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DNTS 1

Functional correlation of genome-wide DNA methylation profiles in genetic and teratogenic neurodevelopmental disorders

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An expanding range of hereditary genetic syndromes are characterized by genome-wide disruptions in DNA methylation profiles referred to as episignatures. Episignatures, detectible in peripheral blood, are distinct, highly sensitive, and specific biomarkers that have recently been applied in clinical diagnosis of genetic syndromes. Episignatures are contained within the broader disorder-specific genome-wide DNA methylation changes which can share significant overlap amongst different episignature conditions. In this presentation, we describe disorder-specific and overlapping genome-wide DNA methylation in over 100 genetic syndromes. We expand by presenting emerging evidence of genome-wide disruption of DNA methylation and diagnostic episignatures in patients affected by teratogenic exposures. These changes show significant enrichment for gene promoters and CpG islands and disproportionally affect gene pathways and networks related to neurodevelopment, including neuronal generation and differentiation, and axon guidance. This work advances beyond the demonstrated diagnostic utility of DNA methylation and provides evidence of episignatures as a key functional element in the molecular etiology of genetic and teratogenic neurodevelopmental disorders.

DNTS 2

MR fingerprinting (MRF), feasibility of a novel technology to image infants with prenatal opioid exposure (POE)

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Introduction: The use of magnetic resonance imaging (MRI) has increased rapidly as a means of assessing medical conditions that impact the developing brain, including prenatal opioid exposure (POE). Although powerful, MRI presents challenges to imaging infants for research due to long scan times and motion artifacts. Magnetic resonance fingerprinting (MRF) is a novel quantitative scanning method with advantages for infant studies. MRF is a single fast scan that simultaneously quantifies multiple critical MR contrasts, decreasing motion sensitivity and increasing objectivity and reproducibility. Objective: We conducted imaging on a small series of opioid exposed infants with NOWs scanned with both MRI and MRF to assess the feasibility of using MRF scan and comparability with MRI for infant developmental studies. Method: Eleven infants with POE, from 1 - 12 months in age were scanned covering the whole brain on a Siemens Vida 3.0T MRI scanner without sedation. Total sequence acquisition time was < 40 minutes for the standard protocol with an additional 5-10 minutes for MRF. Results: We present figures for three of the infants illustrating comparisons between MRI and MRF in motion artifact on the same infant, and two cases where MRI failed but acceptable images were obtained from MRF. Conclusions: MRF is an effective scan to image challenging infants when motion is problematic. Both 2D and 3D MRF scans are short and often effective in obtaining useful images when standard MRI fails. We plan to optimize the 3D MRF scans for improved motion tolerance and evaluate various quantitative values acquired from the MRF scans.

DNTS 3

Polysubstance use in pregnant opioid using mothers at delivery and infant birth outcomes

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Background: Opioid use disorder (OUD) and polysubstance use during pregnancy have increased, raising concerns about infant developmental outcomes. Identifying the full range of substances used by pregnant women is important to understand effects of OUD/polysubstance exposure on infant outcomes.
Objective: Assess polysubstance use among pregnant women with OUD and compare infant birth outcomes between women with and without medication assisted therapy (MAT).
Methods: Medical record data was extracted from two hospitals to identify pregnant women with OUD. Urine/meconium screen results, maternal psychiatric and health comorbidities, infant hospital stay (LOS), birth outcomes and medical problems were compared using t-tests.
Results: Of 146 women (93% white, age 20-42 years), 81 (55%) were in MAT. Women also used tobacco (61%), antidepressants (10%), marijuana (20%), benzodiazepines (7%), heroin/fentanyl (33%). Co-diagnoses included depression (28%), hepatitis C (26%), anxiety (23%), pain (18%), bipolar disorder (13%), and ADD (9%). More women in MAT had hepatitis C (21% vs. 5%, p <.005), greater use of legal amphetamines (16% vs. 6%), delivered fewer preterm infants (4% vs. 9%, p<.001); infants had more feeding problems (38% vs. 18%, p<.01) longer LOS (23.5 vs. 12 days, p<.001). Respiratory problems (14%) were common.
Conclusions: Polysubstance use, including illegal substances, was common in pregnant women with OUD, independent of MAT. Depression appears to be undertreated and may contribute to poorer developmental outcomes of infants with prenatal opioid exposure. More research,

including assessment of alcohol exposure not evident in urine screens, is needed to understand developmental sequelae of prenatal opioid exposure.

DNTS 4

Persisting neurobehavioral consequences of developmental benzo[a]pyrene and cadmium exposure in zebrafish and rats

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Neurotoxic exposures are often modelled using individual compounds, yet co-exposures are quite common. Several mixtures of concern contain both polycyclic aromatic hydrocarbons and heavy metals, although their potential interactions remain largely unknown. In parallel studies, zebrafish and rats were developmentally exposed to the PAH BaP and/or the heavy metal Cd. Zebrafish were exposed to BaP (0-3 µM), Cd (0-0.3 µM) or both from 5-120hpf. Single-exposures to BaP caused impaired dark-induced motility at 6dpf and hyperactivity in adolescence. Co-exposure with Cd blocked both of these BaP effects. However, Cd also led to a mixture-specific BaP-effect, inhibiting motility in the light at 6dpf. For comparison, we exposed female Sprague-Dawley rats to BaP (0.03 mg/kg/day), Cd (0.3 mg/kg/day) or both via osmotic minipumps (sc) from pre-mating to the neonatal period. Offspring were assessed for bodily and reflex development, as well as locomotor, emotional and cognitive function. Preliminary results show that these doses are subthreshold for impairing fertility or offspring weight gain. However, anogenital distance was decreased in males with BaP exposure. CD-exposed rats showed faster negative geotaxis than controls on the initial day of testing (PND7), but co-exposure with BAP reversed this effect. BAP-treated offspring also showed greater novelty-induced suppression of eating compared to controls. These studies show that persisting neurobehavioral effects are seen in both zebrafish and rats after chronic developmental exposure to BaP and Cd. However, these effects can differ between single-exposures and mixtures, indicating a need for greater clarity on interactions within mixtures.

DNTS 5

Reproductive and developmental outcomes following deployment to the Gulf War

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Reproductive outcomes, such as preterm birth, miscarriage/stillbirth, and pre-eclampsia, are understudied in veterans, particularly in veterans who served in the 1990-1991 Gulf War

(GWVs). During deployment, women GWVs were exposed to a broad spectrum of toxicants, including ones associated with adverse reproductive and developmental outcomes.
The study population consisted of 239 veterans from the Gulf War Women's Health Cohort. Questionnaires asked about service history, current and past general health, reproductive and family health, demographics, and exposures during deployment. We computed odds ratios with 95% confidence intervals, between exposures in theatre and health outcomes.
GWVs had high rates of adverse reproductive outcomes following GW deployment: 25% had difficulty conceiving and 25% had a pregnancy that ended in a miscarriage or stillbirth. This is almost double the rate in the general population of the US, where the rates are 12% and 16%, respectively. The rate of GWVs with pregnancy complications was three times as high as the general population: 28% were told they were a high-risk pregnancy and 15% were diagnosed with pre-eclampsia. Almost half of the children (42%) born to GWVs had a disability, ADHD, or frequent behavioral problems. Use of pesticide cream during deployment was associated with higher odds of all outcomes.
GWVs have higher rates of adverse reproductive and developmental outcomes among their children compared to the US population. Exploratory analyses suggest exposures during deployment are associated with higher odds of adverse outcomes. Future studies, especially longitudinal studies, should prioritize examining pregnancy and children's health outcomes.

DNTS 6

YoungMoms: A prospective mixed methods cohort study of prenatal cannabis and tobacco use

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Prenatal cannabis use (PCU) has increased in recent decades and is more prevalent among younger people. We present baseline data from the YoungMoms study (R01DA04640), an ongoing mixed-methods study of pregnant people < 22 years old. Participants were recruited before or during OB visits (n = 236; mean age = 19.7 years, range = 14-21; 78% Black or Biracial) and asked to complete an online survey and provide access to their clinical urine sample, medical records, and Department of Human Services records. Participants who complete the baseline survey < 14 weeks gestation are recruited for the longitudinal study for assessment during the second and third trimesters and at 6-18 months postpartum. Twenty-nine percent of the sample reported PCU and 48% had a positive urine screen for THC (r = 0.45). Blunts were the predominant mode of administration (24%), followed by joints (7%), bowls (5%), edibles (4%), bongs (3%), and vapes (3%). Two percent reported medical marijuana use. Prenatal cannabis use was not related to race or severity of morning sickness. Depressive symptoms (M = 18.8 PCU vs M =15.2 no PCU), prenatal tobacco use (70% PCU vs 16% no PCU), and household cannabis use (45% PCU vs 20% no PCU) were significantly associated with PCU (self-report or positive screen) in a logistic regression. Results highlight the prevalence of PCU and co-use with tobacco in younger populations. Depressive symptoms may represent a modifiable factor related to prenatal tobacco and cannabis use that can be addressed to promote perinatal smoking cessation.

DNTS 7

Scoping review on environmental enrichment: Are critical periods and sex differences adequately studied?

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Decades of research have shown the robust behavioral, structural and molecular effects of environmental enrichment (EE) for a host of neuropathological conditions with most conditions improving following EE. However, the roles of stage of life and sex differences in response to EE have been understudied. We conducted a scoping-style of review to evaluate where sex differences (or similarities) are described and whether critical developmental periods are addressed in reviews of EE effects. Critical developmental periods included prenatal, neonatal, post-weaning, adolescent, adult or ageing. Review articles published during the period of 2011 to 2021 focusing on animal studies and indexed in Pubmed and Psychinfo were used. Results show that most studies examined EE during adulthood such as following an injury or following addictive drug exposure. However, overall, little attention is paid to effects of EE at various stages of the lifespan. Also, few reviews acknowledge sex differences. Confounding issues include: lack of systematic reporting; status of the "control group"; the use and reporting of proper statistical analyses such that no more than a single subject from a litter contributed to a group. In conclusion, review articles reflect a lack of integration of information on age and sex differences in response to EE. We call for future studies of EE to examine both sexes equally and consider critical periods of the lifespan when applying EE in experimental models. This will facilitate the translation of the potentially powerful effects of environmental enrichment, a non-pharmaceutical intervention, on human health and well being.

