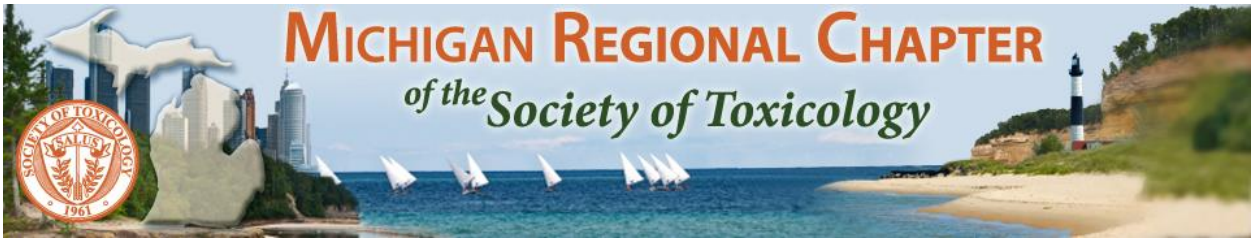


MISOT FALL 2021 VIRTUAL MEETING

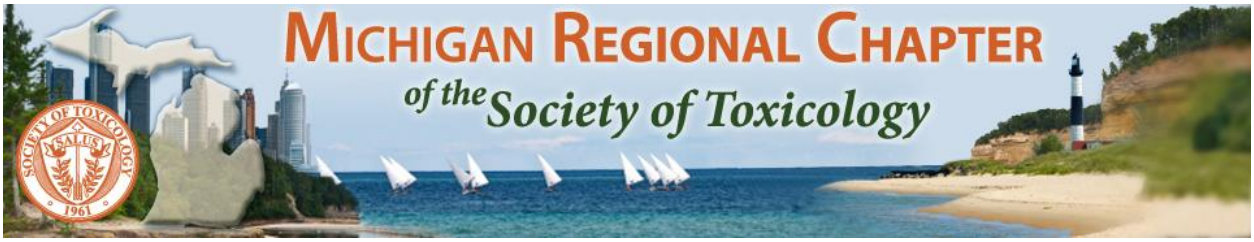
Friday October 15, 2021

"Microphysiological Systems: Recent Advances and Future Directions in Toxicology"

Registration Information:
https://www.toxicology.org/groups/rc/misot/meetings.asp

Agenda:

9:00-9:10	Welcome and Introduction
9:10-10:00	Dr. Brian Johnson, Michigan State University *"Harnessing digital manufacturing and automation to construct and test microphysiological models of development and disease"*
10:00-10:10	Break
10:10-11:00	Dr. Geeta Mehta, University of Michigan *"Precision oncology models for gynecologic cancers"*
11:00-11:50	Dr. Edward Kelly, University of Washington *"Utility of microphysiological systems for disease modeling and safety testing"*
11:50-12:30	Lunch Break
12:30-1:30	Careers in Toxicology Panel Discussion



1:30-2:40 Online Poster Breakout Sessions

2:40-2:50 Break

2:50-3:50 Trainee Platform Presentations

- Diana Pacyga, Michigan State University
  "*Higher quality maternal diet attenuates negative associations of maternal paraben concentrations with newborn weight and length*"

- R. Berube, Institut National de la recherche scientifique (currently at Wayne State University)
  "Comparison of conventional and non-conventional oil toxicity on three freshwater fish species: molecular, developmental, and global health effects"

- Zimu (Christine) Wei, Michigan State University
  "Thrombin-catalyzed fibrin polymerization controls fibrin(ogen) solubility dynamics in early acetaminophen hepatotoxicity"

3:50-4:00 Closing Comments
(Awards will be announced by email and on our webpage after meeting concludes)

Graduate Student Posters (Room 1):

1. **Ebenezar Okoyeocha**, Lung toxicity in rats from vesicating and nettle agent phosgene oxime inhalation: a pilot study
2. **David Filipovic**, Interpretable Predictive Models of Genome-wide Binding of the Inducible Transcription Factor Aryl Hydrocarbon Receptor
3. **Luca Kaiser**, Arsenic trioxide suppresses expression of activation markers and antibody production by B cells in response to influenza A virus
4. **Saamera Awali**, Inhibition of dendritic cell activation by the Nrf2 activator, tBHQ
5. **Allison Boss**, The Nrf2 effects of tBHQ on activated murine NK cells
6. **Brad Ryva**, Associations between midlife urinary phthalate concentrations and prior fibroids diagnosis
7. **Anna-Katherine Fournier**, Macrophage receptor with collagenous structure (MARCO) promotes liver repair following acetaminophen overdose

Graduate Student Posters (Room 2):

8. **Tomoko Ishikawa**, Sex-specific effects of PFOA on cardiogenesis and cardiac function
9. **Omid Madadgar**, Analysis of inflammatory cytokines after C57BL/6 mice skin exposure with chemical threat agent phosgene oxime
10. **Lisa Koshko**, Gestational Benzene Exposure Predisposes Offspring to Metabolic Syndrome through Alterations in Hypothalamic Development
11. **Samantha Heldman**, The Endocrine Disrupting Activities Associated with Liquid Crystal Monomers and their Mixtures
12. **Katelyn Polemi**, Identifying the Link Between Chemical Exposures and Breast Cancer in African American Women via Integrated in Vitro and Exposure Biomarker Data
13. **Rachel K. Morgan**, Exploring the Role of piRNA in Neural Differentiation and Its Susceptibility to Lead Exposure
14. **Russell R. Fling**, Dose-dependent aryl hydrocarbon receptor (AhR) activation by TCDD shifts gut microbiome consistent with the progression of steatosis to steatohepatitis with fibrosis

Undergraduate, Post-Bac and Postdoc Posters:

15. **Karina Orlowska (Postdoc)**, Sulfasalazine, an inhibitor of the cystine/glutamate Xc- antiporter, diminished 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced glutathione oxidation while increasing cytotoxicity in primary mouse hepatocytes

16. **Dinesh G Goswami (Postdoc),** Mechanisms mediating the Phosgene oxime induced skin toxicity in the SKH-1 hairless mouse

17. **Lucas Kniess Debarba (Postdoc),** VOC metabolic reprograming of microglia in the regulation of the IKK/NF-κB inflammatory response

18. **Rebekah Petroff (Postdoc),** Neurotoxicity of Prolonged, Low-Level Exposure to the Marine Toxin, Domoic Acid, in Macaques

19. **Yu-Ting Tiffany Chiang (Post-bacc)**, The Effects of Contraceptive Chemicals and Mixtures on Adipogenesis and Hormone Receptor Signaling

20. **Eleanor Scheeres (Undergraduate)**, Toxicant-Pathogen Interactions During Pregnancy: Pilot Study of Pregnant Murine Co-exposure to Trichloroethylene (TCE) and Group B Streptococcus (GBS)

21. **Nicholas Cemalovic (Undergraduate)**, “High-throughput high-content imaging of environmental toxicants reveals novel morphometric phenotypes”

Poster Presentation Abstracts

(1) Lung toxicity in rats from vesicating and nettle agent phosgene oxime inhalation: a pilot study.

