The 48th Annual Meeting of the Developmental Neurotoxicology Society (DNTS)

Held in Conjunction with the 64th Annual Meeting of the Society for Birth Defects Research and Prevention
Wyndham Grand Pittsburgh, Pittsburgh, PA, June 22-26, 2024

Oral Presentations

DNTS 1 Warkany Lecture

Understanding of Mechanisms of Environmental Teratogenesis: From Mercury to Nutrition
Christina D. Chambers
University of California San Diego, San Diego CA, USA

DNTS 2-5 Patterns and Effects of Polysubstance Exposure Symposium

DNTS 2

Patterns and Correlates of Polysubstance Use among Younger Pregnant and Birthing People
Natacha M. De Genna[1], Lidush Goldschmidt[2], Gale A. Richardson[1]
[1]University of Pittsburgh, Pittsburgh, PA, USA. [2]University of Pittsburgh Medical Center (UPMC), Pittsburgh, PA, USA

Most prenatal drug studies report on ANY substance use across pregnancy or focus only on the third trimester, when substance use decreases. We describe the prevalence of nicotine or tobacco use only (T), cannabis only (C), and co-use of tobacco and cannabis (CT) during each trimester and 6-12 months postpartum in an ongoing high-risk prenatal cohort (M = 19 years, range: 14-22; 65% Black). Participants completed online surveys and provided urine samples that were screened for cotinine and THC during each trimester and 6-12 months postpartum. In addition to patterns and correlates of polysubstance use, we will discuss how the use of different types of substances relates to concordance between self-report and biospecimens. For example, there was greater concordance for cannabis than for tobacco use and tobacco non-reporters were more likely to have used cannabis blunts than participants whose results were concordant. At every time point, polysubstance use (CT) was more prevalent than T or C in isolation. During the first trimester, T=12%, C=13%, and CT=27%. C and CT participants reported more depressive symptoms than T participants. CT participants were more likely to be Black than T participants. CT decreased substantially by the third trimester (13%), with more modest decreases in rates of C (8%) or T (10%). By 6 months postpartum, rates of polysubstance use increased (CT=42%), while rates of T (9%) and C (8%) remained relatively stable. Preliminary results from this cohort highlight the elevated rates of polysubstance use among younger pregnant and birthing people.

DNTS 3

Substance Use and the Maternal Brain
Helena Rutherford
Yale University, New Haven, CT, USA

Maternal substance use, abuse, and dependence remain a significant concern, impacting the health and well-being of birthing parents, their developing child, and the broader family system. Theoretical work suggests that reward neural circuits co-opted by addiction include those thought to foster motivation for affiliative relationships and social interactions. Specifically, it has been hypothesized that stimuli biologically programmed to be rewarding, such as infant faces, may not provide the same level of reward in parents who use substances, thereby decreasing the salience of infants and the pleasure of caregiving. Stress also plays an important role in the down-regulation of reward processes, and emerging research indicates that caregiving may be more stressful for mothers using substances. In this presentation, neuroimaging findings will be shared that evidence differences in the neural processing of infant stimuli in mothers currently using, and not using, substances. Advancing this work will be recent efforts to move beyond associations between categorical distinctions of substance use (present or absent) and measures of the maternal brain, instead introducing a more inclusive, continuous approach to the assessment of substance use.

DNTS 4

Marijuana & Tobacco Co-Use in Pregnancy: Biobehavioral Pathways & Impact on Offspring Neurobehavioral Development

Laura Stroud[1], Janet O. Joseph[1], Katelyn Borba[1], Todd M. Everson[2], Carmen J. Marsit[2]
[1]Brown University, Providence, RI, USA, [2]Emory University, Atlanta, GA, USA

Despite evidence for increasing cannabis use and high rates of co-use of tobacco (TOB) and marijuana (MJ) during pregnancy, little is known regarding the joint effects of prenatal TOB+MJ exposure on offspring development or biobehavioral pathways underlying these effects. We investigated the impact of co-exposure to TOB+MJ on infant neurobehavioral development and cortisol response over the first postnatal month, a period of critical importance for parent-infant attachment and early identification and prevention efforts. We then present preliminary proof-of-concept findings highlighting the impact of prenatal (co)exposures on placenta epigenomic activity. Co-exposure was associated with nearly double the impact on infant self-soothing and need for examiner soothing, and with a 42-75% increased impact on infant attention and motor activity vs. TOB-exposure alone. In addition, TOB+MJ-exposed male infants showed 35-36% attenuation of baseline cortisol levels vs. unexposed and TOB-exposed males, while no exposure effects emerged for females. Preliminary findings from ongoing placental epigenomic studies revealed an impact of prenatal MJ exposure on stress- and endocannabinoid-signaling pathways and alterations in genes regulating the placental extracellular matrix. Results highlight the synergistic impact on next generation neurobehavioral development from dual exposure to TOB+MJ vs. TOB exposure alone. It is critical to study prenatal exposures in concert and to adapt interventions to address co-use. Preliminary findings also highlight the value of the placenta as a mechanistic footprint of prenatal (co)exposures.

DNTS 5

Polysubstance Exposure, Caregiving Context, and Child Outcomes

Rina Das Eiden
Pennsylvania State University, State College, PA, USA

Prenatal substance exposure has often been examined with a focus on the effects of individual substances, driven by large public health questions related to the potential causal role of particular substances for fetal and child maladaptations. However, many substances are used in combination with other substances either simultaneously or around the same use period. In addition, there are challenges in measuring dose, timing, and chronicity of exposure, the role of genetic linkages and epigenetic modifications, and effects of pre- to postnatal substance use on parenting and the caregiving context that all play a role in predicting developmental outcomes. Using both developmental psychopathology and behavioral teratology frameworks may allow for examination of some of these complexities while attending to dose, timing, and chronicity effects with multimethod and prospective measurements of exposure. In addition, the developmental psychopathology concepts of equifinality, highlighting multiple developmental pathways to particular child outcomes, and multifinality, or multiple possible outcomes among children exposed to similar risk conditions, allow for consideration of multiple transacting developmental mechanisms, in addition to exposure alone. Data from two longitudinal studies will be presented highlighting the role of polysubstance exposure and the caregiving context on multiple child and adolescent outcomes. The first is a study of prenatal cocaine and other substance exposure and the second is a study of prenatal tobacco and tobacco-cannabis co-exposure. Both studies used a case-control design with group matching on demographic variables and multi-method assessments of prenatal exposure, consideration of continued postnatal exposure, and incorporating mediating and moderating mechanisms across time.

DNTS 6

Dopamine D2 Receptor-Mediated Effects on Glutamatergic Neurons and Responses in the Anterior Cingulate Cortex

Yasmeena Thabet, Devon Graham, Gregg Stanwood
Florida State University, Tallahassee, FL, USA

Dopamine (DA) as a neurotransmitter has critical effects on multiple neurodevelopmental and neuroplastic processes, mediated at least in part through its interaction with the DA D2 receptor (D2R). Manipulating dopamine or D2R signaling in mice during embryonic development has been shown to impact normal neurodevelopmental trajectory, behavior, and cognition later in life. We are investigating how D2R loss in a specific subpopulation of cortical GABAergic interneurons influences neuronal phenotypes and behavior, using Nkx2.1-D2R conditional knockout (cKO) mice. Our findings indicate a statistically significant increase in *Slc17a7*+ glutamatergic projection neuron (~25%) density in the anterior cingulate cortex (ACC) of adult cKO mice and a sharply blunted locomotor response to MK-801. The increase in glutamatergic neuron number varied as a function of the cortical layer and brain region; it was seen in both male and female cKO mice. Ongoing studies are 1) testing when in neurodevelopment the increase in cortical neurons occurs, 2) examining the dose-dependency of the differential responsiveness to MK-801, and 3) determining the glutamate receptor expression and activation patterns within the ACC in cKO mice. These studies highlight the significance of D2R signaling in neurodevelopment and offer insights into the neurodevelopmental disorders induced by early-life chemical and non-chemical stressors.

DNTS 7

Perfluorohexane Sulfonate (PFHxS) Alters the Choroid Plexus Transcriptome and Enters the Developing Rat Brain

Katherine O'Shaughnessy[1], Aubrey Sasser[1,2], Kiersten Bell[1,2], James McCord[1]
[1]United States Environmental Protection Agency, Research Triangle Park, NC, USA. [2]Oak Ridge Institute for Science and Education, Oak Ridge, TN, USA

Per- and polyfluoroalkyl substances (PFAS) are persistent organic pollutants associated with developmental neurotoxicity. Recently, human biomonitoring showed that PFAS are detectable in newborn cerebrospinal fluid (CSF). This suggests that these chemicals may cross the protective blood-brain and blood-CSF barriers. Thus, understanding how PFAS enter the brain is crucial for determining their risk. To confirm whether PFAS can cross the brain barriers and to delineate a potential mechanism, we exposed pregnant rats to perfluorohexane sulfonate (PFHxS, 50 mg/kg/day) or vehicle control during pregnancy and lactation. PFHxS was chosen as the test compound due to its efficacy in entering human CSF. From the day of birth until postnatal day 14 (PN14), PFHxS was measured in pup sera and in perfused brain on PN6. These data show that PFHxS has significant lactational transfer, and brain PFHxS is ~1/100 of serum. This shows that PFHxS enters the brain but not by passive diffusion. Next, we performed RNA-Seq of the PN6 blood-CSF barrier (choroid plexus). We detected 158 differentially expressed genes in PFHxS exposed animals compared with controls (FDR $q<0.05$). Pathway analyses revealed that PFHxS downregulates endoplasmic reticulum stress and alters expression of carboxylic acid transporters. Together, these data suggest that PFHxS cross the brain barriers. Additionally, PFHxS is likely not diffusing into the brain, but may be transported across the brain barriers via endogenous carboxylic acid transporters. As PFHxS and many other PFAS are structurally similar to fatty acids, this mechanism may be biologically plausible. T*his work does not reflect US EPA policy.*

DNTS 8

Developmental Toxicity of Cannabidiol (CBD) In Sprague-Dawley Rats

Timothy Flanigan, W. Drew Gill, Andrew Shen
U.S. FDA/NCTR, Jefferson, AR, USA

Cannabidiol (CBD) is a component of cannabis lacking euphoric psychotropic effects and is the active ingredient in Epidiolex, an FDA-approved drug to treat rare childhood seizure disorders. The availability and consumption of CBD has greatly increased in the U.S. CBD is perceived as natural and safe by some and is claimed to relieve many negative effects of pregnancy such as anxiety, pain, and insomnia. There is concern that pregnant individuals may use CBD despite sparse data regarding its safety. To investigate the impact of CBD use during pregnancy, timed-pregnant Sprague-Dawley rats were treated via oral gavage from gestational day (GD) 6 through the day prior to parturition with vehicle, 15, 100, 250, 300, or 350 mg/kg CBD. Following parturition, pups were treated via oral gavage with the same respective dose as their dams from postnatal day (PND) 1 through PND 21. Higher doses of CBD, 300 and 350 mg/kg/day, were materno- and fetotoxic, respectively. Offspring from lower doses, 15-250mg/kg/day, survived and underwent assessments of

motor function, sensorimotor gaiting, anxiety-like behavior, and cognition from adolescence until adulthood. Analyses of both behavioral and neurochemical tests used here did not reveal dose-dependent effects of perinatal CBD on offspring neurodevelopment. While CBD metabolite profiles differ between rats and humans, this study provides evidence that doses of CBD up to 250 mg/kg, approximately 3.3x the maximum recommended human dose of Epidiolex (20 mg/kg) based on allometric scaling, had little impact on rat pregnancy.