DNTS 8

Mapping neurotoxicological endpoints in a systematic review of lead exposure in animal models

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Lead (Pb) is a toxic metal historically used in a variety of industrial applications and currently results in human exposures from environmental levels. There is concern for public health implications of Pb exposures, and many human and animal studies have assessed the potential for Pb exposures to result in adverse health effects. This scoping review identifies mammalian studies that examined neurological outcomes to recognize areas of robust research, uncertainties, data gaps, and research needs. Studies evaluating Pb toxicity were identified through a literature search. A Population, Exposures, Comparators, Outcomes, and Study Design (PECOS) statement was developed to inform screening and tagging to organize literature into a systematic

evidence map of Pb effects on health, focusing on neurological effects. A comprehensive literature search of multiple databases yielded 131,793 unique records published between 2011 and 2021. We used natural language processing and machine learning methods to prioritize 14,587 studies for title and abstract screening; 841 studies were identified as meeting the PECOS criteria. Refined PECOS criteria were applied to further scope the review, resulting in 150 animal toxicology studies inventoried at full text review. Studies were organized by health effect domain, exposure condition, and species. Relevant study details were extracted into literature inventories to assess database characteristics, potential trends, and issues that would benefit from further study. Evidence mapping illustrated significant diversity in exposure contexts and endpoints for neurotoxicological outcomes examined across the database. Future work will focus on the extraction of data for use in synthesis and integration with epidemiologic evidence.

DNTS 9

Early life adversity: Effects on the caecal microbiome and long-term memory, and effects of intervention with short-chain fatty acid supplementation in rats

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Maternal separation, a rodent model of early life adversity, produces lasting detrimental effects on health and neurobehavioral function. Some of these effects may be due to stress-induced changes in the gut microbiome. In this study, Long-Evans rats separated from their dams for three hours per day from postnatal days (PNDs) 1 through 15 demonstrated transient changes in caecal microbiome beta diversity, including a dramatic decrease in short-chain fatty acid (SCFA)-producing probiotic bacteria of the family *Lachnospiracae.* These changes were no longer evident by PND 28. As young adults, separated rats demonstrated intact short-term memory but impaired long-term memory in an object recognition task and the Morris water maze. Oral supplementation of short-chain fatty acids butyrate, propionate, and acetate in the drinking water from PND21 through behavioral testing partially ameliorated these long-term memory deficits. These results point toward a potential role of gut *Lachnospiracae* and their SCFA metabolites in early postnatal development, stress, and subsequent memory function.

DNTS 10

Neurobehavioral health of children living near coal ash storage sites

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Coal fly ash, a byproduct of coal combustion, contains neurotoxins with the potential to adversely impact child behavioral health. Children may be at particular risk due to the developing brain and behaviors that increase opportunity for exposures inside the home. Based

on previous research on neurobehavioral effects of components of coal fly ash we hypothesized that indoor exposure to fly ash would be associated with increased child behavior problems in children aged 6 to 14 years living near coal ash storage sites. One week of air sampling in the homes of 288 children was used to identify the presence of fly ash in the child's home. Behavior problems were assessed with the Child Behavior Checklist (CBCL). Multivariable Poisson regression models were used to estimate the association of indoor fly ash with conduct disturbance. Approximately 43% of children had fly ash in the home. Indoor fly ash was associated with a higher prevalence of CBCL Rule-Breaking problems (risk ratio = 1.90, CI= 1.06, 3.43, p=0.04) after adjusting for covariates. Increased risk of fly ash exposure was also suggested by increased but non-significant risk ratios for the CBCL Conduct Disorder scale (RR=1.59, p=.08), CBCL Oppositional Defiant scale (RR=1.43, p=.13) and CBCL Aggressive Behavior scale (RR=1.36, p=.25). There was no effect of sex based on the fly ash x sex interaction (p=0.55). Results indicated that fly ash exposure increased risk for conduct disturbance in children and suggest potential neurotoxic effects of by-products of coal burning power plants on the developing brain.

DNTS 11

Impulsive choice in two different rat models of Attention-Deficit/Hyperactivity Disorder: Spontaneously Hypertensive versus *Lphn3* knockout rats

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Impulsivity is a symptom of Attention-Deficit/Hyperactivity Disorder (ADHD). Data from a genome-wide, multigenerational sample linked variants in the *Lphn3* gene to ADHD. While previous research has demonstrated *Lphn3*$^{-/-}$ (i.e., knockout, KO) rats are hyperactive and exhibit deficits in impulsive action compared with *Lphn3*$^{+/+}$ (i.e., wildtype, WT) rats, this project examined the impact of Lphn3 deletion in rats on impulsive choice using a delay-discounting task. “Positive control” measures were also collected in Spontaneously Hypertensive Rats (SHRs), which are an accepted animal model of ADHD that also demonstrate hyperactivity and impulsive action deficits. Rats were placed in an operant chamber and given the option to press one of two levers for either a delayed reward of 3 food pellets or an immediate reward of 1 pellet. Impulsive choice was measured as the tendency to discount the larger, delayed reward for the smaller, immediate reward. We hypothesized the SHR and KO rats would demonstrate more impulsive choice by choosing the small, immediate reward more often and at shorter delays than their control strains, the WT and Wistar-Kyoto (WKY) rats, respectively. While there was not a difference between the KO and WT rats, the SHRs discounted the larger reward significantly more often than the WKY at all delays. When combined with previous results, these results suggest differences among the models. The *Lphn3* KO rat is hyperactive and exhibits deficits on tasks of impulsive action only. The SHRs are also hyperactive, but exhibit deficits on tasks of impulsive action *and* impulsive choice.

DNTS 12

Regulation of mood by interneuron dopamine D1 receptors

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Major depressive disorder is a common mental health disorder estimated to affect more than 264 million people worldwide, at least one-third of whom do not respond effectively to current treatments. Dysregulation of the development of a subset of cerebral cortical inhibitory neurons has been proposed as a crucial substrate for mood regulation and stress-induced neural responses. Importantly, the developmental lineage of this interneuron subpopulation has been described. Using the Cre-loxP system in laboratory mice, we therefore deleted the D1 dopamine receptor (Drd1) from Nkx2.1-expressing interneurons. Conditional knockout (Nkx2.1-D1-cKO) mice exhibited significant anti-depressant-like effects using the forced swim test and novelty-induced hypophagia. Plasma corticosterone levels were also reduced in the mutant mice. Other behavioral characteristics of the animals were largely unaltered, demonstrating a potentially selective effect on mood regulation. Immunoblotting revealed a reduction in the neurotrophin receptor TrkB in the mPFC of Nkx2.1-D1-cKO mice in the absence of changes in the ligand BDNF, and in situ hybridization revealed a downregulation of the neurexin gene contactin-associated protein like 4 (Cntnap4) in the mPFC in Nkx2.1-D1-cKO mice. Ongoing studies are exploring stress-induced behavioral, neuroendocrine and neurobiological substrates in Nkx2.1-D1-cKO mice. Pursuing a deep physiological and neural understanding of the neuroadaptations that accompany Drd1 loss from these GABAergic interneurons will allow us to develop potentially innovative strategies to use in the development of novel therapeutics for the treatment of mood disorders.

DNTS 13

Thyroid hormone action controls cell signaling in the developing ventricular epithelium: Implications for a mechanistic adverse outcome pathway

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Developmental thyroid hormone (TH) insufficiency is associated with some neurodevelopmental disorders in children. Previously, we demonstrated that TH insufficiency alters cell adhesion and migration in the neonatal rat brain. These abnormalities were localized to the ventricular epithelium, a progenitor cell niche, and later resulted in a periventricular heterotopia. The heterotopia now serves as an anchor in a putative adverse outcome pathway (AOP). To further elucidate key events in this AOP, we employed laser capture microdissection and RNA-Sequencing (RNA-Seq) of the ventricular epithelium to evaluate how reduced thyroid action may

affect this cell population. Pregnant rats were treated with propylthiouracil (PTU, 0.0003%) through the drinking water to induce maternal TH insufficiency from gestational day 6 until postnatal day 14 (PN14). This exposure significantly reduced total thyroxine (T4) but not triiodothyronine (T3) in the sera of dams, and both T4/T3 were significantly reduced in the pups. T4/T3 were also significantly reduced in the telencephalon of exposed neonates relative to controls. Next, frozen sections were collected from pup brains, the posterior ventricular epithelium microdissected, and RNA sequenced using Illumina HiSeq. We identified 268 genes that were differentially expressed in the PTU-exposed animals compared to controls (adj. *p*-values < 0.05). Using Ingenuity Pathway Analysis, we show a significant molecular signature related to cell junction maintenance, congruent with our previous studies. These results support the hypothesis that differential expression of genes involved in cell junction structure and function may serve as crucial key event(s) of heterotopia formation. This work does not reflect US EPA policy.

DNTS 14

Proteomic analysis of brain regions of adult Long-Evans rats exposed to kainic acid

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We are using protein signatures produced by prototypical neurotoxicants to better understand of the molecular events produced by these compounds. Adult male Long Evans rats were treated with the excitotoxin kainic acid (KA; 6 mg/mL, s.c.), vehicle (VC; buffered saline, 0 mg/mL), or were non-injected (cage controls, CC). At 3 or 24h post-exposure, rats were perfused, brain regions were collected, and stored at -80°C. Hippocampal samples were assessed for proteomic content using Orbitrap LC-MS, and proteins were identified and processed using Proteome Discover 2.4. Pathway analysis was performed using Ingenuity Pathway Analysis (IPA) software (significant protein cutoffs: $\leq \log2(0.8)$, $\geq \log2(1.2)$, FDR p-value ≤ 0.05). A total of 1894 and 1770 proteins were identified for mapping with IPA, at 3 and 24 h respectively. Of those proteins, 199 and 103 were altered by KA treatment at 3 and 24h, respectively. At 24h, top proteomic inhibited pathways included synaptogenesis signaling pathway, G Beta Gamma signaling, and Ephrin receptor signaling. Activated pathways at 24h included PTEN signaling, semaphoring neuronal repulsive signaling pathway, and autophagy. Histological changes in the hippocampus were examined (H&E stain) in additional rats, treated and sacrificed at 3, 24, or 48h following KA exposure (0 or 6 mg/mL). No hippocampal microscopic lesions were observed. Proteomic signatures revealed several pathways, including neuronal signaling and cell death, to be impacted in the absence of overt histological changes. Additional brain regions are being examined. *Abstract of proposed presentation above does not necessarily reflect US EPA policy.*

DNTS 15.

