

## In vitro dihalobenzene nephrotoxicity in isolated kidney cells

**Jana A. Sherif, Jude A. Sherif, Mia Jarrell, Cade Cole, Dianne K. Anestis, Gary O. Rankin**

*Marshall School of Pharmacey, 1538 Charleston Ave, Huntington, WV 25701; Joan C. Edwards School of Medicine, Marshall University, 1600 Medical Center Dr., Huntington, WV 25701*

Bromobenzene and chlorobenzene are widely used industrial intermediates known to cause organ toxicity, including nephrotoxicity. However, limited data exists on how polyhalogenation (substitution with more than one of the same halogen) or mixed-halogen substitution alters their toxicological profiles, particularly in the kidney. This study evaluated the nephrotoxicity of mono-, di-, and mixed-halogenated benzenes using isolated kidney cells (IKCs) from male Fischer 344 rats as the in vitro model. Compounds tested were bromobenzene, 1-bromo-4-fluorobenzene, 1-bromo-4-chlorobenzene, 1-bromo-4-iodobenzene, chlorobenzene, 1-chloro-4-fluorobenzene, and 1-chloro-4-iodobenzene. IKC (~4 million/ml; 3 ml) were treated with DMSO or 0.25, 0.5, and 1.0 mM of a halogenated benzene for 30 or 60 minutes under oxygenated conditions. Cytotoxicity was measured via lactate dehydrogenase (LDH) release and trypan blue exclusion assays. Nephrotoxicity was both concentration- and time-dependent, with higher toxicity observed at increased concentrations and longer exposures. Among the brominated compounds, 1-bromo-4-chlorobenzene was the most nephrotoxic, followed by 1-bromo-4-fluorobenzene, bromobenzene, 1,4-dibromobenzene, and 1-bromo-4-iodobenzene. Within the chlorobenzene group, 1,4-dichlorobenzene showed the highest toxicity, followed by 1-bromo-4-chlorobenzene, 1-chloro-4-fluorobenzene, chlorobenzene, and 1-chloro-4-iodobenzene. These results suggest that the halogen substitution pattern is a critical determinant of nephrotoxic potential in vitro, with specific halogen types significantly influencing renal toxicity. Mixed-halogenated compounds can be as, or more, toxic than their mono- or dihalogenated counterparts, suggesting possible additive or synergistic halogen effects.

**Abstract 052 – Master Student**

## Drug Repurposing and Pathway Interactions: A Mechanistic Study in Lung Cancer Models

**Mittal Chauhan[1], Anita Thyagarajan[1], Ravi P. Sahu[1]**

*[1]Department of Pharmacology and Toxicology, Boonshoft School of Medicine Wright State University, Dayton, OH 45435*

Drug repurposing has emerged as a promising strategy to treat disease pathophysiology, including human malignancies, due to its potential to target multiple cellular signaling pathways. Arsenic-based compounds have particular interest in lung cancer, the leading cause of cancer-related deaths worldwide. However, the molecular mechanisms underlying their effects in lung cancer remain incompletely defined. The focus of this study is to determine the role of platelet-activating factor receptor (PAFR) signaling and Microvesicle particles (MVP) in regulating the cytotoxic and anti-migratory effects of arsenic compounds in lung cancer cells. PAFR-expressing lung cancer cell lines were treated with arsenic compounds at varying doses and time points. Cell viability, proliferation, migration, and cytotoxicity were evaluated. Data were analyzed using GraphPad Prism and ImageJ software for quantification of experimental outcomes. Our findings demonstrate that arsenic compounds significantly reduce lung cancer cell viability and migration in both dose and time dependent manners. Importantly, activation of PAFR reversed arsenic-mediated suppression of proliferation and migration, indicating that this pathway plays a regulatory role in attenuating arsenic compound efficacy. Moreover, evidence suggests that arsenic compounds may decrease CPAF-mediated MVP release, thereby influencing resistance-related mechanisms in lung cancer. This study highlights that arsenic compounds possess cytotoxic and anti-migratory properties in lung cancer cells and that PAFR signaling and MVP may serve as key modulators of these effects. These findings suggest that targeting PAFR and MVP pathways, in combination with repurposed arsenic compounds, could provide new therapeutic strategies for overcoming resistance and improving treatment outcomes in lung cancer.

**Abstract 053 – Master Student**

## Urinary ACE2 as an Early Biomarker for Diabetic Kidney Disease and Its Long-Term Stability in Biorepositories

**Nadia Bostick, Sridevi Gutta, Unmesha Thanekar, Mohammad Saklayen, Khalid M. Elased**

*Department of Pharmacology & Toxicology, Wright State University, Boonshoft School of Medicine, Dayton, OH, USA*

Angiotensin-converting enzyme 2 (ACE2) and neprilysin (NEP) are renal enzymes that generate the protective peptide angiotensin (1–7). Elevated urinary ACE2 has been observed in diabetic patients with chronic kidney disease (CKD) and kidney transplants. This study tested whether urinary ACE2, NEP, and ADAM17 could serve as early CKD biomarkers in diabetes. Using ELISA, Western blot, and enzyme assays, we analyzed samples from 20 nondiabetics and 40 diabetics with varying levels of albuminuria. ACE2 and NEP were significantly elevated in diabetic groups, even before albuminuria appeared, and correlated with metabolic and renal markers. Growing interest from the National Institutes of Health (NIH) in leveraging archived clinical trial samples have accelerated efforts to identify biomarkers for Type 1 Diabetes and Diabetic Kidney Disease (DKD). However, uncertainty about the long-term stability of stored proteins has limited progress. Most studies assess integrity for only short periods without standardized preservation. To address this, we evaluated human urine and murine kidney samples collected over a decade ago and stored at –80°C with protease inhibitors. Key DKD biomarkers—ACE2, NEP, ADAM17, and albumin—remained readily detectable, confirming that optimized preservation maintains protein integrity for more than ten years. Notably, stable recovery of low-abundance urinary proteins underscore the effectiveness of our stabilization approach. These findings validate urinary ACE2, NEP, and ADAM17 as early CKD biomarkers in diabetic patients and demonstrate that long-term stored samples can reliably support retrospective research. Confirming protein stability beyond a decade enhances the value of NIH and institutional biorepositories, enabling cost-effective biomarker discovery without immediate reliance on new large-scale clinical trials.

**Abstract 054 – Master Student**

## Role of AhR Transactivation Domain in Regulating B-cell Antibody Production

**Prakriti Dhungel, Karen Prarthana Kirloskar, Milli Bhakta Yadav, Eimaan Virani, Courtney E.W. Sulentic**

*Wright State University,3640 Colonel Glenn Hwy, Dayton, OH 45435*

B cells secrete antibodies that play a critical role in immune defense, and antibody production is regulated by both genetic and signaling factors. The aryl hydrocarbon receptor (AhR) is a transcription factor that senses external and cellular signals and modulates immune responses through its transactivation domain (TAD). To investigate the role of AhR TAD in antibody production, we compared a human B- cell line, CL-01 WT (heterozygous for TAD AhR function), with a CRISPR/Cas9- edited 10F10 clone expressing only functional AhR TAD+ alleles. Cells were stimulated with T cell- mimicking signals (CD40L and IL-21), and antibody secretion was measured across Ig isotypes. Preliminary results indicate that CL-01 WT cells exhibit higher antibody production in response to CD40L +IL-21 stimulation compared to 10F10 cells, suggesting that AhR TAD status modulates B- cell responsiveness to T cell derived signals. These findings provide new insight into how AhR-mediated signaling influences antibody production in human B cells.