Ebenezar Okoyeocha[1], Dinesh G Goswami[1], Swati Sharma[1], Maddie Godziela[1], Omid Madadgar[1], Ryan Lewandowski [2], Claire R Croutch[3], Jared M Brown[4], James G Wagner[2], Jack Harkema[2], Neera Tewari-Singh[1]

1. Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI

2. Department of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI

3. MRI Global, Kansas City, MO

4. Department of Pharmaceutical Sciences, University of Colorado Denver, Aurora

Phosgene Oxime (CX; dichloroformoxime), an urticant and vesicating agent, is of special interest as a chemical threat agent due to its high penetrative property and immediate toxic effects. Toxic effects of CX are dependent on its route and duration of exposure. Some previous studies show that CX exposure results in an instant upper respiratory tract irritation and sinus pain at low doses. Higher doses of CX could result in pulmonary edema, dyspnea, fibrosis, and mortality. Molecular mechanisms that lead to these toxic effects are understudied hampering the development of targeted treatments. The objective of our study is to investigate the lung toxicity from CX aerosol inhalation in a rat model and elucidate its pathophysiology. To ensure a solely respiratory effect, we exposed male Sprague Dawley rats using a nose-only inhalation system to CX particulate aerosol at MRIGlobal. Rats were exposed to different doses of CX for 10-30 mins (either 2.0 or 2.5mg/min/m^3). At 24h post-exposure, rats were euthanized and bronchoalveolar lavage (BAL) fluid was aspirated. Lung tissue was harvested and fixed for histopathological analysis. Differential counting of the BAL fluid using Giemsa staining showed hemorrhage and increased neutrophils indicating inflammation. qPCR analysis on BAL fluid showed a 2.9-fold increase in IL-1β upon exposure to 2.5g/min/m^3 CX compared to control. Histopathological analysis of Lung tissue showed that CX induced necrotizing bronchiolitis with intramural edema and hemorrhage in submucosa. A marked exfoliation of airway epithelial cells and mixed inflammatory cell infiltration was also observed upon exposure to 2.5g/min/m^3 CX. Toluidine blue staining showed an increased degranulation of mast cells in the lung tissue after CX exposure. These studies are the first steps toward elucidating lung damage from CX inhalation and the role of inflammatory cytokines and immune cells like mast cells in CX-induced lung toxicity. Further studies are being carried out to determine the signaling pathways and mechanism involved in CX induced lung injury.

(2) Interpretable Predictive Models of Genome-wide Binding of the Inducible Transcription Factor Aryl Hydrocarbon Receptor

David Filipovic[1,2*], Wenjie Qi[1,2*], Suresh Cuddapah[6], and Sudin Bhattacharya[1,2,3,4,5]

[1]Department of Biomedical Engineering, Michigan State University, East Lansing, MI 48824, USA
[2]Institute for Quantitative Heaklth Science & Engineering, East Lansing, MI 48824, USA
[3]Department of Pharmacology & Toxicology, Michigan State University, East Lansing, MI 48824, USA
[4]Institute for Integrative Toxicology, Michigan State University, East Lansing, MI 48824, USA
[5]Center for Research on Ingredient Safety, Michigan State University, East Lansing, MI 48824, USA

[6]Department of Environmental Medicine, New York University School of Medicine, New York, NY, 10010, USA

*These authors contributed equally to the work.

The Aryl Hydrocarbon Receptor (AhR) is an inducible transcription factor (TF) whose ligands include the potent environmental contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). TCDD-mediated toxicity occurs through the activation of AhR and its subsequent binding to the core DNA motif 5'-GCGTG-3', referred to as the Dioxin Response Element (DRE). However, *in vivo* AhR binding in human tissues is highly dynamic and tissue-specific. Approximately 50% of all experimentally verified AhR binding sites do not contain a DRE, and a great number of accessible DREs are not bound by AhR. Identification of the determinants of tissue-specific AhR binding is crucial for understanding downstream gene regulatory effects and potential adverse health outcomes of TCDD exposure, such as liver toxicity and immune suppression. We applied XGBoost, a supervised machine learning architecture, to predict genome-wide AhR binding as a function of DNA sequences immediately flanking the DRE, and local chromatin context features such as DNase-seq, histone modifications (HM) and transcription factor (TF) ChIP-seq signals, as well as proximity of the DRE to gene promoters. We predicted binding of exogenously induced AhR in MCF-7 breast cancer cells, human hepatocytes, and the human lymphoblastoid cell line GM17212, as well as non-induced, basally active AhR in HepG2 hepatocellular carcinoma cells. Our results demonstrate highly accurate and robust models of within-tissue binding, with several specific TFs and HMs identified as predictive of AhR binding within and across tissues. Additionally, we show that tissue-specific AhR binding is driven by a complex interplay of DNA flanking sequence and local chromatin context.

(3) Arsenic trioxide suppresses expression of activation markers and antibody production by B cells in response to influenza A virus

Luca M. Kaiser[€,*], Robert A. Freeborn[*,ω], Allison P. Boss[*,α], Cheryl E. Rockwell[*,Ω]
College of Osteopathic Medicine[€],
Department of Pharmacology and Toxicology*,
Department of Food Science and Human Nutrition[α],
Applied Immunology Center for Education and Research[Ω],
Michigan State University, East Lansing;
Stanford University, Palo Alto[ω]

Arsenic compounds are common environmental toxicants worldwide and particularly enriched in the Northeast and Southwestern United States, the Alps and Bangladesh. Exposure to arsenic is linked with various detrimental health outcomes, including cancer, cognitive decline and kidney damage. Our group has previously shown that arsenic trioxide alters T cell cytokine production. In this study, we demonstrate that exposure to arsenic compounds alters B cell function in an *in vitro* influenza model. Human peripheral blood mononuclear cells (PBMCs) were isolated from blood and cultured with arsenic trioxide (As_3O_2) or sodium arsenite ($NaAsO_3$) and subsequently challenged with Influenza A virus. Cells were then analyzed using Flow Cytometry and ELISA. B cells showed a decreased expression level of CD267 and CD22 and a marked change in the ratio of CD86 and CD80 when treated with arsenic trioxide, but not with sodium arsenite. We also observed an arsenic trioxide-dependent decrease in antibody production. This work was supported by NIH grant R01 ES024966).

(4) Inhibition of dendritic cell activation by the Nrf2 activator, tBHQ

Saamera Awali, Yining Jin, Luca M. Kaiser, Cheryl E. Rockwell

Department of Pharmacology and Toxicology
Applied Immunology Center for Education and Research
Michigan State University, East Lansing

Dendritic cells (DCs) are professional antigen presenting cells that initiate both the innate and adaptive immune responses upon encountering antigen. Through antigen processing, DCs can activate naïve T cells by presenting antigenic peptides on MHC class II molecules. Previous research from our lab demonstrated that *tert*-butylhydroquinone (tBHQ), a potent Nrf2 activator and a widely used food additive, blunts the expression of CD107, fas ligand and CD44, which are markers of activation and effector function, on CD8+ T cells. This suggests tBHQ impedes CD8 T cell activation and effector function, but the mechanism for this is unclear. Since DCs bridge the innate and adaptive immune systems, we hypothesize that exposure of DCs to tBHQ will inhibit expression of MHC class II and other co-stimulatory molecules involved in T cell activation. In our current *in vitro* study, DCs were isolated from female wildtype C57BL/6 mice spleens. The cell culture was activated by LPS, a microbial stimulator, in the presence or absence of tBHQ for 24 hours. The expression of markers for DC maturation, activation, and T cell priming was measured. tBHQ treatment led to a

significant decrease in expression of MHCII as well as CD80, and CD86, markers of DC activation. Overall, our data suggests that tBHQ inhibits the expression of MHC class II and other co-stimulatory molecules expressed by activated DCs, which could impact downstream T cell activation. (This study was supported by NIH R01 ES024966).