Cognitive effects of perinatal exposure to manganese in drinking water, variable stress, and their combination

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Research indicates that the effects of chemicals may be exacerbated by non-chemical factors such as psychosocial and physical stress. These stressors, along with many chemicals, are disproportionately found in lower socioeconomic communities and their prevalence has been negatively associated with neurodevelopmental outcomes in children. To understand the cognitive impact of non-chemical stress in combination with exposure to a known neurodevelopmental toxicant, we developed a rodent model of co-occurring perinatal manipulations and conducted a series of assessments in offspring. Manganese (Mn) was delivered in drinking water (0, 2, or 4 mg/mL Mn) of pregnant rats from gestational day (GD) 7 to postnatal day (PND) 22. A stress paradigm was applied to half of the animals from GD13 to PND9. Short term object memory was assessed in adolescent rats using a novel object recognition task. In adults, spatial memory was assessed using the Morris water maze; associative learning and inhibitory control were assessed using autoshaping and differential reinforcement of low-rates procedures; and attention, inhibitory control, and reaction time were assessed using a cued and uncued choice reaction time task. Individually, Mn and stress both affected object memory, associative learning, and all choice reaction time measures. These effects were often sex-dependent and task-specific. Given in combination, the changes observed with Mn and stress alone were often attenuated and did not lead to greater effects of Mn. The results suggest that non-chemical factors produce lasting changes in brain function and may play a role in differential susceptibility to chemicals in various populations. This is an abstract of a proposed presentation and does not necessarily reflect US EPA policy.

DNTS 16

Attention and memory in behavioral toxicology with adolescent methylmercury exposure

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Early-life toxicity of methylmercury (MeHg) produces permanent changes in behavior in humans and in animal models. While the prenatal period has repeatedly, and robustly, been shown to be sensitive to MeHAg toxicity, other early developmental stages, such as adolescence, are only recently being examined. MeHg interacts with behavior following adolescent exposure in both rats and mice. These disruptions are associated, in part, with altered dopamine neurotransmission and with executive functions. We review work conduced in our lab designed to examine neurobehavioral effects of adolescent MeHg exposure and compare it with those reported following prenatal exposure. The adolescent period is sensitive to methylmercury toxicity with this sensitivity related to altered dopamine neurotransmission. For example, alone, adolescent MeHg does not interact with sustained attention and short-term recall in rodents, as assessed in a modified two-choice visual signal detection task, but acute administration of the dopamine agonist *d*-amphetamine impairs performance in MeHg-exposed animals, producing

baseline-dependent effects in rats and impaired recall, but not attention, in mice. These effects were dopamine-dependent, as administration of the norepinephrine agonist, desipramine, produced no such disruptions in mice. Overall, the effects mostly resemble what has been seen with early developmental exposure but are generally more subtle.

DNTS 17

The last two decades on preclinical and clinical research on inhalant effects

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Inhalants are volatile substances at room temperatures that are intentionally inhaled for recreational purposes. They comprise organic solvents, aerosols, gases, anesthetics, and alkyl nitrites. Inhalant misuse constitutes a significant health problem worldwide. Except for nitrites, inhalants can be found in many household products and produce CNS depressant effects. Most inhalants are positive GABAA receptor modulators and NMDA receptor antagonists. In addition, the anesthetic gas nitrous oxide (aka laughing gas) has some opioid agonist actions. Nitrites lack psychoactive effects; instead, they are volatile vasodilators used to enhance sexual performance. Toluene is the best-studied inhalant and the reference drug among solvents. Its acute effects include irritation of respiratory airways, euphoria, excitation, dizziness, and ataxia, among others. Very high concentrations produce profound CNS depression, and sudden sniffing death can occur. Inhalation of compressed gases can produce frostbite. Chronic inhalant use is associated with liver and kidney toxicity and cognitive deficits, including memory impairment. Neural damage and leukoencephalopathy have also been reported among chronic users. Benzene is also a carcinogenic solvent. Nitrous oxide use can present numbness in hands and feet due to B12 vitamin deficiency, and nitrite chronic use can produce maculopathy and methemoglobinemia. Finally, research agendas must continue studying solvents and their combinations, along with other drugs of abuse, while investigating both age and sex differences of inhalant effects. Abstinence signs and neurochemical changes after inhalant exposure must be researched to help develop strategies to promote recovery for inhalant users.

DNTS 18

Associations of concurrent PCB and PBDE serum concentrations with executive functioning in adolescents

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DNTS 19

Putting the long in longitudinal: Research and mentoring in neurotoxicology

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DNTS 20

Finding a common ground: Translation of the principles of teratology in today's regulatory climate

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Sixty years ago, the Pharmaceutical Manufacturing Association (PMA) set up a series of committees to "seek new knowledge of the predictability of action of drugs in man". One of these committees focused on teratology and was chaired by Dr. Josef Warkany. This committee held two workshops in 1964 and 1965, both chaired by Dr. James Wilson leading to the publication of Dr. Wilson's classic book "Teratology – Principles and Techniques". This work by Dr. Wilson formed the foundation for the regulatory reproductive and developmental toxicity assessments that have been conducted since 1966 to use animal models to predict the hazard of any substance in humans. For more than 5 decades, our testing paradigms have prevented another thalidomide tragedy and helped to better predict which pharmaceuticals and chemicals may be hazardous to human health. Combining this pivotal information with exposure data allows pharmaceuticals to be used at efficacious doses with known side effects and help to set exposure limits for chemicals. Animal testing, based on Wilson's foundational principles, has limits when it comes to translating outcomes for the patient population. Recognizing the limitations of animal testing combined with countless chemicals and environmental contaminants that we are exposed to daily, the desire to reduce the use of animals in research, and the development of pharmaceuticals that may only be active in humans drives us to rethink and reimagine our testing paradigms to meet the needs of the patient population. The future of hazard assessment in nonclinical settings and the translation to clinical outcomes is dependent on a collective understanding of developmental biology, the molecular basis for the action of pharmaceuticals and chemicals, and exposure data. New techniques and advances in biomedical research are paramount as we build on the foundational principles of teratology, better translate non-clinical to clinical outcomes, and continue to navigate 21st century regulatory reproductive and developmental hazard assessments.

DNTS 21

Exploration of disinfection byproduct mixture methods in an epidemiological study of birth defects

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Epidemiological and toxicological studies show associations between disinfection byproducts (DBPs) and birth defects including obstructive genitourinary defects (OGDs) and certain cardiac defects. Quantifying birth defect risks is complicated by the limited number of measured exposure surrogates relative to the number of DBPs (n>600) humans are exposed to. We analyzed 1999–2004 data from a case-control study of birth defects in Massachusetts to examine whether water concentration-based risks for OGDs can be differentiated for DBP mixtures. Joint toxicity combinations were examined for the individual components of four regulated trihalomethanes (THM4) and five haloacetic acids (HAA5). Additional summary measures (HAA9; DBP13) were evaluated based on a kinetic prediction model that predicted the water concentration of more toxic brominated haloacetic acids (HAABr). To compare with unweighted measures, we calculated relative potency factor (RPF) weights for the THM/HAA components using full-litter resorption and/or eye-malformation data from gavage administration in F344 and Long-Evans rats. The highest unweighted DBP exposure categories were associated with elevated adjusted odds ratios (aORs) for OGD (range: 1.31-1.99) including chloroform, bromodichloromethane (BDCM), dibromochloromethane, tribromoacetic acid, chlorodibromoacetic acid, bromodichloroacetic acid, bromochloroacetic acid, a trichloroacetic acid/dichloroacetic acid (TCAA/DCAA) joint exposure, and HAABr, DBPBr, and DBP9 mixture measures. We detected monotonic exposure-response relationships for BDCM and THMBr. The aORs for THM4 and the THM4/TCAA using RPF-based metrics were slightly larger compared to unweighted results. In contrast, RPF-based results were null for the highest TCAA/DCAA quartile. aORs for RPF-based THMBr measure were slightly lower compared with unweighted results, but the monotonic exposure-response relationship remained (quartile 4 aOR=1.68; 95%CI: 1.08,2.61). The unweighted TCAA/DCAA/dibromoacetic acid measure [approximating HAA5 as teratogenicity was not observed for monobromoacetic acid and monochloroacetic acid] showed a slightly increased risk across all exposure quartiles (quartile 4 aOR=1.27) compared to RPF-weighted results (quartile 4 aOR=1.11). The approaches examined here allow for more specificity of potential risks due to better targeting of toxicologically relevant exposure mixture metrics as well as examination of some component DBPs with limited exposure contrasts. *The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the US Environmental Protection Agency.*

DNTS 22

Application of sufficient similarity analyses for complex polychlorinated biphenyl mixtures

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Approaches that consider the potential toxicity of the combined components of a mixture (i.e., whole mixture approaches) are preferred over individual component approaches for risk assessment of chemical mixtures. When toxicological data for a specific mixture are not available, US Environmental Protection Agency (EPA) guidance recommends the use of data from a "sufficiently similar" mixture as an alternative. One method of assessing sufficient similarity uses equivalence testing methodologies that compare the distance between benchmark dose estimates for different mixtures. Polychlorinated biphenyls (PCBs) exist as 209 unique congeners exhibiting diverse biological activity and chemical properties depending on their degree and pattern of chlorination. Humans are exposed to mixtures of PCB congeners by multiple routes, including dietary and inhalation sources. Nursing infants are particularly vulnerable because they can receive large doses of PCBs through human milk. Mixtures present in sources such as breast milk have generally not been evaluated in toxicological studies, whereas toxicological data for commercial PCB mixtures, including Aroclors, are much more abundant. Thus, approaches such as mixtures equivalency testing could be useful to determine if Aroclor mixtures are sufficiently similar to PCB mixtures humans are exposed to in the environment. In this talk, we will use case examples to explore the potential utility of equivalence testing methodologies for predicting the toxicological similarity of environmental and commercial PCB mixtures. *The views expressed in this abstract are those of the author(s) and do not necessarily represent the views or the policies of the US Environmental Protection Agency.*

