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Platform Abstracts (#1-7)

Abstract #1 – Early-Stage Investigator
E-cigarette vapor causes impaired pulmonary and cardiac function in offspring

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Between 7-15% of people in the U.S. report to be regular users of e-cigarettes (ECs), including up to 7% of women that use these products during pregnancy. EC use is viewed as safer than conventional cigarettes, which includes the newest generation of EC-only users that are now reaching childbearing age. Given this prevalence of use, it is imperative to understand how exposure to EC aerosol *in utero* can affect offspring, as several of its components, including particulate matter (PM), are associated with asthma and cardiovascular disease in offspring exposed *in utero*. We have found that EC aerosol exposure during pregnancy causes offspring pulmonary dysfunction in mice, evidenced by increased lung and alveolar stiffness, collagen deposition, and sex-specific goblet cell hyperplasia, even if the e-liquid does not contain nicotine. These effects are further exacerbated by exposing offspring to allergens (dust mite, ragweed, and *aspergillus*; DRA): mice exposed *in utero* to EC vapor, both with and without nicotine, have a remarkable asthmatic phenotype demonstrated by an accentuation in airway resistance in response to methacholine challenge (significantly altered resistance, elastance, Newtonian resistance and tissue elastance via ANOVA), and increased gene expression of inflammatory factors (IL-5, TNF-α, Ccl2) compared to offspring of mice exposed to filtered air (FA) during pregnancy. We also found that these mice have impaired cardiovascular function evidenced by pressure-volume loop analysis (significantly reduced end systolic elastance in male and female mice in EC carrier group vs. FA), and found sex-specific changes in cardiac function in the F2 generation. These phenotypes resemble the results of *in utero* exposure to other contaminants such as particulate matter and cigarette smoke, leading to poor offspring health, and may indicate trans-generational changes as a result of EC use during pregnancy.

Abstract #2 – Doctoral Student
The neurobehavioral effects of chronic perfluorooctanesulfonic acid (PFOS) in mice

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Background and Purpose: Per- and polyfluoroalkyl substances (PFAS) are a group of persistent organic pollutants or "forever chemicals" that are ubiquitously found in the environment and virtually in all living organisms, including humans. Exposure to PFAS has been correlated with sex-specific effects including endocrine disruption and motor behavior deficits. Our previous studies determined out of multiple PFAS, only perfluorooctanesulfonic acid (PFOS) was selectively toxic to dopaminergic neurons in *C. elegans*. A pilot study with female mice dosed with PFOS for 6 months demonstrated decreases in monoamine levels of dopamine and serotonin and their metabolites in the ventral striatum and ventral midbrain. In the hippocampus, PFOS treatment resulted in increased dopamine turnover and decreased serotonin turnover. Given that alteration of neurotransmitter levels and metabolism are implication in neurological diseases and disorders and are associated with functional changes, we hypothesized chronic, systemic PFOS exposure alters neurotransmitter metabolism, resulting in altered neurobehavioral function.

Methods: Here, we quantified changes in neurobehavior in male and female C57BL/6J mice dosed with 1.0 mg/kg/d PFOS or 0.5% tween-20 (vehicle) in drinking water for 16 months, beginning at 1 month of age (n=10-18 per treatment-sex group). Neurobehavioral experiments included open-field locomotor, challenging beam traversal, gait, novel object recognition, and the Morris water maze (MWM). Brains were dissected for future analysis of neurochemistry and PFOS quantification. Open-field locomotor was conducted by placing mice in the activity chamber with photobeams detecting horizontal and vertical movement over 60 minutes. The MWM was performed by placing mice in a tub filled with water covering a platform. Mice were tasked to find the platform by relying on visual cues on the wall. After 5 days of learning the platform location, the platform was removed, and mice were analyzed for distance spent in the platform location.

Results: Analysis of locomotor activity revealed only PFOS-treated male mice (not females) had significant changes compared to vehicle-treated mice. PFOS-treated males demonstrated significant increases in horizontal ($p=0.04$), vertical ($p=0.01$), and total activity ($p=0.009$) compared to vehicle-treated males. PFOS-treated males demonstrated decreased habituation ($p=0.007$) compared to vehicle-treated males. Preliminary MWM results suggest that PFOS-treated mice have decreased distance to the escape platform (increased learning) ($p<0.05$) and during the probe trial, decreased time spent in the platform location (decreased memory) ($p<0.05$).

Conclusion: These results suggest that chronic PFOS treatment causes hyperactivity and decreased non-associative memory in male, but not female mice. Preliminary PFOS-treated mice demonstrated to increase learning and decreased memory. Our study demonstrates that lifelong exposure to PFOS leads to sex-specific neurobehavioral changes and the brain continues to be vulnerable to PFOS neurotoxicity beyond early development. In conclusion, chronic PFOS treatment induces behavior and neurochemical changes that may suggest elevated risk for neuropsychiatric disorders such as attention deficit disorder and major depressive disorder and neurodegenerative diseases including Alzheimer's disease.

Abstract #3 – Doctoral Student
Investigating the sex-specific effects of environmental toxicant mixtures on steatotic liver disease

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Epidemiologic studies have demonstrated that exposure to 'forever chemicals' such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) have been associated with elevated liver enzymes, indicative of liver injury. However, the effects of how 'mixtures' of such chemicals induce liver injury, and underlying sex differences, are still largely unknown. Thus, the objective of this study was to examine the sex-dependent effects of OCPs and PCBs, using chlordane and PCB126 as model compounds, in the context of steatotic liver disease and metabolic disruption in a diet-induced obesity model.

Male and female C57BL/6 mice were fed a high-fat diet (HFD, 42%) and administered either vehicle control or chlordane(20 mg/kg)+PCB126(20µg/kg) over a 12-week period. Tissues and plasma were collected at euthanasia for lipid, histology, oxidative stress, and gene expression analyses. Statistical tests were performed using Two-way ANOVA followed by Fisher's LSD post hoc test for sub-group comparisons (p <0.05 was considered statistically significant).

Female mice generally had lower fat content, but higher spleen and cecal weights *vs.* males. Females displayed lower hepatic lipid content, confirmed by H&E staining and quantified hepatic triglycerides, and could be attributed to estrogenic-protective effects with HFD feeding. However, compared to their sex-matched controls, exposed females exhibited elevated plasma alanine transaminase, increased liver weight and hepatic triglycerides and cholesterol levels, implicating liver injury and dyslipidemia. Moreover, chlordane+PCB126 exposure decreased physical activity (assessed with metabolic chambers) in females. In contrast, exposed males had lower hepatic GSH/GSSG levels, indicating oxidative stress. Chlordane+PCB126 exposure resulted in altered glucose uptake, suggesting insulin resistance in both sexes. Exposure to the mixture also activated hepatic xenobiotic receptors, namely CAR (*Cyp2b10* induction) and AHR (*Cyp1a2* induction) but PXR (*Cyp3a11* induction) was activated only in males, indicating how sex+HFD interactions impact these receptors as potential drivers of toxicant-induced hepatic metabolic disruption.

While females were susceptible to steatosis with exposure, males were more prone to oxidative stress. However, exposure altered glucose metabolism in both sexes. Toxicant-sex-HFD interactions also regulated receptor activation outcomes. Further assessment of off-liver targets including the gut microbiome and adipose tissue will help understand how these toxicant mixtures impact multi-organ toxicity in driving steatotic liver disease.

Abstract #4 – Doctoral Student

Ozone-induced lung injury increases susceptibility to lower respiratory infections by generating a favorable pulmonary microenvironment

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Air pollution exacerbates cardiopulmonary diseases resulting in increased morbidity and mortality. Epidemiological studies have reported that following increased ambient air pollution, there is an increased risk for hospitalization and/or mortality from lower respiratory infections. The biological mechanisms of why this occurs are not well characterized. To uncover these specific mechanisms, we utilized a murine model of ozone (O_3)-exposure followed by a pulmonary infection to test the hypothesis that O_3 increases susceptibility to pneumonia by promoting an airway microenvironment that favors pathogen growth.

Male C57Bl/6J (WT) mice were exposed to filtered air (FA) or 1 ppm O_3 for 3h, and then 24h after either exposure, mice were infected by oropharyngeal aspiration with 2000 colony forming units of the gram-negative bacteria *Klebsiella pneumoniae* (*Kp*). Mice were necropsied 24h post-infection and lung tissue and blood were obtained and used to quantify bacterial burden. Bronchoalveolar lavage (BAL) fluid and cells were evaluated to define the pulmonary immune response. Total protein in the BAL fluid was assessed to quantify lung injury, whereas lung inflammation was assessed by BAL leukocyte differentials, multiplex ELISA and histopathology. Lung tissue was also assayed by bulk transcriptomics to analyze specific pathways that influence host defense. To determine if factors in the O_3-exposed lung directly promotes *Kp* growth, BAL fluid from C57Bl/6J male mice 24h post FA or 1 ppm O_3 exposure was incubated with *Kp* and bacterial growth was measured by spectrophotometry readings over time.

24h following *Kp* infection, O_3-exposed mice had a significant increase in bacterial burden in the lungs and blood when compared to FA controls. O_3-exposed mice infected with *Kp* also had a significant increase in airspace neutrophilia and total BAL protein. Histology of lung tissue identified increased pathology in O_3-exposed mice infected with *Kp*. In addition, O_3-exposed *Kp* infected mice had significantly increased BAL IFN-1β, IL-6 and KC/GRO. When evaluating if the impaired host defense response noted in O_3-exposed mice infected with *Kp* was a result of a favorable lung microenvironment for bacteria, cell-free BAL from O_3-exposed mice significantly increased *Kp* growth when compared to FA BAL.

Taken together, these data suggest that previous exposure to O_3 worsens *Kp* pneumonia by impairing host defense responses and increased lung injury and inflammation. Furthermore, O_3 exposure promotes an airspace microenvironment that facilitates bacterial proliferation. Ongoing studies will identify the specific O_3-induced soluble factors that promote this bacterial growth.

Abstract #5 – Postdoctoral Trainee
Chronic cadmium exposure disrupts metal homeostasis and DNA double-strand break signaling

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Background: Triple negative breast cancer (TNBC) incidence is disproportionately higher in young Black women. Individuals with germline *BRCA1* mutations have a significantly higher risk of developing TNBC. Black women are no more likely to have germline *BRCA* mutations than non-Hispanic white women. Black women are disproportionately exposed to Superfund metals, including cadmium (Cd). Cd is a heavy metal group 1 human carcinogen. Epidemiological studies demonstrate that Cd accumulates in breast cancer tissue and that Cd exposure promotes malignant transformation of breast epithelial cells *in vitro*. Cd displaces zinc (Zn) from Zn finger proteins and several Zn finger proteins are important for DNA double-strand break (DSB) signaling and repair including RAD50, BRCA1 and TP53. Whether Cd exposure promotes TNBC initiation by disruption of DSB signaling and repair remains to be established.

Hypothesis: Cd exposure promotes TNBC initiation by disruption of DSB signaling and repair.

Methods: Immortalized breast epithelial cells (MCF 10A) were exposed to 0 or 2.5 μM Cd for 24 h or 8 weeks. Inductively coupled plasma mass spectrometry (ICP-MS) was used to measure metal accumulation. Neutral COMET assays were performed to detect DSBs. Immunoblotting was performed for DSB signaling and repair proteins, including activated (phosphorylated) and total ATM, DNA-PKcs, CHEK2, P53, BRCA1, RB1, and total CDKN1A. Statistical analyses, including Two-Way ANOVA with Holm-Sidak post hoc test (metallomic analyses), Mann Whitney test (COMET assay), or unpaired student's two-tailed t-test with Welch's correction (immunoblotting), were performed with GraphPad Prism software; p-value <0.05 was considered significant.

Results: Cd accumulates in MCF 10A cells exposed acutely or chronically to Cd. Cd exposure significantly reduces manganese and iron cellular accumulation. Chronic Cd exposure significantly reduces BRCA1 Ser1524 phosphorylation and CDKN1A expression, and significantly increases RB1 Ser807/811 phosphorylation.

Conclusion: Breast epithelial cells chronically exposed to Cd progress through the cell cycle despite cells having higher amounts of DSBs. Thus, Cd exposure may partially explain racial disparities for TNBC by functionally inhibiting BRCA1 and/or TP53 by direct Zn displacement or indirectly by disrupting metal homeostasis.

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Abstract #6 – Postdoctoral Trainee

The role of cardiomyocyte-specific CXCR7 in doxorubicin-induced cardiotoxicity in aging mice

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Background: Doxorubicin (DOX) is an effective antitumor anthracycline antibiotic, but DOX-induced cardiotoxicity (DICT) has limited its use, particularly in aging patients. Atypical chemokine receptor ACKR3/CXCR7 is a recently identified second receptor of stromal cell-derived factor-1/CXCL12. It has been reported to play critical roles in cardiovascular development. However, the pathophysiological roles of CXCR7 in the development of DICT remain unknown. This study aims to determine the role of CXCR7 in the development of DICT in aging mice that mimic elderly cancer survivors.

Methods: The MerCreMer male mice (Stock No: 005657) that express Cre recombinase in cardiomyocytes driven by the mouse cardiac-specific α-MHC promoter were bred into CXCR7flox/flox female mice (Stock No: 033828) to generate CXCR7flox/flox-MerCreMer mice. Six months old male CXCR7flox/flox-MerCreMer mice and age- and sex-matched littermate CXCR7flox/flox controls were treated with tamoxifen (100 mg/kg for consecutive 5 days) to induce cardiomyocyte-specific CXCR7 deletion (CXCR7-icKO). Twelve months after tamoxifen induction, cardiotoxicity was induced by DOX injection (5 mg/kg/week) for consecutive 4 weeks (20 mg/kg cumulative dose). One week after the last DOX injection, mice were euthanized after heart function examination by echocardiography. The cardiac CXCR7 mRNA expression was quantified by qRT-PCR.

Key findings: CXCR7-icKO resulted in approximately 75% reduction of CXCR7 mRNA expression in the whole heart male aging mice. DOX administration decreased the body weights at the end of the experimental period, and heart weights remained unchanged when compared to those without DOX treatment in both CXCR7-icKO mice and littermate controls. Ejection fraction and fractional shortening tended to deteriorate ($p < 0.05$) upon DOX treatment compared with their corresponding controls. A more obviously decreasing tendency of left ventricular mass and posterior wall thickness induced by DOX was observed in CXCR7-icKO mice compared to controls. However, no significant differences in either systolic or diastolic parameters were observed between DOX-induced CXCR7-icKO mice and littermate controls ($p > 0.05$).

Conclusions: Our findings demonstrate that cardiomyocyte-specific CXCR7 deletion does not aggravate DOX-induced systolic dysfunction in aging mice. The precise roles of CXCR7 in the pathogenesis of DICT among mice of different ages need to be further evaluated in future studies.

Abstract #7 – Postdoctoral Trainee

Myeloid heme oxygenase-1 (HO-1) involvement in ozone (O_3)-induced pulmonary inflammation, antioxidant responses, and alterations to heme metabolism

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It is known that O_3 increases HO-1 expression in the lung; however, it is unknown if macrophage expression of HO-1 dampens O_3-induced inflammation and oxidative stress. We hypothesized that HO-1 expression in macrophages is essential for dampening pulmonary inflammation and oxidative stress following exposure to O_3.

We exposed male LysM-Cre driven heme oxygenase-1 knockout (LysM-HO-$1^{-/-}$, KO) and WT mice to either filtered air (FA) or 1 ppm O_3 for 3 hrs (proportional to a human exposure during an O_3 action day). Mice were necropsied at 24 and 48 hrs following exposure to collect bronchoalveolar lavage (BAL) fluid, blood, and lung tissue. We measured cell differentials in BAL samples, as well as total protein, albumin, hemopexin, haptoglobin, and ferritin via enzyme-linked immunosorbent assay (ELISA). We also measured the antioxidant response via mRNA expression in lung tissue.