**Abstract 055 – Master Student**

## Unmasking the Role of Polycyclic Aromatic Hydrocarbon Compounds in Lung Cancer

**Riya Rawal[1], Anita Thyagarajan[1], Ravi P. Sahu[1]**

*Department of Pharmacology and Toxicology, Boonshoft School of Medicine Wright State University, Dayton, OH 45435*

Exposure to environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs) is rising and contributes to lung cancer development. Among these compounds, benzo[a]pyrene (BaP) is well established as a carcinogen, but its impact on therapeutic outcomes in lung cancer has not been clearly defined. This study explores how BaP influences cancer cell behaviour and treatment response, with an emphasis on the roles of platelet-activating factor receptor (PAFR) signaling and microvesicle particles (MVPs). In this work, cancer cells were exposed to BaP alone or in combination with pathway modulators. A series of assays were conducted to assess changes in proliferation, migration, clonogenic assay and MVP release. The data showed that BaP exposure led to enhanced cell migration and stimulated proliferation. The results further suggested that PAFR activity and MVP release are key mediators of these BaP-driven effects. The data was analyzed using GraphPad Prism and ImageJ software for the quantification of the conducted experiments. Collectively, the findings demonstrate that BaP promotes lung cancer progression by activating signaling pathways that support migration and proliferation. This ongoing research underscores the potential for environmental pollutants to alter therapeutic responses and points to PAFR and MVP pathways as promising targets to counteract resistance and improve treatment strategies in lung cancer.

**Abstract 056 – Master Student**

## Hormonal and Environmental Signals Alter Antibody Production in Human B cells

**Sonika Gautam, Harleen Kaur Arora, Prakriti Dhungel, Courtney Sulentic**

*Wright State University. 3640 Colonel Glenn Hwy, Dayton, OH 45435, USA*

The immunoglobulin heavy chain (*IgH*) locus is essential for antibody class switching and secretion, with activity influenced by the 3'*IgH* regulatory region (3'*IgH*RR). This enhancer cluster integrates immune and environmental cues, though its role in human B cells is not fully understood. To investigate this, we treated a Burkitt's Lymphoma B-cell line (CL-01) and CRISPR/Cas9-edited variants (4E10 & 4D5) carrying different hs1.2 enhancer genotypes that carry modifications in the 3'*IgH*RR with hydrocortisone and the aryl hydrocarbon receptor (AhR) ligands TCDD and Indirubin. Hydrocortisone was chosen for its established immunomodulatory role, while TCDD and Indirubin represent environmental and dietary AhR agonists. Antibody secretion was quantified by ELISA. Preliminary results indicate that hydrocortisone suppresses immunoglobulin secretion in CL-01 cells, and this suppression is altered by co-treatment with AhR ligands-TCDD further enhances the inhibitory effects, whereas Indirubin partially reverse it. These findings support the hypothesis that hormonal and environmental pathways converge at the 3'*IgH*RR to influence antibody production in humans.

## Abstract 057 – Master Student

## Effect of Photosensitizing Drug Hydrochlorothiazide on the Release of UV-Photoproduct containing cell free DNA

**Udaya sri Byreddy, Mike G. Kemp**

*Wright State University*

Ultraviolet (UV) radiation comes from the sun and can produce cyclobutane pyrimidine dimers in the DNA of skin cells. Some of this damaged DNA can be shed into the surrounding environment by cells as cell-free DNA (cfDNA) where it serves as a useful marker for the extent of ultraviolet damage. In particular, the way cells react to UV and how much cfDNA is released may shift if certain drugs are used - especially ones that make people more susceptible to light. In this research, we are focusing on hydrochlorothiazide. Human skin keratinocytes (HaCaT cell) were incubated with different concentrations of hydrochlorothiazide. Then cells were irradiated by a small non-lethal dose of solar-simulating light (SSL). We determined cell survival and examined the cell supernatants for total cfDNA and CPD-containing cfDNA. Preliminary results show that combining hydrochlorothiazide with SSL increases the amount of CPD-containing cfDNA released. Detection of CPDs in cfDNA may serve as a novel tool for monitoring how everyday medications shape the skin’s response to sunlight.

**Abstract 058 – Undergraduate Student**

## Functional Inhibitors of Acid Sphingomyelinase Reduce UVB-Induced Erythema in Rosacea via Topical Imipramine and Amitriptyline: A Pilot Clinical Trial

**Aadil Umerani, Craig Rohan, Jade Bryant, Madison Owens, Risha Annamraju, Jeffrey Travers**

*Wright State University Boonshoft School of Medicine (3640 Colonel Glenn Hwy, Fairborn, OH 45324), Wright State Physicians (725 University Blvd, Fairborn, OH 45324)*

Background: Rosacea is a common chronic inflammatory condition often associated with photosensitivity. Previous studies by our group and others have demonstrated that Ultraviolet B (UVB) light exacerbates skin inflammation through the release of subcellular particles such as microvesicle particles (MVPs). The enzyme acid sphingomyelinase (aSMase) is a key driver for MVP generation, and functional inhibitors of aSMase (FIASMs), including the tricyclic antidepressants amitriptyline and imipramine, have been shown to block MVP release. Our study evaluated whether topical FIASMs reduce UVB-induced erythema in subjects with rosacea.

Methods: In a single-center, double-blinded, placebo-controlled clinical trial, rosacea patients and controls received either 4% topical amitriptyline or imipramine on one facial side and vehicle on the contralateral side. Participants were exposed to a low-fluence (300 J/m$^2$) of artificial UVB light. Erythema, pain, and itch were measured at baseline, 30 minutes after applying topical medication, and 10, 60, 120 minutes, and 24 hours post-UVB administration. Based on initial findings with 26 subjects, the trial was escalated to evaluate 10% formulations for enhanced efficacy, with ongoing enrollment.

Results: At the 4% concentration, both amitriptyline and imipramine produced a statistically significant reduction in UVB-induced erythema compared to vehicle (one-tailed t-test, $p < 0.05$). The 10% concentration is currently under investigation, with preliminary data from ten subjects suggesting tolerability and the potential for greater erythema reduction (two-tailed t-test, $p<0.005$). No adverse events/safety concerns were observed.

Conclusion: Topical FIASMs amitriptyline and imipramine significantly reduced UVB-induced erythema in rosacea. These studies provide premise for a novel therapeutic target for photosensitive rosacea.