DNTS 23

Impact of prenatal exposure to phthalate mixture on uterine function in female offspring

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Phthalates are synthetic endocrine-disrupting chemicals that are found in numerous consumer products. People are ubiquitously exposed to phthalates on a daily basis, and gender-specific differences exist in phthalate exposures and toxicity. Specifically, phthalate exposures are reported to be higher in women than in men, likely because of higher personal care product use in women than men. Pregnant women are exposed to phthalates throughout their pregnancy and chemical exposures during pregnancy can have long-lasting health impacts on the offspring. However, few studies have examined the effects of prenatal exposure to phthalate mixture on the reproductive health of the offspring. Thus, our preliminary studies examined the impacts of prenatal exposure to an environmentally relevant phthalate mixture on reproductive parameters in female offspring. We exposed pregnant dams with vehicle control or different doses of phthalate mixture (Mix) and collected uterine tissues from the offspring for analysis. Specifically, female mice were bred and divided into one of four treatment groups: tocopherol-stripped corn oil as vehicle control, 20 µg/kg/day, 200 µg/kg/day, and 200 mg/kg body weight/day. Dams were treated daily with vehicle control or Mix beginning on day 10.5 until birth. Uterine samples from the F1 generation offspring were collected at postnatal day (PND) 8, PND 21, and PND 90 and subjected to morphological assessments. Our studies revealed that morphology of F1 uteri that were prenatally exposed to Mix at 20 µg/kg/day, 200 µg/kg/day, and 200 mg/kg/day was comparable to that of controls at PND 8 or PND 21. However, F1 cycling females at 3 months that were prenatally exposed to 200 µg or

200 mg of Mix exhibited dilated endometrial glands compared to controls. We also determined the pregnancy and offspring outcomes in a small group of Mix-exposed F1 females at 3 months. Our studies revealed no significant alteration in pregnancy and offspring outcome in F1 females upon exposure to phthalate mixture. Interestingly, F1 cycling females at 6 months and 13 months that were prenatally exposed to the Mix, exhibited enhanced uterine fibrosis compared to controls. Future studies will address the impact of Mix on pregnancy and offspring outcomes as F1 females age.

DNTS 24

Prenatal consumer product chemical mixtures and size-for-gestational age at birth

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Deviations in normal fetal growth trajectories may predispose infants to adverse outcomes during childhood and in later life. While fetal growth is a tightly regulated process, it is sensitive to environmental exposures during pregnancy. Many commonly used consumer products contain chemicals that can disturb processes underlying fetal growth. However, associations between mixtures of these compounds and fetal growth have been minimally examined. To address this gap, we investigated associations between prenatal exposure to 33 consumer product chemicals (9 organophosphate ester flame retardant [OPE] metabolites, 12 phthalate metabolites, and 12 phenols) and the odds of a small- or large-for-gestational-age (SGA and LGA) birth using both single- and multi-pollutant approaches. This pilot case-control study comprised SGA (N = 31), LGA (N = 28), and control (N = 31) births selected from the LIFECODES birth cohort. Biomarkers of exposure to consumer product chemicals were quantified in maternal urine collected at three study visits during pregnancy. Quantile g-computation was used to jointly estimate the odds ratios (OR) and 95% confidence interval (CI) of SGA and LGA births per simultaneous one quartile-change increase in all biomarkers within each chemical class. We estimated lower odds of an LGA birth for higher concentrations of OPE (OR: 0.49, 95% CI: 0.27, 0.89) and phthalate (OR: 0.23, 95% CI: 0.07, 0.73) metabolites. Associations with SGA births were largely null. Our findings that OPE and phthalates were jointly associated with LGA births could indicate a specific impact of these exposures on the high end of the birthweight spectrum. Future work to understand this nuance in the associations between consumer product chemicals and fetal growth is warranted. In addition to these pilot study results, findings from the next phase of this study will be presented.

DNTS 25

Association between exposure to a metal mixture and neurobehavior at six to seven years of age

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Background: Children are exposed to an array of essential and nonessential metals throughout their development. Although there is an increase in studies accounting for more than a single metal at a time when assessing their effects on neurodevelopment, there are many unknowns in this field of study. Aim: This study sought to identify trace element mixture-associated deficits in learning behavior using operant testing in a prospective cohort in Mexico City. Methods: The study included 322 participants aged six-to-seven years with complete data on prenatal trace elements measurements (third-trimester blood lead and manganese levels, and urine cadmium and arsenic levels), demographic covariates, and the Incremental Repeated Acquisition (IRA) task, an associative learning task. In the IRA task, the child was required to learn to press the levers in a specific sequence dictated by the illumination of the serial position indicator lights and the measures analyzed for this task included: percent task completed, accuracy of chains completed, accuracy of their search, memory accuracy, effective response rate, and ineffective response rate. Weighted quantile sum (WQS) regression models were used to estimate the joint association of the mixture of all four trace elements and each IRA task measure. Results: Results showed that prenatal exposure to a mixture of four elements was associated with poorer performance on an IRA task. The mixture of Pb, Cd, As, and Mn was associated with a significant decrease in four task performance measures—percent of task completed (β = -1.0; 95% CI = -1.15, -0.85), memory accuracy (β = -1.17; 95% CI = -1.37, -0.97), effective response rate (β = -0.02; 95% CI = -0.023, -0.017), and ineffective response rate (β = -0.01; 95% CI = -0.014, -0.005). Pb, Cd, and As contributed the greatest weights to the mixture effect. Conclusion: The impact of trace element exposures on discrete aspects of task performance allows us to attribute these effects to the dysregulation of memory, motivational, and motoric processes. These findings add to a growing literature that suggests that studies should evaluate co-exposures to multiple neurotoxic elements.

DNTS 26

Children’s exposures to chemical and non-chemical stressors: What have we learned about health and well-being?

Tulve N

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Children, pregnant women, and men are exposed to diverse chemical and non-chemical stressors found in their everyday environment. These stressors, individually and in combination, are thought to contribute to health and well-being during each developmental stage throughout their lifecourse. To understand chemical exposures, we collect and analyze multimedia samples for chemicals of interest including, but not limited to, pesticides, PBDEs, BPA, nanosilver, and other

consumer product ingredients. We also collect supporting information (time activity data and household inventories). Over time, our research has evolved to consider a holistic approach to children's environmental health. Using a systems approach, we collect and analyze data and information on exposures to both chemical and non-chemical stressors, recognizing that these interrelationships may impact the biological response to a chemical agent. Examples of non-chemical stressors include access to recreational amenities, family dynamics, and/or neighborhood characteristics. Our conceptual framework was designed to show how children's health and well-being should consider exposures to chemical and non-chemical stressors from the built, natural, and social environments, activities and behaviors, and inherent characteristics. Our research approach includes distinct research activities with emphasis on an exposure analysis approach that considers how non-chemical stressor information may be used in combination with chemical exposure data. Our research has shown that both childhood obesity and general cognitive ability are influenced by a myriad of chemical and non-chemical stressors. This presentation will explore our research strategy, show how data and information can be considered within the conceptual framework, and highlight public health examples.

DNTS 27

In memory of Dr. Robert L Brent: Tribute to his heritage: Let the yolk sac speak—A small drop in the deep water of Bob's legacy in science

Ornoy A

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Dr. Bob Brent passed away on February 24, 2021. I knew him for many years as one of the founders of the Teratology Society (Birth Defects Research Society) and as a brilliant, innovative, and pioneering scientist. However, I would like to focus on one of his many contributions to the study of birth defects: his work on the development, function, and role of the yolk sac placenta in the etiology of birth defects in rodents. I have special feelings towards these contributions since the time I had spent in his laboratory in Philadelphia and the time he had spent in my laboratory in Jerusalem was mainly devoted to matters related to studies on this unique organ. The visceral yolk sac (VYS), as characterized by Bob and his group, is "the primary source of exchange between the embryo and mother and is involved with nutritional, endocrine, metabolic, immunologic, secretory, excretory and hematopoietic function". He presumed that damage to the yolk sac by anti-VYS antibodies damages the developing embryo, resulting in various congenital malformations and even embryonic death. He indeed proved this concept and published several classic papers 40–50 years ago. In 1967 he published the first paper "Production of congenital malformations using tissue antisera - 3. Placental antiserum". Since then, he explored the role of the yolk sac in embryonic nutrition, oxygen and antibody transfer, and the mechanisms of the teratogenic action of the yolk sac antibodies. I was personally involved in the biochemical and morphological studies of the *in vivo* and *in vitro* yolk sac recovery phases following the exposure of rat embryos to teratogenic anti-VYS antibodies. We found that 24 and 48 hours after exposure there were substantial morphological changes in the VYS epithelium evidenced by Transmission and Scanning Electron microscopy and a significant reduction in the yolk sac endocytosis. Within

96–192 hours after exposure, we observed a gradual recovery with normalization of the ultrastructure and the endocytic index. However, embryonic damage was already produced in spite of the VYS recovery. These and other findings from many of Bob's studies in this area will be presented.

DNTS 28

Radiation risks and information needs of pregnant and lactating women

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The purpose is to review historical and current information regarding risks and effects of ionizing radiation in the context of human pregnancy and in particular the information needed for pregnant women to understand the type and magnitude of risks as well as being able to place them in a realistic context. Much of our understanding comes from early animal studies but has been supported by studies of human exposure to medical radiation, radiation accidents, and nuclear weapons. The work of Robert L. Brent will be emphasized particularly with regard to decisions on whether radiation exposure is appropriate, what types of exposure are important, and whether termination of pregnancy may be warranted under certain very limited circumstances. Exposure of infants from maternal radionuclides will also be covered. Finally, relevant data after *in utero* exposure at Chernobyl and Fukushima accidents will be presented.