(5) The Nrf2 effects of tBHQ on activated murine NK cells

Allison P. Boss*[α], Elizabeth M. Gardner[α], and Cheryl E. Rockwell*[Ω]

Department of Pharmacology and Toxicology*,
Department of Food Science and Human Nutrition[α],
Applied Immunology Center for Education and Research[Ω],
Michigan State University, East Lansing

The transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) is involved in the upregulation of antioxidant, detoxification, and cell stress genes when activated by oxidative stress or exogenous compounds. *tert*-butylhydroquinone (tBHQ) is a potent activator of Nrf2 and is a widely used food preservative. Previously, we have found tBHQ to negatively impact NK cell activation and effector function and alter NK cell maturation. In the current study, we examined the effects of Nrf2 activation by tBHQ in NK cells. Splenocytes were isolated from wild-type (WT) C57Bl/6J mice or Nrf2-deficient mice with a C57Bl/6J background and treated with 0.1 μM, 0.5 μM, 1 μM, or 5 μM tBHQ. Following treatment, NK cells were activated for 24 hours with either phorbol 12-myristate 13-acetate (PMA) and ionomycin or a specific NK cell activator, IL-12/IL-18 cytokines. In WT NK cells activated with IL-12/IL-18 cytokines, the percentage of mature NK cells were significantly increased compared to Nrf2-deficient NK cells. However, treatment with 5 μM tBHQ decreased percentage of CD27+CD11b+ in WT NK cells. In NK cells activated with PMA and ionomycin, terminally differentiated, CD27-CD11b+, NK cells from WT mice were significantly increased suggesting Nrf2 plays a prominent role in NK cell maturation. Additionally, NK cell activation was significantly decreased with 5 μM tBHQ, which was independent of Nrf2. In PMA/ionomycin activated NK cells, expression of FasL was significantly decreased with 5 μM tBHQ in a Nrf2-dependent manner. Production of IFNγ following PMA/ionomycin activation was significantly reduced with 1 and 5 μM tBHQ in WT NK cells and 5 μM tBHQ in Nrf2-deficient NK cells. In conclusion, this study demonstrates the involvement of Nrf2 in NK cell maturation. Additionally, activation of Nrf2 by tBHQ increases terminally mature NK cells and decreases NK cell effector functions, such as FasL and IFNγ expression.

(6) Associations between midlife urinary phthalate concentrations and prior fibroids diagnosis

Brad A. Ryva[1,2], Diana C. Pacyga[2,3], Jodi A. Flaws[4], Rita S. Strakovsky[2,3]

[1]Department of Pharmacology & Toxicology, College of Osteopathic Medicine, [2]Institute

for Integrative Toxicology, and [3]Department of Food Science & Human Nutrition, Michigan State University; [4]Department of Comparative Biosciences, University of Illinois.

Background: Phthalates are endocrine disruptors found in many consumer products, while fibroids are hormonally-mediated abnormal uterine growths associated with adverse health outcomes. Thus, we evaluated associations of phthalates with fibroids diagnosis in midlife women.
Methods: Women (ages: 45–54; n=754) from the Baltimore Midlife Women's Health Study self-reported past fibroids diagnosis, age at diagnosis, and provided 1-4 urine samples over four consecutive weeks. Urines were pooled and analyzed for concentrations of nine phthalate metabolite biomarkers assessed as individual metabolites or molar sums of metabolites from common parents (i.e., di(2-ethylhexyl) phthalate, ΣDEHP), of similar biological activity (anti-androgenic, ΣAA), and of all metabolites (ΣPhthalates). We used logistic regression models, controlling for important lifestyle/sociodemographic factors, to evaluate associations of ln-transformed, specific gravity-adjusted phthalate biomarker concentrations with the odds of having prior fibroids diagnosis. We also explored if associations differed in women who became overweight/obese, remained overweight/obese, or remained under-/normal weight from age 18 to 45-54. Our sensitivity analyses considered whether associations differed in women with recent (<5 years since phthalate assessment) versus earlier diagnosis.
Results: The prevalence of fibroids was 27%, and >99% of women had detectable levels of all phthalate metabolite biomarkers. Overall, some phthalate biomarkers were associated with fibroids. Specifically, women had 22% (OR: 1.22; 95%CI: 1.03, 1.44) or 26% (OR: 1.26; 95%CI: 1.03, 1.54) higher odds of having prior fibroids diagnosis for each two-fold increase in ΣDEHP or ΣAA, respectively. These associations were strongest in women who became overweight/obese from age 18 to 45-54. In sensitivity analyses, associations of ΣDEHP, ΣAA, and ΣPhthalates were strongest in women diagnosed <5 years before phthalate biomarker assessment.
Conclusions: In midlife women, ΣDEHP metabolites were associated with higher odds of fibroids diagnosis, with strongest associations in women who gained weight since age 18. Future prospective studies are needed to corroborate our findings.

(7) Macrophage receptor with collagenous structure (MARCO) promotes liver repair following acetaminophen overdose

Anna-Katherine Fournier[1], Lauren G Poole[1], Holly M Cline-Fedewa[1], James P Luyendyk[1]

[1]Department of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI

Macrophages promote repair following overdose of the over-the-counter drug acetaminophen (APAP). Macrophages play a critical role in clearance of necrotic debris from the APAP-injured liver. We tested the hypothesis that macrophage receptor with

collagenous structure (MARCO), a scavenger receptor expressed on the cell surface of macrophages, promotes liver repair after APAP overdose. Plasma and liver were collected from wild-type and MARCO knockout ($MARCO^{-/-}$) mice 24, 48, and 72 hours after challenge with APAP (300 mg/kg) or vehicle (saline). Compared to vehicle-treated mice, MARCO mRNA and protein expression, determined by qRT-PCR and immunolabeling, were upregulated in APAP-challenged wild-type mice at each time point. Hepatic necrosis was increased in $MARCO^{-/-}$ mice compared to wild-type mice 24 hours after APAP challenge, indicated by analysis of hematoxylin and eosin (H&E)-stained liver sections. Persistent liver injury was evident in APAP-challenged $MARCO^{-/-}$ mice at 48 hours, indicated by elevated serum alanine aminotransferase (ALT) levels. To define the precise changes in macrophage phenotype driven by MARCO, we isolated $F480^{+}$ cells (i.e., macrophages) from livers of wild-type and $MARCO^{-/-}$ mice 48 h after APAP challenge and measured expression of pro-repair genes. Interestingly, expression of one pro-repair gene, *Mmp12,* was increased, whereas another, *Gpnmb,* was reduced in the macrophages from the $MARCO^{-/-}$ mice compared to wild-type macrophages. The results indicate MARCO expression increases in the APAP-injured liver and that MARCO deficiency produces sustained hepatic injury after APAP challenge. The results suggest a critical role of MARCO in repair of the APAP-injured liver.