DNTS 9

The Relationships of Prenatal Cocaine Exposure and Experience of Victimization to Aggression in Adolescence

Duc Khoa Le[1], Jeffrey Albert[1], Sonia Minnes[2], Jung-Yung Kim[3], Meeyoung Min[4], Lynn Singer[1]
[1]Case Western Reserve University, Cleveland, OH, USA. [2]Jack, Joseph, and Morton Mandel School of Applied Social Sciences, Cleveland, OH, USA. [3]University of North Dakota, Grand Forks, ND, USA. [4]University of Utah, Salt Lake City, UT, USA

Prenatal cocaine exposure (PCE) can alter the monoaminergic neurotransmitter system in the fetal brain related to emotional and behavioral regulation. PCE has been associated with a risk of victimization during childhood and adolescence, and heightened rates of aggressive symptoms. However, little is known about the relationships of PCE and specific types of victimization to aggression in adolescence. Objectives: Assess whether adolescents with PCE differ from those without PCE in self-report of lifetime victimization and aggression, and if so, whether specific types of victimization relate to aggression. At age 17, 336 adolescents (50% with PCE, 46% male) reported lifetime victimization across five categories of the Juvenile Victimization Questionnaire, as well as aggression using the Problem-Oriented Screening Instrument for Teenagers. ANOVA, binomial logistic regression, and multiple regression were conducted to examine group differences and relationships among variables while controlling for sex. Adolescents with PCE were more likely to report child maltreatment (p = 0.004), peer and sibling victimization (p = 0.043), and aggression (p = 0.012). PCE (p = 0.005), child maltreatment (p = 0.009), and peer/sibling victimization (p < 0.001) were associated with aggression in adolescence. Girls with PCE reported higher aggression than girls without PCE, while boys did not differ. Adolescents with PCE reported more child maltreatment, peer and sibling victimization, as well as aggression, than those without PCE. Victimization was related to increased aggression for all adolescents. Thus, PCE and victimization both contribute independently to higher aggression, especially for girls with PCE.

DNTS 10

Early Developmental Neurotoxic Exposure Continues to Impact Neurobehavioral Function Across the Lifespan into Aging

Edward Levin
Duke University, Durham, NC, USA

Early life neurotoxic exposure has been shown to cause cognitive deficits and emotional dysfunction in juvenile, adolescent, and young adult life phases, but what about effects expressed during older adulthood? The human lifespan averages many decades. Prospective human studies from early developmental exposure through the lifespan would require considerable time and many grant renewals to complete. Cross-sectional studies with retrospective estimates of early-life exposure can be done, but often estimates of early developmental toxicant exposure are vague. In vitro studies can determine short-term neurotoxicity but have problems extending to effects persisting across the lifespan. Rats and zebrafish with lifespans of a couple of years offer a way to telescope across time to preview what could be in store later in life for humans who have had early developmental neurotoxicant exposure. We have found in rats that early developmental exposure to low levels of the organophosphate pesticides parathion and diazinon cause cognitive impairment that becomes more evident in older adulthood. We have also found that older zebrafish also show persisting neurobehavioral impairments of early developmental diazinon exposure. These models also offer opportunities to develop better understanding of mechanisms of long-term neurobehavioral impairment and its effective treatment. We can provide the evidence of developmental neurotoxic risk that can help remove toxicants from continuing exposure, but we will need to be concerned with the continuing fallout of early-life exposure to legacy toxicants

for many decades across the human lifespan even after these toxicants are removed. Supported by the Duke University Superfund Research Center (ES010356).

DNTS 11 Patricia Rodier Mid-Career Award for Research and Mentoring (joint with BDRP)

Reproductive Toxicology in Industry: Research and Mentoring are the Special Sauce
Christopher J. Bowman
Pfizer, Inc., Groton, CT, USA

DNTS 12 Keynote Lecture – (joint with BDRP)

Putting the Pieces Together: Head to Toe
Wendy K. Chung
Boston Children's Hospital, Boston, MA, USA

Genomics have provided an unbiased way to assess the molecules and processes involved in developmental disorders. The genetic contributions to congenital anomalies include *de novo* variants of large effect, inherited rare variants of modest effect, and common variants of small effect. There are similarities and differences between the genetic architecture and genes involved in different congenital anomalies. By putting this information together, the genetic information can be used to diagnose syndromes prenatally and postnatally, provide improved prognostic information, and potentially tailor clinical care based on the underlying diagnosis.

DNTS 13-16 Congenital Anomalies in Minoritized Populations: An Overview of Factors Affecting Prevalence and Outcomes Symposium (joint with BDRP)

DNTS 13

Using Birth Defects Surveillance Data to Understand and Address Health Disparities
Wendy N. Nembhard
University of Arkansas for Medical Sciences, Little Rock, AR, USA

Birth defects occur in 3% of births in the United States and are associated with increased risk of morbidity, mortality, and disabilities across the lifespan. Studies have demonstrated racial/ethnic, socioeconomic, geographic, and other health disparities in the occurrence, morbidity, mortality, and long-term outcomes among children and adults born with birth defects. Historically, hospital-based and clinic-based studies were used to explore health disparities and inequity in intervention and treatment modalities for children with birth defects. However, increased awareness of inherent biases within these data sources have increased the utilization of data from population-based birth defects surveillance systems to understand and address health disparities. This presentation will discuss current gaps in the literature regarding health disparities in birth defects research, available data sources, and the strengths and limitations of using population-based birth defects surveillance system data to investigate health disparities.

DNTS 14

Congenital Anomalies among American Indian and Alaska Native People in Oklahoma
Amanda E. Janitz
University of Oklahoma Health Sciences, Oklahoma City, OK, USA

Oklahoma has a high percentage of the American Indian/Alaska Native (AI/AN) population (14%) compared to the US overall (2%). Congenital anomalies are the leading cause of death for infants with a death rate of 1.12 per 1,000 live births in the US, with a higher mortality rate in Oklahoma of 1.52 per 1,000 (1.69 per 1,000 Oklahoma AI/AN births) from 2019–2021. We aimed to evaluate the prevalence of congenital anomalies by race/ethnicity and evaluate geographic differences in anomalies in Oklahoma. We conducted a retrospective analysis of congenital anomalies using data from the Oklahoma Birth Defects Registry from 1997–2019, with

more in-depth data available through 2009. We calculated prevalence proportions and 95% confidence intervals of anomalies overall and for specific anomalies, including central nervous system, ear, cardiovascular, orofacial, gastrointestinal, genitourinary, musculoskeletal, and chromosomal, by race/ethnicity and geographic location (county and census tract level). To compare the prevalence by race/ethnic group, we calculated prevalence proportion ratios (PPR) and 95% confidence intervals. We used the Moran's I test to evaluate spatial autocorrelation of anomalies and Getis-Ord Gi* to evaluate local hot spots in Oklahoma at the census tract level. The prevalence of any anomaly in Oklahoma from 1997–2019 was 3.9%, with a higher prevalence among Hispanic children (3.9%) than non-Hispanic (NH) children (3.4%) and higher among White children (3.6%) compared to other racial groups (Black: 3.5%, AI/AN: 3.5%, and Asian/Pacific Islander: 2.7%). Using data through 2009, we observed that NH AI/AN children had a higher prevalence of several congenital heart defects compared to NH White children, although this was based on small numbers of children (Common truncus PPR: 1.45, double outlet right ventricle PPR: 1.59, hypoplastic left heart syndrome PPR: 2.94, single ventricle PPR: 1.76, total anomalous pulmonary venous connection PPR: 2.35, transposition of the great arteries PPR: 1.34). We identified spatial autocorrelation for neural tube defects, with local hot spots identified in western Oklahoma. In summary, evaluating disparities in congenital anomalies by race/ethnicity and geographic regions can identify priority populations for prevention and early identification.

DNTS 15

Voluntary Folic Acid Fortification Policy of Corn Masa Flour and Tortillas in the US and Neural Tube Defects Among Hispanics

Vijaya Kancherla. Emory University, Atlanta, GA, USA

Corn masa is a staple food of many Hispanics in the US; however, mandatory corn masa fortification with folic acid is not implemented in parallel to enriched cereal grain fortification to prevent neural tube defects (NTDs). In April 2016, the US Food and Drug Administration published regulations allowing voluntary fortification of corn masa flour and tortillas. Race/ethnic disparities persist in the US with regard to the prevalence of NTDs. Mandatory fortification of cereal grain products in the US went into effect in 1996 and has been associated with a significant and sustained reduction in the prevalence of NTDs among non-Hispanic Whites and Blacks. However, the prevalence among Hispanics has remained high in comparison to their counterparts. Hispanic women are less likely to consume folic acid supplement pills periconceptionally. Further, data from multiple cycles of the National Health and Nutrition Examination Survey show lower levels of blood folate concentrations among Hispanic women of reproductive age compared to non-Hispanic Whites, pointing to a health disparity. In December 2017, 20 months after the FDA permitted voluntary fortification of corn masa with folic acid, we conducted a market analysis of corn masa and soft corn tortillas in northeast Atlanta that had a large concentration of Hispanic families. Both inspection of nutrition labels and laboratory analysis of products revealed that voluntary fortification was ineffective. In 2019, we conducted a national social media campaign utilizing citizen reports of nutrition labels of corn masa products and found that voluntary fortification policy was ineffective nationally. In February 2023, the Center for Science in the Public Interest published a report to bring attention to this issue once again. Their report recommended manufacturers fortify corn masa and corn tortilla products with adequate levels of folic, retailer efforts to stock more fortified corn masa products, FDA guidance to industry to fortify, and consumer education encouraging women of reproductive age to seek fortified corn masa products and consume daily supplements with 400–800 mcg of folic acid, to prevent NTDs among Hispanics. Efforts to promote mandatory corn masa fortification are ongoing. There is an urgency to address inequities in NTD prevention adversely impacting many Hispanics in the US.

DNTS 16

Neighborhood Sociodemographic Characteristics and Prevalence of Congenital Anomalies in Texas

Jeremy M. Schraw[1]; Elwin Jaime[1]; Rutu Rathod[2]; Charles J. Shumate[3]; Amy E. Hughes[2]; Sandi L. Pruitt[2]; Gary M. Shaw[4]; Philip J. Lupo[1]

[1]Baylor College of Medicine, Houston, TX, USA; [2]University of Texas Southwestern Medical Center, Dallas, TX, USA; [3]Texas Department of State Health Services, Austin, TX, USA; [4]Stanford University, Stanford, CA, USA.

Studies of racial and ethnic disparities in birth defects have largely focused on individual-level maternal factors. Neighborhood characteristics influence pregnancy outcomes through effects on maternal nutrition, chronic health conditions, prenatal care access, and exposure to environmental toxins. Therefore, we evaluated the prevalence of >140 birth defects monitored by the Texas Birth Defects Registry among residents of Hispanic/Latino enclaves (neighborhoods with high proportions of Hispanic/Latino residents, recent immigrants, and Spanish-speaking households) and neighborhoods with low socioeconomic status (low nSES). Our study included all birth defect cases regardless of pregnancy outcome (1999–2018) and a reference population of all live births in Texas. Using tract-level census and American Community Survey data, we calculated Yost socioeconomic index scores and Hispanic/Latino enclave index scores. We used Poisson regression to estimate the prevalence ratio (PR) and 95% confidence interval (CI) of each defect among residents of neighborhoods classified as low nSES/enclave (bottom three quintiles of Yost score, top quintile of enclave index score; 34.5%); low nSES/not enclave (25.6%); high nSES/enclave (4.6%); and high nSES/not enclave (referent; 35.3%). Women were predominantly Hispanic/Latina (47.9%), non-Hispanic/Latina White (35.0%), or non-Hispanic/Latina Black (11.7%). Low nSES was associated with microcephaly, atrial septal defect, and Down syndrome, and inversely associated with male genital anomalies. Offspring of women in low nSES neighborhoods demonstrated an increased prevalence of pulmonary artery anomalies, and this association was stronger among women who lived in enclaves (PR 1.61, CI 1.46-1.76) than women who did not (PR 1.27, CI 1.15-1.40). Offspring of women in low nSES enclaves were at increased risk of ventricular septal defect, pulmonary valve anomalies, and patent ductus arteriosus relative to those in other neighborhoods. Conversely, whereas pyloric stenosis was associated with low nSES among women not living in enclaves (PR 1.16, CI 1.04-1.29), there was no association among women living in enclaves, and we observed the lowest PR of hypospadias, epispadias, and chordee among offspring of women in low nSES enclaves (PR 0.84, CI 0.78-0.90). In summary, nSES and enclave status were associated with the prevalence of several birth defects. These associations are largely unappreciated in the literature.