DNTS 29

Professionally responsible counseling of pregnant women about COVID-19 vaccination

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Physician hesitancy occurs when physicians do not recommend COVID-19 vaccination and is a contributing factor of the low vaccine rate of pregnant women for COVID-19. Physician hesitancy has become a major, unaddressed problem for the quality and safety of obstetric care. We identify three root causes of physician hesitancy and describe how professional ethics in obstetrics should guide reversing these root causes. They are clinical misapplications of key components of professionally responsible obstetric practice: therapeutic nihilism; shared decision making; and respect for patient autonomy. Therapeutic nihilism directs the obstetrician to avoid any clinical interventions during pregnancy, to prevent teratogenic effects that might be unknown. Therapeutic nihilism is misapplied when there is documented net clinical benefit with no evidence of clinical harm. Shared decision directs the obstetrician only offer but not recommend clinical management. Shared decision making plays a major role when there is uncertainty in clinical judgment but is misapplied when it becomes a universal model. Shared decision making does not apply when there is net clinical benefit. When there is net clinical benefit, clinical management should be

recommended, not simply offered. The ethical principle of respect for patient autonomy plays an indispensable role in decision making with patients. It is misapplied when it is assumed that respect for autonomy requires physicians not to make recommendations and to defer to and implement patients' decisions without exception. There is evidence that the obstetrician's recommendations about the management of pregnancy are the most important factor in a pregnant women's decision making. Simply deferring to patient's decisions makes misapplied respect for patient autonomy. Obstetricians must end physician hesitancy about COVID-19 vaccination of pregnant women by reversing these three root causes of physician hesitancy. Reversing root causes of physician hesitancy is an urgent matter of patient safety. The longer physician hesitancy continues and the longer the low vaccine acceptance rate of pregnant women lasts, preventable serious diseases, deaths of pregnant women, ICU admissions, stillbirths, and other maternal and fetal complications of unvaccinated women will continue to occur. Physician hesitancy should not be permitted to influence the response to future pandemics.

DNTS 30

Using supervised learning methods to create prediction models based on narrative descriptions of fetal risk

Ailes E

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Women and healthcare providers lack adequate information on medication safety during pregnancy. Our objective was to develop a list of medications of greatest concern during pregnancy to help healthcare providers counsel reproductive-aged and pregnant women about medication use. Prescription drug labels submitted to the US Food and Drug Administration with information in the Teratogen Information System (TERIS) database and/or the 10th edition of *Drugs in Pregnancy and Lactation* by Briggs and Freeman were included (N=1,186). Each data source (drug labels, TERIS, or Briggs and Freeman) provided its own categorization and narrative summary of fetal risk. We applied two types of supervised learning models, support vector machine and sentiment analysis, to the narrative summaries in each data source. These methods create prediction models from the text to classify medications as "high" or "not high" fetal risk. Our final list of medications of greatest concern during pregnancy included those medications categorized as "high" risk in at least three of four prediction models (if two data sources were used) or at least four of six prediction models (if all three data sources were used). We identified ~ 80 (7%) prescription medications that warrant careful consideration before use in pregnant women and women of reproductive age due to their associations with birth defects, pregnancy loss, or other adverse fetal effects. Most medications were antineoplastic agents, angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonist, and anticonvulsants. This evidence-based list could be a useful tool for healthcare providers in counselling reproductive-aged and pregnant women about prescription medication use. However, providers and patients should weigh the specific risks and benefits of any pharmacologic treatment to manage medical conditions before and during pregnancy.

DNTS 31

Bob Brent: Medical expert, expert medical witness, and expert on medical expert witnessing

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Bob Brent was consulted as an expert witness on the causation of birth defects in hundreds of legal cases over the course of his career. He approached this service as he did everything else—as a scholarly endeavour—and provided his reflections about it in several papers on the legal, ethical, scientific, and academic responsibilities of acting as an expert witness. Among the principles he espoused were: (1) An expert witness should consult or testify *only* in specific areas in which he or she is knowledgeable, experienced, and recognized as an expert. (2) An expert witness should bring objective scholarship to the courtroom and *not* function as a partisan for either side. (3) An expert witness should bring facts and an objective analysis of the allegation and should *not* base his or her opinion on speculation or hypothesis. His involvement in the 1993 Daubert v. Merrell-Dow Pharmaceuticals case, which the US Supreme Court used to establish a standard for admitting expert testimony in federal trials, was both highly influential and controversial.

DNTS 32

Disparities in maternal health and neurodevelopmental outcomes

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In the City of Pittsburgh, twice as many pregnancies to Black women result in fetal death compared to pregnancies to white women. Black women in the US experience pregnancy morbidity attributed to systemic structural, interpersonal, and individual experiences of racism. Structural racism and discrimination (SRD) has also been linked to cannabis use in nonpregnant minoritized adolescents. SRD may increase perinatal stress, depression, and substance use, contributing to inflammation, poor cardiovascular health, and adverse pregnancy outcomes. Prenatal exposure to cannabis is also associated with adverse birth outcomes and neurodevelopmental outcomes. Prior work on the long-term outcomes of prenatal cannabis exposure will be reviewed, with a focus on racial disparities. Current research examining perinatal cannabis use, pregnancy morbidity, and SRD will also be discussed.

DNTS 33

The influence of social disparities on congenital anomalies in the North

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A high rate of congenital anomalies in the Canadian Arctic territory of Nunavut has been well documented for nearly three decades. For example, congenital heart defects in infants born to Inuit (Northern Indigenous) mothers have been reported to have a birth prevalence three times higher than other Canadian populations. Whether this high rate of congenital anomalies is a result of genetic factors, or rather a product of social determinants of health remains to be established. Food insecurity (~42%), low levels of folate intake/red blood cell folate, low rates of vitamin use in women of childbearing years, and persistently high rates of maternal smoking (>80%) are possible contributors. A review of what is known about congenital anomalies in Nunavut, previous studies on genetic factors, and the relevance of social determinants of health will be discussed within a global context of health disparity and congenital anomalies.

DNTS 34

Structural and interpersonal racism and adverse birth outcomes: From associations to advocacy

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The United States Centers for Disease Control and Prevention (CDC) considers racism a serious threat to public health. The CDC defines racism as "a system—consisting of structures, policies, practices, and norms—that assigns value and determines opportunity based on the way people look or the color of their skin." Racism is considered a fundamental cause of racial disparities in health that operates at multiple levels of society, including structurally and interpersonally. Measures of structural racism, such as racial residential segregation, are associated with adverse birth outcomes especially among Black birthing people, as well as racial disparities in adverse birth outcomes between Black and White birthing people. Interpersonal racism is also associated with racial disparities in adverse birth outcomes. Black pregnant women experience interpersonal racism, such as racialized pregnancy stigma—stereotypes stigmatizing Black motherhood—in everyday, health care, and social services contexts, which may contribute to poorer maternal and infant outcomes due to reduced access to quality health care and social services, and poorer psychological health. Approaches for mitigating the influence of structural racism on adverse birth outcomes include improving area-level economic and social factors. Approaches for addressing impersonal racism include anti-bias training for health care and social service providers and diversifying the healthcare workforce by funding training and employment of doulas and midwives of color.

DNTS 35

What improves, and what impairs, executive functions

Adele Diamond

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"Executive functions" (EF) make it possible for us to think before we act, resist temptations, creatively problem-solve, and succeed despite obstacles. I'll invite you to question some things you thought you knew about what impairs, and what aids, EFs. I'll invite you to see the whole person (mind and body, intellect and emotions) as fundamentally interrelated. Simple activities for improving EFs that can be done at home will be discussed.
EFs depend on prefrontal cortex (PFC) and interconnected brain regions. PFC is the newest area of the brain during evolution and the brain area that takes longest to mature during development, making PFC particularly fragile. It's affected first and more severely if you're sad, stressed, lonely, or not physical fit than any other brain region, and thus EFs, too, are affected first and more severely than any other mental function by these things.
Even extremely mild stress impairs the EFs of most people. There are good neurobiological reasons for that. For example, even mild stress increases dopamine in PFC and nowhere else in the brain; that can easily push dopamine past the optimal level in PFC. Despite talk of "good" stress, there's no level of stress that is good for most people's EFs. In particular, feeling stressed because you're worried about what others might think of you or might think of your performance (social evaluative stress) is not beneficial for most people's EFs. Joy and the challenge of pushing one's limits are better motivators than fear or anxiety.
Although PFC is especially vulnerable to environmental and genetic variations, PFC can be key in helping one feel less anxious or stressed. Normally, the amygdala sends out alarm signals whenever there's a risk one might be harmed. It is PFC that calms down the amygdala when there is really no cause for alarm.

DNTS 36

Underlying Conditions and PFAS Exposures: A Risky Mix in Pregnancy

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Per- and polyfluoroalkyl substances (PFAS) are endocrine-disrupting chemicals that have been linked to adverse human health outcomes. The vast majority of the US population are exposed to these chemicals that are persistent; yet certain populations may be particularly sensitive to exposure to PFAS chemicals. For example, epidemiologic and animal studies suggest that pregnancy may be a sensitive window for endocrine-disrupting chemical exposures. Evidence evaluating PFAS has shown associations with a variety of adverse pregnancy outcomes. This talk will: (1) describe a framework for pregnancy as a stress test for environmental chemical exposures and maternal cardiometabolic health across the reproductive life course; (2) present plausible mechanisms by which having higher PFAS chemical concentrations might initiate and sustain a modified cardiometabolic health profile during and following pregnancy; (3) provide examples of epidemiologic studies evaluating associations between PFAS and several adverse cardiometabolic outcomes in the perinatal period, including obesity measures, hypertensive disorders of pregnancy, and gestational diabetes risk; (4) discuss health inequities linked to disparities in PFAS exposure

and related pregnancy outcomes. This presentation will highlight mixtures analytic techniques utilized in epidemiologic studies. In addition, the presentation will provide an example of research evaluating mixtures methods conducted in animal models that may support epidemiologic studies. To conclude, this presentation will discuss the link between maternal PFAS exposure and adverse child health outcomes associated with these pregnancy conditions.

