the hepatic transcriptome in offspring. While the PFHxS exposed animals did not show a change in overall bodyweight, we are currently investigating changes in maternal and offspring body composition throughout the lifespan. *This work does not necessarily reflect US EPA policy.*

3754 Toxicity and Transcriptome Comparisons of Different Firefighting Foam Exposures in Human Renal Proximal Tubule Epithelial Cells

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Firefighters have long used Military Specification aqueous film forming foams (AFFF), containing complex mixtures of per- and polyfluorinated alkyl substances (PFAS), to extinguish liquid fuel fires. Chronic disease concerns following AFFF exposure have arisen in the fire service based on elevated PFAS levels with long half lives in firefighter serum compared to the public. Historically, elevated PFAS serum levels are linked to multiple adverse chronic health outcomes including kidney cancer. With phase out of AFFF as early as 2024 in some municipalities, replacement synthetic 'fluorine-free' foams (SFFF) are quickly being integrated into use despite limited information on potential health effects. An urgent need exists to evaluate past AFFF and near future SFFF exposures for key characteristics of carcinogens (KCCs) and underlying mechanisms of disease. The main objective of the study was to compare toxicological responses, cell signaling pathways, and functions in human renal proximal tubule epithelial cells with over-expressed OAT1 (RPTEC-OAT1) following acute exposure to select AFFF and SFFF to identify potential underlying mechanisms of disease. We hypothesized that AFFF exposure causes known PFAS-associated effects and KCCs while SFFF exposure elicits novel effects not associated with fluorine-based constituents. RPTEC-OAT1 cells were exposed for 2 - 24 hr to increasing doses of different products including five AFFF (A-E), six SFFF (F-H), and seven single PFAS species. High throughput multiplex fluorescent screening was conducted using a high content imager. Next, mRNA libraries were prepared from total RNA from lysed cells and subjected to RNAseq on an Illumina Novaseq. Sequences were preprocessed with adapter trimming and alignment-free quantification with Kallisto using Ensembl v96 transcriptomes. Differentially expressed genes (DEGs) for each treatment were identified with DESeq2 and uploaded to Ingenuity Pathway Analysis to identify and compare major changes in signaling pathways and cell functions. All AFFF caused significantly greater cytotoxicity than legacy PFAS species, including PFOS, on a total fluorine dose basis. Four of the SFFF were more cytotoxic than all AFFF. One SFFF sample (J) caused a dose-dependent decrease in mitochondrial membrane polarization while all other foam responses trended with cytotoxicity. Although only one SFFF sample (F) caused robust increased intracellular reactive oxygen species, several AFFF and PFAS species showed decreased antioxidant capacity. Most AFFF and SFFF caused dose-dependent shifts toward G1 phase indicating slowed proliferation. Initial RNAseq results indicate that AFFF exposure affects cell proliferation, antioxidant response, amino acid metabolism, and molecular transport functions while SFFF caused changes in pathways and functions associated acute phase inflammation and membrane functions consistent with a surfactant irritant-like response. In summary, linking phenotypic responses to transcriptomic profiles allows for phenotypic anchoring to identify modes of action of AFFF and SFFF.

3755 Perfluorooctanesulfonic Acid Alters Procancer Phenotypes in Testicular Germ Cell Tumors

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Poly- and perfluoroalkyl substances (PFASs) are ubiquitous chemicals used in daily life. These chemicals are made from strong carbon-fluoride bonds, causing their persistent and bioaccumulative nature. In several animal and epidemiology studies, PFAS exposure has been associated with an increased risk of several cancers, including testicular cancer. While this association has been noted, there has been a lack of mechanistic studies. Nude mice were injected with testicular germ cell tumor (TGCT) cells, both parental and cisplatin-resistant, and dosed with 10 mg/kg perfluorooctanesulfonic acid (PFOS)/day for 15 days. Tumors were measured and then excised. Also, TGCT cells were dosed with 10 and 1000 nM of PFOS with samples collected for metabolomic and RNA-seq analysis. In nude mice, there were minimal changes in tumor growth in parental cells after PFOS exposure. However, cisplatin-resistant cells, which have significantly diminished basal tumor-forming ability compared to parental cells, had increased tumor growth after PFOS exposure. RNA-seq of cisplatin-resistant tumors showed alterations in DNA methylation and polycomb repressive complex 2 (PRC2) target genes and polycomb alterations linked to the tumor-forming ability of TGCT cells. These alterations were consistent in metabolomics analysis, specifically in metabolites involved with the Warburg effect. Ribose-5-phosphate and 2-phosphoglycerate concentrations were affected by PFOS, while RNA-seq analysis showed alterations in Kras, hypoxia, and spermatogenesis, along with PRC2 target pathways. These results provide novel insight into the proposed PFAS-mediated promotion of TGCTs. Validation and in-depth pathway analysis studies are ongoing.