DNTS 17 Elsevier Distinguished Lecture

Reopening Critical Periods with Psychedelics: Basic Mechanisms and Therapeutic Opportunities
Gül Dölen
University of California Berkeley, Berkeley, CA, USA

DNTS 18-21 Chemicals Altering Signaling Molecules and Developmental Pathways Symposium (joint with BDRP)

DNTS 18

Platelet-derived growth factor receptor alpha (Pdgfra) and mTOR Signaling in Alcohol Teratogenesis
Johann K. Eberhart; Scott K. Tucker
University of Texas, Austin, TX, USA

Prenatal alcohol exposure is a leading cause of birth defects. These defects are collectively termed Fetal Alcohol Spectrum Disorders. However, not all alcohol exposures result in FASD, and exposure outcomes are strongly tied to genetics. We have found that reduction in Platelet-derived growth factor receptor alpha (Pdgfra) signaling sensitizes zebrafish embryos to craniofacial defects following alcohol exposure. Our results show that a reduction in signaling via the Mechanistic Target of Rapamycin Complex 1 (mTORC1) underlies this sensitization. We generated *raptor* mutants, encoding an essential component of mTORC1, and found that Raptor function is required for craniofacial development. Among the functions of mTORC1 is the inhibition of autophagy and we find that elevated autophagy is the cause of the craniofacial defects in *raptor* mutants. We have found that loss of *raptor* exacerbates the craniofacial phenotypes in *pdgfra* mutants. We also demonstrated that inhibition of autophagy partially rescues the ethanol-induced craniofacial defects in embryos with reduced Pdgfra function. Collectively, our results suggest that ethanol induces autophagic stress and ethanol-induced defects occur when this stress cannot be buffered via mTORC1 signaling.

DNTS 19

Alcohol and Cannabinoids Act through Overlapping Pathways Critical for Brain and Face Development
Karen E. Boschen[1,2]; Eric W. Fish[1]; Kevin P. Williams[3]; Scott E. Parnell[1]
[1]University of North Carolina, Chapel Hill, NC, USA; [2]SUNY Upstate Medical University, Syracuse, NY, USA; [3]North Carolina Central University, Durham, NC, USA

Understanding the biological pathways through which drugs, such as alcohol and cannabinoids, affect fetal growth is critical for establishing clinical recommendations and developing therapeutics. While the craniofacial and growth abnormalities associated with prenatal alcohol exposure have been well documented, the pathogenic mechanisms of alcohol are still under investigation. Using transcriptomics and transgenic mice, we have shown that prenatal alcohol interacts with primary cilia, hair-like organelles involved in cell signaling and mitosis. Normal cilia function is required for Sonic hedgehog (Shh) transduction, a morphogen critical for midline face and brain development that is a target of early gestational alcohol exposure. Interestingly, we have evidence that cannabinoids also act through an allosteric CB1-Shh interaction and that this interaction contributes to the craniofacial malformations induced by early gestational cannabinoid exposure. Our results support further consideration of primary cilia and the Shh pathway as possible overlapping mechanisms of alcohol and cannabinoids during CNS development.

DNTS 20

Executive Function Deficits after Prenatal Opioid Exposure Relate to Changes in Microglia
Brittany L. Smith[1]; Brandon Brooks-Patton[1]; Justin L. Bollinger[2]; Samuel C. Woodburn[2]; Tess A. Guzman[2]; Alexander H. Brendle[2]; Anna G. Makela[1]; Eric S. Wohleb[2]; Teresa M. Reyes[2]
[1]Northern Kentucky University, Highland Heights, KY, USA; [2]University of Cincinnati, Cincinnati, OH, USA

Prenatal opioid exposure is associated with an increased incidence of childhood behavioral problems. These problems frequently manifest as deficits in executive function. The prefrontal cortex is the brain region that largely controls executive function. Opioids can activate the toll-like receptor 4 pathway, which initiates an immune response cascade. Microglia, the immune cells of the brain, actively refine synapses during development. We proposed that prenatal opioid exposure would affect the function of microglia in the prefrontal cortex of the offspring brain and that this could potentially relate to executive function deficits that emerge after exposure. To study this, we used a mouse model of prenatal opioid exposure. Female mice were given morphine (MO) or saccharin vehicle control in their drinking water throughout gestation and lactation until offspring were weaned on postnatal day 21. Executive function was assessed in adult offspring using the 5-choice serial reaction time task (5CSRTT). After the 5CSRTT, we collected offspring prefrontal cortex brain samples and FACS-sorted microglia for RNA sequencing. We found that prenatal MO exposure impaired executive function in male and female offspring. Female MO-exposed offspring had an increase in inattentive errors during 5CSRTT training while male MO-exposed offspring had reduced correct responding across 5CSRTT testing. Prenatal MO exposure significantly altered prefrontal cortical microglia phenotypes in adult offspring that were unique to each sex. Microglia in the PFC of adult male MO offspring displayed evidence of increased gene expression of chemokine signaling pathways and increased toll-like receptor 4 protein levels. Notably, microglial gene expression downregulation in the prefrontal cortex of MO offspring correlated to behavioral performance (*sco2* gene in males and *otub2* in females). Overall, prenatal opioid exposure impairs adult offspring executive function and alters gene expression patterns in microglia within the prefrontal cortex. While both sexes displayed executive function deficits, gene expression signatures differed. Notably, we identified genes that may relate to behavioral deficits, which are promising future targets for preventing or reversing deficits after prenatal opioid exposure.

DNTS 21

The Role of the Aryl Hydrocarbon Receptor in Multi-Organ Developmental Disorders
Christine Perdan Curran
Northern Kentucky University, Highland Heights, KY, USA

The aryl hydrocarbon receptor is ubiquitously expressed during development, but its function is not fully understood in all tissues and cell types. This talk will compare what is known about classic AHR-mediated teratogenesis including cleft palate, cardiac malformations, and hydronephrosis to an emerging understanding

of the receptor's role in neurotoxicity and inflammatory pathways. Advances from a variety of model organisms will be discussed from the roundworm *C. elegans* to rodent models.

DNTS 22-25 Neural Tube Defects in a Post-Folate Fortified World Symposium (joint with BDRP)

DNTS 22

Genomic Advances in Understanding the Etiology of Neural Tube Defects

M. Elizabeth Ross[1]; Paul Wolujewicz[1]; Vanessa Aguiar-Pulido[1]; Alokkumar Jha[1]; Andrew Tidball[2]; Richard H. Finnell[3]
[1]Weill Cornell Medicine, New York, NY, USA; [2]University of Michigan, Ann Arbor, MI, USA; [3]Baylor College of Medicine, Houston, TX, USA

Decades of experience in the genetics of naturally occurring Neural Tube Defects (NTDs) in mouse models and human cases have supported that a complex interplay of multiple hypomorphic mutations with the intrauterine environment are needed to result in neural tube closure failure. Unlike common complex genetic disorders such as Alzheimer's Disease, Autism Spectrum Disorder, or Schizophrenia, direct interrogation of human genomes for elucidation of the genetics underpinning NTDs has been historically limited by the comparatively lower prevalence of human NTDs that prevents reaching statistical significance in a genome-wide association study (GWAS) approach. Recent advances including the elucidation of the human genome sequence, improved genome diversity in existing databases, better understanding of the 2-D and 3-D structure of the human genome, and increased power of computational capabilities offer unprecedented opportunities to assess mechanisms and even individual genetic risk for NTD occurrence. This presentation will give examples demonstrating the strategic application of machine learning and deep learning methods to the interrogation of NTDs arising in human populations. Using these tools, it is possible to probe protein-coding and intervening, nonprotein-coding sequence within the human genome and avoid the inherent bias imposed by candidate gene approaches to NTD genetic studies. Insights obtained from our initial investigations highlight critical molecular pathways impacted by single nucleotide variants (SNVs) in protein-coding and regulatory regions of the human genome, as well as contributing influences of structural, copy number variants (CNVs) and topologically associated domains (TADs) that define genomic, 3-D shape and therefore accessibility of genetic loci to transcription. As we accrue larger, although still relatively modestly sized patient populations, the long-sought goal will come within reach to assess the individual vulnerability of couples to have an NTD-affected pregnancy and use that knowledge to optimize their chances for healthy birth outcomes.

DNTS 23

Policy Matters: Folate Fortification, Pharmaceutical Interactions, and Neural Tube Defect

Robert M. Cabrera; Gabriel L. Tukeman; Hui Wei; Linda Lin; Bogdan J. Wlodarczyk; Richard H. Finnell
Baylor College of Medicine, Houston, TX, USA

Maternal folate status is an established modifier of birth defects and neural tube defect (NTD) risk. We have previously reported that dolutegravir, an HIV integrase inhibitor, is a noncompetitive antagonist of folate receptor and results in folate-responsive developmental toxicity in fish and mice. We hypothesize the integrase inhibitor bictegravir (BIC) will produce folate-responsive developmental toxicity in mice. BIC was evaluated for developmental toxicity using pregnant mice fed a normal or low folic acid diet. CD-1 mice were provided a diet with normal (3mg per kg) or low (0.3mg per kg) folic acid. They were treated with water or human therapeutic-equivalent exposures of BIC from mouse embryonic day E6.5 to E12.5. Pregnant dams were sacrificed at term (E18.5) and fetuses were inspected for gross, internal, and skeletal defects. Fetuses with exencephaly, an NTD, were present with human equivalent exposures to BIC in dams fed a low folic acid diet. The research indicates that maintaining recommended dietary folate intake, by fortified, enriched, or nutrient-dense diets, could ameliorate developmental defects arising from BIC exposure. This finding is particularly relevant for individuals living with HIV who have low folate status during pregnancy, resulting in elevated risks for NTDs. The study underscores the need for future research to consider folate status as a modifier for integrase inhibitor-associated developmental toxicity.

DNTS 24

Folic Acid and Birth Defect Prevention: Metabolic Regulation of Biomechanical Forces Explains the Beneficial Effects of Folic Acid in Preventing Preventable Birth Defects

Richard H. Finnell[1]; John Steele[1]; Carlo D. Caiaffa[1]; Yogeshwari S. Ambekar[2]; Robert M. Cabrera[1]; Kirill Larin[2]
[1]Baylor College of Medicine, Houston, TX, USA; [2]University of Houston, Houston, TX, USA

Periconceptional maternal folic acid (FA) supplementation and food fortification programs reduce risks for selected congenital malformations in a manner that remains unknown. The mechanisms through which folate facilitates neural tube closure (NTC) are largely unknown, primarily because we don't understand how the metabolic functions of FA translate into tissue-level mechanics. NTC is initiated by a convergent extension (CE) of neuroepithelial cells (NE) that will define the eventual CNS along its anterior-posterior and mediolateral axes. CE involves rearrangement and intercalation of NE cells driven by biomechanical integration of cell crawling and contraction of cell-cell junctions. The edges of the neural plate elevate and bend through the formation of a single medial and two lateral hinge points, creating the neural folds that will eventually meet along the dorsal midline to form the closed, functional neural tube. Actomyosin dynamics play an important role in neural plate bending. Our objective was to translate folate regulation of developmental pathways into a comprehensive understanding of the biomechanical forces necessary to complete NTC. We utilized Brillouin microscopy coupled with Optical Coherence Tomography to better understand how mechanical properties involved in NTC respond to folate status. We examined multiple folate formulations on cell proliferation of mouse embryonic fibroblasts and assayed F-actin formation in revealing folate concentration-dependent differences in the number of F-actin fibers based on Phalloidin staining. We also determined folate's impact on Vangl2 expression, as it codes for a membrane-bound protein that regulates PCP signaling and modulates actin dynamics critical to CE and other polarized cell movements required for NTC. We observed a reduction in Vangl2 protein at lower folate concentrations secondary to reduced gene expression. Western blot analysis suggested that the folate receptor (FOLR1) was downregulated in response to lower folate concentrations, especially in the 5mTHF groups. As the FOLR1 protein was translocated to the nucleus and could stimulate the Vangl2 promoter, it may be acting as a folate-dependent transcription factor to regulate gene expression required for biomechanical cell remodeling. We hypothesize that perturbation of folate-dependent processes may lead to reduced stiffness of NE tissue and ultimately contribute to failed NTC.