In the WT, BAL total protein increased 24 hrs ($p<0.001$) and 48 hrs ($p<0.001$) after O_3 exposure. Neutrophilia was unchanged at both time points. In the KO, BAL total protein was increased at 48 hrs only ($p=0.001$) and was lower than BAL total protein observed for the WT at 48 hrs ($p=0.008$). Neutrophilia was unchanged at both time points for the KO mouse. At 24 hrs after exposure, O_3 induced greater lung tissue expression of the antioxidant genes HMOX1 ($p=0.042$) and NRF2 ($p=0.014$) in the WT only, as well as NQO1 ($p<0.001$) in the KO only. HMOX1 expression in the KO was reduced compared to HMOX1 expression in the WT. To determine if the antioxidant response was driven by altered heme metabolism, we measured BAL hemopexin, haptoglobin, and ferritin. At 48 hrs after exposure, O_3 increased BAL hemopexin (µg/mL) (WT, $p=0.01$; KO, $p=0.003$), increased BAL haptoglobin (µg/mL) (WT, $p=0.018$; KO, $p=0.001$), and reduced BAL ferritin (pg/mL) (WT, $p<0.001$; KO, $p=0.006$).

Our data indicate that myeloid-derived HO-1 represents a significant portion of HO-1 in the lung but does not significantly affect expression of other antioxidant response genes. We also observed continued increases in BAL total protein and hemopexin at 48 hrs after O_3 exposure, suggesting there may be latent injury during the initiation of the inflammation resolution phase. Future studies will focus on involvement of myeloid-expressed HO-1 in inflammation resolution and altered heme metabolism resulting from O_3 exposure.

Big Picture Science and Posters (#8-64)

Abstract #8 – Early-Stage Investigator
Derivation of tolerable intake for hexamethylene diisocyanate

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Hexamethylene diisocyanate (HDI) is an ink hardening agent heavily used in industrial painting applications. An oral risk assessment was needed to establish tolerable intake under sub-acute conditions (less than 30 days). Limited toxicological data available in the literature related to oral exposure of HDI to humans. A group of albino rats administered with HDI (40, 60 and 300 mg/kg) in peanut oil via gavage for 10 days over a 2-week period was chosen as the key study to develop tolerable dose. The lowest adverse effect level (LOAEL) of 300 mg/kg/day based on the non-adverse effects was selected as the Point-of-Departure. A total uncertainty factor of 1000 (10 for extrapolation from animals to humans, and 10 for human variability) and a modifying factor of 10 (for the use of LOAEL) were applied to the duration-adjusted LOAEL. An allowable daily intake of 0.2 mg/kg/day was derived. The developed tolerable intake has sufficient margin of safety and protective under acute, sub-acute exposure settings.

Abstract #9 – Early-Stage Investigator
Chemical and biological characterization of thirdhand vaping exposure in a mouse model

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Thirdhand smoke (THS) is the accumulation of secondhand smoke on surfaces that ages with time and becomes progressively more toxic. THS exposure is a potential health threat to children, partners of smokers, and workers in environments with current or past smoking, and needs further investigation. In this study, we hypothesized that thirdhand Electronic Nicotine Delivery Systems (ENDS) exposures elicit lung and systemic inflammation due to resuspended particulate matter (PM) and inorganic compounds that remain after active vaping has ceased. To test our hypothesis, we exposed C57BL/6J mice to towels contaminated with ENDS aerosols from vape juice (6mg nicotine in 50/50 PV/VG) for 1h/day, five days/week, for three weeks. We assessed protein levels in serum and bronchoalveolar lavage fluid (BALF) using a multiplex protein assay. We found that the median PM measurements in mouse cages with an ENDS contaminated towel were significantly higher than those with a control (unexposed) towel in the cage. Two compounds: 4-methyl-1,2-dioxolane and 4-methyl-cyclohexanol were detected in vape juice and on ENDS contaminated towels, but not on control towels. In addition, 2,5-dimethylfuran, 2,4-dimethylfuran, dimethyldisulfide, hexanal, and 1-chlorooctane were found only in ENDS- contaminated towel samples, possibly due to novel combustion byproducts or chemical transformations. Mice exposed to ENDS-contaminated towels displayed lower levels of Il-7 in serum ($p=0.02$, $n=7$), and higher levels of Il-13 in the BALF ($p=0.0006$, $n=7$) than those exposed to control towels ($n=6$). This study provides further evidence that thirdhand ENDS aerosols can adhere to and contaminate surfaces and may subsequently influence lung and systemic health upon exposure.

Abstract #10 – Early-Stage Investigator
Hexavalent chromium (Cr[VI]) induced behavioral effects and neurodegeneration in rats after 90 days drinking water exposure

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We are facing an era with an unprecedented geriatric population (65+) that will live as geriatrics for multiple decades. By 2030, 20% of the population in developed nations will reach a geriatric age, and many of them will live to see their 100th birthday. This aged population will be uniquely faced with challenges of environmental pollution far exceeding that of previous geriatric generations, but we currently have a poor understanding of how environmental pollution will impact geriatric health distinctly from younger populations. We approach this new paradigm with a perspective we colloquially refer to as a Toxic Aging Coin. On the heads side, we consider how the age of an individual impacts the toxic outcome of a chemical insult. Whereas on the tails side, we consider how toxic chemicals accelerate aging – or how they act as gerontogens. Hexavalent chromium [Cr(VI)] is a major environmental health concern that induces aging phenotypes and has the best defined genotoxic mechanism of heavy metals. The neurotoxicity of Cr(VI) is poorly described, but it is clear Cr(VI) induces widespread oxidative damage and neurodegeneration in the brain. Studies in human populations demonstrate Cr(VI) can impair a variety of neurobehaviors including child learning and attention, geriatric spatial and social memory, adult olfactory function, and increased risk of motor neuron disease. Here we have addressed the heads side of the Toxic Aging Coin, by assessing neurobehavior effects and neurodegeneration across brain regions in both sexes of young (3 months), middle-aged (7 months), and geriatric (18 months) rats exposed to Cr(VI) in drinking water [0, 0.05, 0.1 mg Cr(VI)/L] for 90 days. Importantly, these drinking water levels reflect the regulations set by the World Health Organization and the U.S. Environmental Protection Agency. Despite these levels being considered “safe” for drinking water consumption, we observed Cr(VI) impaired spatial memory and grip strength, elevated anxiety-like behavior, and induced widespread neurodegeneration, particularly in the hippocampus. Further work will consider oxidative damage, DNA damage, and blood brain barrier damage across Cr(VI) treatments, sexes, and ages. These data will help inform how age and sex impact Cr(VI) neurotoxicity. Hence, our data more thoroughly characterizes Cr(VI) neurotoxicology and begins to identify gerontogenic effects. This work is supported by R21ES033327 (JPWJr) and R35ES032876 (JPWSr).

Abstract #11 – Postdoctoral Trainee

New approach methodologies (NAMs) in predicting thyroid toxicity of crop protection compounds using zebrafish and triculture tissue models

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Endocrine disruptors are a global concern for both human and environmental safety, and the design of safe agrochemical products is critical for sustainability. Hence, it is critical that we have a robust thyroid toxicity testing strategy to develop new crop protection products in the future. Traditionally, mammalian models have been used extensively for thyroid disruption assays. With increased commitment from regulatory agencies to reduce the number of mammals used for toxicity testing, the need for development of assays using new approach methodologies (NAMs) is well recognized. Here we describe two different approaches to assess the modes of action (MoA) contributing to thyroid toxicity. Zebrafish have been established as an excellent model for toxicity testing due to their small and robust size, fast embryonic development, high fecundity, transparent embryos, and a high degree of genetic homology with humans. Furthermore, zebrafish larvae are not considered vertebrates until after 5 days of age and are a medium to high throughput screening model that captures several human and ecologically relevant mechanisms for thyroid toxicity. For initial screening, 5 days old transgenic zebrafish Tg(*tg:mCherry*) larvae were exposed to known thyroid disruptor compounds and fluorescence intensity in the thyroid gland was quantified as a measure of toxicity. Additionally, 5 days old wild type zebrafish larvae were utilized to investigate the MoAs that cause such thyroid disruption via biomarker identification and expression quantification. Changes in whole body thyroxine levels in zebrafish larvae were also examined to validate thyroid disruption. To further support our findings, we have also utilized coculture tissue models using rat and human liver cells to examine clearance of thyroxine from the liver, which is not captured in zebrafish and is not human relevant. Leveraging the zebrafish and coculture tissue models facilitate adopting NAMs for thyroid toxicity testing, while providing comprehensive evidence for potential thyroid disruption in the development of new crop protection products with a more favorable environmental profile compared to the alternatives.

Abstract #12 – Postdoctoral Trainee

Sex differences in airway hyperresponsiveness in a model of ozone exacerbation of allergic asthma

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Asthma is a chronic disease characterized by bronchoconstriction that causes shortness of breath and wheezing. Although typically managed by inhaled steroids, there are ~500,000 deaths per year attributed to acute exacerbations or episodes of airway hyperresponsiveness (AHR). One environmental trigger of AHR is inhalation of ozone (O_3). Th2-high allergic asthma is the most studied disease endotype; yet the mechanisms of acute exacerbations by O_3 are not well defined because management strategies primarily focus on symptom relief. A limitation in basic research is the use of single-allergen models; patients with allergic asthma respond to multiple allergens that contribute to disease pathogenesis. Therefore, we used a clinically relevant mixed-allergen model of "DRA" (dust mite, ragweed, *aspergillus*) followed by O_3 to more closely mimic human disease etiology. DRA sensitization and challenge was performed by intranasal administration in male and female C57BL/6 mice; 24 hr after the last challenge mice were exposed to 2 ppm O_3 for 3 hr and harvested 24 hr later. Studies in male mice revealed that AHR was significantly higher in DRA+O_3 treated mice compared to O_3 or DRA alone. In the lung tissue, DRA prevented induction of *Gpx1 and Gpx2* by O_3 and reduced IL-10 levels and monocyte numbers compared to DRA alone. Suggesting that this exacerbation of AHR was likely due to impaired antioxidant and anti-inflammatory mechanisms. Therefore, we employed scRNA sequencing to identify potential pathways. These data revealed that in the DRA+O_3 group compared to controls, alveolar macrophages (AM) had the highest number of differentially expressed genes compared to DCs and adaptive cell types; upregulated genes were primarily involved in cytokine/chemokine signaling. Biological sex has been shown to influence asthma and O_3 respiratory inflammation and pathway analysis revealed that Estrogen Receptor Signaling through *Esr1* (ERα) was upregulated in AMs from DRA+O_3 treated animals (pooled male and female) compared to controls. Notably, when experiments were performed in female mice, the additive effect of DRA+O_3 was absent; AHR was increased in DRA and DRA+O_3 groups, but there was no difference between the two. Accordingly, preliminary scRNA seq results indicated that the primary pathways found to be different between sexes related to neuroendocrine signaling, likely involved in airway smooth muscle (ASM) contraction. Using sex-based comparisons, this model provides an important opportunity to study mechanisms of asthma exacerbation. Future studies will focus on sex differences in AM-ASM signaling to elucidate mechanisms of susceptibility to O_3 exacerbation of AHR.

Abstract #13 – Postdoctoral Trainee
Serotonin is required for balance development in zebrafish larvae

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The Posterior Lateral Line (PLL) of teleost consists of mechanosensory organs called neuromasts. Neuromasts are structurally, functionally, and molecularly similar to human inner ear hair cells. We screened 292 chemicals from the EPA ToxCast Phase I library using transgenic zebrafish expressing GFP in neuromasts. We found that exposure to 22 of those chemicals altered the number of neuromasts in 4 days old zebrafish larvae. By running the PLL disruptors against ToxCast data through a univariate correlation analysis, we identified 10 ToxCast *in vitro* assays that were affected by sets of the same 22 PLL disruptors. Five of the 10 *in vitro* assays were related to different aspects of serotonin signaling. Furthermore, exposure to known serotonin modulators confirmed that alterations in serotonin levels or function results in PLL disruption. Our results may have implications for human hearing as it has been shown that patients with hearing loss have elevated plasma serotonin levels. We conclude that optimal serotonin levels are required for proper PLL development in zebrafish larvae, and that zebrafish *in vivo* screening data in combination with modeling of ToxCast data can be used to produce testable hypotheses on modes of action of chemical exposures.

Abstract #14 – Postdoctoral Trainee

Polystyrene nanoplastics induce skeletal deformities via ROS-mediated apoptotic pathway in zebrafish (*Danio rerio*)

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Nanoplastics (NPs) are plastic pieces of variable sizes ranging from 1nm to 1mm and are widely distributed in fresh water and marine environment around the world. The threat that larger plastic pieces pose to aquatic species has been well documented, yet there remain data gaps concerning the effect of NPs. Polystyrene (PS) is a common component of plastic debris in aquatic environment and is meticulously problematic from a toxicity perspective. This study assessed the polystyrene nanoplastics (PS-NPs) induced skeletal developmental defects through ROS-mediated signal transduction pathway in embryonic zebrafish. The embryos were exposed to 100 nm of PS-NPs with different concentrations (1, 10, and 100 µg/mL) from 2 hours post fertilization (hpf) to 96 hpf. PS-NPs exposure caused significant developmental deformities in larvae, including short body length, scoliosis, coiled tail, and curved spines. In addition, PS-NPs were found to accumulate at all developmental stages of embryos due to its high-affinity interaction with the biological system of zebrafish. Besides, the reactive oxygen species (ROS) production and apoptosis rates were significantly higher in PS-NPs treated groups. Interestingly, superoxide dismutase (SOD) and catalase (CAT) activities were greatly reduced in zebrafish larvae following PS-NPs exposure. Further biochemical assays demonstrated that PS-NPs suppressed erythrocytes proliferation and ectopic mineralization process in developing zebrafish larvae. Furthermore, a Real-time PCR assay demonstrated that the mRNA levels of *sod1, cat, nrf2, casp 3b, ptgs2a, gadd45ba*, and *tnfa* were altered in zebrafish embryos after PS-NPs exposure. This study suggests that PS-NPs accumulation might be responsible for ROS induction and oxidative damage which would have facilitated the positive regulation of apoptotic signaling pathway resulting in impaired blood-bone axis development in zebrafish larvae. Therefore, our findings illustrate that NPs could induce skeletal developmental defects in zebrafish larvae through activation of the ROS-mediated apoptotic pathway.

Abstract #15 – Postdoctoral Trainee

Lipid regulation of nanoparticle-induced pulmonary inflammation: sex and disease variations

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Metabolic syndrome (MetS) is a significant public health concern that is diagnosed when an individual exhibits at least three out of five risk factors including abdominal obesity, systemic hypertension, insulin resistance, hypertriglyceridemia, and/or low HDL cholesterol levels. Most evaluations of nanoparticle safety have traditionally focused on healthy models, however, recent evidence demonstrates individuals with MetS are more vulnerable to the adverse health effects of particulate air pollution. Our previous research identified a MetS mouse model displayed heightened pulmonary inflammation 24 hours after exposure to silver nanoparticles (AgNPs) when compared to a healthy mouse model which was accompanied by a reduction in specific lipid mediators of resolution. Additionally, our research revealed inflammation remained elevated for up to 21 days after AgNP exposure in MetS while resolved in the healthy model. Notably, the administration of a specialized pro-resolving mediator (SPM), resolvin D1 (RvD1), mitigated the exacerbated effects observed in the MetS model in response to AgNP exposure. Our current study hypothesized other SPMs such as resolvin E1 (RvE1), protectin D1 (PD1) and maresin which are also known to be dysregulated in MetS following AgNP exposure may also be able to treat these enhanced inflammatory responses. Moreover, disparities in the occurrence of respiratory diseases between the sexes have been documented with females demonstrating exacerbated effects. Therefore, we also hypothesize variations in lipid mediators may contribute to differential inflammation and toxicity following exposures. C57BL/6 J male and female mice were fed with either a healthy or high fat western diet. Subsets of mice were exposed to either 50 µl of water (control) or 20 nm AgNP via oropharyngeal aspiration. Twenty-four hours later, subsets of mice were treated with either 40 µl saline (control) or 400 ng of SPMs in 40 µl saline via oropharyngeal aspiration. Endpoints of toxicity and inflammation were evaluated 48 h post-treatment. Sex-specific responses were examined 24-h post-exposure without treatments. The MetS models demonstrated elevated body weights and cholesterol levels compared to healthy models (male and female). Analysis of bronchoalveolar lavage fluid (BALF) revealed an intensified AgNP-induced influx of neutrophils in MetS mice and total protein levels indicative of inflammation and lung injury (males and females). Treatment with resolution mediators demonstrated differential inhibition of inflammation through modulation of BALF inflammatory cells and cytokine/chemokines. Ongoing assessments are examining lipid mediators which may contribute to sex-dependent differences in exposure response and therapeutic interventions targeting lipid dysregulation. Overall, our evaluations demonstrate pulmonary lipids may contribute to MetS susceptibility and can be leveraged for treatments.