DNTS 37

GenX effects in pregnant mice and persistent effects in their offspring

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Hexafluoropropylene oxide-dimer acid (HFPO-DA, commonly called GenX) is a chemical in the family of per- and polyfluoroalkyl substances (PFAS) that is being used to replace perfluorooctanoic acid (PFOA). PFOA is a well-known developmental toxicant and has been associated with fatty liver disease in humans. Through a series of targeted studies in CD-1 mice, we have shown that exposure to 5 mg/kg PFOA or 2 or 10 mg/kg GenX during pregnancy induces significant excess weight gain, increased relative liver size, and increased abnormalities in liver and placental pathology and embryo:placenta weight ratios in exposed dams. We also confirmed dam liver bioaccumulation of PFOA is about 7-fold higher than GenX, even though serum levels were similar. Follow-up studies evaluating effects of $\leq$ 2 mg/kg GenX on offspring health discovered decreased pup weights at weaning and a significantly increased rate of weight gain in male offspring fed regular chow. Males also demonstrated altered clinical chemistry, enlarged liver size at weaning and 6 weeks, and increased body fat mass, body weight, and fasting serum insulin levels at 18 weeks, suggesting cardiometabolic disease. Male and female offspring demonstrated significantly increased liver lesions, with the females most affected. With the fat as a definitive target, there were dose-dependent delays in mammary gland development of female offspring, and stunted growth of mammary stalks in male pups. We have recently implemented whole-transcriptome gene expression analysis to investigate the molecular mechanisms of liver, placenta, and mammary gland toxicity after developmental exposure to PFOA and GenX. We have defined a group of 10 differentially regulated genes each with strong negative or positive ($-0.92<X^2>0.93$) correlations to relative liver weight and demonstrating dose dependent change for both PFOA and GenX. These findings and others suggest the molecular mechanisms of liver toxicity are nearly identical for PFOA and GenX in both maternal and fetal livers in CD-1 mice and provide critical insight into pathways that may indicate risk for adult fatty liver disease. *The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.*

DNTS 38

Cardiometabolic and bone health effects of PFAS exposure in adolescents

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The developing fetus, infant, and child may be especially susceptible to the adverse health effects of exposure to per- and polyfluoroalkyl substances (PFAS), a class of ubiquitous endocrine disruptors with suspected obesogenic and osteotoxic effects. Using data from 200 mother-adolescent dyads from the Cincinnati, OH region followed from the second trimester until age 12 years, we assessed exposure to PFAS five times using serum concentrations, measured peripheral leukocyte DNA methylation at delivery and age 12 years, and evaluated cardiometabolic and bone health at age 12 years. We identified patterns and predictors of PFAS concentrations across the first 12 years of life using linear regression methods and estimated associations of individual PFAS and their mixture with these endpoints using epigenome-wide association methods, multiple informants models, and exposure index methods. Four key findings have emerged from our studies. First, longitudinal measures of serum PFAS concentrations increased from delivery to age three years and declined thereafter. Second, children who were breastfed had higher PFAS concentrations than those who were not, particularly at age three years. Third, serum concentrations of PFOA and PFHxS during gestation, but not at other times, were associated with excess adiposity, increased risk of being overweight/obese, and insulin resistance. Individual concentrations of PFAS during gestation and their mixture were associated with decreased hip and forearm bone mineral density at age 12 years. Finally, gestational PFAS was associated with altered DNA methylation at several CpG sites at both delivery and 12 years; several CpGs were replicated in another cohort. These results suggest that gestational exposure to PFAS may have multiple effects on adolescent health outcomes related to later life chronic diseases and these potential impacts may be due to re-programming of the epigenome. Future studies from this study will quantify potential mediation by the epigenome and investigate the impact of PFAS on the metabolome. Ultimately, these results may help identify at-risk populations and inform interventions to ameliorate the potential effects of PFAS in exposed populations.

DNTS 39

Immunotoxic effects of PFAS in children

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Perfluoroalkyl substances (PFAS) are a group of persistent grease and water-repellent chemicals widely used in consumer products such as food packaging, clothing, and furniture. We are all exposed to PFAS through contaminated food and water as well as dust from treated materials. PFAS are transferred across the placenta and into breast milk, thereby causing peak exposures in infancy. Research has shown that PFAS may damage the immune system with early infancy seemingly being the most vulnerable time of exposure. In low-income countries with poor living conditions and inadequate access to clean water, food, and health care, the consequences of reduced immune function may be particularly severe. We have therefore extended our research on PFAS-associated impacts on antibody responses to routine childhood vaccinations that now focuses on the impacts in West Africa. Using data from a randomized vaccination trial, we analyzed PFAS in serum samples collected at age four–seven months and measured measles antibodies before and after measles vaccination. The infants in this study were exposed at much lower concentrations than seen elsewhere, but even at these low concentrations we found PFAS-associated increases in morbidity and decreases in measles-specific antibody concentrations before and after vaccination.

DNTS 40

Approaches to address concerns about PFAS exposure in susceptible populations

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Per- and polyfluoroalkyl substances (PFAS), a large class of fully or partially fluorinated chemicals, have been manufactured and used for decades. They are highly persistent in the environment and have been detected in drinking water, food grown or raised near places that make or use PFAS, and in some consumer products. Research in humans suggests that exposure to high levels of certain PFAS may increase the risk of health effects including decreased vaccine response in children, small decreases in infant birth weights, and increased risk of high blood pressure or pre-eclampsia in pregnant women. In the United States, federal, state, and local health organizations are working to protect and promote the health of susceptible populations. In particular, the Agency for Toxic Substances and Disease Registry (ATSDR) and the National Institutes of Environmental Health Sciences have enlisted the National Academies of Sciences, Engineering, and Medicine to conduct an independent review of the current evidence regarding human health effects associated with PFAS exposure to inform clinical care of exposed patients, including women and children. Additionally, ATSDR has developed intermediate oral minimal risk levels (MRLs) for four PFAS to enable the public health community to determine if further evaluation of PFAS exposure is needed. To evaluate exposure to children drinking water containing PFAS, ATSDR has translated these MRLs into media-specific comparison values that are based on the body weight and drinking water intake of an infant. ATSDR is also working to update its Toxicological Profile for PFAS to incorporate the most recent scientific literature. The US Environmental Protection Agency is working to develop National Primary Drinking Water Regulations for perfluorooctanoic acid and perfluorooctane sulfonic acid that are based on pharmacokinetic models that consider gestational and lactational exposure to infants. Together,

these efforts and others aim to answer important questions in pursuit of protecting susceptible populations from the risks associated with PFAS exposure. The findings and conclusions in this presentation have not been formally disseminated by the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry and should not be construed to represent any agency determination or policy.

DNTS 41

Fetal alcohol exposure disrupts angiogenesis- and erythropoiesis-related placental gene network modules

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Background: Given the 9.8% global prevalence of alcohol use during pregnancy and fetal alcohol spectrum disorder (FASD) prevalence estimates of 2-5% in US school children, the neurotoxic effects of fetal alcohol exposure remain a major public health problem. Placental surveys present an important opportunity to uncover early biomarkers that may identify those at risk for neurodevelopmental outcomes. Here, we report the first transcriptome-wide evaluation to comprehensively characterize human placental pathways disrupted by fetal alcohol exposure.
Methods: In a prospective longitudinal birth cohort in Cape Town, South Africa, we performed RNAseq to profile transcriptome-wide gene expression in bulk placenta tissue from 34 women reporting heavy drinking during pregnancy (Mean = 7.3 drinks/occasion on 1.5 days/week) and 34 abstainers/light drinkers. Weighted gene coexpression network analysis (WGCNA) and differential gene expression analysis were performed to assess associations between fetal alcohol exposure and placental gene expression patterns at a network-wide and single gene level, respectively. All analyses were conducted using R version 4.1.1.
Results: We observed significant associations between two modules, enriched for erythropoiesis (n=170 genes) and angiogenesis (n=660 genes), and fetal alcohol exposure. Sixty-four genes were differentially expressed in placenta from heavy drinkers vs. abstainers/light drinkers, and these differentially expressed genes were significantly enriched in the erythropoiesis module.
Conclusion: Our findings indicate that erythropoiesis- and angiogenesis-related pathways active in the placenta are uniquely susceptible to fetal alcohol exposure. Follow-up studies are warranted to evaluate these placental disruptions in association with FASD-related neurodevelopmental deficits that present later in childhood.

DNTS 42

Investigating the effects of prenatal alcohol exposure on the human placenta at the cellular level

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Introduction: Fetal alcohol spectrum disorders (FASD) are the most common preventable cause of birth defects and neurodevelopmental disorders worldwide. The Cape Coloured community in South Africa has one of the highest prevalence of FASD in the world. The placenta is the crucial interphase between mother and fetus involved in fetal growth and development. Prenatal alcohol exposure has been shown to influence bulk tissue placental gene expression, but few studies have examined this relation at the cellular level.
Methods: We leveraged a placenta single-cell RNA-seq dataset to perform cell-type deconvolution of bulk placental tissue RNA-seq data from 35 heavy drinking pregnant women and 34 controls in our prospective birth cohort in Cape Town, South Africa. We used bivariate analyses and adjusted linear regression models to assess the effects of PAE on the RNA-seq inferred placental cell-type proportions. Finally, we performed follow-up analyses to assess differential expression of cell-type specific gene markers.
Results: Deconvolution analyses showed heterogeneous composition; stromal, endothelial and cytotrophoblasts were the predominant cell-types. Average ounces absolute alcohol/day consumed around conception was associated with higher percent of Hofbauer cells (ß= 0.10,p=0.03) in linear models adjusted for maternal age, infant sex, and gestational age. 53 myeloid cell gene markers were differentially expressed in alcohol exposed placentas (FDR $p<0.05$).
Conclusion: Our findings suggest that heavy alcohol exposure during pregnancy can influence the proportion of fetal placental villi macrophages (Hofbauer cells). Larger studies are needed to further characterize these effects and to assess the potential role of placental inflammation in FASD.

DNTS 43

A novel prenatal model of fentanyl exposure and its long-term consequences

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Use of illicit fentanyl has skyrocketed in recent years, triggering a surge in overdoses and fatalities. Fentanyl is 50Xs more potent than heroine and triggers receptor modifications and signaling cascades distinct from other opioids. As many people are testing positive for fentanyl at the time of birth, understanding how the drug affects the mother and the offspring is necessary.