**Abstract 059 – Undergraduate Student**

## The RAD50 Zinc Hook Motif as a Vulnerable Target in Heavy Metal-Induced Genotoxicity

**Andrea Arroyo, Alexandra N. Nail, Mayukh Banerjee, J. Christopher States**

*Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, KY*

**Background**: DNA double-strand breaks (DSBs) are linked to development of breast, ovarian, prostate and other cancers. The MRN complex is one of the first responders to DNA double-strand breaks (DSBs), while its disruption impairs DSB repair. The MRN complex consists of the proteins MRE11, RAD50 and NBN. To form functional MRN complex, two RAD50 molecules must bind one zinc (Zn) ion via their respective Zn hooks forming a tetrahedral Zn-S complex (CXXC-CXXC). This Zn binding allows RAD50 to bind damaged DNA and enables DNA repair. Ataxia telengectasia mutated (ATM) is activated by MRN complex. Cadmium (Cd), a non-mutagenic metal, causes suppression of ATM activation in breast epithelial cells. Exposure to another Zn displacing metal, inorganic trivalent arsenic (iAs), also causes suppression of ATM activation in skin keratinocytes. Here, we evaluate whether iAs and Cd directly bind to the RAD50 Zn hook motif and displace Zn, providing a mechanism for the observed inhibition of ATM activation caused by each metal.

**Hypothesis**: iAs and Cd will bind to the RAD50 Zn hook motif and displace Zn.

**Methods**: Synthetic P. furiosus RAD50 Zn hook peptide and UV-Vis spectrophotometry was used to characterize metal-peptide interactions. The peptide (1 Eq, 100 µM) was incubated with increasing concentrations of Zn (0 – 5 Eq; 0 – 500 µM), Cd (0 – 5 Eq; 0 – 500 µM), or iAs (0 – 10 Eq; 0 – 1000 µM) for 30 min at room temperature followed by monitoring absorbance change to assess binding of Zn (220 nm), Cd (240 nm) and iAs (270 nm). For Zn displacement, the peptide (1 Eq, 100 µM) was incubated with Zn (1 Eq, 100 µM) for 30 min followed by incubation with increasing Cd/iAs concentrations (0 – 10 Eq; 0 – 1000 µM) for another 30 min, followed by assessing absorbance changes. Binding/displacement constants were calculated by curve fitting (Michaelis-Menten/allosteric sigmoidal) as appropriate.

**Results**: Allosteric sigmoidal model provided the best fit for all metal binding kinetics. Cd (Khalf = 0.21 Eq) and Zn (Khalf = 0.20 Eq) had similar binding affinity for the RAD50 Zn hook motif, ~10-fold higher than that of iAs (Khalf = 2.16 Eq). Cd displaced Zn from the RAD50 Zn hook motif (Dissociation Khalf = 0.33 Eq, 33 µM).

**Conclusions**: Metal binding to the RAD50 Zn hook motif likely promotes carcinogenesis by inhibition of DSB repair pathways that normally prevent genomic instability. The marked difference in binding affinity of Cd and iAs for the RAD50 Zn hook suggests that Cd and iAs may differentially compromise cellular DNA-repair capacity.

**Grant support:** American Cancer Society PF-23-1153675-01-DMC and NIH-NIEHS grants R01ES027778, P30ES030283, and 1K99ES036286.

**Abstract 060 – Undergraduate Student**

## Sex- and Diet-Specific Effects of a Low-Dose Dioxin Mixture on Adipose-Liver Axis Disruption

**Kassidy Stewart, Gavin Phillips, Oluwanifemi E Bolatimi, Yuan Hua, Banrida Wahlang**

*University of Louisville, Louisville, KY, USA*

Persistent organic pollutants (POPs), including “dioxin and dioxin-like” compounds, are linked to metabolic disorders such as obesity and steatotic liver disease. The combined effects of dioxin mixtures on the adipose-liver axis, and how this contributes to metabolic outcomes, remain unclear. Thus, we examined the impact of chronic exposure to a dioxin mixture in male and female mice in the context of a hypercaloric diet, focusing on adipose and systemic metabolic outcomes.

Male and female C57BL/6 mice were fed a low-fat diet (LFD) or a high-fat diet (HFD) and treated with vehicle or defined mixture of dioxin compounds, TCDD (10ng.kg) + PeCDF (80ng/kg) + PCB 126 (140 ng/kg) over a 12-week period. Body weight was recorded weekly, and body composition was assessed using Echo-MRI. At study completion, liver, adipose tissue, and plasma were collected. White adipose tissue (WAT) morphology was assessed histologically, while hepatic and circulating lipids were quantified by biochemical assays. Hepatic xenobiotic target gene expression was measured by RT-PCR.

Males generally exhibited higher body weight and total fat mass compared to females. Male mice also had greater adipose tissue weights relative to body weight. In terms of adipose morphology, males displayed larger adipocyte area than females, while crown-like structures (CLS) burden showed modest sex differences. Diet exerted strong effects on metabolic endpoints. HFD significantly increased body weight, adiposity, adipocyte hypertrophy, and CLS burden compared to low-fat diet (LFD), regardless of sex. HFD also elevated plasma triglycerides, hepatic lipid accumulation, and markers of liver injury (ALT and AST), confirming its role as a primary driver of metabolic disruption. LFD-fed females exposed to the dioxin mixture displayed elevated hepatic triglycerides, and ALT, suggesting greater susceptibility to hepatotoxicity, while exposed males exhibited suppressed lipogenic gene expression (Fasn and Scd1). Activation of hepatic xenobiotic receptors differed by context: AHR was activated across all exposed groups (Cyp1a1 and Cyp1a2 induction), whereas CAR (Cyp 2b10) and PXR (Cyp3a11) activation were modulated in a sex- and diet-dependent manner.

Exposure to a dioxin mixture produced sex- and diet-specific metabolic effects, along the adipose-liver axis. Males were more impacted in adipose morphology, while females exhibited greater susceptibility to dioxin-induced hepatic injury. These findings demonstrate that both sex and dietary context critically shape toxicant responses and may inform risk assessment of chemical mixtures.