Abstract #16 – Postdoctoral Trainee
2,3-Dibromophenol nephrotoxicity in isolated kidney cells from male Fischer 344 rats

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Brominated benzenes are frequently used chemical intermediates in the synthesis of a wide variety of industrial and agricultural products. Some dibromobenzenes have been shown to be nephrotoxicants *in vivo* and/or *in vitro*, but the mechanism of inducing toxicity is not fully understood. The purpose of this study was to begin to investigate the nephrotoxic potential of 2,3-dibromophenol (2,3-DBP), a metabolite of 1,2-dibromombenzene (1,2-DBB), using isolated kidney cells (IKC) from male Fisher 344 rats as the *in vitro* model and to determine if free radicals or further oxidative metabolism contributed to 2,3-dibromophenol nephrotoxicity. IKC (~4 million cells/ml; 3 ml) from male Fischer 344 rats were incubated with shaking at 37^{o}C under a 95% oxygen/5% carbon dioxide atmosphere with 2,3-DBP (0.25, 0.50 or 1.0 mM) or vehicle (dimethyl sulfoxide) for 30 or 60 min. Cytotoxicity was measured by determining trypan blue exclusion by IKC and measuring changes in lactate dehydrogenase (LDH) release. In some experiments, IKC were pretreated with an antioxidant (glutathione, 1.0 mM, 30 min or ascorbate, 1.0 mM, 5 min) or a non-selective cytochrome P450 (CYP) inhibitor (piperonyl butoxide, 0.1 mM, 15 min) before 2,3-DBP (1.0 mM; 60 min incubation). At 30 min, only 2,3-DBP 1.0 mM induced cytotoxicity, while 2,3-DBP 0.5 mM or greater induced cytotoxicity at 60 min. 2,3-DBP-induced nephrotoxicity was partially attenuated by ascorbate and fully attenuated by glutathione pretreatment. 2.3-DBP cytotoxicity was also markedly reduced by CYP inhibition. These results suggest that 2,3-dibromophenol is a nephrotoxic metabolite of 1,2-DBB and that further metabolism of 2,3-DBP and free radicals contribute to 2,3-DBP nephrotoxicity *in vitro*. Supported in part by P20GM103434.

Abstract #17 – Postdoctoral Trainee

Gene-environment interaction between SORL1 variant and the environmental toxicant atrazine in Alzheimer's disease pathology

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Alzheimer's disease (AD) is a chronic neurodegenerative disorder responsible for 70–80% of all dementia cases. Several variants are identified as genetic risk factors for AD including APOE and APP and most recently SORL1. SORL1 (sortilin-related receptor 1) plays a role in APP processing and trafficking and interacts with several brain metabolites. In recent years multiple studies suggest a potential role of several environmental factors like lead (metal), polystyrene (microplastic), atrazine (herbicide), and other agents in AD but studies are limited studying the gene-environment (GxE) interaction of most of these environmental agents in AD pathology. We chose to investigate the GxE interaction of SORL1 and the agricultural herbicide atrazine (ATZ), which contaminates drinking water sources in areas in which this herbicide is applied. ATZ exposure is associated with neuroendocrine disruption, neurotransmission alterations, and other impacts to the central nervous system. A recently developed sorl1 CRISPR-Cas9 zebrafish mutant was used to study the GxE interaction, which has altered behavior and expression of AD associated genes in larvae, along with sex-specific expression perturbations identified by proteomic analysis in the adult brain of these mutants. Specifically, PSEN1 and MAPT were upregulated in both sexes but APP was female-specific. The sorl1 mutants were then exposed to 0, 0.3, 3, or 30 ppb (µg/L) ATZ throughout embryogenesis (1-72 hours post fertilization, hpf) and larval behavior and expression of AD associated genes compared to the same ATZ treatments in wild type zebrafish. The visual motor response behavior in the larval fish were assessed at 120 hpf. Behavior alterations were more apparent in the sorl1 mutants compared to wild type fish amongst the ATZ treatment groups with hypoactivity in the 30 ppb treatment group in the wild type and in all ATZ treatment groups (0.3, 3, and 30 ppb) in the sorl1 mutants ($p<0.05$). In addition, qPCR analysis of AD associated genes showed variable outcomes when comparing among ATZ treatment groups in the sorl1 mutant and wild type populations. Overall, the sorl1 gene variant exacerbates ATZ neurotoxicity, which could increase risk of AD pathology.

Abstract #18 – Postdoctoral Trainee
The protective role of rhFGF1$^{\Delta HBS}$ against DOX-induced cardiotoxicity in young mice

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Background: We have developed a rhFGF1 partial agonist known as rhFGF1$^{\Delta HBS}$ that carries triple mutations of the heparin-binding sites (HBS). This engineered protein is designed to reduce the hyperproliferative effect of native FGF1 and lower the risk of tumorigenesis. In a recent study, our previous team members found that rhFGF1$^{\Delta HBS}$ effectively prevented doxorubicin (DOX)-induced cardiotoxicity (DICT) in adult mice. We are now interested in testing its ability to prevent DICT in young mice that mimic pediatric cancer survivors.

Methods: A total of 72 male C57BL/6J mice, aged three weeks, were included in the study. The mice were randomly divided into two groups: the control group (n=12), which received only PBS/saline, and the DOX group (n=60), which was intraperitoneally injected with DOX (5 mg/kg) once a week for five weeks. The DOX group was further divided into five subgroups, each receiving a different dose of FGF1$^{\Delta HBS}$ (0.25, 0.5, 1, or 2 mg/kg). The rhFGF1$^{\Delta HBS}$ treatment was administered simultaneously with DOX injection and repeated every other day. Body weight and blood glucose levels were measured. Echo analysis was performed one week after the last DOX injection. The mice were then euthanized, and their internal organs were collected and weighed.

Results: Administration of DOX+rhFGF1$^{\Delta HBS}$ at 1 and 2 mg/kg decreased body weights and blood glucose levels significantly in mice compared to those treated with DOX alone. The mass weight of the heart, liver, lungs, and kidneys was also considerably reduced in the mice that were given rhFGF1$^{\Delta HBS}$ doses ranging from 0.5 to 2 mg/kg compared to the control group. However, the weight of both testes and spleen was significantly decreased at all dose levels and 1-2 mg/kg doses of FGF1$^{\Delta HBS}$, respectively. To determine at which amount the FGF1$^{\Delta HBS}$ offers protection against DOX-induced cardiac dysfunction in young mice, we assessed cardiac function using Echo. Our research showed that treatment of mice with FGF1$^{\Delta HBS}$ at a dosage of 0.25-0.5 mg/kg resulted in increased stroke volume (SV), cardiac output (CO), ejection fraction (EF), and fractional shortening (FS) compared to those treated only with DOX. Though the increase was insignificant, FGF1$^{\Delta HBS}$ could positively affect cardiac function in younger mice.

Conclusion: Based on our observations, 0.25 mg/kg of FGF1$^{\Delta HBS}$ might be appropriate for protecting young mice from DICT. However, additional research is required to determine if lower doses and frequency of FGF1$^{\Delta HBS}$ administration can still provide protection against DICT without causing significant decreases in body weight and blood glucose levels in DOX-treated young mice.

Abstract #19 – MD Student (Poster and Big Picture Science)
Sorafenib modulates lung cancer growth and induces microvesicle particle release in a platelet-activating factor-receptor and acid sphingomyelinase-dependent manner

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Non-small cell lung cancer (NSCLC) remains the leading cause of cancer-related deaths associated with low response rates to current treatment options, including the tyrosine kinase inhibitor, sorafenib (SF). This indicates the need to explore novel mechanisms responsible for impeding the efficacy of such therapeutic agents. Previous studies have implicated the potential role of a platelet-activating factor receptor (PAFR) pathway in favoring tumor growth and impeding the efficacy of therapeutic agents in various cancer models. Besides, previous research has implicated the involvement of tumor-secreted microvesicle particles (MVP), in carrying PAFR agonists, thus allowing survival and enhanced proliferation of cancer cells in response to different therapies. The impact of these MVPs and the enzyme central to their synthesis and release, acid sphingomyelinase (aSMase), on sorafenib, specifically, has remained elusive. Therefore, we aimed to determine the impact of the PAFR and aSMase pathways in SF-mediated cell cytotoxicity and MVP release in NSCLC models. These studies used two NSCLC cell lines, A549 and H1299, which express PAFR. We tested the effects of SF on the growth of these cell lines by sulforhodamine B assay. The results showed that SF inhibits the survival of both A549 and H1299 in a dose- and time-dependent manner. To determine the effects of sorafenib on MVP release, the same cell lines were treated with SF alongside a known PAFR agonist, carbamyl-PAF (CPAF), and a PAFR-independent agonist, phorbol myristate acetate (PMA) as positive controls. We demonstrate that SF induces MVP release in dose-dependent manner, similar to CPAF and PMA. To determine the PAFR dependency and the role of aSMase enzyme in SF-induced MVP release, cell lines were pretreated with PAFR antagonist, WEB2086 or the aSMase inhibitor, imipramine. The results showed that SF-mediated MVP release is blocked by both WEB2086 and imipramine. Our ongoing studies are exploring the effects of the PAFR and aSMase blockade on sorafenib-induced suppression of lung cancer growth. Overall, various therapies have shown improved survival rates in NSCLC patients; however, their efficacy is hampered by several factors. Since MVPs carry PAFR agonists and reduce therapeutic efficacy, we propose that targeting the PAFR-aSMase axis can improve established therapies. Overall, the findings here provide new insights and targets to be considered when using sorafenib for NSCLC.

Abstract #20 – MD Student

The significance of the platelet-activating factor-receptor expression as well as its associated genes in affecting the clinicopathological characteristics of human malignancies

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Platelet-activating factor receptor (PTAFR) is a G protein-coupled receptor known to play a role in metastasis, angiogenesis, tumor transformation, and anti-apoptosis in several cancers. However, its involvement in certain human malignancies has not been thoroughly investigated. Our objective was to identify those malignancies exhibiting significant levels of PTAFR expression and genes correlated with PTAFR that have functional significance in tumorigenesis to find biomarkers for possible future therapeutic targets. Data analysis was performed using The Cancer Genome Atlas Project (TCGA). TCGA includes RNA-seq and clinical data from 31 cancer types, including PTAFR expression across multiple clinicopathologic features. TCGA displayed the genes positively correlated with PTAFR in each cancer type and the effect of gene expression level on patient survival. The cancer types having a highly significant ($p < 0.001$) elevation in PTAFR expression in tumor samples were selected for further analysis. Next, the PTAFR-correlated genes with a Pearson correlation coefficient of 0.5 or higher were examined. Moreover, we examined the functional significance of those selected genes. We identified five cancer types to have significantly higher expression of PTAFR in tumor samples as compared to normal samples: cholangiocarcinoma (CHOL), glioblastoma multiforme (GBM), kidney renal clear cell carcinoma (KIRC), stomach adenocarcinoma (STAD), and uterine corpus endometrial carcinoma (UCEC). We focused our analysis on the top three most significant genes in the four cancer models, whose functions/mechanisms were supported by published literature. Importantly, the gene neutrophil cytosolic factor 1C (NCF1C) has a significant survival profile in both GBM and UCEC, and the gene CD300C has a significant survival profile in both CHOL and KIRC. Of significance, these genes play crucial roles in regulating various oncogenic properties of tumor cells such as apoptosis, cell proliferation, ferritinophagy, tumor infiltration of macrophages, and T cell-mediated immune responses. As PTAFR modulates tumor cell properties, including apoptosis, cell proliferation, and T cell-mediated immune responses, the identification of PTAFR-associated genes that regulate such cancer cells properties can be explored as potential targets for the intervention of these malignancies. Importantly, our analyses showed the relevance of these important genes and their use as biomarkers that could be helpful for personalized therapy.

Abstract #21 – PhD Student

Comparison Between MRI and Histological Measures of Myelination in Rats: A Pilot Study

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Bisphenol-A (BPA) is a ubiquitous endocrine disrupting chemical (EDC) used in the production of plastic products that has been linked to alterations in anxiety behaviors and memory. Exposure to BPA potentially dysregulates myelination during post-natal brain development. Magnetic resonance imaging (MRI) can measure myelination *in vivo* but must be validated histologically. Therefore, we compared *in vivo* MRI and *ex vivo* histological measures of myelination in rats. For this pilot study, Sprague-Dawley rats (Charles River, N = 2-7 per dose level) were treated with BPA (2.5, 25, and 250 µg/kg bw/day) or vehicle-only starting from PND 0. Rats were scanned in a Bruker 7T MRI scanner at PND 90. Upon completion, rats were euthanized, and perfusion fixed with 4% paraformaldehyde. Brains were harvested, post-fixed, cryoprotected (20% followed by 30% sucrose), and cryosectioned at 10µm. Sections were stained with Fluoromyelin (488nm), phalloidin (695nm), and DAPI (460nm), then imaged using fluorescence microscopy at 4x with large image acquisition. From four regions of interest (ROI: corpus collosum, hippocampus, hypothalamus, cingulate tract), we extracted mean myelin water fraction (MWF) and fractional anisotropy (FA) from MRI images and mean luminescence from the 488nm fluorescent channel, in histological sections. Pooled data from all ROIs demonstrated positive correlations between Fluoromyelin and MWF ($r = 0.69$, $p = 4.48e\text{-}5$) and FA ($r = 0.41$, $p = 0.02$). However, there were no statistically significant correlations between Fluoromyelin and MWF or FA within any one brain ROI. Additionally, preliminary results suggest a non-monotonic dose response of myelination to BPA with 2.5 ug/kg BPA dosed rats having the lowest mean fluorescence intensity. However, this may be due to our current small sample size. Our results suggest MRI measures of myelination (MWF, FA) are representative of histological measures of myelination (Fluoromyelin) within the rat brain. Data acquisition and analysis is on-going, however the above findings between *in vivo* and *ex vivo* outcomes provide support for using MRI for assessing the effects of BPA on myelination within the central nervous system.

Abstract #22 – PhD Student (Poster and Big Picture Science)
ChemR23 axis regulates ozone-induced lung inflammation

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Ozone (O_3) is a criteria air pollutant shown to increase morbidity and mortality from chronic lung diseases. O_3 induces lung injury and inflammation through promotion of oxidative stress leading to cell death, and inducing production of pro-inflammatory lipid mediators. Studies have identified a novel class of lipid mediators, termed specialized pro-resolving mediators (SPM) that resolve inflammation following injury by binding to their respective G-protein coupled receptor (GPCR). ChemR23 is a GPCR that binds to ligands, Chemerin and the SPM Resolvin E1 (RvE1). RvE1 is metabolized from the n-3 polyunsaturated fatty acid Eicosapentaenoic Acid (EPA) and binds to ChemR23, facilitating inflammation resolution and tissue homeostasis. We have previously shown O_3 downregulates select SPM in the lung and the SPM receptor ChemR23. From this, we hypothesized the ChemR23 receptor binding to its ligand, RvE1, leads to protection of the lung from O_3-induced pulmonary inflammation and injury.

To test our hypothesis, male C57BL/6J (WT) and ChemR23 deficient ($ChemR23^{-/-}$) mice were exposed to either filtered air (FA) or 1 part per million O_3 for 3hrs. Mice were euthanized at 24hrs post exposure and bronchoalveolar lavage (BAL) fluid and lung tissue were collected to assess inflammation/injury.

Following acute O_3 exposure, $ChemR23^{-/-}$ mice revealed unchanged BAL protein, suggesting comparable lung injury to the WTs. However, $ChemR23^{-/-}$ mice showed increased pulmonary inflammation with higher levels of BAL cell differentials and higher production of pro-inflammatory cytokines and chemokines including IL-6, KC, and MCP-1 24hrs post O_3 exposure. $ChemR23^{-/-}$ mice exposed to O_3 had a significant decrease in RvE1 BAL not due to alterations in pulmonary EPA levels when compared to WT controls.

To further modify the EPA/RvE1:ChemR23 axis, we utilized EPA dietary supplementation. Male C57BL/6J mice were fed a control or EPA-supplemented diet for 4 weeks prior to the FA or 1 part per million O_3 for 3hr exposure with endpoints previously described.