We developed a prenatal model of fentanyl exposure using oral gavage throughout gestation. We hypothesized that early exposure to fentanyl will elicit deficits in motor development, affect, cognition, sociability, and addiction-related behaviors in the offspring.
Overall, we have found few significant differences between treatment groups. Dams treated with fentanyl gained significantly less weight, starting around E15, than controls. There were no significant differences in the number of fetuses, resorptions, or size. We also noted no significant differences in blood O2 levels between dams.
Behaviorally, there were few functional changes in the offspring. Fentanyl-treated mice did not behave differently in the elevated zero maze compared to controls, although in the light/dark assay, a trend emerged such that fentanyl-treated mice spent more time in the darkened chamber. Prenatal fentanyl treatment did not differ in locomotor activity (basal or fentanyl-induced), but in terms of motor coordination, mice exposed to fentanyl had a greater latency to fall (i.e., enhanced motor coordination) on days 2 and 3 compared to controls.
Overall, gestational fentanyl exposure resulted in some behavioral modifications, although further research, such as pharmacokinetic analyses and dose-response curves, is necessary to parse these out.

DNTS 44

The effect of developmental benzo[a]pyrene exposure on brain-derived neurotrophic factor

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Benzo[a]pyrene (BaP) is a polycyclic aromatic hydrocarbon and key component of vehicle exhaust, grilled foods, wildfire and tobacco smoke. BaP is associated with learning and memory deficits in children exposed during pregnancy and early life. Our previous studies found that *Cyp1a2(-/-)* knockout mice were more susceptible to developmental BaP exposure compared with wild type mice. We are now conducting studies on a related line of mice, *Cyp1a1(-/-)* knockouts. All three members of the CYP1 family can metabolize BaP, so we hypothesized that *Cyp1a1(-/-)* mice will also be susceptible since they have reduced capability to clear the pollutant. Pregnant dams are treated with 10/mg/kg BaP in corn oil-soaked cereal from gestational day 10 to weaning at postnatal day 25 (P25). Behavioral testing begins at P60. Following testing, we collected the hippocampi to measure neurotransmitter levels and brain-derived neurotrophic factor (BDNF). BDNF supports brain development and learning and memory. We measured BDNF levels using an ELISA kit and normalized to total protein. In our preliminary study, there were no significant gene x treatment interactions when comparing BaP and control treated *Cyp1a1(+/+)* wild type and *Cyp1a1(-/-)* knockout mice ($P > 0.05$). However, the trends for BaP-treated mice went in the opposite directions. BDNF levels were lower in wild type mice compared with their controls whereas BDNF levels were higher in knockout mice compared with their controls.

DNTS 45

The effect of benzo[a]pyrene on learning and memory in *Cyp1a1(-/-)* knockout and wild type mice

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Exposure to traffic-related air pollution (TRAP) during pregnancy and early life has been linked with lower IQ in exposed children. We used a mouse model to determine if genetic differences affect an individual's risk to the widespread air pollutant benzo[a]pyrene. We are focused on the aryl hydrocarbon receptor (AHR) pathway and the metabolic enzyme CYP1A1 which is regulated by the AHR. Pregnant *Cyp1a1(-/-)* knockout and *Cyp1a1(+/+)* wild type mice were treated with 10mg/kg/day benzo[a]pyrene BaP) from gestational day 10 to postnatal day 25. One male and one female from each litter were tested starting at P60 using novel object recognition and Morris water maze to assess non-spatial and spatial learning and memory. In our preliminary analysis, we found no effect of BaP treatment and no gene x treatment interaction in the novel object test. BaP-exposed mice had longer path lengths on all 6 days of testing in the Morris water maze, but the differences were not significant. There was a significant gene x treatment interaction in the Shift-reduced Probe trial with BaP-exposed Cyp1a1(+/+) knockout mice having more zone crossings than all other groups ($P < 0.01$). There were no sex differences.

DNTS 46

Dopamine and serotonin signaling following developmental benzo[a]pyrene exposure in *Cyp1a1* (-/-) knockout and wild type mice

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Benzo[a]pyrene (BaP) is a widespread pollutant that exerts neurotoxic effects on early brain development. Our recent studies found increased susceptibility to developmental BaP exposure in Cyp1a2(-/-) knockout mice. We are now extending our studies to *Cyp1a1(-/-)* knockouts. For this study, we quantified neurotransmitter levels in adult offspring after behavioral testing. Pregnant dams were treated from gestational day 10 to postnatal day 25 with 10mg/kg/day BaP in corn oil-soaked cereal or the vehicle. On postnatal day 25, one male and one female from each litter were randomly selected for behavioral testing. At postnatal day 120, striatum, hippocampus, prefrontal cortex, and hypothalamus were collected. We used HPLC with electrochemical detection to measure dopamine, serotonin and their metabolites DOPAC and 5HIAA. In the hippocampus, there was a significant main effect of genotype. *Cyp1a1(-/-)* knockout mice had higher DOPAC levels ($P < 0.001$) and dopamine turnover ($P < 0.05$) compared with wild type mice. Knockout mice also had significantly lower 5HIAA levels ($P < 0.001$) and a trend for lower serotonin levels ($P = 0.055$). In the hypothalamus, there was a significant main effect of sex. Females had higher levels of all neurotransmitters and metabolites compared with males ($P < 0.05$). There was a significant gene x treatment x sex interaction ($P < 0.05$) for DOPAC and a trend for gene x treatment x sex interaction for 5HIAA levels ($P = 0.07$). These results suggest that genotype and sex have greater influence on neurotransmitter levels than developmental BaP exposure.

DNTS 47

The aspects of lifestyle that influence ozone induced morbidities: Interactions of non-chemical and chemical stressors on brain

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A critical part of community based human health risk assessment following chemical exposure is identifying sources of susceptibility. Such susceptibilities include lifestyle, life stage, diet, and exercise among many others. The ubiquitous air pollutant, ozone (O_3) induces dysfunction of the pulmonary, cardiac, and nervous systems; conceivably mediated by activation of central stress centers. Therefore, our research group has assessed neurological changes in response to O_3 exposure across these susceptibility factors. Our studies show the O_3 exposure induces systemic effects from the sites of first contact (nasopharyngeal and cardiovascular systems) to distant central centers (various brain regions). One focal point of our studies has been on the impact of subchronic O_3 exposure on mitochondrial bioenergetics. We have shown that O_3 disrupts complex enzyme activities in the brain in a region-specific manner in addition to inducing changes in antioxidants, oxidative damage, and related gene expression. Most interesting are the increases in reactive microglial cells post O_3 exposure. In a separate set of experiments, we have shown that this effect on microglia reactivity was diminished with supplementation of specific PUFAs. These results demonstrate that O_3 induces microglia reactivity within stress centers of the brain and that mitochondrial bioenergetics are altered. Some of these effects may be augmented by diet and exercise, suggesting a role for lifestyle in O_3 effects on brain mitochondrial bioenergetics parameters in agreement with our previous reports on other endpoints. (This abstract does not necessarily reflect USEPA policy).

DNTS 48

Understanding the impact of the environment on maternal health outcomes and disparities: Introduction and future directions

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The environment contributes to a variety of health outcomes, and pregnant women, children, and other sensitive subpopulations can be particularly vulnerable to adverse effects associated with environmental exposures. As the incidence of adverse maternal outcomes such as hypertensive disorders of pregnancy continues to rise and the incidence among Black and Indigenous women increasing at rates two to four times higher than the overall population, it is critical to consider all factors that contribute to disparities in outcomes across racial and ethnic groups to understand risk. The interplay of chemical and nonchemical stressors represents an important consideration in the context of health outcomes and disparities. The development of approaches that consider

and, to the extent possible, model these stressors will allow for a better understanding of the mechanisms that may contribute to health outcome differences across groups.

DNTS 49

Health disparities in pregnancy and postpartum care during public health emergencies

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In recent years, several public health emergencies, including natural disasters and emerging infectious diseases, have threatened the health of pregnant persons and their babies. For example, Hurricane Katrina, a category 5 hurricane that made landfall on the US Gulf Coast in 2005, resulted in significant disruption to health care during pregnancy; an estimated 56,000 pregnancies were directly affected by the hurricane. More recently, during the COVID-19 pandemic, pregnant persons have been shown to be at increased risk for severe disease compared to nonpregnant persons of reproductive age, and SARS-CoV-2 infected pregnancies have been shown to be at increased risk for pregnancy complications such as preterm birth. Even among noninfected persons, the pandemic has disrupted prenatal care and the labor and delivery process. In addition to the effects on pregnant persons, public health emergencies have been shown to exacerbate longstanding health disparities. For example, Hurricane Katrina had varying effects on different communities, with those who were black and poor most severely affected. Likewise, in the COVID-19 pandemic, race-ethnicity and socioeconomic status affected both the prevalence and severity of disease. These issues result in pregnant persons of color facing even greater challenges. During future public health emergencies, early consideration of the effects of pregnancy, race-ethnicity, and socioeconomic status, including the potential drivers of socioeconomic disparities (e.g., living conditions, work settings, and health circumstances), is needed so that disparities can be identified in a timely manner and appropriately addressed.

DNTS 50

Developmental lead exposure, brain and behavioral outcomes: A forty-year prospective study

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The Cincinnati Lead Study is a prospective longitudinal investigation examining the early and late effects of prenatal and childhood lead exposure on growth and development with a particular emphasis on central nervous system outcomes. Data were collected on exposure (blood and bone lead concentrations), neurobehavior, child health, nutrition, environmental nurture, and sociodemographic variables on a quarterly to yearly basis since its inception. Blood lead concentrations were high by contemporary standards with most members of the cohort exceeding 15 ug/dL whole blood during early childhood. Early results indicated an effect of prenatal and postnatal lead exposure on measures of infant and toddler cognitive and neuromotor development

followed by lead-related deficits in IQ in school-age children. Moving beyond IQ, we examined the relationship between early exposure to lead and juvenile and adult criminality. We found associations between prenatal and postnatal blood lead concentrations on measures of juvenile delinquency and repeat measures of adult criminality. On this same cohort of adult subjects, we have conducted neuroradiological studies examining the relationship between childhood lead exposure and a number of parameters of brain anatomy, physiology, and function. Using fMRI we found dose-dependent reductions in brain activation, using HR anatomical MRI we found dose-dependent reductions in cortical gray matter, using DT MRI we found dose-dependent injury to both myelin and axonal structures, and using proton MRS we found dose-dependent reductions in gray matter NAA along with white matter choline declines. Developmental lead exposure appears to have long-term consequences.

