no effects on maternal or fetal bodyweight. PFO5DoA significantly reduced GD22 maternal serum total triiodothyronine (T3) at ≥3 mg/kg and total thyroxine (T4) at ≥10 mg/kg, while fetal serum total T3 and T4 were both significantly reduced at ≥1 mg/kg. GD22 fetal liver glycogen concentration was significantly reduced at ≥0.3 mg/kg. PFO5DoA significantly increased maternal liver weight at ≥3 mg/kg. GD22 fetal livers displayed significantly altered glucose metabolism genes at ≥0.3 mg/kg, including >170-fold upregulation of *Pck1* and >20-fold downregulation of *Ugp2*. GD22 dam clinical chemistry displayed multiple significant dose-related changes at ≥3 mg/kg including elevated ALT and BUN:creatinine and reduced cholesterol, triglycerides, albumin, globulin, and total protein. Fetal serum displayed significantly elevated BUN at ≥0.3 mg/kg and elevated total bile acids and glucose at ≥3 mg/kg. In the GD8-PND2 exposure study PFO5DoA produced no overt maternal toxicity with bodyweights and weight gains similar to control at all doses. Pup birthweight and bodyweight on PND2 were significantly reduced at ≥3 mg/kg. Maternal and pup liver weights were significantly increased at ≥1 mg/kg. Pup survival was significantly reduced at ≥3 mg/kg. Fetal serum PFO5DoA concentrations were approximately 2-fold greater than maternal serum levels on GD22, while PND2 pup serum levels were about 2-fold greater than maternal levels at the low dose but were similar at the high dose. Liver concentrations on PND2 were similar between maternal rats and pups. Ongoing analyses will evaluate maternal and pups serum thyroid hormones, pup liver glycogen concentration, liver gene expression, and liver and kidney histopathology in maternal and neonatal rats. **Conclusions:** PFO5DoA produced toxic key events and adverse outcomes in maternal, fetal, and neonatal rats consistent with other straight chain and ether-linked PFAS carboxylates we have investigated in similar study designs, including PFOA, HFPO-DA (GenX), and PFMOAA. The most sensitive of these effects include reduced serum thyroid hormone concentration, increased liver weights, and multiple alterations to glucose, lipid, and protein homeostasis. The most notable difference was the greater oral potency of PFO5DoA compared to the other carboxylate PFAS we have studied. The rank order of oral dose potency for effects on reduced pup survival were PFO5DoA>HFPO-DA>PFOA>PFMOAA. Despite the presence of multiple ether linkages disrupting the carbon chain, PFO5DoA behaved nearly identical to PFOA and other carboxylate PFAS. This is particularly important for estimating human health risk from exposure to multiple PFAS, which have been shown to produce dose additive joint toxicity. The views expressed in this abstract are those of the author(s) and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

3541 Comprehensive analysis of the effects of PFAS on the cellular pathways and potential toxicities

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Background and Purpose: There are many compounds produced by chemical industry that end up in the environment. The per- and poly-fluoroalkyl substances (PFAS) are some of these compounds. PFAS are synthetic chemicals widely used in industry, such as textiles, chemical manufacturing, and fire extinguishers. PFAS are a major global concern due to their persistence in the environment, propensity to accumulate in mammals, and extensive associations with adverse health outcomes. While certain PFAS like perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been extensively studied (e.g., animals, cells, and mechanistic assays), and many PFAS lack sufficient toxicity information. PFOS and PFOA were ultimately banned in the United States, but increased production of alternative PFAS with limited available safety data represents a crucial gap for environmental health. **Methods:** In this study, we analyzed mechanistic-based screening data from PFAS exposures with Tox21 high-throughput assays surveying, with concentrations ranging from 2nM to 92μM, more than 75 assay endpoints (e.g., nuclear receptors, developmental signaling, stress response pathways, and drug metabolism). Each PFAS was analyzed for its effect in this assay panel, including cell-based assay and enzyme-based assays. **Results:** Our findings revealed several PFAS affected one or more nuclear receptors including androgen receptor, estrogen receptor, progesterone receptor, retinoid X receptor, peroxisome proliferator-activated receptors, and thyroid receptor, which have been previously reported. We also discovered previously unreported interactions between PFAS and estrogen-related receptor α (ERR α), retinoic acid receptor (RAR), and retinoid-related orphan receptor γ (RORγ), which play critical roles in development, immunity, and reproduction. Furthermore, some PFAS exposure levels resulted in cellular stress responses (e.g., oxidative stress repair inhibition) with plausible linkages to human diseases (e.g., diabetes, neurodegenerative conditions, cancer and cardiovascular disorders). In addition, we found that many PFAS markedly inhibited the functional activity of major human drug metabolizing enzymes cytochromes P450s (CYPs), especially CYP2C9, which serve essential roles in cellular metabolism and xenobiotic clearance. These findings have important implications for drug clearance, the combined effects of PFAS mixtures, and their ultimate translation to human health. **Conclusions:** In this study, we used Tox21 screening data to analyze the effects of PFAS on toxicological-related targets and pathways, which are a major public health concern around the world. We identified several PFAS as endocrine-disrupting compounds (i.e., NR agonist/antagonist), inducers of cellular stress, and CYP inhibitors along with their respective potencies. Furthermore, our findings revealed novel biological targets such as ERRα, RAR, and RORγ that warrant further study. Our results provide valuable pieces of the complex PFAS puzzle to enhance our understanding of their biological action in humans and mechanistically inform safety assessments with the larger family of PFAS.