Mice fed the EPA diet revealed similar levels of protein in BAL to mice on the control diet following O_3. However, EPA supplementation decreased O_3-induced airspace neutrophilia. Additionally, EPA supplementation revealed increased EPA-derived lipid mediators and decreased pro-inflammatory-associated lipid mediators in the pulmonary lipidome.

Taken together, our results indicate the EPA/RvE1:ChemR23 axis plays a protective role in O_3-induced pulmonary inflammation, but not injury. Future studies will investigate how EPA supplementation alters O_3-induced cytokine/chemokine expression and production.

Abstract #23 – PhD Student (Poster and Big Picture Science)
Personalized detection of excess Mn accumulation in the brain of welders

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Over-exposure to manganese (Mn) occurs in occupational settings such as welding, leading to excess accumulation of this essential trace metal in the brain. High accumulation of Mn in the brain is known to cause psychological, cognitive, and motor deficits similar to Parkinson's disease (PD), a condition termed manganism. Because the signs of metal-induced toxicity are neurological, it is critical to understand how excess metal deposition affects the brain. Due to the paramagnetic properties of Mn, MRI is the only imaging modality that can non-invasively assess Mn accumulation in the body *in vivo*. Specifically, the longitudinal relaxation rate R1 (R1 = 1/T1) increases as Mn accumulation increases in the brain. This study aims to develop a single-subject comparison method for detecting abnormal relaxation rates because of excess Mn accumulation.

Relaxometry maps of the brain were acquired from 25 healthy non-Mn exposed volunteers. A normative R1 atlas was established by modeling the relaxation rate variability within the healthy cohort (HC) using a linear model that accounts for the impact of age. To perform a subject-specific comparison, z-scores were computed across the brain by comparing the measured R1 values against the established normative atlas. To illustrate the feasibility and good sensitivity of the atlases in detecting abnormal R1 values, the single-subject comparison method was used on 10 selected welders (5 highly exposed (HEX) and 5 low exposed (LEX)).

The established atlas exhibits a low variation in white matter structures (mean RMSE of model equal to 0.13 s^{-1} for R1). The subject-specific method detected increased relaxation rates in the globus pallidus (mean z-score >2.5) and white matter areas in the frontal lobe and brain stem (z-scores between ~3-6) in the HEX group. The z-scores from the LEX group did not show clear areas that deviated from the normal values; however, the pattern was similar.

The proposed study introduces a method for personalized detection and characterization of excess Mn accumulation in the brains of welders. Voxel-wise population-derived norms were established by modeling R1 distribution values within a frequency age-matched HC. This approach departs from the conventional method of group comparisons and offers great potential for studying Mn neurotoxicity.

Abstract #24 – PhD Student (Poster and Big Picture Science)
Trem2 regulates ozone-induced immune cell trafficking and neuroinflammation in the lung-brain axis

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Alzheimer's disease (AD) is the leading cause of dementia, and reports implicate a potential role for disrupted immune cell trafficking in the disease. Evidence supports that environmental factors play a role in AD etiology, and epidemiology reports have linked ozone (O3) exposure to increased AD incidence. O3 is a reactive oxidant that is confined to the respiratory tract after inhalation and is unable to translocate to the brain, highlighting a potential role for the pulmonary immune response in central nervous system (CNS) effects (The Lung-Brain Axis). Recently, AD genome-wide association studies have identified loss of function Trem2 (Triggering Receptor Expressed on Myeloid Cells 2) mutations, indicating myeloid cell-specific mutations can convey AD risk. We have previously shown O3 disrupts the chemotactic microglial response to amyloid plaques and disturbs the disease-associated microglia phenotype in 5xFAD mice. These are processes regulated by TREM2, supporting a role for TREM2 in the CNS effects of O3. However, the role of TREM2 in the lung-brain axis has yet to be directly tested, and whether TREM2 regulates the O3-induced peripheral immune mechanisms that could impact the brain is unknown. To begin to address these questions, male Trem2-/- mice and Trem2+/+ control mice were exposed to either filtered air, 1 ppm O3 or 2 ppm O3, and bronchoalveolar lavage fluid (BALF), plasma, cervical lymph nodes (deep and lateral, CLNs), and brains were collected. Data revealed 2 ppm O3 exposure caused an increase in the percent of neutrophils in the BALF of Trem2-/- mice compared to Trem2+/+ mice. Trem2-/- mice also showed transcriptional changes indicative of modified neuroinflammatory response in the cortex and midbrain. In cortex and midbrain, Tnf expression trended higher in 2 ppm O3-exposed Trem2+/+ mice compared to filtered air controls, but Tnf expression remained unchanged in Trem2-/- mice after O3 exposure. Quantitative analysis of midbrain cDNA showed a genotype-exposure interaction in Il-1β and Nlrp3 expression. CLN transcriptional changes were analyzed and revealed that 1 ppm and 2 ppm O3 exposures changed gene expression patterns indicative of modified immune cell trafficking, which was dependent upon Trem2 genotype. For example, Trem2-/- mice exposed to O3 showed changes in gene expression patterns indicative of an increase in T-cells, which did not occur in Trem2+/+ mice. Collectively, these findings indicate that TREM2 regulates O3-induced immune cell trafficking as well as neuroinflammation in the lung-brain axis, illustrating TREM2's impact in the periphery may very well affect the CNS neuroimmune milieu and regulate CNS health and disease.

Abstract #25 – PhD Student

Developmental Pb exposure increases AD risk via altered intracellular Ca^{2+} homeostasis in hiPSC-derived cortical neurons

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Exposure to environmental chemicals such as lead (Pb) during vulnerable developmental periods can result in adverse health outcomes later in life. Human cohort studies have demonstrated associations between developmental Pb exposure and Alzheimer's Disease (AD) onset in later life which were further corroborated by findings from animal studies. The molecular pathway linking developmental Pb exposure and increased AD risk, however, remains elusive. In this work, we used human iPSC-derived cortical neurons as a model system to study the effects of Pb exposure on AD-like pathogenesis in human cortical neurons. We exposed neural progenitor cells derived from human iPSC to 0, 15 and 50 ppb Pb for 48 h, removed Pb containing medium and further differentiated them into cortical neurons. Immunofluorescence, Western blotting, RNA-sequencing, ELISA and FRET reporter cell lines were used to determine changes in AD-like pathogenesis in differentiated cortical neurons. Exposing neural progenitor cells to low dose Pb, mimicking a developmental exposure can result in altered neurite morphology. Differentiated neurons exhibit altered calcium homeostasis, synaptic plasticity, epigenetic landscape along with elevated AD-like pathogenesis markers, including phosphorylated tau, tau aggregates and Aβ42/40. Collectively, our findings provide an evidence base for Ca dysregulation caused by developmental Pb exposure as a plausible molecular mechanism accounting for increased AD risk in populations with developmental Pb exposure.

Abstract #26 – PhD Student (Poster and Big Picture Science)
Docosahexaenoic acid supplemented diet reduces pulmonary inflammation and enhances resolution responses in an environmental exposure model

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Ozone (O_3) is a toxic air pollutant that exacerbates pulmonary diseases by activating the innate immune response, causing lung injury, and inhibiting resolution of inflammation. Dietary nutrients influence the pulmonary inflammatory response to O_3. In inflammatory conditions, polyunsaturated fatty acids such as docosahexaenoic acid (DHA) reduce inflammation and enhance resolution of inflammation in part by increasing specialized pro-resolving mediators (SPMs). SPMs such as maresin 1 (MaR1) or resolvin D1 (RvD1) are fatty acid-derived metabolites that decrease inflammatory cytokines and improve macrophage phagocytosis of apoptotic cells; a resolution process termed 'efferocytosis'. It is unclear if increased levels of DHA and its metabolites can protect the lung from O_3-induced inflammation. We hypothesize that DHA supplementation will increase SPM production leading to decreased O_3-induced pulmonary inflammation and increased tissue resolution. To test this hypothesis, we provided 5-week-old C57BL/6J male mice a control diet or DHA enriched diet (2% kcal from DHA) for 6 weeks. Mice were then exposed to filtered air or 1 ppm O_3 for 3h (comparable to an O_3 action day for humans) and necropsied 24 or 48h after exposure, representing acute and resolution phases of inflammation. At 24h post-exposure, DHA increased pulmonary concentrations of 14-HDHA (MaR1 metabolism marker), and the SPM resolvin D5 (RvD5), and improved pulmonary macrophage efferocytosis. Then, 48h after exposure, the expression of macrophage recruiting chemokine CCL2 was decreased in lung tissue, and bronchoalveolar lavage (BAL) protein – a marker of lung injury – was decreased in DHA fed mice. We then dosed 10-week-old C57BL/6J male mice with MaR1 or RvD1 1.5h prior to 3h of 1 ppm O_3 exposure. MaR1 decreased BAL macrophages and increased lung tissue CCL2 expression 24h following O_3 exposure, while RvD1 did not alter macrophages or CCL2. Overall, this data indicates that DHA influences the pulmonary inflammatory response by altering pulmonary macrophage concentrations, recruitment signaling, and efferocytosis behavior. Future investigations will focus on the differential effects of DHA-derived SPMs on pulmonary macrophages and how they influence the immune response to environmental toxicants.

Abstract #27 – PhD Student
A review of the effects of nitrogen mustard on corneal epithelium

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Background: Nitrogen sulfur compounds, such as nitrogen mustard (NM), serve as crucial analogs to sulfur mustard, allowing for in-depth exploration of their shared pathophysiological effects. Sulfur mustard, infamous for its use as a chemical weapon, inflicts severe damage on both ocular and systemic levels. Understanding their common pathophysiology and distinct clinical manifestations is essential for medical research and the development of effective treatments. We review recent studies that explore the pathophysiology, clinical manifestations, and treatment for NM exposure.

Methods: Extensive literature review was performed using PubMed and Google Scholar databases. Following search terms and their combinations were input: “nitrogen mustard,” “cornea,” “injury,” and “eye”. Boolean operators “OR,” and “AND” were utilized to further refine the published literature. Inclusion criteria required (1) articles to be in English, (2) studies specifically mentioning corneal injury, and (3) experiments employing human/animal subjects or extracted tissue.

Results: Studies collectively revealed that exposure to NM resulted in a diverse array of ocular injuries, encompassing corneal thinning, epithelial-stromal separation, neovascularization, and inflammatory responses leading to corneal ulceration. These pathologies reflected increased levels of cyclooxygenases, interleukins, vascular endothelial growth factor, and caspase-1 following NM exposure. Following NM exposure, some dysfunctional proteins involved in inflammation, angiogenesis, and cell cycle function showed reversal with dexamethasone administration.

Conclusion: NM exposure results in a range of ocular injuries due to numerous pathological changes, including corneal damage, inflammation, neovascularization, and alterations in protein expression. These manifestations can lead to visual impairment and are associated with biphasic injury. Additionally, NM exposure triggers various molecular responses such as changes in protein expression, DNA damage, and inflammation, contributing to the overall pathogenesis of NM-induced ocular injury. Further investigation into NM exposure should focus on (1) establishing a dose-response relationship to evaluate threshold levels that cause ocular tissue injury, (2) providing temporal relationship of exposure to damage ratio, (3) further elucidating the molecular and cellular pathways, and (4) investigating potential therapies to the demonstrated pathologies. Understanding these diverse manifestations is vital for advancing strategies for diagnosis, treatment, and prevention of NM-related ocular damage.

Abstract #28 – PhD Student (Poster and Big Picture Science)
Investigating PCB 126 impact on intestinal permeability in mice consuming an ethanol diet

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The effects of chronic alcohol consumption on the gastrointestinal tract are characterized in the disease process called Leaky gut syndrome (LGS). LGS is described as increased intestinal epithelium permeability and can result in metabolic and immune system derangements. Polychlorinated biphenyl (PCB) 126 is a persistent and ubiquitous environmental toxicant that also affects multiple organ systems. Although its direct production has been banned since 1979, inadvertent PCB (iPCB) generation is still allowed in various manufacturing processes. To date, there are no reports of how PCB 126 exposure in conjunction with alcohol consumption may enhance disease status.

This study aims to develop an alcohol-plus-PCB 126 model to evaluate the effects of PCB 126 exposure on mice consuming an ethanol diet. It is hypothesized that PCB 126 exposure will exacerbate LGS endpoints.

Male, C57BL/6J mice were orally gavaged 0.2 mg/kg PCB 126 or corn oil vehicle four days before ethanol feedings using the chronic-binge (ten-plus-one) model. Gene expression was acquired via qPCR. Villus height and crypt depth were determined in Adobe Photoshop (v23.2) by measuring pixel distance. All numerical data was analyzed via a 2-way ANOVA with significance threshold set at a p-value of <0.05.

Ileal Ahr activation was observed by increased Cyp1a1 gene expression by PCB 126 exposure; however, Ahr gene expression was downregulated due to ethanol feeding. Zonula occludin genes, Tjp1, Ocln, Cldn2, Cldn3, and intestinal barrier gene, Muc2, expression were decreased by ethanol feeding. Adherens junction gene (Cdh5) expression was decreased by PCB 126 exposure. Pro-inflammatory cytokine gene, Tnf-α, expression was downregulated, while another pro-inflammatory cytokine gene, Il-6, expression was unaltered. Further, anti-inflammatory cytokine gene, Tgf-β, expression was downregulated with ethanol feeding. Finally, ileal morphological disruption was observed where villi length-to-crypt depth ratio was decreased due to PCB 126 exposure and ethanol feeding.

Ethanol feeding was observed to be the primary driver of increased intestinal permeability. PCB 126 exposure did not exacerbate alcohol-induced gut permeability in this model. However, PCB 126 did impact adherens junction and intestinal morphology. Therefore, chronic studies are needed to assess the full extent of PCB 126's influence on ethanol's toxicological effects in the gut.

Abstract #29 – PhD Student (Poster and Big Picture Science)
Classification of the toxicity in early-life exposure to lead in zebrafish sorl1 mutants

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Exposure to lead (Pb) is a public health problem responsible for several adverse health outcomes dependent on exposure dose and life stage of exposure. Besides the classical clinical signs of Pb exposure (e.g., developmental learning impacts, mental confusion, cardiac output), other pathologies like those related to Alzheimer's disease are suggested to be associated with Pb exposure. In recent studies, an increased incidence and severity of Alzheimer's disease cases was observed in patients exposed to Pb and other heavy metals. In addition, laboratory studies in multiple biological models are reporting alterations in molecular pathways and pathologies related to Alzheimer's disease with Pb exposure. Based on these findings, in this study exposure to low doses of Pb [1, 10, or 100 ppb (µg/L)] during embryogenesis (1 to 72 hours post-fertilization, hpf) in wild-type (5D) and a sorl1 deletion mutant strain was evaluated. SORL1 is important in the processing of the beta-amyloid protein and was recently identified as a genetic variant associated with Alzheimer's disease. The sorl1 deletion mutant was used in this study to investigate the gene x environment interaction of the sorl1 variant with Pb exposure. During the exposure period (1-72 hpf), spontaneous movement rate at 24 hpf, heartbeat rate at 48 hpf, and hatching rate up to 72 hpf was evaluated. Additionally, at 72 hpf, the zebrafish were rinsed, Pb exposure removed, and organisms continued to develop until 168 hpf when the aversive stimulus test (AST) was performed. Lethality was also recorded throughout. No increase in lethality was observed throughout the experiment (i.e., above 97% survival in all treatment groups), along with no significant differences in time to hatch. The rate of spontaneous movement and the rate of heartbeat were altered in the sorl1 mutant in the group exposed to 10 ppb Pb (two-way ANOVA, Tukey's post-hoc, $p<0.05$), indicating increased susceptibility and a gene x environment interaction. Furthermore, the assessment of the larvae's cognitive capacity by the AST was mostly impaired in the sorl1 mutant treated with 10 ppb Pb (two-way ANOVA, Tukey's post-hoc, $p<0.05$). In this test, there was no difference between the 10 ppb Pb wild-type and sorl1 mutant groups, but the cognitive impairment was accentuated in the treated mutant groups overall. These conclusions indicate that Pb alone is capable of affecting cognition but the sorl1 genetic variant background results in more pronounced impairment.