3756 Development of a B Cell Bioenergetic Profile for Immunotoxicants: Focus on Effects of PFAS on *Ex Vivo* B Cell Stimulation

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Per-and poly-fluoroalkyl substances (PFAS) are synthetic, highly persistent chemical contaminants that have been detected in the environment, especially in drinking water. Despite their industrial and consumer applications, PFAS exposure has been associated with many adverse effects in humans, including suppression of vaccine antibody responses. Our laboratory's studies with animal models indicate that PFAS exposure reduces the T-cell dependent antibody response (TDAR), which is a functional outcome analogous to the vaccine response in humans. Our previous studies with a well-studied PFAS, perfluorooctanoic acid (PFOA), indicate that suppression of the TDAR is linked with impacts on B cell functions. Given that PFAS, including PFOA, are known to affect basal metabolic functions, and that naïve B cells undergo several metabolic shifts and experience high metabolic demands when transitioning to antibody-secreting plasma cells, we hypothesize that suppression of the TDAR arises from B cell metabolic impairment. Adult male and female C57BL/6 mice were given PFOA (0 or 7.5 mg/kg) via gavage for 30 days. This dose is known to be immunosuppressive in this strain of mice in the absence of systemic toxicity. One day after dosing ended, naïve B cells were isolated from spleens by negative bead selection, and then these enriched B cells were stimulated *ex-vivo* by the addition of α-CD40 and IL-4 antibodies. After 24 hours in culture, the enriched B cells were assessed for mitochondrial function by a mitochondrial stress test to measure basal, maximal, and reserve respiratory capacities. Our data demonstrate increases in basal, maximal, and reserve capacities between unstimulated and stimulated groups in both males and females of exposed and control groups, supporting proof of concept for *ex vivo* stimulation. These preliminary data indicate that *in vivo* PFOA exposure shifted the bioenergetic profile of B cells and thus may affect B cell development and antibody responses through alteration of mitochondrial function. Additional studies will explore mitochondrial function across a range of B cell subtypes and assess energy usage within both proliferating and differentiating B cells.

3757 Toxicity Mechanisms of Legacy and Novel Sulphonated Perfluoroalkyl and Polyfluoroalkyl Substances: PFAS

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Perfluoroalkyl and polyfluoroalkyl Substances (PFAS) are a broad chemical class of highly complex synthetic compounds used in products and processes that are in direct contact with the environment or with humans, such as food packaging and textiles. Due to their stability, PFAS are omnipresent in the environment, including in the remote Arctic regions, and extremely persistent. The current understanding is that these substances will remain in the environment forever. Besides, PFAS are bioaccumulative in human and animal tissues, and plants. As PFAS became increasingly regulated, other fluorinated alternatives were developed and introduced on the global market. Although the toxicological implications of human exposure to certain (legacy) PFAS are well-studied and recognised, data on lesser-characterised and/or novel PFAS are still very limited. In fact, only a small fraction of PFAS has been assessed for toxicity. Such a knowledge-gap causes uncertainties regarding the possible human-health risks associated with exposure to PFAS and subsequently hampers proper risk management actions. Therefore, there is a dire need to collect experimental toxicity information of poor-data/alternative PFAS. The high serum-binding of PFAS may influence their cytotoxic activity. Therefore, the influence of foetal bovine serum (FBS) and bovine serum albumin (BSA) on the cytotoxic activity of one legacy (Perfluorooctanesulfonic acid (PFOS)) and seven strategically selected novel/alternative PFAS, all with a sulfonate functional group attached, was investigated. Two simple epithelial *in vitro* models, i.e. A549 (lung) cells and a differentiated co-culture of Caco-2 HTB-37 and HT-29 MTX cells were exposed to the compounds in the presence of zero, low (1%) and high (10%) FBS, or BSA (1 mg/mL). Cells exposed to PFOS in the presence of BSA displayed an increased susceptibility to the cytotoxic actions of PFOS than cells exposed in the presence of any or no level of FBS. This was observed for some, but not all alternative PFAS. In contrast, cells exposed to the alternative PFAS 11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (8:2 Cl-PFESA) in the presence of no or low FBS tolerated a lesser concentration than cells exposed in high FBS and BSA. Furthermore, a large discrepancy in sensitivity between the cellular models was observed, with the intestinal epithelium being the less sensitive system. This could in part be attributed to the models' inherent properties, yet it warrants further investigations into the specific toxicity mechanisms in different organ systems. Future studies encompass single-cell transcriptomics of PFAS exposed advanced *in vitro* 3D models representative of the main PFAS exposure routes and contact sites, including the intestinal barrier, the alveolar barrier and the skin. These results are expected to inform on potential apical effects that can be measured in high throughput assays, and so contributing to a testing toolbox to improve and address the hazard of PFAS exposure to human health and ultimately, facilitate read-across actions between legacy and data-poor compounds.

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