(8) Sex-specific effects of PFOA on cardiogenesis and cardiac function

Tomoko Ishikawa, Todd Heron, Andre Monteiro De Rocha, Jeff Creech, Christopher Wayne, Laurie Svoboda

University of Michigan, Ann Arbor, MI

Background: Cardiovascular diseases (CVDs) are a leading cause of morbidity and mortality, and numerous environmental exposures have been shown to influence CVD risk. Marked differences exist between men and women in cardiac physiology, as well as pathogenesis, symptoms, and prognosis of CVDs. Perfluoroalkyl substances (PFAS) are chemicals that have been widely used in foams for firefighting, linings for food containers, and coatings for grease- and water-proofing consumer products. Exposures to PFAS have been linked to CVDs, including hypertension and coronary heart disease. However, the effects of PFAS on cardiogenesis are unknown, and how their effects differ by sex are unclear. To address this question, we will use human induced pluripotent stem cells (hiPSCs) from age-matched male and female donors to study the sex-specific effects of PFOA exposure on early cardiac differentiation and function.
Methods: We are using an established small molecules protocol for differentiating hiPSCs to ventricular cardiomyocytes. Briefly, hiPSCs are plated into 48 well plates at a density of 2.4×10^4 cells/cm^2. Once cells are approximately 90% confluent, mesoderm induction is initiated by applying a GSK3 inhibitor to activate the Wnt pathway. After 48 hours, Wnt inhibitor is applied to achieve specification of cardiac mesoderm. Cardiac progenitor cells arise 8 days after mesoderm induction and mature to cardiomyocytes on day 15. We will treat cells with different concentrations of perfluorooctanoic acid

(PFOA), a PFAS that is ubiquitous in the environment and in the human body, or control from differentiation day 0 through day 15. Cells will be collected at several differentiation milestones: day 3 (pre-cardiac mesodermal cells), day 5 (cardiac mesodermal cells), day 8 (cardiac progenitors), and day 15 (cardiac myocytes) for analysis of gene and protein expression and DNA methylation. In order to investigate the effects of developmental PFOA exposure on cardiac function, cells differentiated in the presence of PFOA or control will also be purified and plated in 96 well plates for optical mapping of sodium and calcium currents.
Conclusions: Ultimately the findings of this study will increase our understanding of the sex specific effects of PFAS on the heart and may identify opportunities for targeted interventions.

(9) Analysis of inflammatory cytokines after C57BL/6 mice skin exposure with chemical threat agent phosgene oxime

Omid Madadgar[1], Satyendra K Singh[2], Anna Monson[1,3], Dinesh G Goswami[1], Poojya Anantharam[4], Claire R Croutch[4], Neera Tewari Singh[1]

1- Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI
2- College of Veterinary Medicine, Michigan State University, East Lansing, MI
3- School of Pharmacy, University of Pittsburgh, Pittsburgh, PA
4- MRIGlobal, Kansas City, MO

Abstract: Phosgene oxime (dichloroformoxime; CX), an urticant grouped with vesicating agents, is a potential chemical threat agent. Its exposure causes rapid and painful dermal injury and systemic toxic effects. CX toxicity could result from its different characteristics, such as nucleophilic or possible alkylating properties and the effect of chlorine, oxime, or carbonyl groups. CX exposure can result in enzyme inactivation, corrosive injury, and cell death with rapid tissue destruction, and induce the recruitment and activation of immune cells. However, the mechanism of CX-induced skin toxicity is not known. Studies in our lab have shown that CX exposure causes mast cell degranulation and release of inflammatory mediators like COX-2, MMP9, MPO, and an inflammatory response in the skin. In the present study, we analyzed the inflammatory cytokine profile in C57BL/6 mouse skin following dermal CX exposure (neat CX for either 0.5 or 1.0 min using two 12 mm vapor caps on the dorsal skin at MRIGlobal). Cytokine array analyses showed an increase in the pro-inflammatory cytokines IL-6 (Interleukin 6) and TNF-α (Tumor Necrosis Factor Alpha) in both male and female mice. KC (neutrophil chemoattractant), MCP-1 (Monocyte chemoattractant), MCSF (Macrophage colony-stimulating factor) and RANTES (T cell, monocyte attractant) increased in both sexes, showing involvement of immune cells in the injury/toxicity. IL-13, an allergic inflammation and several diseases' mediator was found to be increased at later time point in female mice and at 2 hours after 1min CX exposure in male mice. IL-4 and IL-5 levels decreased in first 24 h but were increased after 1day post 0.5 min CX exposure. Altogether, our results show immune cell infiltration with inflammation and

changes in inflammatory cytokines. Inflammatory cytokine changes from CX exposure are parallel in the male and female mice; however, slightly different patterns were observed in some cytokines which could be due to shorter inflammatory phase and earlier healing process observed in the female mice. Further molecular analysis is underway to determine the signaling pathways in CX-induced dermal inflammation and toxicity.

(10) Gestational Benzene Exposure Predisposes Offspring to Metabolic Syndrome through Alterations in Hypothalamic Development

L. Koshko, LK. Debarba, M. Sacla, P. Fakhoury, I., Ayyar, M. Sadagurski

Department of Biological Sciences, Integrative Biosciences Center, Wayne State University

The hypothalamus is essential in the regulation of metabolism, notably during critical windows of neurodevelopment. An abnormal hormonal and inflammatory milieu during development can trigger persistent changes in the function of hypothalamic neurocircuits, which leads to long lasting effects on the body's energy homeostasis and metabolism. Benzene, a volatile organic compound (VOC), is a known carcinogen capable of crossing the blood-brain barrier. We recently demonstrated that gestational exposure to low concentrations of benzene, induces a severe metabolic imbalance in both male and female offspring (Koshko et al, 2021). However, the mechanisms behind these outcomes have yet to be resolved. The hypothalamus undergoes growth beginning in the embryonic period, and alterations in perinatal environment can affect various developmental processes, including axon growth and microglia priming, which can lead to abnormal hypothalamic development. Metabolic hormones, particularly leptin, are capable of transmitting signals to the developing hypothalamus in response to alterations in the perinatal environment and may underlie potential maladaptive responses to early metabolic perturbations. We hypothesize that gestational exposure to low concentrations of benzene induces hypothalamic stress, contributing to the adverse metabolic effects seen later in life. We exposed pregnant C57BL/6JB dams to benzene or filtered air for 5 days/week (6h/day from gestational day 1 to birth), and analyzed the neural outcomes of young offspring. We assessed neuroinflammation and found a significant increase in the microglia-specific Iba1 marker, in the arcuate nucleus of the hypothalamus in benzene-exposed male offspring at P21. Microglia morphology was significantly impaired in benzene-exposed offspring, indicated by increased process length and density. Quantification of fiber density in the anterior paraventricular hypothalamus revealed decreases in both orexigenic AgRP and anorexigenic α-MSH projections in benzene-exposed offspring indicating impairment in hypothalamic development. In support, maternal benzene exposure reduced hypothalamic activation of STAT3 after leptin administration. Our new data provide the evidence and mechanistic basis that gestational VOC exposure is a potential risk factor for late-life metabolic disorders.

(11) The Endocrine Disrupting Activities Associated with Liquid Crystal Monomers and their Mixtures

Samantha Heldman[1] and Christopher Kassotis[1]

[1]Institute of Environmental Health Sciences, Wayne State University, Detroit, MI 48202

Rationale and scope:
Mixtures of liquid crystal monomers (LCMs) are an essential part of liquid crystal devices such as televisions, computers, and smartphones. Several LCM biphenyls and analogues are predicted to be persistent and bio-accumulative, and have been reported in household dust and other environmental samples, suggesting potential human exposure. Recent research exposed chicken embryonic hepatocytes cells to a mixture of LCMs and reported significant modulation of several genes associated with adipogenesis. To determine potential mechanistic effects, we investigated the activity of a subset of LCMs on hormone receptors involved in adipogenesis.

Methods:
10 LCMs (5 fluorinated, 5 non-fluorinated) as well as two mixtures containing: all fluorinated and all non-fluorinated LCMs, were tested at environmentally relevant concentrations for either agonism or antagonism of thyroid receptor beta (TRβ), peroxisome proliferator activated receptor gamma (PPARγ), or retinoid X receptor alpha (RXRα) using a transient transfection, luciferase reporter gene assay in human embryonic kidney cells (HEK-293T/17).

Results:
At 10 μM, 4-methyl-4'-pentyl-1,1'-biphenyl exhibited significant (12.72%) PPARγ agonism and weak (3% and 2%) TRβ and RXRα agonism (percent activation relative to maximal positive control responses). Additive agonism for this LCM was much greater for PPARγ, TRβ, and RXRα bioactivities (60%, 43%, and 37%, respectively) when co-exposed with half maximal concentrations of positive control agonists. Significant RXRα antagonism (≤20% inhibition of added agonists) was observed for 3 LCMs tested. No other LCMs exhibited significant agonism, additive agonism, or antagonism of PPARγ, TRβ, or RXRα.