DNTS 25

Precision Medicine Approaches for Treating Spina Bifida

Bruna Corradetti[1]; Crisiane R.S. Fonteles[2]; John Steele[1]; Bogdan J. Wlodarczyk[1]; Lin Ying[1]; Robert M. Cabrera[1]; Richard H. Finnell[1]
[1]Baylor College of Medicine, Houston, TX, USA; [2]Universidade Federal do Ceara, Fortaleza, Brazil.

Clinical trials have recently shed light on the effectiveness of patches functionalized with placental stem cells for the *in utero* surgical treatment of spina bifida. While promising, *in utero* repair performed at mid-pregnancy to reduce the ongoing damage of the exposed spinal cord still represents an invasive procedure to close the fetus' malformation. Here we propose the use of mesenchymal stem cells (MSCs) from the amniotic fluid to develop less invasive intervention strategies able to create a pro-regenerative environment within the amniotic cavity and induce repair. It is widely established that MSCs act as trophic mediators, modulating the function of surrounding endogenous cells by releasing paracrine signals (growth factors, cytokines, chemokines, and extracellular vesicles, EVs). EVs retain parental molecular moieties and display inherent targeting capabilities, which make them an interesting tool to develop effective cell-free therapeutic systems for precision medicine. Our laboratory has recently developed an efficient approach to utilize EVs as reconfigurable systems for the delivery of bioactive factors and established a platform based on a cell extrusion approach to increase the production of EV-based therapeutics. We called nanoparticles obtained from this approach exosome mimetics (MIMs). In this study, we tested the potential of natural EVs isolated from amniotic fluid-MSCs, and their mimetic counterparts, as valid, stable, and minimally invasive therapeutic alternatives. Molecular and physiochemical characterization showed no differences between natural EVs and MIMs, with MIMs determining a 3-fold greater yield. When applied as potential RNA therapeutics, MIMs delivered a more intense and prolonged expression of mRNA encoding for green fluorescent protein in macrophages and fibroblasts. An *ex-vivo* whole embryo culture demonstrated that MIMs mainly accumulate at the level of the yolk sac, while EVs reach the embryo. Present data confirm the potential application of EVs for the prenatal repair of neural

tube defects and suggests MIMs as prospective vehicles to prevent congenital malformations caused by *in utero* exposure to drugs.

DNTS 26 Robert L. Brent Lecture – (joint with BDRP)

Everywhere All at Once: The Prenatal Chemical Exposome and Influences on Health and Health Inequities

Tracey Woodruff
University of California San Francisco, San Francisco, CA, USA

DNTS 27

Remembering Don Hutchings

Diana Dow-Edwards
SUNY, Downstate Health Sciences University, Brooklyn, NY, USA

DNTS 28-31 The East Palestine Train Derailment: A Complex Environmental Disaster Symposium

DNTS 28

Train Derailment in East Palestine, Ohio: A Complex Environmental Disaster

Lynn Singer
Case Western Reserve University, Cleveland, OH, USA

On February 3, 2023, A Norfolk Southern train, carrying hazardous chemicals, derailed in East Palestine, Ohio, causing immediate evacuation of half its residents. Subsequently, a controlled combustion was conducted to prevent explosion of toxins, including vinyl chloride and butyl acrylate. Volatile compounds immediately affected air and water quality, and reports by citizens of acute respiratory and other symptoms raised concerns about the long-term impact despite EPA assurances of safe contaminant levels. Chemicals were identified by the NIEHS as high priority but with limited human health information. In response, a multi-university - community consortium was formed to develop objective summaries of the state of the science regarding the contaminants released relative to type, toxicological profiles, locations, and reported health information. The consortium serves as an independent group of scientists to assist with communicating data-informed summaries to the community. In December, the National Academies convened a panel to explore potential human health impacts and to develop a research agenda responsive to community concerns. NIEHS has now funded 5 exploratory grants to begin to address the complexities of the extent and nature of exposure effects of the derailment. In this panel, Fred Shumacher presents a novel plan to use somatic mutation rate to measure possible human effects of exposure based on geocoding. Jim Fabisiak and Laura Dietz will address health effects from the mixture of chemicals released and psychosocial responses, including heightened concern for children. Tim Ciesielski will present consortium findings regarding difficulties of quantitating risk from complex toxic mixtures from East Palestine.

DNTS 29

Healthy Futures Research Study: Utilizing Somatic Mutation Rate as a Novel Biomarker for Environmental Exposures

Fredrick Schumacher
Case Western Reserve University, Cleveland, OH, USA. Case Comprehensive Cancer Center, Cleveland, OH, USA

On February 3, 2023, a Norfolk Southern train carrying hazardous chemicals, including *vinyl chloride and butyl acrylate,* derailed in the village of East Palestine, Ohio. A subsequent controlled combustion of five tankers was undertaken, resulting in the release of phosgene and hydrogen chloride into the atmosphere. While initial air sampling did not show evidence of vinyl chloride or hydrogen chloride concentrations above air quality standards, testing is ongoing. Contaminated run-off was detected in two surface water streams. For residents

of East Palestine and the surrounding communities, concern about the long-term environmental and health impacts of these exposures remains high. Due to the multi-pollutant nature of this chemical exposure, and the potential for pre-existing exposures, a quantitative approach based on biospecimens is crucial to guide subsequent disease surveillance. While chemical exposure assessment is typically done via interrogation of biospecimens in blood and urine for specific contaminants, this approach only provides a snapshot of short-term chemical exposure. The somatic mutation rate (SMR), however, provides a global overview of chemical exposures, as demonstrated by previous research, and serves as a proxy for environmental chemical exposures. Here, we propose utilizing SMR to establish a baseline for acute chemical exposure and long-term monitoring with respect to health and disease risks. Our time-sensitive response, *"Healthy Futures Research Study: Linking somatic mutation rate with baseline exposure in East Palestine"*, establishes the baseline of a chemical exposure utilizing a novel genomic biomarker as a surrogate for direct chemical concentration levels given the mixture of potential contaminants.

DNTS 30

Identifying Chemical Disaster Hazards after the EP Derailment in Real Time: Some of Our Limitations and Thoughts for Addressing Them

Timothy Ciesielski
Case Western Reserve University School of Medicine, Cleveland, OH, USA

Quantitating risk in the East Palestine disaster is not currently feasible. Even if we had a single well-characterized chemical hazard, quantitative risk assessment would still require assumptions about human exposure. However, we do not have a single hazard of concern. We do not even have a full accounting of what the chemicals of concern might be. We can get a manifest of substances on the train, but what existed after combustion, remediation interventions, passive degradation, and transport in the environment over time? What contaminants were present before the event? The relevant matrices and potential exposure pathways are daunting: outdoor air via combustion or stream aeration, indoor air via outdoor air or vapor intrusion, ground water via advection-dispersion-reaction, etc. Additionally, we have very little biomarker data and a limited snapshot for health surveillance. Finally, there is the hurdle of benchmarking this complex mix against the toxicological knowledge that we have on its individual components. Even for single compounds, CompTox searches reveal deficits in our understanding of the relevant health endpoints, and the database does not facilitate the identification of sensitive-endpoints. Having said all this, there are some feasible steps to take as we prepare for the next chemical disaster, and a few of these are being supported through NIEHS rapid funds. We could develop: 1) greater capacity for mobile nontargeted chemical assessment, 2) rapid symptom and biomarker surveillance protocols, 3) guaranteed free assessments with Environmental Medicine boarded physicians, and 4) a moratorium on claims of safety when the evidence is insufficient.

DNTS 31

Chemical and Psychosocial Stressors as Toxic Exposures after the East Palestine Derailment

James Fabisiak[1], Laura Dietz[2]
[1]University of Pittsburgh, School of Public Health, Dept. Environmental & Occupational Health, Pittsburgh, PA, USA. [2]University of Pittsburgh, School of Health & Rehabilitation Sciences, Dept. of Counseling and Behavioral Health, Pittsburgh, PA, USA

On February 3, 2023, a 150-car Norfolk-Southern freight train carrying hazardous chemicals partially derailed within the town of East Palestine, Ohio. A controlled deliberate burn was conducted to presumably prevent explosions from cars that had caught fire, increasing the emission of volatile compounds into air and water, and forcing the evacuation of residents within a 1-mile radius of the site for one week. Despite results from the EPA suggesting contaminant levels in water and air to be safe, this environmental disaster has resulted in long-term psychosocial stress for residents who have experienced adverse health effects and have continued concerns about chemical contamination. In this presentation, we will review the complex relationship between environmental and psychosocial risks unfolding in the aftermath of the East Palestine disaster. We will examine the health effects of the complex interactions between chemicals carried by the Norfolk-Southern train including vinyl chloride, 2-butoxyethanol, 2-ethylhexyl acrylate, isobutylene, butyl acrylate, and benzene, as well as the chemicals likely released by the controlled burn such as hydrochloric acid, phosgene, acrolein, and

dioxins, as well as PFAS/PFOS compounds in fire-fighting foam. Next, we will review the psychological experiences of many of the East Palestine residents, including stress regarding their health and safety, the viability of remaining in their communities, and concerns about the long-term effects on their children's development and adjustment. Our presentation will conclude with a holistic model of understanding the interplay between environmental exposures, health outcomes, and mental health, and how environmental disasters impact community response and resiliency.

DNTS 32 Richard Butcher New Investigator Award

DNTS 33-36 Developmental Neuropathology in Rodents: Chemicals versus Pediatric Pharmaceuticals Workshop - joint with BDRP

DNTS 33

Regulatory Requirements for the Assessment of Developmental Neuropathology Following Chemical and Pharmaceutical Exposure

Prägati S. Coder
Charles River Laboratories, Ashland, OH, USA

Safety assessment studies for chemicals as well as human pharmaceuticals require a detailed assessment of the effects of these substances on peri- and postnatal development. Effects on development can be studied for the organism as a whole (systemic toxicity) or as effects on specific organ systems (e.g., the developing nervous system). An assessment of developmental neuropathology (DNP) includes an evaluation of the brain and central and peripheral nervous system tissues, often in neonatal, weanling, and adult animals. Depending upon the clinical indication, pharmaceuticals may be administered to pediatric patients for brief periods (potentially during critical developmental windows) or chronically through adulthood. The mechanism of action of these products is often well understood and the dose achieved in the brain and CNS/PNS can often be accurately determined depending on the route of administration, treatment duration, and known total dose. In contrast, for industrial, agricultural, and environmental chemicals, achieved doses can vary significantly, depending upon potential exposure, and often little is known about the timing of exposure or the consequences of the effects of cumulative long-term exposure during development and through adulthood. As such, the requirements for neuropathological evaluations for chemicals vs human pharmaceuticals can be substantively different. The developmental neurotoxicity study guidelines (OECD 426 and US EPA 870.6300) and associated guidance documents describe the conduct of studies for the testing of chemicals and are fairly comprehensive with regards to study-specific requirements. In contrast, the ICH S11 guidelines supporting the development of pediatric pharmaceuticals do not specify the functional or structural assessments that should be performed and instead direct registrants to a weight-of-evidence approach to support study objectives. Thus, significant consternation exists regarding the best approach for developmental neuropathology assessment. The goal of this workshop is to bring together industry experts in the field of developmental and adult neuropathology assessment for both chemicals and human adult and pediatric pharmaceuticals.