**Abstract 061 – Undergraduate Student**

## Hexavalent Chromium [Cr(VI)] Induces Blood-Brain Barrier Dysfunction and DNA Damage in Endothelial Cells: Using a Toxic Aging Coin to Assess Cr(VI) Neurotoxicity

**William Buchanan[1], Samuel T. Vielee[1], Haiyan Lu[1], Jamie Lynn Wise[1], Idoia Meaza[1], Aggie Barnell[1], J. Calvin Kouokam[1], Sandra S. Diven[1], Jun Cai[1], Lu Cai[1], John P. Wise, Jr[2].**

*[1]University of Louisville School of Medicine, Louiville, KY; [2]Department of Pharmacology and Experimental Therapeutics, University of Toledo, Toledo, OH*

Hexavalent chromium [Cr(VI)] is a widespread environmental pollutant and was identified as the #1 chemical hazard in U.S. drinking water. Concurrently, we are facing an increase in the proportion of the geriatric population (age 65+) and associated increasing prevalence of aging-related diseases, nearly all of which are linked to metal exposures. The blood-brain barrier (BBB) protects the brain parenchyma and regulates the exchange of exogenous materials but naturally deteriorates with age and/or toxicant exposure, underlying many age-related neurodegenerative diseases. DNA damage is a key part of biological aging and cellular senescence. Cr(VI) is a potent genotoxicant in peripheral tissues, but Cr(VI)-induced DNA damage in the BBB has never been investigated. We suspect Cr(VI) disrupts the BBB to reach the brain. We exposed Sprague-Dawley rats (both sexes) at three ages (3-, 7-, and 18- months) to Cr(VI) in drinking water at WHO and U.S. EPA maximum contaminant levels (0.05 and 0.1 mg/L, respectively) for 90 days. Brain tissues were fixed and sectioned to assess BBB integrity, DNA damage, and neuroinflammation in the dorsal hippocampus by immunofluorescence. Glial fibrillary acidic protein (GFAP, an astrocyte marker), tissue plasminogen activator (tPA, a marker for BBB stress), and 8-hydroxy-2'-deoxyguanosine (8-OHdG, a marker of oxidative DNA damage) expression were quantified by confocal microscopy and ROI analyses of individual blood vessels (2 – 20 μm diameter). Significance was assessed by the Mann-Whitney U test. tPA expression in blood vessels significantly decreased in young females exposed to 0.05 mg/L Cr(VI), middle-aged females exposed to 0.1 mg/L Cr(VI), and geriatric females exposed to both Cr(VI) concentrations. GFAP expression significantly increased in middle-aged females exposed to 0.05 mg/L Cr(VI) but decreased in geriatric females. In both young and geriatric male rats, 8-OHdG levels exhibited a concentration-associated increase following Cr(VI) exposure. Increased GFAP expression in middle-aged females may be due to astrocyte activation, while decreased GFAP expression in geriatric females may be a result of end-foot retraction. Decreased tPA expression may indicate accelerated biological aging or impaired biosignaling of the plasmin activation system, which regulates BBB permeability and the plasmin response. Together, these results indicate Cr(VI) may accelerate the aging-related decline of BBB integrity. Further research will examine the effects of Cr(VI) on other BBB structures such as tight junctions as well 8-OHdG levels in females. Funding from NIEHS R21-ES033327 (JPW), T32-ES011564 (STV), and the University of Louisville Summer Research Opportunity Program (WJB).

**Abstract 062 – Undergraduate Student**

## Effects of developmental benzo[a]pyrene exposure on cardiac function in adult mice

**Alexandria Easton, Abby Hepp, Emma Lallai, Gabi McCauley, Mackenzie Feltner, Amanda Honaker, Kaya Abbaraju, Mickayla Kowalski, and Christine Perdan Curran**

*Department of Biological Sciences, Northern Kentucky University*

**Background and purpose**: Benzo[a]pyrene (BaP) is a polycyclic aromatic hydrocarbon (PAH) that can be found in grilled foods, car exhaust, cigarette smoke, and any form of air pollution. In studies performed all over the world, BaP and other air pollutants are known for causing heart defects and cardiovascular diseases in infants and adults. In this study, we were interested in identifying genetic differences that affect cardiotoxicity in adults following exposure to BaP during gestation and lactation.

**Methods:** We compared mice *Cyp1b1(-/-)* knockout and *Cyp1b1(+/+)* wildtype, as well as *Cyp1a2(-/-)* knockout and *Cyp1a2(+/+)* wildtype to determine if there was a genotype effect. Dams were treated with 10mg/kg/day of BaP in corn oil or the corn oil vehicle. Dams were exercised for two weeks prior to meeting and until gestational day 10 (GD10). Offspring exercised from postnatal day 30 until 60 when behavioral testing began. Both groups received 1h daily of voluntary exercise on running wheels. We recorded blood pressure and heart rates when the mice were young adults at 2 months of age and followed the mice out to 6 months of age. We used the CODA non-invasive blood pressure monitoring system to record blood pressure and heart rate every week, as well as weight measurements. Data is collected automatically by the system software, which verifies when a reliable measurement was made.

**Results:** Offspring exercise had a positive effect on both blood pressure and heart rate when comparing *Cyp1b1(-/-)* knockout and *Cyp1b1(+/+)* wildtype mice ($P<0.01$). There was an exercise x genotype interaction when comparing *Cyp1a2(-/-)* knockout and *Cyp1a2(+/+)* wildtype mice. Wildtype mice had greater reductions in blood pressure and heart rate compared with knockout mice; however, *Cyp1a2(-/-)* knockouts had overall lower blood pressure and heart rates regardless of exercise treatment.

**Conclusion:** Regular exercise improved cardiac function in both *Cyp1b1(-/-)* knockout mice and wildtype mice regardless of BaP treatment. Effects may have been muted in *Cyp1a2(-/-)* knockouts, because their blood pressure and heart rates were already lower than the other two genotypes.

**Abstract 063 – Undergraduate Student**

## Persistent metabolic changes in the liver of mice exposed to benzo[a]pyrene during gestation and lactation

**Taylor Easybuck, Mackenzie Feltner, Christine Perdan Curran and Joshua Cooper**

*Department of Biological Sciences, Northern Kentucky University*

**Rationale:** Benzo[a]pyrene (BaP) is a polycyclic aromatic hydrocarbon (PAH) and a widespread pollutant that is created through incomplete combustion reactions. The metabolism of tryptophan is an important pathway that is mediated by the gut microbiome, with several tryptophan metabolites having been linked to activation of the aryl hydrocarbon receptor (AhR).One metabolite, kynurenine, can be further metabolized to either neuroprotective or neurotoxic compounds in the brain. In this study we used a mouse model with genetic differences in the CYP1A2 enzyme to see how exposure to BaP during gestation and lactation affects the tryptophan-kynurenine pathway.

**Experimental design:** We compared *Cyp1a2(-/-)* knockout mice with wild type C57Bl/6J mice. Pregnant dams were treated from gestational day 10 to postnatal day 25 (P25) with either BaP in corn oil or the corn oil vehicle. One male and one female were randomly selected for neurobehavioral testing, and their livers were collected following those tests at approximately P120. Untargeted metabolomics was performed at a KY-INBRE core facility at the University of Louisville to allow us to investigate a wide range of metabolites beyond the Trp-Kyn pathway.

**Results:** Our preliminary analysis uncovered changes in kynurenine levels and several other metabolites, but no significant differences in tryptophan. There were also significant differences in the level of oxidized v reduced glutathione. A pathway analysis uncovered consistent changes in histidine metabolism and purine synthesis as well as some changes in pathways critical for energy metabolism.

**Conclusions**: Developmental benzo[a]pyrene exposure has significant and persistent effects on multiple pathways in the livers of adult mice.