Conclusion:
Several LCMs individually exhibit significant agonism, additive agonism, or antagonism of key receptors involved in adipogenesis. These results suggest that LCMs deserve further investigation as potential contaminants of concern, particularly given likely co-occurrence with diverse mixtures of other endocrine active contaminants. Ongoing research is assessing disruption of other hormone receptors and determination of adipogenic activity using *in vitro* pre-adipocyte models.

(12) Identifying the Link Between Chemical Exposures and Breast Cancer in African American Women via Integrated *in Vitro* and Exposure Biomarker Data

K. M. Polemi[1], V. K. Nguyen[1,2,], J. Heidt[1], A. Kahana[1], O. Jolliet[1,3], J. A. Colacino[1,3,4]

Affiliations: [1] Department of Environmental Health Sciences, University of Michigan, Ann Arbor, MI, USA [2] Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI, USA [3] Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI, USA [4] Department of Nutritional Sciences, University of Michigan, Ann Arbor, MI, USA

Among women, breast cancer is the most prevalent form of cancer worldwide and has the second highest mortality rate of any cancer in the United States. The breast cancer-related death rate is 40% higher in non-Hispanic Black women compared to non-Hispanic White women. The incidence of triple negative breast cancer (TNBC), an aggressive subtype of breast cancer for which there is no targeted therapy, is approximately three times higher for Black relative to White women. The drivers of these differences are poorly understood. Here, we aimed to identify chemical exposures which play a role in breast cancer disparities. Using chemical biomonitoring data from NHANES and biological activity data from the EPA's ToxCast program, we assessed the toxicological profiles of chemicals to which US Black women are disproportionately exposed. We conducted a literature search to identify breast cancer targets in ToxCast to analyze the response of chemicals with exposure disparities in these assays. Forty-three chemical biomarkers are significantly higher in Black women. Investigation of these chemicals in ToxCast resulted in 32,683 assays for analysis, 5,172 of which contained nonzero values for the concentration at which the dose-response fitted model reaches the cutoff considered active. Of these chemicals BPA, PFOS, and thiram are most comprehensively assayed. 2,5-dichlorophenol, 1,4-dichlorobenzene, and methyl and propyl parabens had higher biomarker concentrations in Black women and moderate testing and activity in ToxCast. The distribution of active concentrations for these chemicals in ToxCast assays are comparable to biomarker concentrations in Black women NHANES participants. Through this integrated analysis, we identify that multiple chemicals, including thiram, propylparaben, and p,p' DDE, have disproportionate exposures in Black women and have breast cancer associated biological activity at human exposure relevant doses.

(13) Exploring the Role of piRNA in Neural Differentiation and Its Susceptibility to Lead Exposure

RK Morgan, KM Polemi, LK Svoboda, JA Colacino, DC Dolinoy

University of Michigan Department of Environmental Health Sciences, Department of Nutritional Sciences

Early life neurotoxicant exposures can have long-lasting implications for cognition and health, due in part to impacts on epigenetic mechanisms governing neurodevelopment. In addition to the classically studied epigenetic mechanisms of DNA methylation and

histone modifications, small, non-coding RNA serve as an epigenetic link between environmental exposures and adverse health outcomes. PIWI-interacting RNA (piRNA) associate with PIWI proteins to silence transposable elements (TEs), and this complex has been shown to interact with DNA methylation machinery as a part of TE regulation. While piRNA were long thought to be exclusively expressed in the germline, work from our group has overturned this conclusion with somatic studies in both mice and humans. In early developmental human tissue (gestational day 90-105), *PIWIL1* and *2*, while expressed to a lesser degree than in the germline, showed some of the highest relative expression in the brain when normalized to the gonads. The relative expression of *PIWIL3* and *4* in the brain exceeded that of the germline, with 182% and 294% normalized to the gonads, respectively. Our group has demonstrated that perinatal lead (Pb) exposure disrupts TEs within the mouse brain, with 3.86%, 2.83%, and 1.77% less DNA methylation at intracisternal a particles (IAPs) 110, 236, and 506, respectively. Given the presence of the piRNA system as well as Pb-associated hypomethylation at TEs in the brain, we hypothesized that piRNA plays a mechanistic role in this relationship. We tested this in the SH-SY5Y cell model, wherein we found *PIWIL1* and *4* expression increased significantly during the course of neural differentiation, 323% and 299% on Day 15 relative to Day 3, suggesting an active role in neurodevelopment. While *PIWIL* expression did not change with Pb exposure under static conditions, we are assessing if Pb exposure disrupts the baseline changes in *PIWIL1* and *4* during differentiation. Understanding the mechanisms of early epigenetic programming and those of Pb-induced epigenetic disruption will provide opportunities for targeted therapeutics and will produce valuable data for future models of toxicity.

(14) Dose-dependent aryl hydrocarbon receptor (AhR) activation by TCDD shifts gut microbiome consistent with the progression of steatosis to steatohepatitis with fibrosis

Russell R. Fling[a,b], Tim Zacharewski[b,c]

[a]Department of Microbiology & Molecular Genetics, Michigan State University, E. Lansing, MI 48824; [b]Institute for Integrative Toxicology, Michigan State University, E. Lansing, MI 48824;
[c]Department of Biochemistry & Molecular Biology, Michigan State University, E. Lansing, MI 48824

Gut dysbiosis with disrupted enterohepatic bile acid metabolism is commonly associated with non-alcoholic fatty liver disease (NAFLD) and recapitulated during the progression of hepatic steatosis to steatohepatitis with fibrosis elicited by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in mice. TCDD induces hepatic fat accumulation and serum levels of primary and secondary bile acids including taurolithocholic acid and deoxycholic acid, both of which are microbial metabolites that regulate bile acid, glucose, and lipid

homeostasis. To investigate the effects of TCDD on the gut microbiota, the cecum contents of male C57BL/6 mice orally gavaged with sesame oil vehicle or 0.3, 3, or 30 µg/kg TCDD every 4 days for 28 days were examined using shotgun metagenomic sequencing. Taxonomic analysis identified a dose-dependent increase in Turcibacter sanguinis and Lactobacillus species (i.e., Lactobacillus reuteri). Enriched species were also associated with the dose-dependent increases in bile salt hydrolases responsible for the first deconjugation reaction in secondary bile acid metabolism. Increased L. reuteri levels were further associated with the mevalonate-dependent isopentenyl diphosphate (IPP) biosynthesis, a key intermediate in menaquinone (a.k.a., vitamin K2) and peptidoglycan biosynthesis. Analysis of a publicly available gut microbiome dataset of patients with fibrosis identified increased abundance of the mevalonate-dependent IPP pathway with Streptococcus and Lactobacillus species contributing to the pathway, consistent with the results observed in the mouse cecum metagenomic analysis. These results extend the association of lactobacilli with the AhR/intestinal axis in the progression of hepatic steatosis to steatohepatitis with fibrosis and highlight similarities in the pathogenesis of TCDD-elicited NAFLD phenotype in mice to human disease.

