2480 Differential Neurotoxicity in Three Generations of Brominated Flame Retardants

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Brominated Flame Retardants (BFRs) are ubiquitously utilized to reduce flammability in a wide range of household products including carpets, upholstery, and paints. While useful chemicals, BFRs also migrate from their products into the environment. This has resulted in continuous, population-level exposure that has been correlated to impaired learning and memory. To determine the effects of multiple BFRs on different stages of neuronal development, human Neural Stem Cells (NSCs) and mouse hippocampal HT-22 cells were exposed to tetrabromobisphenol-A (TBBPA), hexabromocyclododecane (HBCD), or 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) (current, phasing out, and phased out BFRs, respectively). Cell viability analysis was assessed by MTT staining, as well as nuclear morphology after 48 hr of exposure. HBCD exposure resulted in lower IC_{50} values in NSCs (3 μM IC50) and HT-22 cells (15 μM IC_{50}), as compared to TBBPA (NSC 20 μM, HT-22 50 μM) and BDE-47 (NSC 9 μM, HT-22 60 μM). HT-22 cellular and nuclear morphology suggested the presence of apoptosis after exposure to the IC_{50} for each BFR at both 24 and 48 hr exposure. Flow cytometry provided further support for a time and concentration-dependent increase in apoptosis as indicated by increases in annexin V staining in cells. Both 24 and 48 hr exposure to HBCD (50 or 100 μM) or BDE-47 (100 μM) induced significant increases in apoptosis, although 100 μM TBBPA only induced apoptosis and necrosis after 48 hr. Interestingly, these chemicals also induced cell cycle alterations with increasing exposure time. Upon 24 hr exposure, HBCD (50 or 100 μM) induced significant S-phase arrest which was maintained upon 48 hr exposure. However, only upon 48 hr exposure did BDE-47 (50 and 100 μM) and TBBPA (100 μM) induce significant S-phase arrest. These data demonstrate that BFRs can induce chemical-dependent toxicity in neural cells *in vitro*, possibly by multiple mechanisms. Further study is needed to determine if BFR-induced neural cytotoxicity would adversely affect learning and memory *in vivo*.

2481 Exploring Responses to Neuroactive Chemicals through Inter-Individual Variation

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The degree of response to diseases, stressors, but also to toxic chemicals is dependent on the susceptibility of the individual; however, the underlying reasons for these differences remain largely unclear. Inter-individual differences in responses to chemicals can have widespread consequences, as they could tip the scales between efficient drug therapy and life-threatening toxicities. Similarly, in environmental toxicology such differences decide between succumbing or adapting to a chemical threat, potentially extinguishing populations or giving rise to tolerant ones. In particular, for neuroactive chemicals individual effects are difficult to predict, due to the complex etiology of neurological and mental disorders and the multitude of unknown molecular targets. By investigating inter-individual differences in zebrafish larvae, we aim to identify molecular targets of neuroactive chemicals, whilst exploring the reasons underlying variability in responses to these chemicals. We use behavioral measures to sort chemically exposed individual zebrafish larvae based on their sensitivity. Larval locomotor behavior is widely used as a read-out for the assessment of external challenges to the nervous system; however, it is highly variable and difficult to predict at the individual level. Yet, in an initial analysis of unexposed larvae, we found that locomotor activity of an individual becomes consistent from 6 to 7 days post fertilization, with variability lowest when fish encounter sudden darkness. Using this information, we carried out exposures to neuroactive chemicals and sorted the larvae into tolerant and susceptible populations based on their response. Individuals from the different sensitivity categories are currently being subjected to transcriptome analysis to explore the molecular mechanisms that underpin these sensitivity differences. In addition, differentially regulated genes might point to potential molecular targets of the chemicals. Overall, these data will provide mechanistic understanding of toxicological responses that can benefit drug discovery processes and human health, whilst additionally supporting environmental risk assessment, as high quantities of neuroactive chemicals and other man-made chemicals are frequently detected in the aquatic environment.