**Abstract 064 – Undergraduate Student**

## Community-engaged research to identify sites with heavy metal contamination in Newport, KY

**Elliot David Glos, Nirmal Pandit, Abilene Morgan, Cody Fry and Christine Perdan Curran**

*Department of Biological Sciences, Northern Kentucky University*

Students in a Northern Kentucky University service learning course (Environmental Toxicology) in collaboration with the City of Newport, Kentucky and two local citizens groups collected soil metal data around a suspected point source of pollution using X-ray fluorescence (XRF) and X-ray diffraction (XRD). Collection sites were private properties whose owners had become worried about the potential health effects due to contamination along with public sites in the area. Multiple XRF measurements were taken on each site, including technical replicates. Soil samples from those locations were returned to the lab for drying, screening and re-testing with XRF and additional testing with XRD. Once measurements were completed, individual reports with plots containing the average site value for each metal of concern (arsenic, cobalt, lead, and mercury) were created for the community partners and property owners. The plots contained established Environmental Protection Agency and National Institutes of Health screening levels, so property owners could make informed decisions on whether follow-up actions were needed to be taken. To help quantify whether the point source is a source of contamination at sites, location data was used along with the average site values for analysis.

**Abstract 065 – Undergraduate Student**

## Can Exercise Mitigate Motor Deficits Following Developmental Exposure to Neurotoxicants?

**Christina Gogzheyan, Mackenzie Feltner, Brooke Wetzel, Sei Lillard, Leah Bishop, Emma Lallai, Deaysha Fox, Taylor Easybuck, Elliot Glos, Renn Spigelmyer and Christine Perdan Curran**

*Department of Biological Sciences, Northern Kentucky University*

**Background**: Benzo[a]pyrene (BaP) is a neurotoxic polycyclic aromatic hydrocarbon that can be found in grilled foods, air pollution, car exhaust, and more. Pregnant women can be exposed to this carcinogen, which has been shown to cause major health deficits in their offspring. Exercise is known to improve brain function, but little is known about how exercise during pregnancy and early life can affect the developing brain. In this study, we wanted to find out if exercise could protect against exposure to BaP and its potential effect on motor function.

**Methods:** We compared *Cyp1b1(-/-)* knockout and *Cyp1b1(+/+)* wild type mice, because our previous studies showed the knockouts were more susceptible to developmental BaP exposure. We treated the dams with either 10mg/kg/day BaP in corn oil-soaked cereal or corn oil-soaked cereal alone from gestational day 10 (G10) to postnatal day 25 (P25). Dams were exercised for two weeks prior to meeting through G10. Offspring exercised from P30-P60 when behavioral testing began. Both groups received 1hr daily of voluntary exercise on running wheels. We used the Rotarod to determine if exercise improved motor function and motor memory.

**Results:** Our results represent the findings from the first seven cohorts of mice tested. We did not find a significant effect of treatment or dam exercise; however, offspring exercise significantly improved rotarod performance on Days 2-5 of testing. $P < 0.01$.

**Conclusion:** Our preliminary results suggest that offspring exercise improved both motor function and motor memory in both genotypes of mice, regardless of treatment. We will continue testing until we have n=15-20 litters per group at which point we will also be able to test for sex differences.

**Abstract 066 – Undergraduate Student**

## Using Cell Culture to Explore Chromium Exposure in Astrocytes

**Elizabeth Evans[1], Samuel T. Vielee[1], Morgan Delnicki[1], Lu Cai[1], John P. Wise, Jr.[2]**

*[1] Department of Pharmacology and Toxicology, University of Louisville School of Medicine; [2] Department of Pharmacology and Experimental Therapeutics, University of Toledo*

Hexavalent Chromium [Cr(VI)] is a known environmental toxicant that can be found in drinking water across the United States. In a national study assessing drinking water quality, chromium was named the #1 health hazard among drinking water contaminants. Sources of drinking water pollution include industrial effluents, geogenic sources, and cast-iron pipes. Cr(VI) exposure is closely monitored in occupational settings due to its distinction as a known lung carcinogen and there are many negative health outcomes associated with oral Cr exposure. Despite this, current Cr(VI) drinking water regulations are based on dermal exposure to Cr(VI). Much of the literature focuses on the effects of Cr(VI) exposure in the lungs through inhalation. The effects of chromium exposure on the brain are not well understood. Preliminary data from our lab indicated astrocyte activation in rat brains following 90 days exposure to Cr(VI) in drinking water. This study aims to characterize Cr(VI) effects on astrocytes. Astrocytes are the most abundant cell type in the brain with significant relevance to the function and protection of the central nervous system. To examine the effect Cr(VI) has on astrocytes, we assessed cytotoxicity, Cr uptake, and genotoxicity in CTX-TNA2 cells. CTX-TNA2 astrocyte lines are a type 1 cell line isolated from the cortex of 1-day-old rats. To determine what concentrations of Cr(VI) are cytotoxic to CTX-TNA2 cells, we performed a clonogenic assay. Astrocytes seeded in 100mm dishes were grown for 24h, followed by a 24h treatment with Na2CrO4 (0, 0.5, 1.0, 2.0, 3.0, or 4.0 μM Cr(VI)). Cells were harvested and plated at colony forming density for 7-8 days, stained with crystal violet, and scored for colonies formed. Cells exposed to greater than 1.0 μM Na2CrO4 exhibited a dose-dependent cytotoxic effect. To determine the effects of Cr(VI) on genomic stability, CTX-TNA2 cells were treated at the same concentrations for 24h before being arrested in metaphase with demecolcine and stained with Giemsa stain and quantifying for the quantification of chromosome damage and aneuploidy. We observed a linear dose-dependent effect for the percent of metaphases with Cr(VI)-induced damage but observed a logarithmic curve when considering total chromosome damage and observed a threshold effect for aneuploidy after 1 uM. Ongoing studies aim to expand upon these data to quantify DNA damage and repair using fluorescent microscopy techniques as well as investigate cellular senescence over longer exposures through growth curves and other assays. Overall, our data suggests 24h Cr(VI) exposure induces genomic instability and aneuploidy in a dose-dependent response. Funding provided by T32-ES011564 (STV) and R21-ES033327 (JPW).