Abstract #30 – PhD Student

Particulate hexavalent chromium targets genes in lung cancer and DNA repair pathways

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Hexavalent chromium [Cr(VI)] is a well-established human lung carcinogen with widespread environmental and occupational exposure. Cr(VI) causes DNA double-strand breaks and loss of DNA break repair, leads to chromosome instability, which is a driving mechanism in Cr(VI) carcinogenesis. However, the ability of particulate Cr(VI) to inhibit DNA break repair across repair pathways is poorly understood. Our previous data show Cr(VI) alters the expression of mRNAs related to DNA double-strand break repair; however, these studies focused on individual genes and not the broader impact on repair genes across the genome. Thus, the aim of this study was to characterize the global transcriptional changes in mRNA expression and specifically consider DNA repair pathways in human lung cells after acute (24 h) and prolonged (>72 h) particulate Cr(VI) exposure. Cells were exposed to various concentrations (0.1, 0.2 and 0.3 ug/cm^2) of zinc chromate, a representative particulate chromate compound. Cells were harvested, and RNA was extracted, followed by a library preparation step and next generation sequencing. Our results show exposure to particulate Cr(VI) induced a time- and concentration-dependent increase in the total number of upregulated and downregulated genes. Using software MetaCore, we compared our list of Cr(VI) targeted genes with disease databases and found Cr(VI)-induced differentially expressed genes were involved in lung cancer. Pathway analyses revealed differentially expressed genes were involved in key DNA repair pathways and DNA maintenance. Thus, we generated heatmaps for genes involved in 8 different DNA repair pathways: 1) homologous recombination repair, 2) non homologous end joining, 3) microhomology-directed end-joining, 4) single strand annealing, 5) mismatch repair, 6) base excision repair, 7) nucleotide excision repair and 8) crosslink repair. Our data show global downregulation of genes involved in all eight DNA repair pathways compared to untreated controls indicating particulate Cr(VI) modulates all of them. Altogether, our data show Cr(VI) exposure in human lung cells leads to differentially expressed genes involved in lung cancer and induces a global downregulation of genes involved in high fidelity DNA repair pathways, which can lead to chromosome instability and cancer. This work was supported by NIEHS grants R01ES016893, R35ES032876 (JPW), and T32ES011564 (RMS and J.P.W.).

Abstract #31 – PhD Student
AhR activation differentially alters the expression profile of IGH constant regions

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a persistent environmental contaminant that inhibits Ig gene expression and antibody secretion in animal models. This inhibition is mediated through the aryl hydrocarbon receptor (AhR) for which TCDD is a high-affinity ligand. Most studies evaluating Ig expression have been limited to assessing IgM using animal models. The current study focuses on determining the effects of TCDD and AhR activation on the expression profile of human Ig isotypes (IgM, IgG1-4, IgA1-2, IgE) utilizing a human B cell line, CL-01 that can be activated to secrete Ig and undergo class switch recombination from IgM to IgG, IgA or IgE. Our results suggest that TCDD has little to no effect on IgM secretion, but significantly inhibits total IgG secretion, an effect reversed by the AhR antagonist CH223191 (AhRA). The effect of TCDD on the expression of the Ig heavy chain (IGH) constant regions (i.e. μ, γ1-4, α1-2, and ε, encoding for the heavy chain proteins in IgM, IgG1-4, IgA1-2, and IgE, respectively) was also evaluated. At the transcript level, TCDD significantly inhibits γ1-4 and ε transcripts but not α1-2 transcripts. Notably, the AhRA reversed these TCDD-induced effects on IGH expression. Interestingly, the effects of TCDD and AhRA on μ, γ1-4, and ε expression were independent of the AhR having a functional transactivation domain. In contrast, the effect of the AhRA on the expression of α transcripts was greatly dependent on the AhR transactivation domain. These results suggest that AhR activation differentially alters the expression profile of *IGH* constant regions in both a transactivation-dependent and independent manner.

Abstract #32 – PhD Student

Low-dose PFAS exposure as an environmental risk factor for neurodegenerative diseases

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PFOA and GenX belong to the family of Per- and Polyfluorinated Substances (PFAS), a group of chemicals widely used for water repellent products, non-sticky cookware, and firefighting foams. GenX is a widely used alternative of PFOA starting from 2005. PFOA and GenX are widely detected in various water supplies, including surface and ground water as well as air emission. In 2022, the US Environmental Protection Agency (EPA) issued a health advisory on PFOA with a recommended level of 0.004 parts per trillion (ppt) in drinking water; for GenX, the health advisory is 0.01 parts per billion (ppb). Increasing studies have associated PFOA and GenX with the development of cancers and neurodegenerative diseases; however, few of them have systematically assessed low-dose exposure of PFOA and GenX, and their possible mechanism to disease onset. Utilizing both SH-SY5Y, a human cell line that can be differentiated into dopaminergic (DA)-like neurons and have been widely adopted to examine neurotoxic effects, and human induced-pluripotent stem cell (hiPSC)-derived cortical neuron culture, we investigated the effect of PFOA and GenX exposure prior to differentiation and assessed changes in epigenome, transcriptome, neuronal characteristics, and neurodegeneration markers in DA like-neurons and cortical cultures. After prior exposure of low-dose PFOA and GenX, we perceived changes in neuron-specific features including changes in neuronal network complexity and alteration intracellular Calcium. We also observed changes in α-Synuclein level in neurites of DA-like neurons and pTau181 level in neurites of both culture systems, which are common markers of neurodegenerative diseases Parkinson's Disease (PD) and Alzheimer's Disease (AD). RNA sequencing performed in cortical cultures revealed transcriptomic changes in AD-related genes, including APP and APOE. These results altogether revealed persistent deficits induced by low-dose PFOA and GenX exposure in DA-like neurons and cortical cultures, suggesting potential neurotoxicity of PFOA and GenX that can contribute to disease onset.

Abstract #33 – PhD Student
Polychlorinated biphenyl 126 alters the hepatic transcriptome to enhance alcohol-associated liver disease

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Alcohol-associated liver disease (ALD) pathology is initially characterized by hepatic lipid accumulation which can further progress with inflammation and fibrotic scarring. ALD development can be impacted by other factors including hypercaloric diets and smoking. Previously, environmental pollutants, namely persistent organic pollutants (POPs), have demonstrated to promote dyslipidemia in diet-induced obesity models. However, not much research has been produced investigating how POPs may promote or enhance ALD. We recently characterized a novel model for how POP, polychlorinated biphenyl (PCB) 126, can disrupt lipid metabolism and exacerbate steatosis and hepatomegaly in an alcohol feeding model. Because excessive alcohol consumption is a major cause of preventable death and humans are inevitably exposed to POPs, it is important to understand how exposures may modify this lifestyle related disease. Therefore, we performed RNA sequencing (RNA Seq) to suggest mechanisms that may elucidate PCB126's promotion of ALD. We hypothesize that PCB126 exposure prior to alcohol feeding will result in unique differentially expressed genes (DEGs) and enriched pathways will implicate altered metabolism and signaling disruption.

Male C57BL/6 mice were exposed to 0.2mg/kg PCB126 or vehicle by oral gavage. Mice were then fed a 5% ethanol (EF) or 0% ethanol (PF) diet for ten days followed by 5g/kg ethanol binge. Liver RNA was isolated and prepared for RNA Seq. DEG analysis was performed with DESeq2 for pairwise comparisons for $q \leq 0.05$.

Transcriptomic analyses indicated that *PF(Veh. v PCB126)* had 503 (339↑; 164↓) DEGs. Importantly, *EF(Veh. v PCB126)* had 907 (536↑;371↓) DEGs. Among the top 20 upregulated genes in our *EF(Veh. v PCB126)* comparison included genes involved in xenobiotic metabolism and molecular transport. Within the top 20 downregulated genes in this group were involved in lipid metabolism and zinc-related proteins were prevalent. Four genes (*Abcb10*, *Slc46a3*, *Tuba8*, and *Ugt1a6b*) were validated by RTPCR. Top enriched Gene Ontology (GO) processes involved peptidyl-tyrosine modifications. This indicates that signaling, by altered phosphorylation, may have been disrupted specifically in this group. Ongoing research is investigating this hypothesis by phosphoproteomics and immunoblotting methods.

These analyses suggest that PCB126 exposure modifies the hepatic transcriptome to, in part, disrupt signaling processes. Based on our enriched GO processes, tyrosine modifications may be a key mechanism related to PCB126's ability to promote ALD pathology. This project signifies that pollutants can also promote ALD, which was previously unreported.

Abstract #34 – PhD Student

Understanding the toxicological profiles of dicamba and glyphosate to evaluate the effect of Roundup Xtend on zebrafish (*danio rerio*) embryo-larval model

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Glyphosate is the most widely used agricultural herbicide in the US and over the last 20 years, usage and production has tripled, particularly in the midwestern states. High usage over the past 20 years has resulted in weed species that are glyphosate resistant. To combat this issue, alternative formulations such as Roundup Xtend containing a mixture of glyphosate with another agricultural herbicide dicamba is now being marketed and the approval for usage has been granted in 36 states. Glyphosate can move into drinking water sources and is regulated at 700 parts per billion (ppb, µg/L) by the US EPA. While glyphosate is highly used in the US, it is banned in several other countries around the world based on health concerns including risk of carcinogenicity (Group 2A, likely carcinogenic to humans) and developmental toxicity. Dicamba, on the other hand, has not historically been used at high rate but is changing with the introduction of this new herbicide formulation. Based on historic low use there are no drinking regulatory levels for dicamba currently in the US but with increased use comes additional concern for contamination risks. At this time there are limited toxicity studies with dicamba but those that exist report genotoxicity and biochemical toxicity in lower models. The current study was designed to study the sublethal effects of glyphosate and dicamba exposure alone and also in a binary mixture to evaluate developmental and neurotoxicity effects using the zebrafish (*Danio rerio*). Zebrafish embryos were collected and exposed between 0 and 2 hours post fertilization (hpf) and exposed to varying concentrations of glyphosate (0.7 – 7000 ppb), dicamba (0,01 - 1000 ppb) and binary mixtures of the two (700 gly/100 dic, 7000 gly/100 dic). They were exposed until 120 hpf and then taken out of the exposures to perform the neurobehavioral assessments and developmental malformations. The developmental malformations were observed after staining with Alcian Blue and Alizarin Red co-staining and were measured for head size (lower jaw length, ceratohyal cartilage length, intraocular distance), brain length and body length. The neurobehavior was assessed using Daniovision tracking software which altered the light phases into 3 dark and 2 light phases alternatively and tracked the movement of the embryos. Results indicate that glyphosate and dicamba alone can cause severe developmental malformations such as a significant reduction in the lower jaw length, ceratohyal cartilage length, and brain length, which are all parameters used to evaluate head size or circumference, when compared to control larvae. An increased body length of larvae exposed to glyphosate alone was also observed. In the behavior assay, glyphosate exposure led to a significant reduction in the distance moved, velocity, and time spent moving. A similar trend was observed in the dicamba groups with a reduction in distance moved and velocity of movement. The binary exposures had a greater significant reduction in the same parameters as compared to the control or the individual exposures for both the herbicides. Overall, results show developmental and behavioral alterations at environmentally relevant concentrations for single and binary exposures.

Abstract #38 – PhD Student
Lipid-mediated inflammation contributes to metabolic syndrome-associated pulmonary susceptibility nanoparticles

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Metabolic Syndrome (MetS) is a combination of abnormal metabolic disturbances including dyslipidemia, obesity, hyperglycemia, and hypertension enhancing the risk of cardiovascular disease, asthma, diabetes, and other chronic diseases. Several epidemiological assessments demonstrate individuals with MetS exhibit exacerbated inflammatory responses following inhalation of particulates. The mechanisms of these inflammatory responses remain unelucidated and prevent the development of strategies to protect this vulnerable and increasingly prevalent population. Nanomaterials are increasingly utilized in numerous applications and processes resulting in nanoparticles exposures during product use and development. Additionally, a significant portion of ambient particulates are within the nano-size range. Since lipids are dysregulated in MetS and are intricately involved in inflammatory regulation, we hypothesize particulate exposure-induced modifications in bioactive lipids mediate MetS susceptibility to inflammation. To evaluate this hypothesis, mice were fed either a control diet or a high-fat western diet for 14-weeks. Mice were exposed to silver nanoparticles (AgNPs) via oropharyngeal aspiration or water (control). Acute pulmonary toxicity endpoints were evaluated 4 hours after exposure to capture potential variations in pro-inflammatory mediators. Analysis of bronchoalveolar lavage fluid (BALF) demonstrated AgNP exposure resulted in neutrophilia in both health and MetS mouse models which was exacerbated in MetS mice. Gene expression of the inflammatory markers chemokine ligand-1 (CXCL1) and macrophage inflammatory protein-2 (CXCL2) were upregulated equivalently at 4 hours post-exposure in both healthy and MetS mice. Gene expression of interleukin-6 (IL-6), IL-1β, and monocyte chemoattractant protein-1 (CCL2) were decreased in control MetS mice compared to healthy controls. Healthy mice exposed to AgNPs demonstrated down-regulation of IL-1β and CCL2 compared to controls. Pulmonary lipids were evaluated utilizing an MRM profiling approach which demonstrated unique induction of pro-inflammatory lipid mediators in the MetS mouse model following AgNP exposure compared to the healthy model. Specifically, arachidonic acid (AA), prostaglandin-E2 (PGE2), 12-hydroxyeicosaterraenoic acid (12-HETE), leukotriene-B4 (LTB4) and others were determined to be elevated in the MetS model following exposure, supporting the contribution of lipids to exacerbated inflammation. Further, pulmonary gene expression demonstrated distinct upregulation of arachidonate 15-lipoxygenase (ALOX-15) and prostaglandin-endoperoxide synthase 2 (also known as cyclooxygenase-2) (PTGS2) in MetS mice following AgNP exposure while no modifications in arachidonate 5-lipoxygenase (ALOX-5) were observed in either group. Overall, our data suggests dysregulation of pro-inflammatory lipid mediators contributes to early exacerbations in inflammatory responses observed in MetS following particulate exposures. This may be a mechanism that could be targeted for therapeutic interventions to address MetS susceptibility to exposures.

Abstract #39 – PhD Student
Metals in toenails as biomarkers for assessing chronic exposure to welding fumes

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Chronic overexposures to metals in various occupational settings, including welding, brazing, and soldering, have been linked to a range of diseases. While some metals such as manganese (Mn) are known to cause neurotoxic effects and hexavalent chromium (Cr) compounds are well-known carcinogens, the dose-response of different metals in chronic exposure settings is not fully understood. Therefore, biomarkers for chronic exposure are crucial in identifying the deleterious impacts of these exposures. In a previous study, we found that toenail Mn concentration was an excellent biomarker for chronic exposure to Mn. However, the use of concentrations of other metals in toenails as biomarkers to estimate exposure to corresponding metals has not been explored. In this study, we collected welding fumes and toenails from 14 welders and 13 controls to investigate the relationship between metal concentrations in welding fumes and toenails for Mn, iron (Fe), zinc (Zn), Cr, and copper (Cu). We measured metal concentrations in welding fumes and toenails using inductively coupled plasma-optical emission spectrometry. Additionally, we calculated the area under curves (AUCs) of the receiver operating characteristics (ROC) curves to assess the ability of toenail metal levels to distinguish between welders and controls. Our results showed that metal concentrations in welding fumes and toenails were significantly higher in welders than controls. Metal concentration ($\mu g/m^3$) from air samplings for welders and controls were Mn (156.8, 2.9), Fe (1258.6, 24.0), Zn (21.1, 4.7), Cr (2.7, 0.0), and Cu (18.3, 0.1). Metal concentrations in toenails (µg/g) for welders and controls were Mn (3.9, 1.5), Fe (62.3, 22.1), Zn (73.1, 79.9), Cr (2.6, 0.4), and Cu (4.7, 5.8). Mn concentrations in toenails had the most capability to distinguish between welders and controls (AUC = 0.91), followed by Fe (0.73), Cr (0.58), Zn (0.54), and Cu (0.45). These findings highlight the potential of toenail metal concentrations as a biomarker for chronic exposure to metals.

Abstract #40 – PhD Student
Study of the effects of cadmium on pulmonary arterial hypertension

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Pulmonary arterial hypertension (PAH) is characterized by the inflammation, proliferation, and remodeling in endothelial and smooth muscle cells of pulmonary arteries, and cardiomyocytes of the right ventricle (RV). Cadmium (Cd) exposure causes toxicity by damaging mitochondria to produce reactive oxygen species and apoptotic cell death. Previously we reported that PAH patients had elevated Cd levels in both blood and urine samples, therefore this study aimed to test whether Cd directly induces PAH or facilitates PAH pathogenesis induced by a SU5416 and hypoxia (SuHx) mouse model.