3542 Systemic and immunotoxicity induced by topical application of perfluoroheptane sulfonic acid (PFHpS) in a murine model

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Background and Purpose: Per- and polyfluoroalkyl substances (PFAS) comprise a broad class of synthetic surfactants used in many industrial and consumer products, from firefighting foams and stain-resistant textiles to food packaging, non-stick cookware, cosmetics, and personal care products. The hydrophobic and hydrophilic moieties of PFAS give them both oil and water-repellent properties, and their carbon-fluorine bonds make them highly stable biologically and environmentally. Due to these properties, PFAS are used widely, increasing the potential for human exposure through oral, dermal, and respiratory routes. Although multiple PFAS are detectable in human serum, research is needed to identify and characterize the potential biological effects of each PFAS and the specificity pf these effects to the exposure route. One such PFAS commonly detected in human serum is perfluoroheptane sulfonate (PFHpS). This study investigates the systemic and immune effects of dermal exposure to PFHpS in a murine model (B6C3F1, female mice). **Methods:** Following a 28-day sub-chronic dermal exposure to PFHpS (0.3125%, 0.625%, and 1.25% or 7.8-15.6mg/kg/day) organ weight, serum chemistry, histology, immune phenotype, and gene expression were analyzed. To further evaluate adaptive immune responses following exposure, a second study with a 10-day sub-chronic dermal exposure to PFHpS (0.625%, 1.25%, and 2.5%) was performed. **Results:** Supporting the relevance of dermal contact, PFHpS levels increased in serum and urine with increasing PFHpS exposure concentration. Exposed skin demonstrated epidermal thickening supported by gene expression changes associated with barrier function and wound healing. Regarding hepatic effects, PFHpS significantly increased liver weight, serum alkaline phosphatase, and serum alanine aminotransferase. Significant decreases in serum glucose and significant increases in serum cholesterol were observed. Liver histological findings included hypertrophy and immune cell infiltration. PFHpS exposure altered the expression of genes associated with steatosis, fatty acid metabolism, hepatotoxicity, lipid transport, and necrosis. Suggestive of immunotoxicity, exposure to 1.25% PFHpS for 28 days decreased spleen weight, thymus weight, and spleen cellularity. By histology, the spleens of PFHpS-treated mice were thinner and had smaller lymphoid aggregates. The IgM response to sheep red blood cells was significantly reduced following the 10-day exposure to 1.25% and 2.5% PFHpS. Immune phenotyping of the spleen identified that PFHpS altered immune cell subtype abundance, including CD11+ myeloid-derived cells, CD4 T cells, and B cell numbers. **Conclusions:** These findings suggest that dermal PFHpS exposure results in hepatotoxicity and altered immune function. However, further work is necessary to characterize the mechanisms of these alterations and their relevance to human health outcomes.

3543 POLYFLUORINATED ALKYL SULFONATES AS ORPHAN NUCLEAR RECEPTOR 4A1 (NR4A1) AGONISTS THAT INDUCE CANCER CELL GROWTH

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Background and Purpose: Polyfluorinated alkyl substances (PFAS) are an important class of industrial compounds that have been used extensively in a broad array of commercial products including non-stick cookware, textiles, food packing materials and personal care products. Various PFAS congeners have been detected in the environment as contaminants in fish, wildlife, humans, and in food products and this is due, in part, to their lipophilicity chemical and environmental persistence and stability. Polyfluoro-1-octanesulfonate (PFOS) has been widely used and is frequently identified in human samples and epidemiological studies have shown correlations between levels of PFOS and other PFAS and increased incidence of many diseases including cancer. Cell culture and *in vivo* studies of PFAS show that these structurally diverse compounds act through multiple pathways and bind several receptors, however, correlations between PFAS-induced biological effects and the association of these compounds with cancer and other adverse health outcomes are limited. For example, most studies in cancer cell lines show that PFAS inhibit cell growth and are cytotoxic. In this study, we found that among several cancer cell lines, Rh30 rhabdomyosarcoma cells were the most sensitive to PFAS-induced cancer cell proliferation and were used primarily as a model for investigating the pro-oncogenic activity of PFOS and structurally-related

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