**Abstract 067 – Undergraduate Student**

## Mechanistic Insights into Cr(VI)-Induced Neurotoxicity in the Hippocampus through ROI Analysis of 8OHdG and RAD51

**Natalie G. Besaw[1], Samuel T. Vielee[1], William J. Buchanan[1], Idoia Meaza[1], Haiyan Lu[1], Jamie Lynn Wise[1], Sandra S. Diven[1], John P. Wise, Jr.[2]**

*[1]Department of Pharmacology & Toxicology, University of Louisville School of Medicine; [2]Department of Pharmacology & Experimental Therapeutics, University of Toledo*

Hexavalent chromium [Cr(VI)] is a naturally occurring toxicant and was identified as the leading chemical hazard in U.S. public drinking water, exposing millions daily. While Cr(VI)'s systemic toxicity is well documented, its effects on the central nervous system remain poorly understood. In peripheral tissues, Cr(VI) induces DNA damage and genomic instability. Previously, we demonstrated that Cr(VI) exposure at the U.S. EPA maximum contaminant level (0.1 mg/L) altered rat behavior after just one week. After 90 days, Cr selectively accumulated in the hippocampus, induced essential metals dyshomeostasis, and induced marked neurodegeneration. Despite these findings, mechanisms driving Cr(VI)-induced neurotoxicity remain unclear. Here, we quantified DNA damage as an early event in Cr(VI) neurotoxicity, likely contributing to cellular senescence and neuroinflammation. We used brain tissues from 7-month-old female Sprague-Dawley rats exposed to 0.05 or 0.1 mg/L Cr(VI) in drinking water for 90 days. We used immunofluorescence (IF) and confocal microscopy to assess neuronal oxidative DNA damage (8-OHdG) and astrocytic DNA double strand break repair (RAD51) in the dorsal hippocampus. Further, we used IF to quantify astrocytic p16 expression and regional GFAP expression to determine if Cr(VI) induces senescence or astrocyte activation. Normality was tested using the Anderson-Darling test, and statistical significance was determined via T-test with Welch's correction (parametric) or Mann-Whitney U test (nonparametric). Correlations were evaluated using Spearman's test, with significance reported at $p < 0.05$. 8-OHdG expression did not differ significantly with Cr(VI) exposure, but we observed a significant positive correlation between hippocampal Cr levels and neuronal 8-OHdG expression in rats exposed to 0.05 mg/L. Preliminary data in astrocytes suggest aberrant localization of RAD51 from the nucleus to the cytoplasm, increased p16 expression, and increased hippocampal GFAP expression. These findings suggest hippocampal Cr accumulation may contribute to oxidative DNA damage in neurons and that impaired DNA damage repair mechanisms in astrocytes may induce senescence and neuroinflammation. Future studies will confirm effects in astrocytes and extend observations to other brain cell types. Overall, our work provides new mechanistic insight into Cr(VI)-induced neurotoxicity and reinforces the need for stricter environmental regulation of Cr(VI) exposure. Funding from a UofL School of Medicine grant, NIEHS R21-ES033327 (JPW) and T32-ES011564 (STV).

**Abstract 068 – Undergraduate Student**

## Caffeine and Diphenhydramine for Evaluation of Anxiety and Sleep Patterns in Zebrafish Larvae

**Ashilyn Joseph, Wagner Tamagno, Jennifer Freeman**

*School of Health Sciences, Purdue University, West Lafayette, IN*

Zebrafish (Danio rerio) are a valuable model for studying neurobiological processes with key neurotransmitter pathways involved in anxiety regulation, like those found in humans. This study specifically investigated how caffeine (CAF), a stimulant, and diphenhydramine hydrochloride (DPH), an antihistamine with sedative effects, affect zebrafish larvae's anxietylike behavior. An open field test (OFT) was used to evaluate exploratory and anxiety-like behavioral changes following chemical exposure. Based on earlier studies, fish were exposed to CAF 30 mg/L, CAF 40 mg/L, CAF 50 mg/L, DPH 0.45 mg/L, or DPH 0.60 mg/L and compared to a negative control (0 mg/L). According to chemical half-lives, the larvae were exposed to DPH for 30 minutes and CAF for 15 minutes prior to evaluation. The OFT was performed in 6-well plates, where larvae were filmed for 5 minutes. EthoVision XT 17.5 software was used to track and evaluate movement patterns. Multiple outcomes related to locomotor activity and anxiety were assessed (n=6, with 12 subsamples per treatment). The data was first analyzed for normality and homoscedasticity. Given non-normal distribution, statistical analysis was completed using Kruskal-Wallis test followed by Dunn’s post-hoc test. In the two highest caffeine exposures, evidence of hypomotility was observed. DPH, on the other hand, had no discernible effect on zone preference, anxiety-related behaviors, or movement. Although more investigation is needed to further understand the underlying mechanisms, the observed anxiety-like behavior brought on by caffeine may be connected to changes in histaminergic and dopaminergic processes.

**Abstract 069 – Undergraduate Student**

## Exercise reduces anxiety-like behavior in two genotypes of mice

**Deaysha Fox, Mackenzie Feltner, Kalyani Abbaraju, Alexandria Easton, Taylor Easybuck, Duong Pham and Christine Perdan Curran**

*Department of Biological Sciences, Northern Kentucky University*

**Background and Purpose:** Benzo[a]pyrene (BaP) is a polycyclic hydrocarbon (PAH) that can be found in cigarettes smoke, grilled foods, car exhaust and many other air pollutants. Developmental exposure to these toxicants has been observed to cause adverse effects on the cognitive and behavioral health of children through adolescence. Exercise has numerous benefits on cognitive function and behavior. In our studies we hypothesized that regular daily exercise would mitigate the effects of developmental benzo[a]pyrene neurotoxicity.

**Methods:** We used a mouse model with genetic differences in benzo[a]pyrene metabolism to identify individuals that are at higher risk. Our previous studies found a greater susceptibility in mice lacking the CYP1A2 enzyme. The follow-up studies were designed to investigate if maternal or offspring exercise could mitigate some of the adverse effects. Pregnant wild type *Cyp1a2(+/+)* and knockout *Cyp1a2(-/-)* dams were treated with 10/mg/kg/day of BaP in corn oil-soaked cereal from mid-gestation (G10) until weaning on postnatal day 25 (P25). Dams had free access to running wheels for 1h a day for two weeks prior to mating and until G10. Offspring exercised from P30 until P60 when behavioral testing began. We assessed anxiety-like behavior using elevated zero maze and marble burying testing.

**Results:** There was a significant main effect of offspring exercise in both tests. Offspring that exercised buried significantly fewer marbles than offspring that did not exercise ($P<0.01$). In the zero maze test, offspring that exercised had more head dips over the side of the maze and more zone crossings from one closed quadrant to the other ($P<0.01$). Interestingly, offspring that didn't exercise spent significantly longer times in the open quadrant ($P<0.001$).

**Conclusion:** Together, these data suggest that regular exercise reduces anxiety-like behavior in mice regardless of genotype or treatment.