Forty male C57/6J mice were initially divided into two groups: control (n=20) and 5ppm Cd in drinking water (n=20) for 8 weeks. Diastolic and systolic functions of both ventricles (left ventricle (LV) and RV) were examined via transthoracic echocardiography (echo). Next, 10 mice in both groups had PAH induced, resulting in 4 subgroups: Control (CTRL), Cd Only (Cd), PAH, and Cd+PAH. Echo was performed 4 weeks post PAH induction, followed by the euthanasia of 7 mice/group to collect heart and lung tissue for histological and biochemical analyses. The remaining 12 mice (3 mice/group) were used for RV systolic pressure (RVSP) measurements. A 2-way ANOVA with a Tukey comparison was performed for echo and pressure analyses while unpaired student t-tests were performed for histological and biochemical analyses. A p-value of less than 0.05 was considered statistically significant.

Echo results show 8-weeks Cd exposure alone did not significantly alter RV structure, cardiac output, or systolic function, but 12-week Cd exposure significantly increased RV systolic function. RV cardiac output and RVSP measurements were significantly decreased in Cd+PAH compared to PAH, indicating RV failure. Heart histological analyses show 12-week Cd exposure alone significantly increased collagen content in both ventricles and increased LV cardiomyocyte area size compared to CTRL. There weren't any further significant RV changes in terms of collagen content or hypertrophy when comparing Cd+PAH to PAH. Lung histological analyses show Cd alone significantly elevated collagen content and the number of abnormal pulmonary arteries, wall area, and medial thickness when compared to CTRL. However, no significant changes were noted regarding wall area and medial thickness when comparing PAH to Cd+PAH.

12-weeks chronic Cd exposure alone caused RV systolic dysfunction and significant LV/RV/lung inflammation and pulmonary vascular pathology.

Abstract #41 – PhD Student
Environmental exposures and aging

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In recent years, research into air pollution has shown that exposure to certain components in air pollution, primarily $PM_{2.5}$ can accelerate biological aging and thereby lead to increased susceptibility to cardiovascular diseases, cancer, diabetes and neurodegenerative disorders. This premature aging may be investigated by studying some of the hallmarks of aging which include telomere shortening, epigenetic alterations, and cellular senescence. We hypothesize that prolonged exposure to air pollutants can lead to increased telomere shortening which in turn can result in chromosomal instability and senescence of multiple cell types leading to extensive tissue dysfunction, arterial stiffness and susceptibility to other diseases. To examine the effects of air pollution exposure on tissues, we exposed mice to concentrated ambient particles (CAP) for 9, 15, and 21 days, then measured the telomere lengths of peripheral blood mononuclear cells (MNC's), endothelial progenitor cells (EPC's), ckit positive cells and crude bone marrow cells via RT-PCR. Secondly, we measured β-Galactosidase activity via flow cytometry as a measure of cellular senescence in stem cells, stromal cells, MNC's and EPC's. Finally, we investigated epigenetic alterations by measuring histone methylation patterns via western blots in EPC's and MNC's. Statistical analyses for all experiments were done via the unpaired student's t-test in Graphpad to generate if the results were statistically significant. We found consistently decreasing telomeres in groups exposed to air pollution across all of the examined cell types. Additionally, we found significantly higher amounts of β-Galactosidase associated cellular senescence in stem cells, MNC's, and EPC's. Lastly, we found several histone sites that had increased levels of trimethylation upon exposure to air pollution suggesting that air pollution can cause the same epigenetic alterations as advanced aging. Our investigation into the three indices of aging shows us that upon exposure to air pollution, telomeres decrease consistently across multiple cell types, which leads to increased cellular senescence. Additionally, increased exposure to air pollution can lead to epigenetic changes that are identical to changes that arise as a result of aging.

Abstract #42 – PhD Student

Exosomal miRNAs in biofluids of the rotenone-induced rat model of Parkinson's Disease induce dopaminergic neuron cell death in primary midbrain neurons

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Parkinson's Disease (PD) is a chronic and progressive neurodegenerative disorder characterized by degeneration of dopaminergic neurons in the brain, particularly in the nigrostriatal pathway. Both environmental as well as genetic factors contribute to the development of PD. Rotenone, which is a naturally occurring substance that has been used extensively as an insecticide and pesticide. Rotenone is a potent inhibitor of mitochondrial complex I, which is a key pathogenic pathway in PD. Several studies have indicated that experimental rat models treated with rotenone replicate the cardinal features of PD, including dopaminergic neurodegeneration, α-synuclein aggregation, activation of microglia and oxidative alterations. The concept of PD pathology propagation emphasizes the pivotal role of intercellular communication, which is mediated through a subset of extracellular vesicles, the exosomes. Numerous studies have demonstrated that the cargo transported by exosomes exerts a discernable influence on the physiological outcomes in the recipient cells, hereby affecting PD pathology. Exosomal miRNAs have been identified as regulators of the molecular pathways and biological processes in the recipient cells. Since exosomal miRNAs are detected in various biofluids, like serum and cerebrospinal fluid (CSF), not only do they act as promising biomarker candidates for diagnosis of PD at an early stage, but it's also imperative to study the molecular mechanisms through which they modulate the pathways implicated in the pathogenesis of PD. In pursuit of these objectives, our study tested the hypothesis that exosomal miRNAs circulating in the CSF and serum of rats undergo modifications in response to acute rotenone exposure. We conducted experiments on 3-month-old male Sprague Dawley rats, administering acute rotenone doses (3mg/kg) for 8h and 24h, subsequently extracting serum and CSF. Preliminary data suggest that exosome concentration is higher in serum of rats treated with rotenone. Amongst many miRNA alteration patterns, notably miR-181c-5p is specifically altered, with high levels in CSF and low levels in serum at 8h, while the opposite results are observed at 24h, where levels drastically drop in CSF and rise significantly in serum at 24h. The effect of serum exosomes on primary neurons was studied using cell viability assays. Data suggested that significant loss in cell viability is observed in primary midbrain neurons, along with specific toxicity to dopaminergic neurons on treatment of serum exosomes from rotenone-administered rats. Our study holds promise not only for the early detection of the disease through exosomal miRNA biomarkers, but also modulation of cellular functions and viability which may be used for forecasting prognosis and evaluating a suitable treatment response.

Abstract #43 – PhD Student
The impact of chronic manganese on glutamate excitotoxicity in human IPSC-derived cortical model of Alzheimer's disease

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Manganese (Mn) is an essential metal widely present in the environment, but in excess is a neurotoxicant with broad impacts on neurological systems. Alzheimer's disease (AD) is a chronic multifactorial neurodegenerative disorder wherein the vast majority of AD cases cannot solely be attributed to familial inheritance and involve contributions from environmental risk factors. An important overlapping pathological feature shared by Mn neurotoxicity and AD is perturbed glutamate neurobiology. Enhanced neuronal excitability has been reported in AD patients and animal models and studies have shown that acute exposure to high levels of Mn can inhibit synaptic glutamate uptake. However, the effects of chronic exposure to physiologically relevant levels of Mn and its implication in AD pathology remain unknown. Hence, we hypothesized that chronic Mn exposure increases susceptibility to glutamate excitotoxicity in a manner that is altered by an individual's genetic risk for AD. Here, we utilize cortical neurons and astrocytes generated from induced pluripotent stem cells derived from neurotypical and AD patients. Cells were cultured for approximately 100 days to ensure neuronal maturation and astrogliogenesis and subsequently exposed to Mn (vehicle, 0.5 or 5 µM) for up to 40 days. Alterations in glutamate uptake were quantified using ^{14}C-labeled glutamate wherein we observed a significant 30~40% decrease in glutamate uptake in AD patient-derived neurons/astrocytes, an effect that was not seen in the neurotypical controls. To investigate cell type-specific transcriptomic alterations that may, at least in part, contribute to the observed differential pathophysiology, we performed single cell RNA-sequencing following a 43-day Mn exposure. Bioinformatic analyses revealed several significantly altered pathways that share at least partial overlapping pathology in Mn and AD such as 14-3-3, EIF2, glutamate receptor, and mTOR signaling. Finally, preliminary analyses of functional multielectrode array recordings showed a significant increase in mean spike rate following 5 µM Mn exposure. In summary, we provide valuable insight into discerning the transcriptomic and functional alterations caused by chronic Mn and interrogating how an individual's genetic predisposition to AD may alter this pathophysiology. NIH/NIEHS R01 ES031401 (FEH/ABB)

Abstract #44 – PhD Student
The effects of gestational lead exposure on the number of bipolar and amacrine cells in the mammalian retina

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Lead (Pb) is an environmental neurotoxin with adverse effects on the developing brain including the visual nervous system. In both children and animal models, low-level exposures to lead have been shown to negatively impact cognitive and visual function. However, our understanding of the specific retinal cells impacted by Pb exposure remains incomplete.

Previous studies, based on gene expression alone, suggested an increase in the generation of bipolar cells and decrease in amacrine cells. In this study we aimed to quantify the effects of Pb exposure on bipolar and amacrine cell during retinal neurogenesis. To test the effects of Pb on neurogenesis, we administered either 13ppm Pb-Acetate, 27ppm Pb-Acetate, or 27ppm Na- Acetate to the drinking water of pregnant dams from embryonic day (E0) to postnatal day 14 (P14). Retinal sections from P14 mice were assessed using immunohistochemical markers for distinct cell types; Chx10 and TFAP2, for bipolar cells and amacrine cells respectively. Our results revealed no changes in the number of bipolar cells or amacrine cells between control and Pb-expoused animals. Additionally, we observed no changes in other retinal neurons, such as the photoreceptors. Based on our findings, we suggest that lead exposure does not affect neurogenesis and cell specification during retinal development.

Abstract #45 – PhD Student
Elucidating the biological effects of EV-associated adduct-containing DNA

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On a daily basis, the human body is exposed to various genotoxic agents including environmental carcinogens such as UVB radiation and chemotherapeutic compounds like cisplatin. UVB radiation and cisplatin are similar in that both induce the formation of crosslinks between adjacent bases in DNA. These DNA adducts are potentially mutagenic and lethal to cells, and cells therefore use the nucleotide excision repair (NER) machinery to excise these adducts from the human genome as small oligonucleotides. In the absence of efficient NER, these adducts can be found in larger apoptotic DNA fragments. The final fate of these unrepaired DNA adducts is not yet revealed; however, we have recently detected damaged DNA in association with extracellular vehicles (EVs) present in the culture media of UVB- and cisplatin-treated cells. Moreover, we have found that this release occurs in a caspase-dependent manner. Our ongoing work has shown that DNA containing UVB photoproducts or cisplatin-DNA adducts can be taken up by non-damaged bystander cells where it may activate DNA damage checkpoint and/or innate immune signaling pathways. Because EVs have an important role in intercellular communication, our data indicate that damaged DNA has the potential to be transferred to diverse organs throughout the body. Further research in this direction can help us understand the role of EVs containing UVB photoproducts and cisplatin-DNA adducts in the toxicity and systemic effects caused by genotoxic agents.

Abstract #46 – PhD Student
Laboratory test of a novel method to detect airborne *Legionella pneumophila*

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Bioaerosols are aerosols of biological origin that can cause adverse health effects. For example, airborne *Legionella pneumophila (L. pneumophila)* can develop Legionnaires' disease (LD) once inhaled. Starting with symptoms such as headache and muscle pain, LD develops into pneumonia and in some cases is fatal. To assess the exposure to and protect people against airborne *L. pneumophila*, a rapid detection method is necessary. In conventional methods, airborne *L. pneumophila* is collected and then transported to the lab for analysis. However, these processes are time-consuming and not practical in the field. Therefore, an alternative method was developed in this study. The developed method is a combination of a lab-made inertial impactor and a latex agglutination test assay. The inertial impactor was designed to collect bioaerosols larger than 0.5 µm on the substrate used in the latex agglutination assay. To evaluate the collection efficiency and cut-off diameter of the inertial impactor, concentrations of test particles up and downstream of the impactor were measured using a scanning mobility particle sizer (SMPS) and an optical particle sizer (OPS). The limit of detection (LOD) of the latex agglutination assay kit was also determined using a known concentration of *L. pneumophila* suspension. In the lab sampling test, *L. pneumophila* suspension was aerosolized in an exposure chamber. The sampler was in the exposure chamber and particle number concentration was monitored using an OPS. The LOD of the test kit was 4.54×10^{1} cells of *L. pneumophila* per test kit. In the results of the lab sampling test, *L. pneumophila* was detected when the number of deposited particles in the kit exceeded 2.73×10^{1} 2,728,637. The number of particles collected on the latex agglutination kit was measured lower than that of LOD because two or more *L. pneumophila* may be agglomerated in the suspension and contained in one droplet sprayed from the vibrating mesh nebulizer. The results show that the developed method can rapidly detect airborne *L. pneumophila* and overcome the limitations of conventional methods. Considering LD can be developed by low levels of *L. pneumophila,* future experiments will reduce the concentration of *L. pneumophila* suspension and proceed for a longer time.

Abstract #47 – PhD Student

The kisspeptin system in the developing zebrafish and differential gene alterations following two exposure periods to the agricultural herbicide atrazine

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Atrazine is an herbicide used to control broadleaf and grassy weeds on agricultural fields in the US but this herbicide has been banned from use in the European Union since 2003, based mainly on risk of contamination of surface and groundwater. Atrazine is categorized as an endocrine disrupting chemical (EDC), altering release of luteinizing hormone from the pituitary through gonadotropin-releasing hormone in the hypothalamus. The specific mechanism that leads to this disruption is not yet clearly defined. In this study, molecular targets in the kisspeptin signaling system within the neuroendocrine system were explored to elucidate a mechanism that coincides with the multiple observed adverse health outcomes along the endocrine axes. Using the zebrafish model, expression of genes associated with the kisspeptin system (*kiss1, kiss2, kiss1ra, kiss1rb*) were first examined during different stages of development (24, 48, 72, 96, or 120 hours post fertilization, hpf) using qPCR. Expression of the four genes significantly increased throughout development ($p<0.05$). Second, it was determined if a developmental atrazine exposure perturbed expression of these genes using qPCR comparing two developmental exposure periods [1-72 hpf or 72-120 hpf]. Atrazine treatments included 0, 0.3, 3, or 30 ppb (µg/L) to represent concentrations around the current US EPA regulatory level in drinking water of 3 ppb. Results indicated a preferential increase in *kiss1* and *kiss2* expression from the embryonic (1-72 hpf) atrazine exposure ($p<0.05$) with no changes observed for the receptors (*kiss1ra, kiss1rb*) ($p>0.05$). Future studies are needed to further investigate the association of the kisspeptin signaling system and atrazine endocrine disruption.

Abstract #45 – MS Student
Arsenic exerts anti-cancer effects in non-small cell lung cancer

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Non-small cell lung cancer (NSCLC) is the most prevalent type which accounts for 80-85% of lung cancer cases. Besides, 70% of lung cancer patients have locally advanced tumors at the time of diagnosis. While there are several treatment options for NSCLC, most remain ineffective. In addition, other difficult issues in the effective treatment of NSCLC include drug resistance and tumor recurrence. To that end, other compounds with anti-cancer properties are being explored as a drug repurposing candidates. Notably, arsenic compounds such as arsenic trioxide, arsenic sulfide, and tetra arsenic hexoxide represent a novel strategy for treating both drug resistance and advanced-stage NSCLC. Previous studies revealed that arsenic compounds are potent anti-cancer agents resulting in the remission of acute promyelocytic leukemia (APL) and significantly reducing the ability of APL stem cells to self-renewal. Furthermore, it was discovered that arsenic compounds target various oncogenes and oncogenic proteins such as Gli1, N-myc, GAS1, ABC transporters, PDL-1 along with signaling pathways such as hedgehog signaling pathway, NF-κB pathway, and oncogenic-driven mutations such as L858R/T790M. These various mechanisms have been shown to be involved in the suppression of NSCLC growth via regulating various properties of cancer cell, including cell cycle arrest and apoptosis. Arsenic compounds such as arsenic trioxide, arsenic sulfide, and tetra arsenic hexoxide exert promising anti-carcinogenic activity against NSCLC. Arsenic combined with other drugs provide synergistic anti-tumor effects than arsenic alone. Hence arsenic compounds could be explored as a new approach for both treating NSCLC as well as overcoming drug resistance. However, clinic trails are needed to extend/validate the experimental findings.