DNTS 51

Race differences in phthalate exposure and risk of preterm birth: Findings from sixteen US cohorts

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Environmental phthalate exposure is ubiquitous in pregnancy, and non-Hispanic Black women experience the highest exposure levels in the US compared to women of other racial and ethnic groups. Concurrently, Black women have the highest rates of preterm birth. Our previous findings from the Phthalates and Preterm Birth Pooled Study Team show that urinary levels of some phthalate exposure biomarkers, such as mono-iso-butyl phthalate (MiBP) are associated with increased odds of preterm birth. Thus, this exposure disparity may be an important contributor to the elevated rates of preterm birth experienced by Black women. In this analysis, we used data on prenatal urinary phthalate metabolites measured in 16 US cohorts (N=6042). We investigated differences in urinary phthalate exposure biomarkers by maternal race and ethnicity and examined how those differences in exposure levels contributed to preterm birth. Self-identified race/ethnicity of participants was categorized as non-Hispanic white (43%), non-Hispanic Black (13%), Hispanic/Latina (38%), or Other groups (5%; based on categories too sparse for individual analyses). Pregnant participants who were non-Hispanic Black, Hispanic/Latina, and Other racial/ethnic groups had the highest urinary phthalate metabolite levels. For example, after adjusting for relevant confounders, MiBP concentrations were 80%, 49%, and 33% higher in non-Hispanic Black, Hispanic/Latina, and Other participants compared to non-Hispanic white participants. Also from adjusted models, the predicted probability of preterm birth was highest among non-Hispanic Black (9.3%) participants compared to non-Hispanic white (7.3%), Hispanic/Latina (7.4%), or Other (6.9%) participants. In adjusted logistic regression models for the overall study population, several phthalate exposure markers were associated with increased odds of preterm birth. For example, an interquartile (IQR) increase in MiBP was associated with 16% higher odds of delivery preterm (odds ratio [OR]=1.16, 95% confidence interval [CI]=1.00, 1.35). Certain associations exhibited differences in models stratified by race. For MiBP, odds ratios

were higher among non-Hispanic Black (OR=1.82, 95% CI=1.19, 2.78) and Hispanic/Latina (OR=1.29, 95% CI=1.01, 1.63) participants compared to non-Hispanic white participants (OR=0.92, 95% CI=0.73, 1.16). Dramatic differences in exposures to environmental chemicals, such as phthalates, could play an important role in racial and ethnic disparities in adverse pregnancy outcomes such as preterm birth.

DNTS 52

Association of prenatal acetaminophen exposure with attention at 2 and 3 years

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Background: Acetaminophen is the most common drug ingredient in the U.S., and 50-65% of women take acetaminophen during pregnancy, but little is known about how prenatal exposure may impact neurodevelopment.
Methods: Participants reported their medication use six times during pregnancy, and the number of times acetaminophen was taken by trimester and throughout pregnancy were calculated. Mothers completed the CBCL when their child was 2 and 3 years of age. CBCL data were available for 254 children (119 males,135 females) at 2 and 215 children (101 males,114 females) at 3. Associations of acetaminophen exposure by trimester and throughout pregnancy with externalizing behavior, attention, and ADHD problems scores at each age were assessed using generalized linear models adjusted for acetaminophen formulation, delivery type, number of older siblings, and maternal age, education, and income. Child sex was included as a potential modifier.
Results: Most mothers were non-Hispanic white, college educated, and had an annual household income ≥$50,000. At 2 and 3 years, higher acetaminophen exposure during the second trimester was associated with more externalizing behavior (β=0.32,95%CI:0.07,0.57; β=0.44,95%CI:0.13,0.75), attention problems (β=0.07,95%CI:0.02, 0.12; β=0.09,95%CI:0.04,0.14), and ADHD problems (β=0.09,95%CI:0.02,0.15; β=0.14,95%CI:0.07,0.22). Higher acetaminophen exposure throughout pregnancy was also associated with more externalizing behavior (β=0.40,95%CI:0.05,0.75; β=0.50,95%CI:0.09,0.91), attention problems (β=0.09,95%CI:0.02,0.15; β=0.10,95%CI:0.04,0.17), and ADHD problems (β=0.11,95%CI:0.02,0.20; β=0.16,95%CI:0.06,0.26) at both ages.
Conclusions: Acetaminophen exposure during the second trimester and throughout pregnancy were consistently associated with more externalizing behaviors, attention problems, and ADHD problems at 2 and 3 years, indicating that the safety of acetaminophen use during pregnancy should be reassessed.

DNTS 53

Transgenerational transmission of aspartame induced anxiety-like behavior and amygdala GABA-A receptor signaling mechanisms

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Aspartame is among the most widely-used artificial sweeteners. Aspartame's CNS effects are attributed to changes in monoamine neurotransmission because phenylalanine, precursor of brain monoamines, is a metabolite of aspartame. However, aspartame consumption is not associated consistently with changes in brain monoamine content or monoamine-associated behaviors. In fact, non-monoaminergic mechanisms may contribute to aspartame's CNS effects. For example, aspartame alters the gut microbiome, which in turn affects CNS function. We found that male and female C57BL/6 mice when exposed to 0.015% aspartame in drinking water, a dose equivalent to only 50% of the FDA-recommended maximum daily intake for humans, developed robust anxiety-like behavior. RNA sequencing and RT-PCR showed a significant downregulation in expression of the GABA-A receptor associated protein (GABArap) gene in the amygdala, a key center in brain's anxiety circuitry. A single administration of diazepam, a positive allosteric modulator of the GABA-A receptor reduced aspartame-associated anxiety. When the aspartame-exposed male mice were bred with exposure-naïve females, male and female offspring (F1 generation) showed the anxiety-phenotype, behavioral response to diazepam and downregulation of amygdala GABArap mRNA. When the F1 males were bred with exposure-naïve females, all the phenotypes were found in male and female F2 offspring as well. Thus, aspartame consumption at levels well below those considered "safe" may produce anxiety via downregulation of GABA-A receptor signaling in the amygdala, not only in the aspartame-consuming individuals but also in at least 2 generations of descendants.

DNTS 54

Polymorphisms in choline metabolism genes predict severity of fetal alcohol-related memory deficits in two South African birth cohorts

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Background: Animal and human studies have demonstrated the potential for the nutrient choline to ameliorate teratogenic effects of prenatal alcohol exposure (PAE), including growth and recognition memory deficits. We hypothesized that the presence of maternal SNPs in choline metabolism-related genes may modify fetal vulnerability to PAE.

Methods: Mothers from two prenatally recruited birth cohorts in Cape Town, South Africa (discovery cohort: *N*=149; validation cohort: *N*=153) were genotyped for 315 choline metabolism gene SNPs. Primary outcomes were: height/length z-scores (disc. age 9 yr; val. 5 yr) and recognition memory (disc.=CVLT-C recognition discrimination score, age 9 yr; val.=Fagan Test of Infant Intelligence novelty preference score, 6.5 and 12 mo). Linear regression models were constructed using OLS: outcome ~ PAE + gene dose (# effective alleles) + PAE x gene dose; PAE-gene interaction was tested using 2-sided Wald test on the PAE-gene dose interaction term with Benjamini-Hochberg (BH) correction.
Results: PAE (drinking days/wk) was related to shorter height and poorer recognition memory in both cohorts. Gene-PAE interaction for recognition memory was seen in both cohorts for rs12262538 (5' flanking region of Stearoyl-CoA Desaturase (SCD) gene; discovery BH-adj. p = .0015; validation unadj. p = .004); for rs1043261 (3' flanking region of choline dehydrogenase (CHDH) gene), discovery BH-adj. p = .0235 and validation unadj. p = .0903.
Conclusions: We identified two maternal SNPs that appear to confer higher fetal risk for teratogenic effects of PAE. These findings support the potential protective role of choline in fetal alcohol spectrum disorders prevention.

DNTS 55

Parental preconception and prenatal chlorpyrifos exposure effects on the offspring's gene expression and epigenetic regulations: An avian model

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Parental insult exposure was considered until recent years relatively safe if stopped prior to conception. As was shown in our recently published review, maternal and/or paternal preconception insult exposure could affect the offspring leading to numerous neurobehavioral deficits. Our current investigation focuses on the molecular alterations observed in hatching chicks exposed to the neuroteratogen chlorpyrifos (CPF) prenatally and in chicks whose dams or sires were exposed to CPF prior to conception. The investigation includes analysis of several neurogenesis, cholinergic and serotonergic genes expression, in addition to various epigenetic genes. Sex-specific decrease in the expression of the vesicular acetylcholine transporter (SLC18A3) in the three studied models; paternal (57.7%, $p<0.05$), maternal (36%, $p<0.05$) and prenatal (35.6%, $p<0.05$) models was observed as it was only altered in the female offspring. Paternally exposed female offspring showed significant increase in brain-derived neurotrophic factor (BDNF) gene expression (22.3%, $p<0.0005$), while its targeting microRNA, miR10a, was similarly decreased in both female (50.5%, $p<0.05$) and male (56%, $p<0.05$) offspring. Doublecortin's (DCX) targeting microRNA, miR-29a, was decreased in the offspring after maternal preconception CPF exposure (39.8%, $p<0.05$). Finally, prenatal CPF exposure led to a significant increase in protein kinase C beta (PKCß; 44.1%, $p<0.05$), methyl-CpG-binding domain protein 2 (MBD2; 44%, $p<0.01$) and 3 (MBD3; 33%, $p<0.05$) gene expression. Future directions involve performing next generation sequencing (mRNA seq and miRNA seq) on the

offspring paternally exposed to CPF. Understanding the mechanisms of parentally induced deficits may provide means for the reversal of these deficits towards future clinical application.