2482 CSF Proteome as Fluidic Biomarkers of Neurotoxicity

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Neurotoxicity has been linked to exposure to a number of drugs and chemicals, yet efficient, predictive, and minimally-invasive methods to detect it are lacking. Fluid-based biomarkers such as those found in serum, plasma, urine, and cerebrospinal fluid (CSF) have great potential due to the relative ease of sampling, but at present, data on their expression and translation are lacking or inconsistent. Here, we present data on biomolecules that have promise for detection and characterization of neurotoxicity induced by the known neurotoxic agent, trimethyltin (TMT). A single dose of TMT (7 mg/kg, ip) to the rat led to significant alterations in markers of neuroinflammation detectable in CSF, and a proteomic analysis reflected significant alterations in signaling molecules related to neurotoxicity with TMT treatment. TMT samples contained between 29-237 proteins that were significantly different from controls. Network analysis determined that TMT treatment resulted in higher levels of proteins associated with neurological disease and cellular assembly and lower levels of proteins associated with cell survival as compared to controls. These findings provide an opportunity to explore the correlation of these fluid biomarkers with traditional neuropathology and magnetic resonance imaging (MRI) that serve to define TMT-induced neurotoxicity. Our data demonstrate a comprehensive correlation of TMT-induced neuropathology with potential neurotoxicity biomarkers and MRI-based endpoints, findings suggestive of an involvement of specific pathways that can be assessed using peripheral fluids. *Supported by NCTR Protocol E0758001. Disclaimer: This presentation does not represent US EPA or FDA policy.*

2483 Neurodegeneration Induction in Dopaminergic Neurons of *Caenorhabditis elegans* Exposed to Electronic Cigarette (E-cig) Constituents

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Electronic cigarettes (e-cigs) are battery-powered devices, that generate an aerosolized vapor from a liquid and are a popular alternative to tobacco products, particularly among the youth. While the neurotoxic effects of cigarette smoke are well-characterized, little is known about the effects of e-cig constituents on neuronal damage and neurodegeneration. Thus, we investigated the potential neurodegenerative effects of the e-cig constituents, propylene glycol (PG), vegetable glycerin (VG) and PG/VG in combination. *Caenorhabditis elegans* (*C. elegans*) were used to assess the neurodegenerative effects of the e-cig constituents, as they have conserved neurons, are green fluorescent protein (GFP) enabled, and are a successful alternative model to study neuronal morphology and degeneration. For this study, *C. elegans* at the first larval stage (L1) were exposed to dilutions (0-10%) of either PG, VG or PG/VG in nematode growth medium (NGM) agar for 48 hr. Worms were visually observed at 0, 24 or 48 hr. to assess any changes in development and movement compared to normal worms. We observed slower development and movement rates in worms exposed to 5% and 10% of PG, VG and PGVG, with more dramatic effects with PG alone. Neurodegeneration was evaluated after 48 hr. using a fluorescence microscope, to visualize GFP tagged dopaminergic (DAergic) neurons for morphological changes or loss. DAergic neurons are linked to movement and cognition behaviors. Each worm was scored for absence (0) or presence of morphological changes representing degeneration including: thinning of neuron projections (1); 2-3 bleb formations (2); &gt3 bleb formations or shrunken soma (3); and loss or breaks in GFP (4). Notable morphological changes were observed in the neurons upon exposure to PG, VG and PG/VG at 5% and 10%; PG exposure alone had the most severe effects, as worms exhibited the greatest amount of neurodegeneration, with average degenerative percentage at 72.86% compared to controls based on the numbering criteria. Overall, findings indicate that individual and combined constituents of e-cigs adversely affect the DAergic pathways of *C. elegans*, initiating neurodegeneration in this species. This data suggests that like traditional cigarettes, constituents from e-cigs affect neuronal pathways and cause neurodegeneration. *Supported by NYU Dept. funds and NIEHS R01ES10563.*

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