Abstract #46 – MS Student

Towards understanding drinking toxicity as our infrastructure transitions to plastic: cured-in-place-pipe liners for drinking water quality

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As water utilities address leaking infrastructure many are beginning to consider cured in place pipe (CIPP) lining for potable water application, but no studies have yet examined their drinking water quality impacts. Examined how chemical water quality impacts differed when CIPP products were manufactured under optimum and sub-optimum conditions. One epoxy bisphenol resin and two different hardeners from the same manufacturer, a standard cure and a quick cure system, were examined. Learned and applied gas chromatography-mass spectrometry (GCMS) and identified a variety of compounds in the resin, hardeners, and cured composites not listed on the safety data sheets. Discovered BPA, a monomer degradation product, along with other VOCs and SVOCs were extractable from the new CIPPs. Thermogravimetric analysis revealed 3.2-4.8% wt. volatile material in the new composites. Found that composites marginally impacted total organic carbon levels immediately after manufacture (<3 mg/L, 24 hr.). Several individual chemicals (BPA, BADGE, etc.) leached into drinking water, but on a mass basis, and 0.43% of the total chemical mass leached was identified. Advised the East Bay Municipal District (Oakland, CA) on drinking water quality concerns associated with CIPP. Writing up results for publication; My study will be the first globally to evaluate CIPP use for drinking water contact under controlled conditions. Results highlight the importance of post-CIPP flushing and rigorous testing of water before products are placed into use. Future work is recommended to assess chemical mixture drinking water toxicity.

Abstract #47 – MS Student

Time-course of systemic inflammation with co-exposure to particulate matter and stress

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Exposure to ambient particulate matter (PM) increases risk for cardiopulmonary health problems. In situations such as those encountered by deployed military personnel, firefighters, and disaster workers, PM exposure is encountered alongside psychological stress. Thus, the health impacts of PM exposure may be exacerbated with co-exposure to stress due to shared mechanisms such as inflammation. To understand the mechanisms and time-course of the health consequences following burn pit exposure, we exposed adult C57Bl/6 mice to varying levels of ambient PM less than 2.5 μM in diameter ($PM_{2.5}$) alone or in combination with psychological stress using a social defeat model for up to 3 weeks. We analyzed multiple organs (heart, lungs, kidney, spleen, liver) for expression of inflammatory genes, including Il-1b, Il-6, and TNFα and found altered inflammatory progression with PM exposure. We have also seen changes in reactive oxygen species, including SOD1 expression. These data indicate that exposure to $PM_{2.5}$ with stress causes systemic alterations to various systems, but many of these changes are also seen in inflammatory marker expression of the mice exposed to $PM_{2.5}$ without subjection to the social defeat model. We believe this model is well-suited for the study of military and other occupational exposures, and future work will identify potential mechanisms in order to direct prediction of health outcomes as well as treatment for these populations.

Abstract #48 – MS Student
Characterization of adduct-containing DNA release from cisplatin-treated cancer cells

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Cisplatin is an effective chemotherapeutic agent that is used to treat lung, breast, esophageal, ovarian, and pancreatic cancers. Despite being effective in treating a wide variety of cancer types, its cytotoxicity to off-target healthy tissues restrict its therapeutic application. The platinum atom of cisplatin interacts with DNA and results in the formation of inter-and intra-strand crosslinks, where are often referred to as DNA adducts. These adducts are mutagenic and potentially lethal to cells. The sole pathway for cells to excise intra-strand cisplatin-DNA adducts from the genome is through nucleotide excision repair. The ultimate fate of unrepaired DNA adducts is not understood fully. However, using a combination of differential centrifugation and DNA immunoblotting, we have detected cisplatin adduct-containing damaged DNA in associated with small extracellular vesicles (SEVs) that are released into the cell culture medium. Moreover, the inhibition of caspase signaling blocks this release. Our observation that this response holds true with multiple different cancer cell lines (U-2 OS, HeLa, HEK-293, A375) suggests that it is a general phenomenon of cancer cell response to cisplatin. Because SEVs are involved in intercellular communication and can transmit their contents throughout the body, this work has important implications for the systemic effects of DNA damage-based anti-cancer therapies. Moreover, detection of cisplatin adduct-containing DNA could be useful as a marker of cancer cell killing or toxicity.

Abstract #49 – MS Student
Using mesocosms to study the interactive effects of atrazine and chlorothalonil on an Aquatic community

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The growing human population has created a diversity of anthropogenic stressors including habitat loss, climate change, and chemical contaminants that have led to a rising impact on natural systems. Given that multiple anthropogenic stressors occur together in nature, there has been an increasing emphasis on understanding their combined effects on species, communities, and ecosystems. Researching interactive effects of combined stressors will help extrapolate laboratory results to natural communities, which is crucial for making accurate predictions of how stressors alter ecosystems. Since the rise of conventional agriculture, pesticides have been an increasing concern for many ecotoxicologists. While many pesticides are known to be harmful to organisms and communities, their effects when combined with other stressors (anthropogenic or natural) are just starting to be appreciated. Previous research has a heavy focus on studying the effects of a single pesticide on an individual species, often neglecting pesticide interactions and community-level effects. However, there is accumulating evidence that pesticides can have synergistic interactive effects when combined with abiotic factors, biotic factors, and other chemical stressors.

We explored interactive effects of the herbicide atrazine and the fungicide chlorothalonil on an aquatic community. We conducted a 5 x 5 factorial mesocosm experiment with 5 chemical treatments: control, solvent, atrazine (50ppb), chlorothalonil (50ppb), and the combination of atrazine (50ppb) and chlorothalonil (50ppb) for a total of 25 experimental units. All experimental units were stocked with larval bullfrogs, larval dragonflies, aquatic arthropods, gastropods, zooplankton, periphyton, and phytoplankton. Following a 4-week exposure period, samples were collected and preserved of all elements within the aquatic community. Preliminary results suggest that some of the response variables (gastropods, larval dragonflies, and aquatic arthropods) show a significant interactive effect with the combination of atrazine and chlorothalonil. We believe the findings from this experiment will help bridge a gap in knowledge of how pesticides may interact within aquatic communities, further distinguishing the harm pesticides may pose to these ecosystems.

Abstract #50 – MS Student

Differential susceptibility to benzo[a]pyrene exposure during gestation and lactation in Mice with genetic variations in the aryl hydrocarbon receptor and Cyp1 genes

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Polycyclic aromatic hydrocarbons (PAHs) are complex organic molecules formed during combustion processes, the ubiquity of which results in widespread human expo-sure. The most common sources of exposure are traffic-related air pollution, fossil fuel burning, cigarette and wildfire smoke, and ingestion of grilled foods. Our studies were designed to determine if genetic differences in the aryl hydrocarbon receptor (AHR) could affect susceptibility following developmental exposure to the well-characterized PAH benzo[a]pyrene. We used mice in allelic variations in the AHR and the three CYP1 enzymes. Pregnant dams were treated orally with 10mg/kg/day from gestational day 10 (G10) to weaning at postnatal day 25 (P25). We found unexpectedly high neonatal lethality in high-affinity *AhrbCyp1b1(-/-)* knockout mice compared with all other genotypes. Over 60% of BaP-exposed pups died within their first 5 days of life. There was a significant effect of BaP on growth rates in surviving pups, with lower weights observed from P7 to P21. Again, *AhrbCyp1b1(-/-)* knockout mice were the most susceptible to growth retardation. Independent of treatment, this line of mice also had impaired development of the surface righting reflex. We used high-resolution mass spectrometry to measure BaP and metabolites in tissues from both dams and pups. We found the highest BaP levels in adipose from poor-affinity *AhrdCyp1a2(-/-)* dams and identified three major BaP metabolites (BaP-7-OH, BaP-9-OH, and BaP-4,5-diol), but our measurements were limited to a single time point. Future work is needed to understand BaP pharmacokinetics in the context of gestation and lactation and how differential metabolism leads to adverse developmental outcomes.

Abstract #51 – MS Student
Validation of a human B-cell line model to identify potential immunotoxicants

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B-lymphocytes are an important immune cell that provides protection or immunity against pathogens. B-lymphocytes produce antibodies, which bind and help clear the body of pathogens. The COVID pandemic illustrated the importance of antibodies in maintaining immunity against the SARS-CoV-2 virus. However, exposure to environmental, dietary, or pharmaceutical chemicals may dysregulate B-lymphocyte antibody production. Previous animal studies identified effects on immune function by jet-fuel, polyfluoroalkyl substances (PFAS, known as forever chemicals), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin or TCDD), and the asthma drug terbutaline. Since the gene that makes antibodies is very different in humans compared to animals, this project aims to evaluate the effect of these chemicals on antibody production from a human B-lymphocyte cell line. Additionally, humans have genetic variations in the antibody gene, which may result in differences in sensitivity to chemical exposure. Our cellular model incorporates some of these genetic differences and will allow us to determine the impact of genetics and the environment on antibody production. Our studies will evaluate both human antibody gene and protein expression under different chemical exposures and genetic backgrounds. If the genetic differences we are evaluating do lead to altered chemical sensitivity, this will lead to improved risk assessment and identification of at-risk populations.

Abstract #52 – UG Student

Impacts of the benzo[a]pyrene, ultraviolet B, and solar simulated light radiation exposure on microvesicle particle generation from murine skin

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The human skin is the first layer of defense against external and environmental insults that increase the potential for developing skin cancer. Human skin is constantly exposed to two major environmental insults, namely solar UV radiation also known as simulated solar light (SSL), a known carcinogen, and pollutants such as benzo[a]pyrene (BaP), a suspected carcinogenic chemical which can be found in smoke originating from forest fires, cigarettes and burnt food. Due to the prevalence of these hazards in daily life, it is important to understand the effects of BaP, SSL, and ultraviolet B (UVB) exposure on skin tissue, and the mechanisms by which BaP and UVB/SSL mediate their effects in order to design relevant strategies to mitigate their effects. Epidemiological studies indicate that increased exposure to these environmental hazards simultaneously can augment the risk of contracting human malignancies such as skin, mucosal, and lung cancers. Our previous studies demonstrated that exposure to UVB radiation induces the generation of microvesicle particles (MVPs) from human and murine skin in a dose-dependent manner. Notably, our studies have also discovered that these MVPs carry a potent phospholipid mediator, Platelet-activating factor-receptor (PAFR) agonists. Importantly, these PAFR agonists play crucial roles in mediating the effects of pro-oxidative stressors, including UVB and therapeutic agents such as systemic immunosuppression and cancer growth in experimental models. To that end, our current studies sought to determine the effects of topical exposures to BaP, UVB, and SSL on MVP release from PAFR-expressing murine skin. The data demonstrated that these environmental hazards induce increased MVP release. While studies to determine the combined effects of BaP+UVB and BaP+SSL as well as the involvement of the PAFR and MVP are ongoing in the PAFR-deficient and acid sphingomyelinase enzyme deficient mice, the current studies are in agreement with this assumption that PAFR agonists-laden MVP release is one of the possible mechanisms in mediating these hazards-induced effects.

Abstract #53 – UG Student (Poster and Big Picture Science)

Chronic exposure to aqueous film-forming foams leads to evolutionary responses in *Daphnia magna*

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Per- and polyfluoroalkyl substances (PFAS) have historically been a key component in aqueous film-forming foams (AFFF) used in fire suppression. With the increasing emphasis on phasing out PFAS use due to health and environmental concerns, several new chemical technologies have been used to create PFAS-free AFFF. Recent research has demonstrated that these replacement formulations are more acutely toxic to aquatic species than the traditional PFAS-containing AFFF. Given their relatively high toxicity, frequent exposure to the formulations could lead to evolutionary responses (i.e., evolved tolerance) in exposed populations. In this study, we examined the effects of chronic exposure to seven AFFF formulations (6 PFAS-free and 1 PFAS-containing) on the evolution of tolerance in the water flea *Daphnia magna*. Following an 84-day exposure to different concentrations of each formulation, we used a series of laboratory lethal concentration (LC50) tests on a subset of populations to examine the potential change in tolerance. We found that chronic exposure to three AFFF formulations led to a change in tolerance in exposed populations as compared to those with no previous exposure; two populations displayed increased tolerance and one showed decreased tolerance. This work is the first to examine evolved responses to AFFF formulations. Our results highlight the frequently overlooked evolutionary effects of contaminant exposure, particularly on keystone species in aquatic ecosystems, as well as the need to understand the effects of PFAS-free alternative AFFF on the environment.

Abstract #54 – UG Student

Assessing how parasite exposure frequency and dosage influences infection risk in PFAS-exposed grey tree frogs

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants known to bioaccumulate in the environment and adversely affect health and development across many taxa. PFAS exposure in wetland systems occurs alongside stressors, such as parasitism. While previous studies identified the effects of PFAS on host-parasite interactions, little information is available regarding how the frequency and dosage of trematode exposure impacts infection risk in PFAS-exposed tadpoles. We assessed this limitation using a two-stage experimental design with larval gray tree frogs (*Hyla versicolor*) and trematodes (echinostomes). Our first stage included a 10-day exposure to 10 ppb perfluorohexanesulfonic acid (PFHxS). During stage two, we exposed individual tadpoles to 50 or 100 echinostomes at three timing intervals: 0-day, 2-days, and 5-days post-PFAS exposure. Additionally, we exposed a treatment group to either 10 or 20 echinostomes daily for 5 days to assess the effects of PFHxS on parasite loads in more ecologically representative conditions. While infection analyses are still in progress, results have been obtained for several treatment groups. In particular, tadpoles exposed to echinostomes continually for 5 days yielded higher parasite loads than tadpoles in the 0-day group within both the control and PFAS treatments. This suggests that the number of parasites in the initial exposure can influence infection outcomes possibly through interference competition among the parasites at high exposure levels. Additionally, within the 0-day trematode exposure group, we observed higher parasite loads in PFAS-exposed tadpoles compared to the control group. These results are consistent with previous research suggesting that PFAS exposure suppresses immune function and increases infection risk. Lastly, parasite loads did not differ between the control and PFAS exposure groups when tadpoles received echinostomes continually for 5 days. Given that tadpoles depurate PFAS quickly (within days), it is possible that their immune function was able to rebound to levels of unexposed tadpoles. These findings emphasize the importance of assessing the influence of contaminants on amphibian-parasite dynamics at multiple timings and doses, along with suggesting that echinostomes may be able to infect tadpoles more effectively at lower concentrations, possibly due to decreased competition.

Abstract #55 – UG Student

Assessing motor function in mice exposed to benzo[a]pyrene during early brain development

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Benzo[a]pyrene is a known carcinogen and widespread pollutant. Human exposures can occur through inhalation of tobacco smoke, fossil fuel combustion and other wildfire smoke as well as from ingestion of foods cooked at high temperatures. Previous studies implicated bioactivation by CYP1B1 as a factor in BaP immunotoxicity and carcinogenesis. Our studies were designed to determine the role of CYP1B1 during pregnancy and lacation. We used *Cyp1b1(+/+)* wild type and *Cyp1b1(-/-)* knockout dams treated with 10mg/kg/day of BaP in corn oil-soaked food or the corn oil vehicle from gestational day 10 to postnatal day 25 when offspring were weaned. One male and one female per litter were used for behavioral testing starting at postnatal day 60. We use the Rotarod test to compare motor function and motor learning. After two days of acclimation, mice went through 5 days of acceleration testing with the rotating rod increasing in speed from 0-20 rpm over 3 min. The maximum test time was 5 min. We found a significant gene x treatment x sex interaction on Days 2 and 3 of testing with BaP-treated wild type males having shorter latencies to fall compared with wild type and knockout BaP-treated females ($P < 0.05$). There was a treatment x sex interaction on Day 4 with BaP-treated wild type males again having shorter latencies to fall ($P < 0.05$). There was a significant gene x treatment interaction on the last day of testing with BaP-treated *Cyp1b1(-/-)* knockout mice having the shortest latencies to fall compared with all other groups. Together, these data suggest developmental BaP exposure has adverse effects on both wild type and knockout *Cyp1b1* mice.