(15) Sulfasalazine, an inhibitor of the cystine/glutamate Xc- antiporter, diminished 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced glutathione oxidation while increasing cytotoxicity in primary mouse hepatocytes

Karina Orlowska[1,2] and Tim Zacharewski[1,2]

[1]Department of Biochemistry & Molecular Biology, Michigan State University, East Lansing, MI, 48824
[2]Institute for Integrative Toxicology, Michigan State University, East Lansing, MI, 48824

Aryl hydrocarbon receptor (AhR) activation by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induces steatosis and its progression to steatohepatitis with fibrosis. The etiology of these pathologies has been associated with increased oxidative stress and disruption of metabolic pathways such as glutathione (GSH) metabolism. The effects of TCDD on GSH metabolism and cytotoxicity were examined in primary mouse hepatocytes. Although 10 nM TCDD induced *Cyp1a1* 743-fold, intracellular GSH levels were not changed while GSSG levels increased 2.9-fold at 48 hours decreasing the *in vitro* GSH/GSSG ratio from 1282 to 842. Unlike in control liver samples where *Slc7a11*, which encodes for the cystine/glutamate Xc^- transporter, expression was negligible and induced ~900-fold by 30 µg/kg TCDD, levels in control primary mouse hepatocytes were markedly higher following isolation and plating. This did not affect cell viability or functionality which was evaluated by assessing morphology and albumin secretion. TCDD was found to diminish *de novo* intracellular cysteine synthesis following the inhibition of the transsulfuration pathway consistent with decreased cystathionine β-synthase and cystathionine ɣ-lyase mRNA and protein expression as well as hydrogen

sulfide levels. In response, the influx of extracellular cystine following induction of the cystine/glutamate Xc^- transporter becomes an alternative source of cysteine to support GSH synthesis. Co-treatment of primary hepatocytes with sulfasalazine, a cystine/glutamate Xc^- antiporter inhibitor, increased TCDD cytotoxicity, and lowered GSH levels. Collectively, these results suggest that in response to TCDD-elicited oxidative stress, cystine import is increased by the induced cystine/glutamate Xc^- transporter to provide cysteine for increased GSH biosynthesis in order to minimize ROS induced cytotoxicity. *Funded by R01ES029541.*

(16) Mechanisms mediating the Phosgene oxime induced skin toxicity in the SKH-1 hairless mouse

Dinesh G Goswami[1], Satyendra K Singh[2], Omid Madadgar[1], Holly Wright[1], Anna Monson[1,3], Claire R Croutch[4], Erica L Noland[5], Rajesh Agarwal[6], Neera Tewari-Singh[1]

1. Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI
2. College of Veterinary Medicine, Michigan State University, East Lansing, MI
3. University of Pittsburgh School of Pharmacy
4. MRIGlobal, Kansas City, MO
5. Department of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI
6. Department of Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO

Phosgene Oxime (dichloroform oxime; CX), is a potent chemical warfare agent. CX penetrates the skin quickly and causes erythema, urticaria, blanching, itching hives, necrosis, and systemic toxic effects. The mechanism of CX induced toxicity is not known and there are no effective therapies. We have shown that dermal CX exposure in SKH-1 hairless mice causes clinical lesions, epidermal cell death, mast cell degranulation, infiltration of immune cells, and increase in the level of DNA damage and inflammatory markers. In this study we examined the mechanism of CX-induced inflammatory response in SKH-1 mice. Dorsal skin of mice was exposed to neat CX for 0.5 or 1.0 min using two 12 mm vapor caps at MRI Global. CX exposure led to mast cell degranulation within 30 min of exposure and was associated with increased tryptase and chymase levels in the skin. CX exposure also led to increased expression of cyclooxygenase-2 (COX-2), matrix metallopeptidase-9 (MMP-9), and myeloperoxidase (MPO). Inflammatory cytokines and chemokines were analyzed in the skin tissue lysates using inflammatory cytokine array and confirmed by qPCR analyses. CX exposure caused increase in several pro-inflammatory cytokines and chemokines including IL-6, CXCL-1, and a decrease in anti-inflammatory cytokines IL-4 and IL-10. Role of nuclear factor erythroid 2- related factor 2 (Nrf2) pathway, which plays a key role in defense against oxidative stress and inflammation, was also investigated by qPCR analyses. Increase in Nrf2 pathway genes, hemeoxygenase-I (HO-1), NAD(P)H quinone dehydrogenase (NQO-1) was observed upon exposure to CX. Together, these results show signaling pathways related to mast cell activation, Nrf2 and inflammation, could be important contributors in CX-induced

dermal inflammation and toxicity and warrants further exploration of these pathways for designing intervention strategies.

(17) VOC metabolic reprograming of microglia in the regulation of the IKK/NF-κB inflammatory response

Debarba LK[1], Jayarathne HSM[1], Koshko L[1], Mestareehi A[2], Yi Z[2], Sadagurski M[1]

Department of Biological Sciences[1], Department of Pharmaceutical Sciences[2] IBio (Integrative Biosciences Center), Wayne State University

There is growing evidence linking exposure to volatile organic compounds (VOC) in ambient air with insulin resistance and metabolic disease in humans. Benzene is a prominent VOC present in water, food, paint, detergents, vehicle exhaust, tobacco smoke, and e-cigarette vapors. However, is not established a direct link between VOC exposure and metabolic imbalance. Our recent studies provided strong evidence that chronic exposure to benzene, modeling human exposure to environmental pollution, induced significant insulin resistance and glucose intolerance in the mouse model. We further show that acute or chronic benzene exposure promotes robust hypothalamic microglia and astrocytes activation and elevation in the hypothalamic inflammatory IKKβ/NF-κB signaling pathway in microglia followed by the induction of endoplasmic reticulum (ER) stress response. This effect is associated with dysregulated insulin signaling in astrocytes and impaired lactate-glutamate shuttle pathway in hypothalamic astrocytes exposed to VOC. Reactive astrocytes also contributed to blunted responses to glucose stimulation in hypothalamic glucose-sensing neurons. High-performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS)-based proteomics have identified 92 proteins in various pathways with changed protein abundance, including inflammatory IKK/NF-κB and insulin signaling pathways in the hypothalamic nuclei critical for regulation of glucose metabolism. To determine the role of microglia inflammatory mechanism in a VOC-induced metabolic imbalance, we genetically ablated IKK/NF-κB signaling specifically in microglia. Selective inhibition of IKKβ in microglial cells leads to a massive improvement in the astrocytes reactive state with reduced expression of inflammatory cytokines, improved cellular morphology, and reduced neurotoxicity in response to VOC exposure. Furthermore, hypothalamic inhibition of the microglia IKKβ pathway protected from central insulin resistance and whole-body metabolic imbalance induced by VOC exposure. Our data provide evidence that neuroinflammatory responses of the hypothalamic microglia precede and mechanistically contribute to whole-body metabolic imbalance and identify air pollution-induced cellular and molecular targets in the hypothalamus that lead to metabolic disease.