DNTS 34

Neuropathology Evaluation of Olney Lesions in Regulatory Drug Development Toxicology Studies

Deepa Bandi Rao
Greenfield Pathology Services, Inc., Ellicott City, MD, USA

Acute neuropathology findings were first described by John Olney et al. (1989) following the administration of NMDA receptor antagonists (such as phencyclidine, MK-801, and ketamine) in adult rats, although findings in other species have been reported. Initial descriptions of the lesion included neuronal vacuolation in the cingulate and retrosplenial cortex with subsequent characterization by others to include gliosis and/or neuronal cell death. Given the potential of NMDA receptor antagonists to induce Olney lesions, the FDA often requires that sponsors submit, as a part of their IND submission, additional nonclinical safety data for therapeutic candidates with NMDA receptor antagonist properties. Such nonclinical safety data rest primarily on conducting a specialized "Olney lesion" neurotoxicity study in rats. For target populations that include infants and/or children, juvenile animal studies are often required. With the Pediatric Research Equity Act (PREA) and

Best Pharmaceuticals for Children Act (BPCA), safety assessments in juvenile animals have become common. This presentation will focus on aspects of study design and histopathology evaluation in “Olney lesion” studies in adult rats and comparative evaluations in juvenile animal studies. First, the fundamental variables impacting study design [species, strain, sex, age, number of animals per group, control groups (including details on MK-801 employed as a positive control), route of administration, duration of exposure, and sacrifice timing] will be discussed. Second, factors impacting histopathology evaluation (brain-trimming, fixation, embedding, staining, sectioning, and neuroanatomical areas) will be highlighted. Interpretation of neuropathology findings in the context of positive controls and risk assessment perspectives will be included. The objective is to provide guidance towards the optimal evaluation of therapeutic candidates requiring an Olney lesion neurotoxicity study.

DNTS 35

Developmental Neurotoxicity and Brain Morphometric Evaluations for Chemicals

Catherine A. Picut
Charles River Pathology Associates, Durham, NC, USA

Developmental Neurotoxicity (DNT) studies are designed to assess the potential for environmental chemicals to interfere with the development of the central and peripheral nervous systems. Minimum requirements for DNT studies are set forth by the US Environmental Protection Agency (EPA 870.6300) and by the Organization for Economic Cooperation and Development (OECD 426). DNT studies can also be incorporated as an arm of the Extended One Generation Reproductive Toxicity Studies (OECD 443), adding significantly to the complexity of these developmental and reproductive toxicology studies. Whether stand-alone or as an arm of the OECD 443, the DNT study design requires dosing rat dams from gestation day six to lactation day 21, in order to span the entire timeframe for neuronal development of the offspring brain. Pathology endpoints in DNT studies include brain weights, gross brain measurements, qualitative histopathology, and quantitative (morphometric) pathology. These endpoints are designed to detect adverse effects on neural cell proliferation, migration, differentiation, and myelination, but do little to detect effects on synaptogenesis. Each step in achieving these endpoints, starting from necropsy, must be done in a counter-balanced and/or blinded manner to minimize personnel and environmental bias and achieve highly homologous sections upon which the morphometry measurements are based. This session will provide a broad overview of nervous system development in the rat to help understand the reasoning behind the DNT study design and to help interpret certain pathology findings. The session will include a discussion of the Society of Toxicologic Pathology’s “best practices” to achieve dependable and reproducible morphometry data; provide guidance on determining adversity; and provide parameters to assist the pathologist in concluding whether a test article is neurotoxic from a developmental standpoint. The session will conclude with a brief overview of special stains and methodologies that may be used to further characterize or investigate lesions in the brain or other nervous system tissues.

DNTS 36

Neuropathology Evaluations in Juvenile Animal Toxicity Studies for Pharmaceuticals

Brad Bolon
GEMpath, Inc., Longmont, CO, USA

The design of neuropathology evaluations for juvenile animal studies (JAS) to develop pharmaceuticals depends on multiple factors including the nature of the test article (TA); the intended clinical use; the exposure scenario (e.g., dose, duration, and route); and prior knowledge about potential target organs—of which the central (CNS) and peripheral (PNS) nervous systems represent two among many possible targets. For TA with no known or unknown neurotoxicity profiles, neurotoxicity screening in JAS involves macroscopic observations, brain weights, and light microscopic evaluation of routine hematoxylin and eosin (H&E)-stained sections from selected neural tissues: brain (seven coronal sections for all test species), spinal cord (cervical and lumbar divisions), and sciatic nerve. Where the TA is intended to be neuroactive and/or interact with a molecular pathway in neural cells, neurotoxicity is a possible liability. In such cases, expanded neuropathology evaluation (ENHP) should be considered, which involves more sampling of the brain (1–2 sections for rodents, usually 3–8 sections for nonrodents); spinal cord (thoracic ± sacral divisions); and nerves (tibial ± fibular and maybe

others) while adding ganglia (dorsal root and often trigeminal and/or autonomic [including attached nerve roots]) and/or special neurohistological methods such as cell type-specific markers to detect cell death, glial reactions, axon and myelin integrity, and/or inflammation. Conventional tissue processing for JAS is based on fixation by immersion in neutral buffered 10% formalin and embedding in paraffin for neural tissues, so quantitative analyses like morphometric and stereological measurements are generally inappropriate. Typically, ENHP is performed only to answer particular questions (e.g., a more detailed characterization of a potential neuroactive effect) or to fulfill regulatory requests. Special procedures used for dedicated neurotoxicity studies, including fixation by intravascular perfusion and hard plastic embedding of selected tissues (to better stabilize myelin and fine cytoarchitectural details), is unnecessary. Advance consultation with an experienced pathologist is often helpful in choosing the most appropriate design for the neuropathology evaluation in JAS.

Poster Presentations

DNTS P1

Invertebrate DNT: Evaluating a PFAS Mixture from Pittsboro, NC in *Artemia franciscana*

Desiree Batangan, Sheila Cazares, Areeha Khalid, Cassandra Repole, Matt Pytynia, Andrew Hawkey
Midwestern University, Downers Grove, IL, USA

Per- and polyfluoroalkyl substances (PFAS) are among the most detected water pollutants in the United States, with each location showing unique mixtures of PFAS. However, little is known about the toxicity of PFAS within such complex and unique mixtures. The current study used an aquatic invertebrate model to evaluate the developmental neurotoxicity of the PFAS mixture found in the water supply of Pittsboro, NC (PittMix). Newly hatched *Artemia franciscana,* a native American saltwater crustacean, were housed in clean saltwater or artificial PittMix (Top 6 PFAS, Pittsboro levels to 10,000x higher concentrations) for 48h. At the end of the exposure, survival was recorded. Then, 12 shrimp per replicate completed the open field test and 20 underwent morphology analysis. Preliminary results show that PittMix did not impair survival (%) or body length (mm). For body area (mm^2), the 100-1000x concentrations reduced body size ($p < 0.05$), and this attenuated by the highest (10000x) concentration. For swimming speed in the open field test, the environmental concentration caused a reduction in swim speed ($p < 0.05$). This effect was reversed by increasing the concentration 10x ($p < 0.05$), and additional increases (100-10000x) reduced swim speed again ($p < 0.05$). Overall, these initial data suggest that PFAS mixtures can produce complex, non-monotonic effects on development and neuromotor function, potentially due to conflicting effects across compounds or the presence of effects with differing dose-response thresholds. Follow-up studies will investigate single-compound effects and interactions between PFAS, which may contribute to this non-monotonic pattern. Work supported by internal MWU start-up funding.

DNTS P2

Impulsive Behavior in Rats Perinatally Exposed to Delta-9-tetrahydrocannabinol (Δ9-THC)

Monica Carbajal[1], Laura Billings[2], Chelsea Dixon[1], Victoria Williams[1], Helen Sable[1]
[1]University of Memphis, Memphis, TN, USA. [2]Christian Brothers University, Memphis, TN, USA

Many women believe there is slight or no risk associated with using cannabis once or twice a week during pregnancy. Prenatal cannabis exposure has been linked to externalizing behaviors in offspring, including impulsivity. Most preclinical neurotoxicology studies focus on exposure to the psychoactive constituent of cannabis, delta-9-tetrahydrocannabinol (Δ9-THC). Δ9-THC is lipophilic so it crosses the placental barrier and is secreted in maternal milk, thereby exposing the fetus/neonate during gestation and lactation. Perinatal Δ9-THC exposure has altered synaptic plasticity and cell-excitability in rat prefrontal cortex (PFC) slice preparations. The PFC is involved in different facets of impulsivity and research on the effects of perinatal THC exposure on impulsive behavior is limited. Therefore, we orally exposed Wistar rat dams to 5.0 mg/kg/day Δ9-THC or vehicle starting 14 days prior to breeding and continuing until postnatal day (PND) 14. Δ9-THC did not affect maternal behavior, but postnatal weight gain (PND 0-21) of pups exposed to Δ9-THC was decreased. When male/female littermate pairs reached adulthood (PND ~65), they were assessed for impulsive action using a

differential reinforcement of low rates (DRL) task that required 15 sec between lever presses to earn a reinforcer. Δ9-THC resulted in deficits in task acquisition. During earlier sessions, Δ9-THC rats had a lower ratio of reinforced:non-reinforced trials and had a lower cumulative response latency than control rats. In addition, Δ9-THC males, but not females, earned fewer reinforcers overall than their same-sex controls. Such results suggest perinatal cannabis exposure may increase impulsive behavior in exposed offspring.

DNTS P3

Prenatal Tobacco Exposure: Pathways to Aggression and Social Competence in Kindergarten

Makenna Luzenski, Madison Kelm, Kristin Perry, Rina Das Eiden
Pennsylvania State University, State College, PA, USA

Prenatal tobacco (PTE) and tobacco-cannabis co-exposure (PTCE) are associated with children's aggressive behavior, which is negatively associated with social competence at school age. In addition, prenatal substance use is associated with maternal affective dysregulation and continued substance exposure postnatally, which may be additional pathways to risk for increased aggression and lower social competence at school age. We hypothesized that mothers who used tobacco/cannabis would report higher maternal affective dysregulation and postnatal substance exposure, and children would display higher aggression and lower social competence. Low-income, diverse mother-child dyads were recruited in their first trimester (N=247). Substance exposure was assessed using biomarkers and self-report. Mothers reported affective dysregulation at infant-toddler ages and child aggression at kindergarten. Teachers reported child aggression and social competence. All measures were reliable. The path analysis provided an excellent fit to the data, $\chi^2(6)=6.18$, $p=0.40$; CFI=0.99; $SRMR$=.03; $RMSEA$=.01. PTE predicted more maternal affective dysregulation during early childhood (β=0.50, p=.002), which predicted higher maternal-reported child aggressive behavior (β=0.38, p<.001), but not teacher-reported aggressive behavior. However, higher teacher-reported child aggressive behavior predicted less social competence (β=-0.37, p<.001). PTCE was associated with continued postnatal tobacco and cannabis exposure, but postnatal substance exposure was not predictive of child outcomes. Early childhood interventions that target aggressive behavior could be especially helpful for substance-exposed children. Future work could explore sex differences in the direct link between prenatal substance exposure and kindergarten outcomes, as well as shared-method variance in the measurement of child outcomes across contexts.