Abstract #56 – UG Student

Effect of lead and arsenic on cerebral vasculature of zebrafish viewed through confocal imaging

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Lead (Pb) and arsenic (As) are naturally occurring toxic materials. Additionally, Pb was historically used in paint and as a gasoline additive , whereas As was used widely as a pesticide. The toxicological concern for both is blood vessel alterations in the brain that could lead to various neurological issues. The biological model system, the zebrafish, is used to evaluate chemicals for their potential developmental neurotoxicity and relate them to humans due to their shared genetic and biological system similarities. Zebrafish embryos of the fli1 transgenic line were treated with As, Pb, or mixtures of the two metals. The treatment occurred at three different concentrations: 0 ppb (µg/L) (control), 10 ppb, or 100 ppb of the single metal or a 10 ppb or 100 ppb mixture of each metal. After exposure through the end of embryogenesis (1-72 hours post fertilization), the larvae were washed and prepared for confocal imaging with the midbrain and hindbrain being points of interest. These fli1 larvae have their blood vessels fluorescently labeled to distinguish endothelial tissue versus vasculature. The number of vasculogenic sprouts, complete branches, length of choroid vascular plexus, and the length of the basal artery were measured. It was hypothesized that the As and Pb mixture exposure will have an additive effect on vasculature development in the larval zebrafish brain seen through the number of sprouting blood vessels and the number of complete blood vessels. Results showed that there were significant decreases in branching, sprouting, and total vasculature in several concentrations in both the midbrain and hindbrain ($p<0.05$). Overall, this study represented the connection of As and Pb to cerebral vasculature alterations.

Abstract #57 – UG Student

In vitro nephrotoxicity induced by bromobenzene and dibromobenzene isomers in isolated rat kidney cells

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Bromobenzenes are common starting materials used in the synthesis of numerous commercial products. *In vivo* exposure to bromobenzenes can induce organ-related toxicities, including hepatotoxicity and nephrotoxicity. Nephrotoxicity appears to be the result of hepatic biotransformation of the parent brominated benzene to oxidative metabolites that are carried to the kidney to induce toxicity. However, there is little information on the direct effects of the parent bromobenzenes on the kidney. The purpose of this study was to determine the nephrotoxic potential of bromobenzene (BB) and the three dibromobenzene (DBB) isomers in isolated kidney cells (IKCs) from male Fischer 344 rats. IKC (~4 million cells/ml; 3 ml) were incubated with shaking at 37ºC under a 95% oxygen/5% carbon dioxide atmosphere with BB or a DBB isomer (0.25, 0.50 or 1.0 mM) or vehicle (dimethyl sulfoxide) for 30 or 60 min. General cytotoxicity was measured by determining trypan blue exclusion by IKC and measuring changes in lactate dehydrogenase (LDH) release. BB induced cytotoxicity at 0.5 and 1.0 mM at both 30 and 60 min. Both 1,3- and 1,4-DBB induced cytotoxicity at 0.5 mM or 1.0 mM at 30 and 60 min, while 1,2-DBB was not toxic at 30 min but induced cytotoxicity at all concentrations at 60 min. Pretreatment of IKC with piperonyl butoxide (0.1 mM, 15 min), a non-selective cytochrome P450 inhibitor, reduced 1,2-DBB (1.0 mM) toxicity at 60 min. These results suggest that BB and the three DBB isomers are directly toxic to kidney cells and that renally-produced metabolites may contribute to nephrotoxicity induced by bromobenzenes. Supported in part by NIH grant P20GM103434.

Abstract #58 – UG Student

Evaluation of cell viability and inflammatory response in human primary gingival keratinocyte cells exposed to oral nicotine pouches

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Oral nicotine pouches (ONPs) are contemporary smokeless tobacco products, available from various brands in the U.S., constituting a substantial portion of the overall sales of non-combustible nicotine-containing products. These smokeless tobacco products are promoted for widespread use, containing either tobacco-derived nicotine (natural) or tobacco-free nicotine (synthetic) and positioned as alternatives to traditional tobacco products. Among adolescents and young adults, ONPs have gained significant popularity. Specifically, ONPs are the second most prevalent nicotine product used by adolescents The Zyn brand of ONPs is the current market leader and ONPs with a nicotine content of 6 mg are the most popular. The majority of young adult ONP users prefer flavored varieties, including fruit, mint, desserts, and other flavors. The surge in popularity of these products has prompted concerns about potential adverse oral health effects. This arises from the interaction and absorption of nicotine and other by-products from ONPs through the oral mucosa and into the circulatory system. While nicotine pouches are considered to be less harmful compared to traditional tobacco products, the associated risks related to toxicity remain unknown. We hypothesize ONPs induce oral toxicity that is dependent on concentration and flavor. To test this hypothesis, we assessed ONPs with flavors of citrus, mint, cinnamon, coffee, smooth (tobacco) at nicotine concentrations of 3 or 6 mg. ONPs The ONPs were incubated in artificial human saliva or cell culture media for 1 hour and extracted components were characterized. Nicotine levels were quantified utilizing a targeted mass spectrometry approach while other components were identified and relatively quantified using a metabolite profiling approach. This characterization confirmed nicotine concentrations and determined unique components based on flavors and extraction media. Human primary gingival keratinocytes (PGK) were exposed to extracts from flavored ONPs generated from incubation in cell culture media at 0 (controls), 50% or 100% for 3 or 24 hours. Dose- and time-dependent alterations in cell death were observed via the MTT assay. Cellular uptake of nicotine was quantified at 3 hours post-exposure utilizing targeted mass spectrometry methods for nicotine and its primary metabolite cotinine. Expression of genes related to inflammation were measured 3 hours after exposure to 0 (controls) or 50% extracts. Specifically, IL-6 was upregulated following ONP extract exposure. Assessment of inflammatory pathways is ongoing, however, our results demonstrate cellular toxicity associated with ONP exposure.

Abstract #59 – UG Student
Tribromobenzene nephrotoxicity in isolated kidney cells from male Fischer 344 rats

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Brominated benzenes are widely used in the manufacture of many commercial products. Previous studies have demonstrated that mono- and dibromobenzenes are toxic to liver and kidney, primarily through toxic metabolites. There is evidence that tribromobenzenes (TBBs) are also hepatotoxicants, however, there is very little information on the nephrotoxic potential of TBBs. The purpose of this study was to determine the nephrotoxic potential of the three TBB isomers and explore the role of bioactivation and free radicals in TBB nephrotoxicity *in vitro*. Isolated kidney cells (IKC) (~4 million cells/ml; 3 ml) from male Fischer 344 rats were incubated with shaking at 37^{o}C under a 95% oxygen/5% carbon dioxide atmosphere with a TBB isomer (0.25, 0.50 or 1.0 mM) or vehicle (dimethyl sulfoxide) for 30 or 60 min. General cytotoxicity was measured by determining trypan blue exclusion by IKC and measuring changes in lactate dehydrogenase (LDH) release. In some experiments, IKC were pretreated with an antioxidant (glutathione, 1.0 mM, 30 min; ascorbate, 1.0 mM, 5 min; or α-tocopherol, 1.0 mM, 5 min) or a cytochrome P450 (CYP) inhibitor (piperonyl butoxide, 0.1 mM, 15 min) before 1,3,5-TBB (1.0 mM; 60 min incubation). Among the TBBs, only 1,2,4-TBB induced cytotoxicity at 0.5 mM or greater at 30 min and 0.25 mM or greater at 60 min. 1,3,5-TBB induced cytotoxicity at 0.5 mM or greater at 30 and 60 min and 1,2,3-TBB only induced cytotoxicity at 1.0 mM at 30 and 60 min. The cytotoxicity induced by 1,3,5-TBB (1.0 mM, 60 min) was attenuated by all three antioxidants and the CYP inhibitor. These results indicate that the order of decreasing nephrotoxic potential was 1,2,4- > 1.3.5- > 1,2,3-TBB, and that free radicals play a role in the mechanism of nephrotoxicity induced by a 1,3,5-TBB metabolite. Supported in part by NIH grant P20GM103434.

Abstract #60 – UG Student

Pulmonary lipid alteration patterns and inflammation following silver nanoparticle exposure

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Silver nanoparticles (AgNPs) are commonly used in manufacturing processes and consumer/biomedical products. Inhalation is a primary route of nanoparticle exposure and AgNPs have demonstrated lung toxicity including oxidative stress, inflammation, and pulmonary injury. Pulmonary inflammation is associated with the development of diseases including fibrosis, asthma, and cancer. Bioactive lipids govern the initiation and resolution of inflammation. Currently, there is little understanding regarding pulmonary lipid-mediated mechanisms of inflammation following nanoparticle inhalation. This knowledge gap impedes our ability to treat exposures and diseases where inflammation is a primary component. Within this study, we hypothesize AgNP exposure will induce a pulmonary inflammatory response via the dysregulation of lipid mediators. To test this hypothesis, mice were exposed to 50µg of AgNPs or vehicle (control) via oropharyngeal aspiration. Three days following exposure, bronchioalveolar lavage fluid (BALF) and the right lung lobes were collected while the left lung lobe was fixed in carboxymethyl cellulose. BALF analysis demonstrated increased total protein levels and neutrophils following AgNP exposure compared to controls demonstrating pulmonary inflammation and injury. AgNP exposure increased gene expression of inflammatory genes including interleukin-1β (IL-1β), macrophage chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and C-X-X motif chemokine ligand 1 (CXCL1) while no alterations were observed for genes associated with resolution of inflammation interleukin-4 (IL-4) or interleukin-10 (IL-10). Assessment of BALF cytokines demonstrated elevations of the pro-inflammatory mediators macrophage inflammatory protein-2 (MIP-2) and macrophage chemoattractant protein-1 (MCP-1) and no alterations in IL-10 in mice exposed to AgNPs. Fixed lung lobes were sectioned and evaluated via a variety of imaging techniques. Hyperspectral darkfield imaging was utilized to determine AgNP localization while staining with hematoxylin and eosin histologically evaluated inflammation within the lung. Desorption electrospray ionization mass spectrometry (DESI-MS) was employed to assess spatial alterations in lipid mediators and demonstrated AgNP-induced alterations in lipid mediators. Hematoxylin and eosin staining and hyperspectral darkfield microscopy allowed for cross referencing of areas of inflammation and AgNP deposition with MassLynx data from DESI imaging to select regions of interest. A workflow was developed for processing, analyzing, and attributing the mass spectra data to compare the various metabolites between the AgNP exposed and control samples. Overall, our study demonstrates lipid dysregulation may contribute to AgNP-induced inflammation following particulate inhalation. This information can be utilized to identify disruptions of bioactive lipid mediators to better inform therapeutic strategies regarding inflammatory-mediated diseases resulting from exposures.

Abstract #61 – UG Student

In utero exposure to e-cigarette vapor effects inflammatory genes in developing fetal immune system

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For nearly ten years, e-cigarettes (ECs) have dominated the tobacco product market in the United States. As the younger generations are most likely to use e-cigarettes reach childbearing age, this leaves questions of the effects of ECs on fetal development. *In utero* exposure to e-cigarette vapor has links to growth and developmental changes of the fetus. Studies on adult mice exposed to ECs show increased cytokines leading to increased inflammation. The chemical components of EC vapor into fetal circulation may cause both short- and long-term developmental effects on the heart, lung, and placenta of the fetus. To test this, pregnant mice were exposed to: (a) EC vapor with nicotine (PV + Nic; 2% Nic in 50:50 propylene glycol: vegetable glycerin), (b) EC vapor without nicotine (PV; (50:50 propylene glycol: vegetable glycerin)), or (c) HEPA filtered air (FA). At 18 days, just prior to birth, the dams were removed from exposure and heart, lung, and placental tissue from the pups were collected. We found alteration in factors including upregulation of Il-13, and downregulation of of Il-6, which may be linked to decreased immune responses and increased chances for asthma in offspring. The necessity for further research into the effects of e-cigarettes on fetal development arises from the potential dysregulation of inflammatory cytokines in expectant mothers who use these devices.

Abstract #62 – UG Student

Using the Morris Water Maze to test learning and memory in mice exposed to benzo[a]pyrene

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Benzo[a]pyrene is a widespread pollutant linked to several health problems in humans, including cancer and neurological deficits in children exposed during early brain development. Sources include air pollution from forest fires and fossil fuel burning or ingestion of grilled foods. To test how the developing brain is affected by BaP exposure, pregnant *Cyp1b1(+/+)* wild type and *Cyp1b1(-/-)* knockout mice were mated and randomly assigned to treatment groups. Starting on gestational day 10 (G10), the treatment group is given 10mg/kg/day BaP dissolved in corn oil-soaked cereal until the offspring are weaned at postnatal day 25 (P25). Control mice receive only corn oil-soaked cereal. One male and one female from each litter begin neurobehavioral testing when they are young adults at P60. We used the Morris water maze to test hippocampal dependent spatial learning and memory. We found a significant gene x treatment interaction in the final, most difficult phase. All groups showed impairments compared to control *Cyp1b1(+/+)* wild type mice. There were significant differences on 4 days of testing and a trend toward significance on the other two days.

Abstract #63 – UG Student

Pre-differentiation short-chain PFAS exposure induce neurotoxicity via altering ER vulnerability in dopaminergic-like neurons

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Per- and polyfluorinated alkyl substances (PFAS) are a large group of surface-active compounds, which are commonly used in industrial processes and everyday consumer products, affecting the majority of the population. The adverse health effects associated with conventional long-chain PFAS, include increased risks of cancer immune system dysfunction, and neurodegenerative diseases, which collectively led to the replacement of long-chain PFAS such as PFOA and PFOS with short-chain alternatives such as PFBA and PFBS. The health implications of short-chain PFAS, particularly neurotoxicity, however, remains understudied posing long-term health risk in the exposed population. In this study, we exposed progenitor like cells, SH-SY5Y, to 0.4 and 4 µg/L of PFBS or PFBA for four days. We then removed the PFAS upon the onset of differentiation. After 14 day differentiation, we characterized neuronal network and assessed the intensity of tyrosine hydroxylase (TH). Decreased TH intensity was observed after prior exposure to short-chain PFBA or PFBS. In addition, chemicals known to target mitochondria (MPP+) and endoplasmic reticulum (ER) (Tunicamycin) were used to test vulnerability after developmental PFBA or PFBS exposure. Our results revealed that cells previously exposed to PFBA exhibited modified sensitivity to ER stimulation. We also assessed changes in epigenetic markers to understand the potential molecular targets contributing to the establishment of a persistent neurotoxic state in DA-like neurons after prior PFBA or PFBS exposure. Collectively, our results identified neurotoxicity of low-dose PFBA or PFBS exposure in human DA-like neurons following a developmental exposure scheme.

Abstract #64 – HS Student

Investigating the effects of estradiol on antibody production in human CL01 B-lymphocytes

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Systemic Lupus Erythematous (SLE) is an autoimmune disorder characterised by the immune system's attack on healthy tissues and organs. SLE disproportionately affects women, raising questions about the role of sex hormones, particularly estrogen, in the disease's pathogenesis. This study investigates the immunomodulatory effects of a range of E2 concentrations, from 1×10-11M to 1×10-6M, and their effects on IgM, IgG, and IgA production compared to vehicle-treated Human CL01 B-cells. The study involved three trials, totalling 144 treatment cycles. Human CL01 B-lymphocytes were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were maintained at 37°C in a humidified 5% CO2 atmosphere. For experimental treatments, cells were exposed to a range of Estradiol (E2) concentrations (0.01-10 nM) for 48 hours. To assess antibody production after the treatment period, enzyme-linked immunosorbent assays (ELISAs) were performed to quantify immunoglobulin levels (IgG, IgM, and IgA) in cell culture supernatants. Statistical analysis was performed using 95% confidence interval for a one-way ANOVA followed by post-hoc tests to compare antibody levels between the E2-treated and untreated groups. Contrary to the hypothesis, our findings suggest that there is no statistically significant difference in antibody concentration between 1×10-11M to 1×10-6M E2-treated cells and the vehicle population. This study challenges past perspectives and indicates that Estradiol may not have significant immunomodulating effects on Human CL01 B-cells. While this study did not yield significant results, it highlights the importance of exploring the interactions between sex hormones and various immune cell types to gain a comprehensive understanding of autoimmune diseases. Further research in different cell lines and with different forms of estrogen is warranted to elucidate the complex relationship between estrogen and antibody production, ultimately paving the way for more precise treatments of autoimmune disorders like SLE.