**Abstract 070 – Undergraduate Student**

**Effect of Electron-donating Groups on 1,4-Dichlorobenzene Nephrotoxicity in Vitro.**

**Jude A. Sherif, Jana A. Sherif, Linh Tran, Mia Jarrell, Cade Cole, Dianne K. Anestis and Dr. Gary O. Rankin.**

*Department of Biomedical Sciences, Marshall University, Huntington, WV*

Chlorinated aromatic compounds are common environmental and industrial chemicals with variable nephrotoxic potential influenced by their substituent groups. This study investigated the nephrotoxicity of three chlorinated compounds: 1,2,4-trichlorobenzene (1,2,4-TCB), 2,5dichlorophenol (2,5-DCP), and 2,5-dichloroanisole (2,5-DCA), focusing on the role of electrondonating substituents on their nephrotoxic potential. Isolated kidney cells (IKCs) (approximately 4 million cells/mL; 3mL) from male Fischer 344 rats were exposed to dimethyl sulfoxide (DMSO) or 0.25, 0.5, or 1.0 mM of each compound, for 30 or 60 minutes at 37 °C with shaking under oxygenated conditions. Cytotoxicity was determined by measuring lactate dehydrogenase (LDH) release. Among the compounds tested, the decreasing order of nephrotoxicity was 2,5DCP $\geq$ 1,2,4-TCB > 2,5-DCA. This conclusion was based on the time and concentration cytotoxicity induced by the three compounds. These compounds are all derivatives of 1,4dichlorobenzene (1,4-DCB), and the hydrogen atom at the 2 -position results in a compound that is more nephrotoxic than the 2,5-DCA, yet less toxic than 2,5-DCP and 1,2,4-TCB. This trend highlights how the type and position of substituent groups can influence cytotoxicity. These findings suggest that, in general, electron-donating groups may enhance nephrotoxicity, possibly by facilitating metabolic activation or formation of reactive intermediates with the exception being the methoxy group, which reduced cytotoxicity. (Supported by NIH Grant: P20GM103434)

**Abstract 071 – Undergraduate Student**

## Zinc Unavailable: Arsenic Toxicity In Progress

**Leah Alfalah and Mayukh Banerjee**

*Department of Pharmacology and Toxicology, University of Louisville*

**Background:** Zinc is an essential trace element involved in numerous essential homeostatic cellular processes. A growing body of research suggests that dietary zinc insufficiency predisposes individuals to many disease outcomes, while making them more prone to the deleterious effects of other toxicants. One such toxicant, inorganic arsenic (iAs), is a naturally occurring metalloid widely present in contaminated groundwater and industrial waste. Chronic iAs exposure causes multi-organ cancers. Epidemiological data associate low plasma zinc in iAs exposed individuals with higher likelihood of developing skin lesions and cancer. The role of intracellular zinc levels in modulating cellular sensitivity to iAs toxicity is not understood. I aim to address this knowledge gap utilizing preclinical cell line model of iAs-induced skin toxicity.

**Hypothesis:** I hypothesize that zinc insufficiency would make skin cells more vulnerable to arsenic-induced toxicity causing enhanced cell death.

**Methods:** I performed N,N,N′,N′-tetrakis(2-pyridinylmethyl)-1,2-ethanediamine (TPEN; zinc chelator) dose-response and time course (0-5 mM; 24-72h) in HaCaT keratinocyte cell line to optimize non-toxic TPEN concentration by alamar blue assay. I determined if TPEN treatment depleted intracellular free zinc using 6-methoxy-8-p-toluenesulfonamido-quinoline (TSQ) fluorescence assay. Subsequently, I investigated if cellular zinc depletion increased toxicity of iAs in HaCaT cells employing iAs dose-response (0-100 mM) and time course (24-72 h) in presence of TPEN (0/2.5 mM; 24-72 h) and alamar blue assay.

**Results:** TPEN at 2.5 mM concentration was non-toxic to HaCaT cells up to 72 h treatment, while significantly reducing the intracellular free zinc concentration. TPEN treatment at non-toxic 2.5 mM concentration significantly increased iAs toxicity in HaCaT cell line at all three time points tested.

**Conclusion:** My data supports the hypothesis that zinc insufficiency heightens human skin cell sensitivity to iAs toxicity. My work provides a possible mechanism to explain the observation that individuals with dietary zinc deficiency are more prone to develop iAs-induced skin lesions and skin cancer.

**Abstract 072 – Undergraduate Student**

## CHEMR23 Signaling Protects Against Ozone-Induced Airspace Inflammation through RvE1 Signaling

**Nishi Suresh[1], Anushka Mehta[1], Evangeline Schott[1], Sanjay Varikuti[1], Michael Yaeger[1], Ian Cumming[2], Grace Hutton[1], Tyson Ngatikaura[1], Brita Kilburg-Basnyat[3], Robert M. Tighe[2], Saame Raza Shaikh[4], Hannah B. Lovins[1], and Kymberly M. Gowdy[1]**

*[1] The Ohio State University Wexner Medical Center, [2] Duke University Medical Center, [3] Arcus Biosciences, [4] The University of North Carolina at Chapel Hill*

Ground-level ozone ($O_3$) is a criteria air pollutant known to trigger exacerbations of chronic respiratory diseases. Inhaled $O_3$ induces an acute pulmonary inflammatory response involving leukocyte recruitment, proinflammatory cytokine release, and proinflammatory lipid mediator production via n-6 polyunsaturated fatty acids (PUFAs). Specialized pro-resolving mediators (SPMs) are bioactive lipids that are produced during inflammation to promote resolution and restore tissue homeostasis, in part by binding to their corresponding G-protein coupled receptors. Most SPMs are derived from n-3 PUFAs, including Eicosapentaenoic Acid which can be metabolized into Resolvin E1 (RvE1). Prior research from our lab showed that $O_3$ exposure reduces the pulmonary expression of the RvE1 receptor CHEMR23. CHEMR23 is predominately expressed on macrophages, which play a role in initiating and resolving inflammation. Therefore, we hypothesize that CHEMR23 expression on pulmonary macrophages alters $O_3$-induced lung inflammation. Male C57BL/6J wild-type (WT) and CHEMR23-knockout (KO) mice were exposed to filtered air or 1 part per million $O_3$ for 3 hours. At 24 hours post-exposure, bronchoalveolar lavage fluid (BAL) and lung tissue were collected to assess injury and inflammation as well as immune cell quantification via flow cytometry. To evaluate macrophage specific functions of CHEMR23, bone marrow was collected from WT and KO mice and differentiated into bone marrow derived macrophages (BMDMs). BMDMs were treated with lipopolysaccharide (LPS, 100 ng/mL) or PBS followed by RvE1 (100 nM) or vehicle control. After a 4-hour incubation, cell supernatants and lysates were collected to quantify production of pro-inflammatory cytokines. Following $O_3$ exposure, KO mice had increased airspace inflammation compared to WT mice with elevated airspace neutrophilia and proinflammatory cytokines. Furthermore, KO mice exhibited increases in pulmonary lymphocytes after $O_3$ exposure. RvE1 levels decreased post $O_3$ exposure in the airspace of WT mice which were further reduced in KO mice. Using BMDMs collected from WT and KO mice, RvE1 treatment following LPS stimulation reduced cytokine expression in WT BMDMs, however, there was no change in LPS induction of cytokines in KO BMDM following RvE1 treatment. Taken together, our results indicate that the absence of the CHEMR23 receptor augments $O_3$induced airspace inflammation. LPS-induced inflammation was reduced with RvE1 treatment in WT BMDMs, but not in KO BMDMs, suggesting RvE1's anti-inflammatory treatment is mediated through CHEMR23 signaling. Future studies will analyze primary macrophages in the lung after $O_3$ exposure to determine if CHEMR23 signaling drives resolution responses.