DNTS P4

Trajectories of Prenatal Tobacco and Cannabis Exposure: Implications for Child Inflammation

Madison Kelm, Kristin Perry, Rina Das Eiden
Pennsylvania State University, State College, PA, USA

Chronic, heavy prenatal substance exposure may alter child inflammation (IF). Changes in substance use across pregnancy may be more predictive of child IF than exposure at any single timepoint. We examined trajectories of tobacco and cannabis use from preconception-third trimester, and associations with child IF at kindergarten age. Mothers (N=262; M_{Years}=24.09; 51.91%=Black/African American; 46.56% girls), oversampled for tobacco exposure and matched with a demographically-similar comparison group, reported their substance use at preconception and each trimester of pregnancy using a validated calendar-based interview. At Kindergarten-age, child saliva samples were assayed for three markers of IF, interleukin-6 (IL-6), C-reactive protein (CRP), and secretory immunoglobulin A (SIgA). Trajectories of substance use were assessed using latent class growth analysis; associations with child IF were evaluated using the BCH approach and one-way ANOVA. The four-class model provided the best fit to the data. The patterns identified included: Low/No Tobacco+Low/No Cannabis (Class 1, n=178), Medium Tobacco+High Cannabis (Class 2, n=10), High Tobacco+Low/No Cannabis (Class 3, n=50), and High Tobacco+Medium Cannabis (Class 4, n=24). There were no group differences for CRP and SIgA, however, there were differences for IL-6, $\chi 2=34.33$, $p < .001$. Children in Class 2 had lower levels of IL-6 (M=5.79) than those in Class 1 (M=48.69, p<.05), Class 3 (M=29.92, p<.01), and Class 4 (M=73.65, p<.001). Results suggest that high levels of prenatal cannabis exposure in combination with medium levels of tobacco exposure are associated with lower salivary IL-6, which may indicate altered oral immune function.

DNTS P5

Sex-Specific Behavioral Changes Following Early Life Nicotine Exposure

Mia Trupiano[1], Deirdre McCarthy[1], Devon Graham[1], Loren Peeters[2], Russell Brown[2], Yuan Wang[1], Gregg Stanwood[1], Pradeep Bhide[1]
[1]Florida State University, Tallahassee, FL, USA. [2]East Tennessee State University, Johnson City, TN, USA

Maternal smoking during pregnancy is a major public health concern due to potential adverse effects on both the mother and offspring. For example, early life nicotine exposure is associated with an increased risk for ADHD, learning disabilities, depression, and epilepsy in the offspring. Preclinical models provide valuable insights into the behavioral and neurobiological effects of nicotine exposure. While most studies use inbred mouse strains, data on the behavioral consequences of early life nicotine exposure on outbred strains are limited. Outbred strains may represent the human populations more closely than inbred strains. Therefore, we examined the behavioral consequences of early life exposure to nicotine in the outbred Swiss Webster strain of mice. Female mice were exposed to plain drinking water or water containing nicotine (200 µg/ml) beginning 3 weeks prior to conception and continuing throughout pregnancy and nursing. A battery of behavioral assays was performed in adult male and female offspring. Early life nicotine exposure produced sex specific deficits in multiple measures of motivated behavior, demonstrating long-term consequences of early life nicotine exposure in an outbred mouse strain.

DNTS P6

Unveiling the Effects of Nicotine and E-Cigarettes on GABA Neuron Migration

Amber Parnell, Deirdre McCarthy, Pradeep Bhide
Florida State University, Tallahassee, FL, USA

E-cigarettes are marketed as a safer alternative to combustible cigarettes despite accumulating evidence to the contrary. The increase in the popularity of e-cigarettes among pregnant women necessitates an investigation into the impact of e-cigarette use during pregnancy on the developing fetus. The e-cigarette aerosol contains nicotine as well as a cocktail of chemicals (the e-liquid), which alone can produce adverse health effects. Using a Swiss Webster mouse model of whole-body e-cigarette aerosol exposure, we show that prenatal exposure to e-cigarette aerosol, but not e-liquid aerosol (i.e., without nicotine), reduces the density of GABA neurons in the embryonic dorsal forebrain, the future cerebral cortex. To gain mechanistic insights into nicotine's effects, we exposed explants of the embryonic day 15 basal forebrain (ganglionic eminence), the source of GABA neurons of the dorsal forebrain, to nicotine (2, 20 or 200 µM) or PNU282987, a selective α7 nicotinic acetylcholine receptor agonist (0.1, 1 or 10 µM). Our findings support the hypothesis that prenatal e-cigarette exposure alters GABA neuron development and changes in the development of cortical GABA circuits may contribute to the neuropathology associated with developmental disorders such as ADHD, autism spectrum disorder, and schizophrenia.

DNTS P7

The Impact of Prenatal and Postnatal Substance Use and Early Adolescent Processes on Late Adolescent Substance Use

Rachel Marcus, Rina Das Eiden
Pennsylvania State University, State College, PA, USA

We examined a developmental cascade model from prenatal polysubstance exposure (PSE) to late adolescent (LA) substance use (SU), with postnatal SU, and early adolescent (EA) parent monitoring, peer delinquency, and SU as mediating processes. Caregiver-child dyads oversampled for pregnancy SU (n = 216) were recruited at birth and assessed until LA (15-17 years). The sample was low-income and diverse (72% Black). PSE was measured using biomarkers and self-reports, postnatal SU using calendar-based self-reports from birth to EA, adolescent reports of caregiver's knowledge of their behavior (parent monitoring); and peer group engagement in delinquent behaviors (peer delinquency) were measured at EA. Both EA and LA SU were measured with biomarkers and self-reports to create a sum of the number of substances used. All measures were reliable. Path analysis indicated no direct associations between PSE and adolescent SU; PSE predicted

postnatal SU (b = .47, p <.001), but postnatal SU did not predict any EA processes. EA SU predicted LA SU (b = .57, p < .001). Higher levels of EA parental monitoring were associated with lower EA SU (r = -.26, p = .001) and lower peer group delinquency behaviors (r = -.29, p < .001). Peer group delinquency behaviors were not associated with EA SU (r = .14, p = .07). There were sex differences within EA, such that females had higher EA SU than males (b = .38, p = .01). Thus, for children with PSE, EA factors may influence LA SU more than caregiver SU.

DNTS P8

Neurodevelopmental Vulnerability to Gestational Ozone Exposure

Vishnupriya Alavala[1], Sarah Brent[1], Christopher Canal[1], Joseph Wang[1], Russell Hunter[2], Matthew Campen[2], Andrew Ottens[1]
[1]Virginia Commonwealth University, Richmond, VA, USA. [2]University of New Mexico, Albuquerque, NM, USA

Ambient air pollution accounts for about 4.2 million premature deaths annually. Ozone is a reactive air pollutant that induces cellular damage, but little is known about its neurodevelopmental effects. To inquire about ozone on the fetal brain, pregnant Sprague-Dawley rats were exposed once to 0.3 ppm of O3 or filtered air (FA) via whole-body inhalation at gestational day (GD) 10 or GD20, during which key neural processes are occurring, while control animals received a sham FA exposure at both time points. Brain tissue was collected at GD21. Discovery neuroproteomics were interrogated through Ingenuity Pathway Analysis and immunohistochemistry of emblematic markers. Results demonstrate ozone's time-dependent gestational effect on the fetal neuroproteome and changes to cell proliferation-migration and circuit formation. GD10 exposure may represent an abnormal trigger of reactive oxygen species, affecting mitochondrial signaling and cell proliferation/differentiation regulation. Proteomics and microscopy show decreased neuritogenesis, although differences between GD10 and GD20 from proteomics could not be confirmed with Gap43 and Map2 staining. Ozone exposure appears to have reduced apoptosis, potentially leading to proliferation and haphazard migration. Cytochrome C responses decreased only at GD20 by staining but may have decreased with time post-O3. Decreased Caspase 3 outside of the subventricular zone may be due to its role in neurite and synapse maturation. GD10 showed excessive mitogenesis, a potential maladaptive response to oxidative stress that can lead to juvenile behavioral disorders. Future studies will employ microscopy and neuroproteomics data to elucidate potential long-term neurodevelopmental consequences of ozone on urban children.

DNTS P9

Dopamine And Serotonin Signaling Following Developmental Benzo[a]pyrene Exposure in *Cyp1b1(-/-)* Knockout and Wild Type Mice

Mackenzie Feltner, Asia Good, Katelyn Clough, Emma Foster, Christine Perdan Curran
Northern Kentucky University, Highland Heights, KY, USA

Benzo[a]pyrene (BaP) is an aryl-hydrocarbon receptor (AHR) agonist known to exert neurotoxic effects on the developing brain. Deficits in learning, memory, and dopaminergic pathways have been linked to BaP exposure in animal studies. We modeled human genetic differences at the AHR and CYP1 loci using mice to determine susceptibility to developmental BaP neurotoxicity. Pregnant *Cyp1b1(-/-)* and *Cyp1b1(+/+)* dams were treated from gestational day 10 to postnatal day 25 with 10mg/kg/day BaP in corn oil-soaked cereal or the corn oil vehicle. One male and one female from each litter were randomly selected for behavioral testing. We quantified neurotransmitter levels in adult mice following neurobehavioral testing. At P120, striatum, hippocampus, prefrontal cortex, and hypothalamus were collected. High-Performance Liquid Chromatography with Electrochemical Detection was used to measure dopamine, serotonin and their metabolites DOPAC and 5HIAA. We found that BaP exposure significantly decreased dopamine ($P < 0.05$) and DOPAC ($P < 0.001$) in the striatum. There were no differences in the hippocampus for dopamine, but significantly higher serotonin levels in BaP-treated mice ($P < 0.05$). Genotype was highly significant in the hypothalamus with lower levels of DOPAC in *Cyp1b1(-/-)* mice ($P < 0.01$) and a trend for significance for dopamine ($P = 0.058$). Serotonin levels were significantly lower in *Cyp1b1(-/-)* mice, but there was no difference in the levels of 5-HIAA. There was no effect of BaP treatment in the hypothalamus. Together, these data suggest that BaP exposure during early brain development can have persistent effects on monoamine neurotransmitters in adults.

DNTS P10

Gut Microbiome Differences in *Cyp1a1(+/+)* Wild Type and *Cyp1a1(-/-)* Knockout Mice With and Without Developmental Exposure to Benzo[a]pyrene

Duong Pham, Katelyn Clough, Mackenzie Feltner, Jade Perry, Joshua Cooper, Christine Perdan Curran
Northern Kentucky University, Highland Heights, KY, USA

Changes in the gut microbiome are now strongly associated with changes in neurodevelopment and neuroinflammation. Our previous work uncovered genetic susceptibility to benzo[a]pyrene (BaP) neurotoxicity in *Cyp1* knockout mice. Since BaP has been associated with changes to the gut microbiome, we hypothesized that these changes could be at least partially responsible for the adverse effects we found. Pregnant dams were treated with 10 mg/kg/day of BaP in corn oil-soaked cereal or the vehicle from gestational day 10 to weaning postnatal day 25. We collected feces from *Cyp1a1(+/+*) wild type and *Cyp1a1(-/-)* knockout offspring when they reached young adulthood at P60. Using shotgun metagenomics, we found striking differences at the species level when comparing *Cyp1a1(+/+)* wild type and *Cyp1a1(-/-)* knockout mice. All mice microbiomes had high levels of *Muribaculaceae* (Phylum Bacteroides). However, wild type mice were colonized by the beneficial species *Akkermansia muciniphila*, but not the knockouts. In contrast, *Helicobacter* was only found in the knockouts and levels were highest in BaP-exposed *Cyp1a1(-/-)* knockout offspring. Together, these findings suggest that the balance of beneficial vs. deleterious species is more favorable in wild type mice.