(18) Neurotoxicity of Prolonged, Low-Level Exposure to the Marine Toxin, Domoic Acid, in Macaques

Rebekah Petroff[1]*, Christopher Williams[2], G. Jean Harry[2], Jian-Liang Li[3], James W. MacDonald[1], Theo Bammler[1], Todd Richards[4], Audrey Baldessari[5], Brenda Crouthamel[1], Noelle McKain[1], Kimberly S. Grant[1,5], Sara Shum[6], Jing Jing[6], Nina Isoherranen [6,7], Thomas M. Burbacher [1,5,7]

[1] Department of Environmental and Occupational Health Sciences, University of Washington,
Seattle, Washington, [2] Mechanistic Toxicology Branch, Division of National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, [3] Epigenetics & Stem Cell Biology Laboratory, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, [4] Department of Radiology, University of Washington, Seattle, Washington, [5] Washington National Primate Research Center, Seattle, Washington, [6] Department of Pharmaceutics, University of Washington, Seattle, Washington, [7] Center on Human Development and Disability, Seattle, Washington, USA

*Current Affiliation: Department of Environmental Health Sciences, University of Michigan, Ann Arbor, MI

ABSTRACT

Background: Recent epidemiological studies suggest memory deficits associated with prolonged dietary exposure to domoic acid (DA) at levels near the current regulatory limit (20 ppm, ~0.075-0.1 mg/kg). DA acts via glutamatergic receptor activation to induce neuronal excitotoxicity in the hippocampus. The absence of clinical signs of excitotoxicity at these levels and the expression of glutamatergic receptors on astrocytes, suggests involvement of non-neuronal cells in the neurological effects.
Objectives: To determine if chronic exposure to low-level DA induces neuronal pathology and glial reactivity with a correlative transcriptome change in the hippocampus.
Methods: Female *Macaca fascicularis* were orally exposed to 0, 0.075, and 0.15 mg DA/kg/day ~ 2years. Magnetic resonance imaging (MRI) assessed volumetric and tractography changes in the hippocampus and thalamus. Histological evaluation of neurons and glia in the fornix, fibria, internal capsule, thalamus, and hippocampus was conducted. The transcriptome of the hippocampus was evaluated from RNASeq using Ingenuity Pathway Analysis (IPA) and Gene Set Enrichment Analysis (GSEA) for network analysis.
Results: In the hippocampus, there was no evidence of ongoing excitotoxicity but increased staining for GFAP+ astrocytes accompanied by slight increase staining in the fornix. Microglia activation was observed only as rare focal events. Transcriptome analysis yielded 748 differentially expressed genes (>1.5-fold change; $p<0.05$). GSEA showed enrichment in Inflammatory/glia, neuronal health, and excitotoxicity networks.
Discussion: In the absence of overt neuronal excitotoxicity, chronic exposure to human relevant levels of DA induced alterations in structural and molecular aspects of astrocytes that may be related to protective adaptive pathways.

(19) The Effects of Contraceptive Chemicals and Mixtures on Adipogenesis and Hormone Receptor Signaling

Yu-Ting Tiffany Chiang[1] and Christopher Kassotis[1]

[1]Institute of Environmental Health Sciences, Wayne State University, Detroit, MI 48202

Approximately 65% of women aged 15-49 in the U.S. use birth control. A common reported side effect associated with birth control use is weight gain. In some instances, this particular side effect is sufficient to deter women from using birth control altogether. This is especially problematic for those who suffer from endometriosis or irregular menstruation, where birth control is often used as a treatment. We investigated whether the progestins and estrogens used in birth control formulations were potentially disrupting adipocyte development, and if they were, testing to determine the mechanisms promoting the effects seen in the adipocytes. To test this, we conducted a literature search and compiled a list of ten progestins, three estrogens, and seven progestin-estrogen combinations, all of which are found in commonly used birth control formulations. We tested these hormones and mixtures for agonistic and antagonistic activity with the progesterone, estrogen, glucocorticoid, retinoid X, thyroid, and peroxisome proliferator activated receptors using transient transfection reporter gene assays in human cells. We also assessed pro-adipogenic activity using the 3T3-L1 murine pre-adipocyte model, and after ten days of exposure, measured triglyceride accumulation and cell proliferation. We found that all the progestins, both individually and in combination with estrogens, promoted substantial triglyceride accumulation. Medroxyprogesterone Acetate, Etonogestrel, and Nesterone promoted >100% total triglyceride accumulation relative to the maximal positive control response. Additionally, each of the progestins also promoted proliferation. Medroxyprogesterone Acetate, Etonogestrel, and Nesterone promoted >50% cell proliferation relative to differentiated solvent controls at 1 μM. Estrogens were overall less active in promoting effects than progestins, and when combined, muted effects were observed relative to the individual progestin responses. These data suggest that commonly used progestins may interfere with adipocyte development and could promote weight gain through triglyceride accumulation and cell proliferation. Weight gain, a common concern for women taking birth control, has insufficient information available to appreciate potential risk. These data provide improved insight into which contraceptives may have less undesirable effects on weight management.

(20) Toxicant-Pathogen Interactions During Pregnancy: Pilot Study of Pregnant Murine Co-exposure to Trichloroethylene (TCE) and Group B Streptococcus (GBS)

Eleanor Scheeres[1], Emily Schellenboom[1], Sean Harris[2], Erica

Boldenow[1]

[1] Department of Biology, Calvin University, Grand Rapids, Michigan
[2] Department of Environmental Health Sciences, University of Michigan, Ann Arbor, Michigan

Trichloroethylene (TCE) is a widespread environmental contaminant that interferes with immune defenses against pathogens, but little is known about how TCE affects these immune processes during pregnancy. Previous *in-vitro* data showed the bioactive TCE metabolite, *S*-(1,2- dichlorovinyl)-L-cysteine (DCVC), inhibited pathogen stimulated cytokine release, indicating the potential for immune disruption and increased risk of uncontrolled infections. These immunological disruptions may lead to adverse pregnancy outcomes, such as preterm birth.
However, little *in-vivo* data exists on toxicant-pathogen interactions during pregnancy. This pilot experiment investigated co-exposure to TCE and GBS in a pregnant murine model. Timed pregnant Wistar rats (n=4 per treatment) were exposed to either TCE (480 mg/kg) on a wafer for gestational days (GD) 6-17 or no treatment wafers. At GD 15, rats were vaginally inoculated with Group B Streptococcus (GBS) or saline. Rats were euthanized on GD 18 and examined for gross pathological changes. In addition, amniotic fluid and gestational tissues were analyzed for parturition markers (cytokines, MMPs, and prostaglandins)—these protein levels were highly variable among treatment groups. However, distinct patterns of expression were observed in different tissues. As expected, GBS inoculation increased several cytokines, MMP-9, and PGE2 in the amniotic fluid, as well as uterine and placental tissues. Notably, TCE appeared to inhibit GBS induced CXCL-2, CXCL-3, and MMP-9 in co-exposed amniotic fluid. Furthermore, fetal resorptions were increased in TCE exposed rats, but not in co-exposed rats. While statistical significance was not observed, these data provide a foundation for the feasibility of future studies and highlight the importance of better understanding TCE and pathogen co-exposure during pregnancy.

(21) High-throughput high-content imaging of environmental toxicants reveals novel morphometric phenotypes

Nicholas Cemalovic[1], Anagha Tapaswi[1], Justin Colacino[1,2]

[1]Department of Environmental Health Sciences, University of Michigan School of Public Health

[2]Department of Computational Medicine and Bioinformatics, University of Michigan Medical School

High content imaging represents an emerging set of methods to assess total cell

response to pharmaceuticals and environmental toxicants in a high-throughput manner. In particular, the ability to derive a chemical morphometric phenotypic “fingerprint” in an unbiased manner may be useful in identifying the mode of action (MOA) of poorly characterized chemicals. Here, our goal was to apply these methods to derive a chemical’s morphometric phenotypic “fingerprint”, classifying the mode of action for over 15 environmental toxicants previously identified as racially disparate in the US population via NHANES in a dose-dependent manner. MCF10A breast epithelial cells were first exposed to 4 doses of toxicant and a reference panel of 20 small molecules of known target and MOA for 48 hours before being fixed and stained. Following CellPainting, a high-content image-based assay, and automated microscope image processing, 3300 morphometric features were calculated for over 200,000 cells. Integrating BMDExpress and cell viability data, phenotypically active doses and features were selected and fed into generalized linear models to rank each feature for its chemical-specific significance and dose-dependent directionality, followed by unbiased clustering and heatmap visualization. Such analyses demonstrated distinct morphometric fingerprints for each compound, identifying feature clusters with distinguishable structure-activity relationships, modes of action, and dose-dependent behaviors. Specifically, we identified the phenotypes of Copper and Cadmium to be enriched in the nuclear region and to cluster significantly with all 4 histone deacetylase inhibitors (HDACi), suggesting that both chemical classes act upon similar targets related to histone acetylation - an essential mechanism for epigenetic stability previously unexplored with traditional single-cell and high-throughput techniques. These findings support and complement MOA and structure-activity relationship studies, providing distinct morphological targets of future characterization.