**Abstract 073 – Undergraduate Student**

## Glutamatergic-Driven Behavioral Alterations in Lead-Exposed Larval Zebrafish

**Saraf J. Bhuiya[1], Wagner A. Tamagno[1], Jennifer Freeman[1]**

*School of Health Sciences, Purdue University, West Lafayette, IN, United States*

Lead (Pb) exposure is a known neurotoxicant associated with cognitive dysfunction even at low concentrations, making it a relevant model for studying neurodevelopmental and neurodegenerative diseases. This study utilizes larval zebrafish (*Danio rerio*) to investigate the impact of lead exposure on behavioral patterns, particularly anxiety-response and habituation learning. Zebrafish larvae were exposed to 0, 1, 10, or 100 parts per billion (ppb) of lead acetate from 1-hour post-fertilization (hpf) to 72hpf and assessed at 168 hpf using the Acoustic Startle Response (ASR) test. This test evaluates habituation by analyzing the frequency and magnitude of C-bends, a characteristic startle response, as well as total distance moved post-stimuli. Given the critical role of the glutamatergic system in learning and memory, glutamate and glutamine levels were measured to examine potential correlations between neurotransmitter alterations and behavioral adaptations. Disruptions in glutamate-glutamine homeostasis may contribute to impaired habituation and heightened anxiety responses, which could serve as indicators of cognitive deficits. By characterizing these behavioral and neurochemical changes, this study aims to provide insight into the mechanisms of lead-induced neurotoxicity and its potential implications for neurodegenerative disease models. The habituation of startle-response behaviors of lead-exposed groups demonstrated altered patterns, suggesting potential neurotoxic effects on learning and memory processes related to the cycling of glutamine and glutamate, where measured levels of glutamate correlated most with these changes.

**Abstract 074 – Undergraduate Student**

## Investigating the effects of benzo[a]pyrene on mouse learning and behavior using Morris Water Maze and Novel Object Recognition Tests

**Minerva Shakya, LeaAnn King, Christina Gogzheyan, Leah Bishop, Deaysha Fox, Taylor Easybuck, Alexandria Easton, Kalyani Abbaraju, Duong Pham, Mackenzie Feltner and Christine Perdan Curran**

*Department of Biological Sciences, Northern Kentucky University*

**Background**: Benzo[a]pyrene (BaP) is a polycyclic aromatic hydrocarbon and a common environmental pollutant generated through vehicle smoke, burned coil, grilled foods, combustion of organic matter such as wood, coal, tobacco etc. It is a carcinogen that also causes several neurological defects in humans. Chronic exposure to BaP during early brain development has been associated with cognitive deficits, impaired synaptic plasticity, and altered neurotransmitter functions. Its neurotoxic properties especially affect the hippocampus and disrupt its functions. So, in order to determine the effects of BaP on learning and memory, we used the Morris Water Maze experiment and Novel Object Recognition test to analyze their hippocampal-dependent learning and memory.

**Method**s: We mated *Cyp1a2(+/+)* wild type mice with *Cyp1a2(-/-)* knockout mice and assigned them randomly into two groups. The treatment group received 10 mg/kg/day BaP dissolved in corn-oil-soaked cereal from gestational day 10 (G10) to postnatal day 25 (P25). The control group was given cereal soaked in corn-oil. We selected one male and one female mouse from each litter and conducted neurobehavioral tests on P60.

**Results:** There were no significant differences in Novel Object Recognition. BaP-exposed offspring that exercised for 30 days prior to behavioral testing outperformed BaP mice that did not exercise on 5 of 6 days of testing in the most difficult Reverse and Shift phases, but the differences were only statistically significant on one of the days. $P < 0.05$

**Conclusion:** Offspring exercise had a modest effect on learning and memory in both genotypes of mice exposed to BaP during early brain development.

**Abstract 075 – High School Student**

## Hexavalent Chromium Alters GABAergic Neuron Morphology, Impairs GABAergic Function, and Accelerates Biological Aging in a Juvenile C. elegans Model

**Amelia Hassett[1], Samuel T. Vielee[1], Haiyan Lu[1], Sana Turkmani[1], Michael Aschner[2], John Wise, Jr.[3]**

*[1]Department of Pharmacology & Toxicology, University of Louisville School of Medicine; [2]Department of Molecular Pharmacology, Albert Einstein College of Medicine; [3]Department of Pharmacology and Experimental Therapeutics, University of Toledo*

Hexavalent chromium [Cr(VI)] is a naturally occurring metal and widespread pollutant, currently ranked as the 17th greatest chemical threat to human health. Millions of people are exposed to Cr(VI) in drinking water on a daily basis, yet mechanisms of neurotoxicity remain understudied. We previously demonstrated exposure to low concentrations of Cr(VI) in drinking water altered behavior, disrupted essential metals homeostasis, and induced widespread neurodegeneration in a rat model. We have also shown Cr(VI) preferentially targeted GABAergic neurons and accelerated biological aging in adult *Caenorhabditis elegans*; however, no groups have considered Cr(VI) developmental neurotoxicity in *C. elegans*. Here, we consider the effects of Cr(VI) on GABAergic neurons and biological age in L1 *C. elegans*. We hypothesize Cr(VI) will induce dose-dependent neurotoxicity in *C. elegans* GABAergic neurons and perturb biological aging. We synchronized and exposed L1 CZ13799 worms (GFP-tagged GABA neurons) to sodium chromate (0, 1, 10, 100, or 1000 mg/L) with 20 µL OP50 for 24 hours. We assessed survival to determine if Cr(VI) was lethal at these concentrations. We assessed GABAergic morphology with a semiquantitative method and tested GABAergic function with a levamisole sensitivity assay. We assessed gut autofluorescence (421 nm) as a measure of biological age. Normality was assessed by an Anderson-Darling test ($\alpha = 0.05$). Significance was assessed by a Student's T-Test with a Welch's Correction or Mann-Whitney U test for parametric and non-parametric data, respectively. Significance was assigned at $p < 0.05$. Comparisons where $p < 0.1$ are noted. Gut autofluorescence decreased with Cr(VI) concentrations, suggesting disrupted metabolic homeostasis. Cr(VI) was only lethal after exposure to 100 (75% survival) and 1000 mg/L (57% survival). Changes in GABAergic morphology increased after exposure to 10 and 100 mg/L. Levamisole sensitivity assays revealed impaired GABAergic signaling, with near-complete paralysis at concentrations ≥100 mg/L. These results demonstrate Cr(VI) altered GABAergic morphology and function in an L1 *C. elegans* model. Cr(VI) exposure in L4 worms caused sublethal but progressive neurodegeneration that initially targeting GABAergic neurons before spreading to cholinergic neurons, whereas in L1 worms, Cr(VI) produced dose-dependent lethality, disrupted metabolic homeostasis, and selectively impaired GABAergic neurons. This model provides a platform for evaluating Cr(VI)'s developmental neurotoxicity and its potential relevance to human disease. Support from SOT GIFT (STV), T32-ES011564 (STV), R21-ES033327 (JPW).