DNTS P11

Associations of Prenatal Maternal Psychosocial Stress and Depression with Neurodevelopmental Outcomes in 7.5-Month-Old Infants in the ECHO.CA.IL Prospective Birth Cohorts

Nicholas Cragoe
University of Illinois Urbana-Champaign, Urbana, IL, USA

Exposure to maternal stress and depression during pregnancy can increase the likelihood of preterm birth, lower birth weight, and impair various domains of physical and neurodevelopment. The ECHO.CA.IL cohort includes the Chemicals in Our Bodies (CIOB; San Francisco, CA) and Illinois Kids Development Study (IKIDS; Urbana-Champaign, IL) prospective cohorts. In this analysis, we examined associations between two prenatal measures of maternal stress (perceived stress scale (PSS) and stressful life events (SLE)), as well as prenatal maternal depression (IKIDS: Edinburgh Postnatal Depression Scales; CIOB: Center for Epidemiologic Studies Depression Scale), and five domains of neurodevelopment during infancy via the Ages & Stages Questionnaire (ASQ) administered at 7.5 months (N=428). Covariate-adjusted multivariable linear regression models were used to identify patterns of association. CIOB mothers were racially/ethnically diverse (mainly Asian, Hispanic and white, although relatively few Black mothers), while IKIDS mothers were disproportionately white. Cohort demographics were otherwise similar. CIOB mothers reported higher levels of prenatal stress compared to IKIDS mothers. Negative associations of prenatal stress with ASQ domains were found. Two of the more robust findings included the association of PSS scores with fine motor skills (β-0.2595, CI=-0.5219; 0.0029) and SLE with communication (β=-2.9245, CI=-6.1643; 0.3152). Depression showed no clear associations with ASQ scores. These results suggest that higher prenatal stress was associated with delayed motor skills and communication development. Additional research is needed to better understand the relationship between prenatal maternal stress and depression and overall infant development.

DNTS P12

Prenatal Substance Exposure, Intergenerational Maltreatment, and Internalizing and Externalizing Symptoms in Early Adolescence

Meeyoung Min[1], Sonia Minnes[2], June-Yung Kim[3], Lynn Singer[2]
[1]University of Utah, Salt Lake City, UT, USA. [2]Case Western Reserve University, Cleveland, OH, USA.
[3]University of North Dakota, Grand Forks, ND, USA

Few studies examined the impact of intergenerational continuity of maltreatment in the context of prenatal substance exposure (PSE). This study investigates associations between PSE, intergenerational maltreatment, and internalizing and externalizing symptoms. Participants were 273 birth mother-child (44% male) dyads,

enrolled at birth. PSE (amount of alcohol, tobacco, marijuana, cocaine), maternal history of childhood maltreatment, and maternal child maltreatment (MCM) at child age 10 were all specified as latent variables. Maternal psychological distress at child age 10 and mother- and child-reported internalizing and externalizing symptoms at child age 12 were assessed. Structural equation modeling evaluated MCM and maternal psychological distress as mediators linking maternal childhood maltreatment and PSE to maternal and child-reported symptoms, adjusting for covariates (maternal education, child race and sex, violence exposure, ecological assets). More than half of the mothers (n=152) reported childhood maltreatment. Although PSE was related to MCM (p= .025), maternal childhood maltreatment was not (p= .066). MCM was related to child-reported internalizing (p= .01) and externalizing (p= .03), and mother-reported externalizing (p= .003) symptoms. PSE had a direct effect on child-reported externalizing symptoms (p=.009), while maternal childhood maltreatment was marginally related to child-reported internalizing symptoms through the two mediators (p= .078). Both maternal childhood maltreatment and PSE were indirectly related to mother-reported internalizing and externalizing symptoms via maternal psychological distress (all p < .05). PSE and maternal childhood maltreatment increase the vulnerability of offspring psychopathology, reinforcing the need for trauma-informed perinatal care to improve postpartum parenting.

DNTS P13

Parvalbumin as a Target for Thyroid Hormone Mediated Developmental Neurotoxicity

MaryAnn Hawks[1,2], Jermaine Ford[1], Ryne Thomas[1,2], Mary Gilbert[1]
[1]US Environmental Protection Agency, Durham, NC, USA. [2]Oak Ridge Institute for Science and Education, Oak Ridge, TN, USA

Thyroid hormones (TH) are critical for neurodevelopment. Parvalbumin (Pvalb), a calcium binding protein in a large population of cortical inhibitory interneurons has been shown to be sensitive to TH disruption. Pvalb-expressing interneurons are critical to neural network formation and disruption during brain development has been linked to neurodevelopmental disorders. To determine if Pvalb-expression is altered by moderate TH perturbations, we exposed pregnant rats to low doses of a known TH-synthesis disruptor, propylthiouracil (PTU, 0, 0.5, 1, 3ppm drinking water). We aimed to characterize, quantitatively, Pvalb-expression in different brain regions using proteomic and immunohistochemical (IHC) techniques and determine the utility of these approaches for the detection of TH-dependent neurotoxicity. Dams were exposed from gestational day 6 through postnatal day (PN) 22 and brains were collected from offspring on PN 14 and PN22 (2 males/litter/dose/age). One brain at each age was immersion fixed for Pvalb IHC, the other dissected, anterior cortex removed, flash-frozen, and prepared for Pvalb targeted proteomics using LC-MS. Clear age-dependent increases in Pvalb-expression were noted by IHC, a reduction in Pvalb-expression notable in all treatment groups at both ages relative to controls within the neocortex (n=2/dose group). Targeted proteomics proved to be less sensitive at PN14, however at PN22 peak areas of Pvalb were measurable showing a dose dependent Pvalb protein reduction in the 1 and 3ppm groups compared to controls (n=6-8/dose group). These findings extend previous observations of TH-dependent neurotoxicity to milder levels of TH insufficiency, revealing negative impacts on this critical neuronal population. *Does not reflect EPA policy.*

DNTS P14

Alterations in Behavior and Proteomics Following Perinatal Agonism of GABA-Gated Chloride Channels in Long Evans Rats

Emily Pitzer[1], Tracey Beasley[1], Garyn Jung[1], Kathy McDaniel[1], Renee Fisher[2], Charan Ganta[2,3], Jean Harry[4], Ronald Herbert[2], Heather Jensen[2], Robert Sills[2], William Padgett[1], Witold Winnik[1], David Herr[1]
[1]NETB, PHITD, CPHEA, ORD, US EPA, RTP, NC, USA. [2]CMPB, NTP, NIEHS, RTP, NC, USA. [3]Inotiv, RTP, NC, USA. [4]MTB, NIEHS, RTP, NC, USA

The period of neurodevelopment is vulnerable to various insults, including those produced by environmental compounds. Here we used prototypical neurotoxicants to explore both behavioral and molecular alterations that can occur following chemical exposure during development. Pregnant Long Evans rats were gavaged with emamectin benzoate (EB; 3.78 mg/kg in 5 mL/kg DI water) a positive allosteric modulator to GABA-gated chloride channels, or vehicle, from gestational day 6 to postnatal day (PND) 21. For proteomic experiments, pup region-specific brain tissues were collected throughout the postnatal period of exposure (PND2, 8, 15, 22).

Cortex and cerebellum samples were assessed using Orbitrap LC-MS and Proteome Discover software, and further analyzed in Ingenuity Pathway Analysis. Behaviors were assessed throughout the experimental period, starting as early as PND2 as well as into adulthood. Behavioral assays included pup righting, a modified functional observational battery, locomotor activity, novel object recognition, acoustic startle response, and the Morris water maze. Proteomic analyses showed that protein signatures for EB-treated rats differed by region, sex, and age, indicating alterations in neuronal signaling and neurodegeneration pathways emerging at later timepoints (PND15v22). EB-exposed offspring displayed altered ontogeny of locomotor activity, decreased startle response, and uncoordinated hindlimb movements that began in early postnatal weeks and persisted into adulthood. Overall, we observed both behavioral and proteomic alterations following perinatal EB exposure, which can aid in understanding how environmental compounds can impact neurodevelopment. Follow-up measures include assessing EB treated animals for histopathology. *This abstract does not necessarily reflect US EPA policy*.

DNTS P15

The Cincinnati Combined Childhood Cohort (C4): Data Harmonization Across Longitudinal Cohorts

Melinda C. MacDougall[1], Yingying Xu[1], Jeff Burkle[1], Patrick H. Ryan[1,2], Kim M. Cecil[1,2], Kimberly Yolton[1,2]
[1]Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA. [2]University of Cincinnati, Cincinnati, OH, USA

The Cincinnati Combined Childhood Cohort (C4) pools data from two longitudinal cohorts in the Cincinnati, Ohio region to increase analytic power to examine environmental exposures and outcomes in adolescence and young adulthood. The Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) enrolled children aged ~6 months between 2001-2003. The Health Outcomes and Measures of the Environment (HOME) Study enrolled pregnant women between 2003-2006. The harmonized dataset includes 1182 participants (762 CCAAPS, 420 HOME). Previously collected data from both cohorts from baseline to age 12 years were aligned using the crosswalk-cataloging-harmonization process. Candidate variables were listed for each cohort, variables were mapped to categories of interest, and response levels between cohorts were calibrated. Variables harmonized include demographics, biomarkers, air pollution exposure, growth measures, and neurobehavioral assessments including IQ, behavior, and mental health. Overall, there are slightly more males (52%) than females. Participants self-identified as 70% white, 23% black, and 7% other/multi-racial. Mean age of mothers at delivery was 28.9 years, and 92% of participants were born full-term. At baseline, 44% families had an annual household income of $40,000-89,000, and 49% of mothers had a college degree. At age 12, children self-reported depression (23%) or anxiety symptoms (9%). Mean child IQ was 101 (measured at age 12, 8, or 5 years). Multiple analyses are published or in progress linking early life exposures with age 12 outcomes. Data collection is ongoing at ages 17-22 years and focuses on exposure to air pollution and other environmental toxicants and mental health and neuroimaging outcomes.

DNTS P16

Identification of Studies that Evaluate Effect Modification of Methylmercury (MeHg) Developmental Neurotoxicity (DNT) by Nonchemical Stressors

Deborah Segal, Rebecca Nachman, Onyemaechi Nweke, Elizabeth Radke, Leonid Kopylev
USEPA, Washington, DC, USA

Systematic review methods were applied to identify studies with information on susceptibility factors, such as nonchemical stressors, that may modify the health effects of MeHg DNT. The results presented here pertain to the subgroup of studies that provided information on possible effect modification by nonchemical stressors, such as low socioeconomic status (SES), psychosocial stress, depression, and other social or psychological conditions. The first step was a literature search that was conducted as part of the development of an updated MeHg IRIS Assessment utilizing PubMed, Web of Science, Toxline, Science Direct, and SCOPUS. Literature search results were then filtered using SWIFT Review to capture references on human studies. The resulting studies underwent title/abstract and full-text screening in DistillerSR and were considered further if they contained information on human susceptibility factors for MeHg DNT as outlined by PECO criteria. Effect modification was generally assessed in these studies by looking for stratified analyses or the inclusion of interaction terms in analyses. The literature search yielded 17,661 references. Filtering for potential

epidemiology references in SWIFT Review resulted in 7,423 studies being screened in DistillerSR. As a result, 17 studies were identified that considered susceptibility factors related to SES, stress, or other social and psychological conditions. The majority of these studies found evidence of effect modification, although the direction of the modification was inconsistent. *The views expressed are those of the authors and do not necessarily represent the views or policies of the USEPA*.