Platform Presentation Abstracts

Higher quality maternal diet attenuates negative associations of maternal paraben concentrations with newborn weight and length

Diana C. Pacyga[1,2], Susan L. Schantz[3,4], Rita S. Strakovsky[1,2]

(1) Department of Food Science and Human Nutrition & (2) Institute for Integrative Toxicology, Michigan State University; (3) Department of Comparative Biosciences & (4) Beckman Institute, University of Illinois, Urbana-Champaign.

Background/Aim: We and others previously observed sex-specific negative associations of maternal paraben concentrations with birth weight (BW) and length (BL). Here, we evaluated whether maternal diet quality modifies these associations.
Methods: Pregnant women ages 18-40 years from Champaign-Urbana, IL provided 5 first-morning urines across pregnancy, which we pooled for analysis of butylparaben, ethylparaben, methylparaben, and propylparaben concentrations. We collected BW and BL data within 24hrs of birth and calculated sex-specific BW-for-gestational-age z-scores (BWz). Women completed 3-month semi-quantitative food frequency questionnaires in

early and mid-to-late pregnancy, which we used to calculate mean Alternative Healthy Eating Index 2010 (AHEI-2010) – reflecting foods predictive of chronic disease risk. Multivariable linear regression models evaluated whether associations of parabens with BWz (n=403) and BL (n=429) were modified by AHEI-2010 (dichotomized at the median) and whether the modification varied by fetal sex. We modeled ethylparaben, methylparaben, and propylparaben as continuous variables and butylparaben as zero/non-zero.
Results: This predominately non-Hispanic white, college-educated population had lower urinary paraben concentrations than other U.S. women. Median (range) AHEI-2010 was 55.8 (28.1–82.8) out of 110, while BW and BL were 3.5kg (2.2–4.9) and 50.0cm (43.9–55.9), respectively. Associations of parabens with birth size only emerged in female newborns whose mothers consumed a poorer diet (AHEI-2010<median). In these newborns, each 2-fold increase in maternal methylparaben was associated with 0.1 (95%CI: 0.02, 0.2) lower BWz and 0.2cm (95%CI: 0.02, 0.3) shorter BL, with similar associations observed for propylparaben. Similarly, female newborns of women who ate a poorer diet and had non-zero butylparaben concentrations had 0.4 (95%CI: 0.1, 0.8) lower BWz and 0.9cm (95%CI: 0.1, 1.6) shorter BL than women with zero butylparaben concentrations.
Conclusions: Healthier maternal diets may effectively minimize negative associations between parabens and birth size in female newborns. Additional studies in diverse cohorts are needed to confirm these findings.

Comparison of conventional and non-conventional oil toxicity on three freshwater fish species: molecular, developmental, and global health effects

Bérubé R., Langlois V.S. and Couture P

Institut National de la recherche scientifique, 490 rue de la Couronne, Québec, Canada

In Canada, 63% of the oil production comes from the oil sands region in Alberta. The bitumen extracted from the oil sands is transported by pipelines, in which it needs to be diluted (i.e., diluted bitumen or dilbit) with natural gas condensate, to increase its fluidity and allow its transport. The increase in oil production and circulation on the North American continent leads to a higher risk of oil spills in freshwater ecosystems. This project aimed to evaluate and compare the toxicity of a dilbit versus a conventional oil. Water accomodated fractions (WAF) of both oils were prepared and serially diluted. Fathead minnow, rainbow trout, and Atlantic salmon were exposed during their early life stages, and various biomarkers were measured (i.e., mortality, growth, gene expression and activity of detoxifying enzymes). The response of those biomarkers were then compared to the chemistry of the WAFs of both oils. Our first observation was that dilbits increased mortality and were more toxic than conventional oil, presumably because of higher concentrations of low molecular weight components from the added

diluent. This project also determined that early life stages (i.e., fish embryos exposed from fertilization) were the most sensitive stages to oil exposure. We also observed that fish returned to a clean environment (after being exposed from their early development) had a reduced survival rate and a higher malformation rate. In addition, *cyp1a* mRNA levels increased in relation to WAF concentrations, while there was no clear relation with EROD activity. Interestingly, we observed a saturation of glutathione s-transferase (GST) activity, which may lead to an accumulation of lipid peroxidation products and cellular damage. At last, Atlantic Salmon was the most sensitive species of the three fish species tested. We hypothesized that this may be caused by their respective number of AhR genes, since Atlantic salmon has six AhR genes, while the rainbow trout has three and the fathead minnow has only two. Future studies should focus on AhR crosstalk with developmental processes to increase understanding of how petroleum product exposures can disrupt normal embryonic fish development.

Thrombin-catalyzed fibrin polymerization controls fibrin(ogen) solubility dynamics in early acetaminophen hepatotoxicity

Zimu (Christine) Wei[1], Kevin Baker[1], Dafna Groeneveld[1], Lauren Poole[1,2], Holly Cline-Fedewa[1], Matthew J. Flick[4], James P. Luyendyk[1,2,3]

[1]Department of Pathobiology & Diagnostic Investigation, Michigan State University, East Lansing, MI, USA.[2]Institute for Integrative Toxicology, Michigan State University, East Lansing, MI, USA. [3]Department of Pharmacology & Toxicology, Michigan State University, East Lansing, MI, USA. [4]University of North Carolina at Chapel Hill Department of Pathology and Laboratory Medicine, Chapel Hill, NC, USA.

Acetaminophen (APAP) hepatotoxicity is associated with rapid activation of the coagulation protease thrombin and hepatic accumulation of its primary substrate fibrinogen. Inhibition of thrombin-mediated fibrin clot formation enhanced peak APAP hepatotoxicity in mice. Surprisingly, the magnitude and timing of traditional fibrin formation in the APAP-injured liver is not fully understood. In fact, recent studies suggest at peak liver injury, the majority of fibrin(ogen) in the APAP-injured liver accumulates through a unique thrombin-independent mechanism. We tested the hypothesis that traditional thrombin-mediated fibrin clot formation occurs early after APAP challenge in mice. Fibrinogen^{AEK} (Fib^{AEK}) mice, which express a mutant fibrinogen that cannot support thrombin-mediated fibrin formation, and wild-type mice were challenged with a hepatotoxic dose of APAP (300 mg/kg) and liver and plasma samples collected 6 hours later. APAP-induced hepatic injury was similar in wild-type and Fib^{AEK} mice, indicated by serum alanine aminotransferase activity and area of hepatic necrosis. Immunohistochemistry revealed similar fibrin(ogen) accumulation within areas of necrosis in both wild-type and Fib^{AEK} mice challenged with APAP. Western blotting revealed robust fibrinogen accumulation in the insoluble protein fraction in livers of APAP-challenged wild-type mice. In contrast, fibrinogen in livers of APAP-challenged Fib^{AEK} mice did not partition to the insoluble fraction and was instead

retained in the soluble protein fraction. The results indicate rapid formation of insoluble fibrin in the APAP-injured liver does not contribute to APAP hepatotoxicity. Alongside prior studies, the results imply early thrombin-mediated fibrin polymer formation precedes and pairs with thrombin-independent pathways to drive fibrinogen accumulation in the injured liver.