DNTS P17

Cognitive and Behavioral Effects of Whole Brain 18 Gy Single Fraction Proton Irradiation in Adult Sprague Dawley Rats at 1 Gy/S, 60 Gy/S, or 95 Gy/S (FLASH)

Michael Williams[1,2,3], Samantha Regan[1,2], Adam Fritz[2], Brooke Gollaway[2], Anthony Mascia[1,3], Ralph Vatner[1,3], John Perentesis[1,2,3], Charles Vorhees[1,2,3]
[1]University of Cincinnati College of Medicine, Cincinnati, OH, USA. [2]Cincinnati Children's Research Foundation, Cincinnati, OH, USA. [3]Cincinnati Children's/University of Cincinnati Proton Therapy and Research Center, Cincinnati, OH, USA

Ultra-high dose rate radiation (FLASH) may result in cognitive protection. To test this hypothesis, adult male Sprague Dawley rats received whole brain irradiation with 18 Gy protons at a conventional dose rate of 1 Gy/s (Conv), FLASH at 60 Gy/s (FLASH-60) or 95 Gy/s (FLASH-95), or sham irradiated (Control) (n = 22/group). Rats were tested in open-field, acoustic startle (ASR) and tactile startle (TSR) with or without prepulses, novelty preference, radial water maze (RWM), Morris water maze (MWM) acquisition and reversal, Cincinnati water maze configuration-A (CWM-A), MWM shift and shift reversal, CWM configuration-B (CWM-B), and novel object recognition. Compared with the Control group: 1) Irradiated rats weighed less. 2) Irradiated rats had decreased locomotion and an elevated TSR. The FLASH-95 rats also had an elevated ASR. 3) In the MWM, the Conv and FLASH-60 rats demonstrated decreased path efficiency, while the FLASH-95 group was intermediate. 4) In MWM shift and shift reversal, irradiated rats took longer and had reduced path efficiency. 5) In the CWM-A, irradiated rats took longer and made more errors, but there were no differences in the CWM-B. 6) No differences were found in novelty tests or the RWM. 7) The FLASH-95 group exhibited dopaminergic and cytokine changes. Brain irradiation at both conventional and FLASH dose rates impacted the striatum and hippocampus but reduced cognitive toxicity from FLASH irradiation was not found at 18 Gy. Future investigations should focus on determining the optimal FLASH dose and dose rate for observing neuroprotection after brain irradiation.

DNTS P18

Pathways From Prenatal Cocaine Exposure to Regular Marijuana Use During Emerging Adulthood

Sonia Minnes[1], Meeyoung Min[2], Sun Kyung Kim[1], Lynn T. Singer[1]
[1]Case Western Reserve University, Cleveland, OH, USA. [2]University of Utah, Salt Lake City, UT, USA

Few studies have investigated pathways to regular marijuana use in prenatally drug exposed emerging adults. This study considers the indirect effects of executive function (EF), externalizing behavior (EXT) and 15-year marijuana use on the relationship between prenatal cocaine exposure (PCE) and regular marijuana use at 21 years. Participants were 317 (161 PCE, 156 non-prenatally cocaine-exposed (NCE)) youth enrolled at birth. Frequency of marijuana use at age 21 (regular $\geq$ 1-7 times/week vs none or maximum <1 time/week) was assessed using the Substance Abuse Module (SAM). Caregiver report of EF (Global Executive Composite (GEC)) and youth-reported EXT behaviors were assessed at age 12 and marijuana use at 15 years. Structural equation modeling evaluated GEC, EXT, and marijuana use at 15 as mediators linking PCE to marijuana use at age 21, adjusting for covariates. Forty-three percent of emerging adults reported regular use of marijuana. PCE was related to GEC (p=.008), EXT (p=.02), and 15-year marijuana use (p=.014). Two indirect pathways of PCE on 21-year regular marijuana use were identified: one via 12-year GEC and 15-year marijuana use and another via 15-year marijuana use. Externalizing behavior was not related to 15-year or to 21-year marijuana use. Early identification of EF problems may assist in preventing substance use at age 15 and regular use at age 21. Given the negative consequences of marijuana use, efforts to address early cognitive factors may prevent initiation and regular use of marijuana among prenatally cocaine-exposed emerging adults.

DNTS P19

Maintaining Balance: Examining Deiodination of Thyroid Hormones in the Developing Brain

Ryne Thomas, Jermaine Ford, Carter Keuhn, MaryAnn Hawks, Mary Gilbert
US EPA, Durham, NC, USA

Thyroid hormones (TH) regulate development, growth, and metabolism. Deiodinases (DIOs) are metabolizing enzymes that activate (Dio2) and deactivate (Dio3) THs, exerting the precise local control over TH action that is critical for brain development. In vitro assays have identified chemicals with DIO activity, yet little is known of their potential effects in intact mammalian systems. An LCMS-based assay we previously developed to assess DIO in liver was optimized to examine DIO activity in developing rat brain. Microsomes were prepared from cortex of rat pups on postnatal days (PN)2, 14, and 60. To examine the selective action of Dio2, Dio3 was blocked with desethylamiodarone. Similarly, morin hydrate isolated action of Dio3 by blocking Dio2. Separate aliquots of equal protein content were spiked with T3, T4, or rT3, incubated in phosphate buffer and TH analytes measured by LCMS. We then investigated if alterations in DIO activation would be measurable ex vivo in cortical tissue after in vivo exposure to iopanoic acid (IOP), a pan-inhibitor of DIOs. Our three main findings include: successful isolation of functional microsomes from rat brain, demonstration of age-dependent differences in DIO activity, and observation of altered DIO activity in tissue derived from IOP-exposed rats. Dio2 activity peaked on PN14, presumably enhancing TH action during a period of rapid brain development. Inversely Dio3 activity was highest on PN2, limiting TH action at this young age. This assay enables the assessment of xenobiotics effects on DIO activity and potential neurotoxic impacts on the developing brain. *Does not reflect EPA policy*.

DNTS P20

A Faster, Greener Approach to Thyroid Hormone Analysis in Brain

Carter Kuehn[1,2], Mary Gilbert[2], Jermaine Ford[2]
[1]Oak Ridge Institute for Science and Education, Oak Ridge, TN, USA. [2]Environmental Protection Agency, Research Triangle Park, NC, USA

Across all stages of life, thyroid hormones (THs) are important physiological regulators and play critical roles in brain development. Predicting potential neurotoxicity of TH disruptors would be markedly improved by examining hormone concentrations directly in the brain. Unfortunately, measurement of TH in tissue, especially the lipid-laden brain, is rife with challenges. To properly measure these low level THs, extensive sample cleanup is required to ensure that all matrix effects from lipids are fully removed to not impact liquid chromatography mass spectrometry (LCMS) analysis. The current in-house TH extraction procedure from rat brain performs reasonably well, yet the multi-step protocol can be prone to analyte loss and analyst error, in addition to undesirably requiring the use of hazardous chloroform. In this work, we sought to improve this procedure by mitigating the removal of lipids from brain tissue using the commercially available product, Cleanascite™, to develop a greener and more streamlined approach. Cleanascite™ is a non-ionic, solid phase lipid removal agent that can be added to homogenized tissue and centrifuged to separate trapped lipids. Using rat brain tissue homogenized in methanol and spiked with ^{13}C-labeled THs, we developed an extraction procedure utilizing Cleanascite™ and solid phase extraction (SPE) that achieved promising TH recoveries (N=6, average recovery 3,3' T2: 98.4%, T3: 95.1%, rT3: 96.0%, T4: 93.6%), as measured by LCMS. Future work will involve optimizing SPE parameters to minimize matrix effects while continuing to maximize recovery of THs from brains of fetal and neonatal rats. *Does not reflect agency policy*.

DNTS P21

Advancing Computer Vision in High-Throughput Larvae Zebrafish: Developmental, Morphological, and Mortality Screening

Adrian J. Green[1], Arpit Tandon[1], Lisa Truong[2], Brian Howard[1], Robyn L. Tanguay[2], Ruchir Shah[1]
[1]Sciome, LLC, Research Triangle Park, NC, USA. [2]Oregon State University, Corvallis, OR, USA

The advent of deep learning has revolutionized the field of bioinformatics, particularly in the classification of images. We have extended these advancements through a novel approach that leverages convolutional neural network (CNN) model architectures from ResNet and EfficientNet families in the analysis of high-throughput toxicological screening of zebrafish larvae. This study employed a substantial dataset comprising

approximately 41,000 dorsal images to train its classification models. The study found that classification models for mortality at 24 hours post-fertilization (hpf), mortality at five days post-fertilization (dpf), and abnormalities at five dpf had F1 scores of 0.97, 0.94, and 0.81, respectively. These results underscore the models' effectiveness in certain areas, while also highlighting the challenges of accurately predicting developmental abnormalities. Addressing the challenge of limited annotated datasets and the subtle differences between phenotypes, our method demonstrates an impressive average accuracy in mortality classification. Our classification of morphological abnormalities can still be improved, but the initial results are highly encouraging, marking a significant improvement over other machine learning methods. This study not only showcases the potential of deep learning to automate morphological screening of larval zebrafish, but also paves the way for more advanced research into environmental influences on embryonic development.

DNTS P22

Artificial intelligence (AI)-Driven Morphological Assessment of Zebrafish Embryo for Developmental Toxicity Chemical Screening

Arpit Tandon[1], Brian Howard[1], Adrian J. Green[1], Ruchir Shah[1], Alex B. Merrick[2], Keith Shockley[2], Helen Cunny[2], Kristen Ryan[2], Jui-Hua Hsieh[2]
[1]Sciome, LLC, Research Triangle Park, NC, USA. [2]National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA

The Systematic Evaluation of the Application of Zebrafish in Toxicology (SEAZIT) project aims to provide a scientific basis for the routine use of zebrafish in toxicological evaluation of chemicals. To support this effort, we developed deep-learning models using labeled image data to classify over 20 changes in larval morphological and 11 segmentation areas collected from embryos exposed to various test substances for five days. We developed a customized multi-view convolutional neural network (MVCNN) classification model trained on the 46,706 distinct dorsal and lateral views of each embryo from the Vertebrate Automated Screening Technology (VAST) BioImager platform. The study found that MVCNN classification models (14 out of 20) had very good F1 scores of over 0.80. Furthermore, we developed segmentation models targeting 11 specific organs for more comprehensive analysis. Our segmentation models exhibit higher accuracy, with 9 out of 11 organs achieving an Intersection over Union score surpassing 0.80. The classification models show high accuracy for several common malformations evaluated. This enables researchers to quickly screen the bulk of zebrafish embryo images exposed to different chemicals, while allowing expert screeners to focus on more ambiguous or unusual images. Additionally, our segmentation models are very good at identifying most organs. This lets users more accurately measure the effects of a chemical exposure and do a more in-depth analysis of the morphological changes that happen as a result.

DNTS P23

Persisting Neurobehavioral Toxicity after Early Developmental Exposure to Lead and Ethanol in Zebrafish

Kala Nwachukwu, Xiaduo Cheng, Namrata Pradeep, Kennedy Hill, Asha Barnes, Edward D. Levin
Department of Psychiatry and Behavioral Medicine, Duke University, Durham, NC, USA

Developmental lead and ethanol exposures are still too common and cause persisting neurobehavioral toxicity. However, little is known about their co-exposure. This study evaluated in zebrafish the interaction of lead and ethanol in early development, assessing neurobehavioral effects during juvenile, adolescent, and adult life phases. Zebrafish were exposed to water, 3μM lead, 0.1% ethanol, or both during the first five days post-fertilization. No significant effects were seen during the juvenile 30-day-old motility test in light and dark. During adolescence, lead effects were detected. In the novel tank diving test, there was a significant lead x minute interaction. Early developmental lead exposure caused more diving early in the test session, indicative of greater anxiety-like behavior. In the tap startle test, there was a significantly greater startle response in lead treated fish than controls. In adult testing, there were effects of both lead and ethanol exposure. In the novel tank test, there was a significant ethanol x lead interaction. Ethanol-exposed fish had a greater anxiety-like phenotype, swimming significantly closer to the bottom of the tank. Interestingly, co-exposure with lead significantly attenuated this effect. Additionally, the predator response test revealed developmental ethanol exposure significantly increased fleeing relative to the fish without ethanol exposure, while both lead and

ethanol caused significant hypoactivity in predator response. Altogether, our findings indicate that lead and ethanol individually have negative persisting neurobehavioral impacts that become more prominent in adulthood versus adolescence with important interacting influences. *Supported by the Duke University Superfund Research Center ES010356.*