Developmental exposure to a PFAS mixture: PFOA, PFOS and PFHxS alters the neonatal liver transcriptome in mice

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STEEP Sources, Transport, Exposure & Effects of PFASs
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ABSTRACT

Perfluoroalkyl substances (PFAS) are persistent man-made environmental toxicants known to cause adverse health effects. Perfluorooctanoic Acid (PFOA), Perfluorooctanesulfonic Acid (PFOS) and Perfluorohexanesulfonic Acid (PFHxS) were used for decades and most frequently detected in the United States population. PFOA, PFOS, and PFHxS can cross the placental barrier, distribute to the fetus, and induce developmental toxicity in animal models. Most rodent PFAS studies highlight singular PFAS, yet human samples often have multiple PFAS present. Thus, understanding the simultaneous effects and elucidating mechanisms is needed to understand whether PFAS act additively, antagonistically, and/or synergistically. The aim of this study was to examine the effects of gestational and lactational PFAS exposure on the pup liver transcriptome to explicate mechanisms of developmental toxicity. Timed-pregnant CD-1 dams were randomly assigned to a standard chow (SD) or 60% kcal high fat diet (HFD). From gestational day (GD) 1 until postnatal day (PND) 20, dams were orally gavaged with: 0.5% Tween 20 (VEH), 1 mg/kg either PFOA, PFOS, PFHxS, or a PFAS mixture (3 mg/kg = 1 mg/kg of PFOA, PFOS, and PFHxS each). Pup livers were collected, RNA was isolated, and samples were prepared for transcriptomic analysis. Data revealed significant modulation in VEH SD vs PFAS Mix SD, showing upregulation of Cidec (2.44-fold), Cyp4a10 (2.23-fold), and Cyp4a12a (2.00-fold). Pathway analysis will be presented.

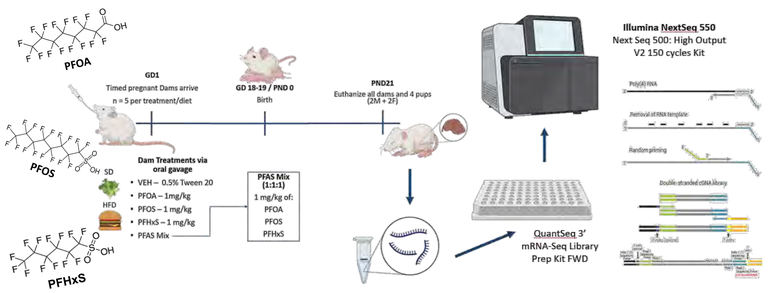
INTRODUCTION

PFAS are persistent man-made environmental toxicants, that are currently minimally regulated regarding the production and manufacturing of these compounds. The full array of adverse health impacts seen as a resultant of exposure is still yet to be completely understood. Perfluorooctanoic Acid (PFOA), Perfluorooctanesulfonic Acid (PFOS) and Perfluorohexanesulfonic Acid (PFHxS) are commonly known and studied chemicals in this group of compounds. PFOA, PFOS and PFHxS induce hepatocyte peroxisome proliferation, liver hypertrophy, vacuolization, and hyperplasia in rats and mice. Additionally, exposure causes elevated liver enzymes, liver enlargement, and hepatic steatosis in adult mice. PFOS and PFOA-induced hepatotoxicity has also been observed in models of developmental exposure. PFOS and PFOA have been identified in umbilical cord serum and human breast milk as potential routes of exposure to the fetus and neonate. Additionally, exposure to PFOS has been shown to alter the adult and pup liver transcriptome. While PFOA, PFOS, and PFHxS effects are well described in adult mice, few studies have examined the effects to liver during developmental exposure. There are minimal studies investigating the impact of PFAS mixtures on the pup liver transcriptome. Most literature available highlights the impacts and effects of individual PFASs. While it is important to understand the individual effects of these toxicants, human samples have shown multiple PFASs to be present within tissues. To further understand the simultaneous effects of these chemicals and elucidate mechanisms, it is imperative to look at how these compounds may act, either additively, antagonistically, and/or synergistically.

HYPOTHESIS

Pup exposure to PFOA, PFOS, PFHxS, and a PFAS Mixture, via the dam - both in utero (gestational) and after birth (lactational) - will significantly alter the pup transcriptome at postnatal day 21 (PND 21) and modulate genes involved in metabolism, inflammation, and lipid transport, storage and synthesis. The PFAS mixture will have additive/synergistic/antagonistic effects when compared to the treatments alone.

MATERIALS and METHODS



Treatment paradigm. Treatments were administered via oral gavage (10 mL/kg) from GD1 to PND 21. The pup exposure was gestational (GD 1 to GD 18/19) and lactational (GD 18/19 to PND 21). On PND 21, all dams and 4 pups (2 M and 2 F) per dam were euthanized and livers were collected for analysis **(Figure 1).**

RNA Isolation and Library Prep. Livers snap-frozen at time of necropsy were cut into 15-25 mg pieces and prepared according to the IBI Total RNA Mini Kit (IBI Scientific, Dubuque, Iowa, USA) instructions. RNA was quantified using the ThermoFisher Nanodrop 1000 and diluted with DEPC water to equal concentrations for library prep input. The QuantSeq 3'mRNA-Seq Library Prep Kit (Lexogen, Vienna, Austria, EU) was used for library preparation and followed according to protocol. The prepared library was processed using the Illumina NextSeq 550 sequencer and Next Seq 500: High Output V2 150 cycles Kit (Illumina, San Diego, CA, USA).

Analysis. Sequencing data after the run was imported into the BaseSpace Sequence Hub (Illumina, San Diego, CA, USA) and the BlueBee Genomics Platform (Lexogen, Vienna, Austria, EU) was used to trim, align and count reads, and perform differential expression analysis. Further pathway analysis was conducted using the IPA software loaded with the Log2FC, p-values, and q-values for each comparison. Additionally, analyses comparing all comparisons combined was run to look at overarching pathways and upstream regulators between treatments.

RESULTS

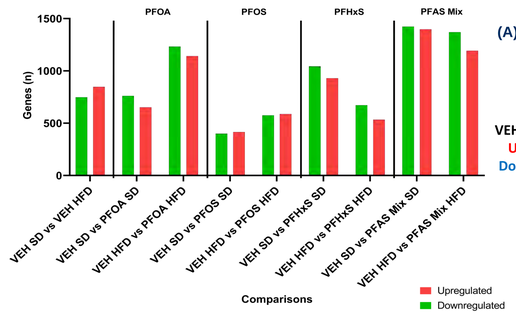


Figure 2. The number of genes up/downregulated within each diet and treatment. Gene number based on significant (p <0.05) Log2FC values, filtering out all insignificant Log2FC values. VEH SD vs PFAS treatment SD* and VEH HFD vs PFAS treatment HFD
*PFAS Treatments include: PFOA, PFOS, PFHxS, and PFAS Mixture

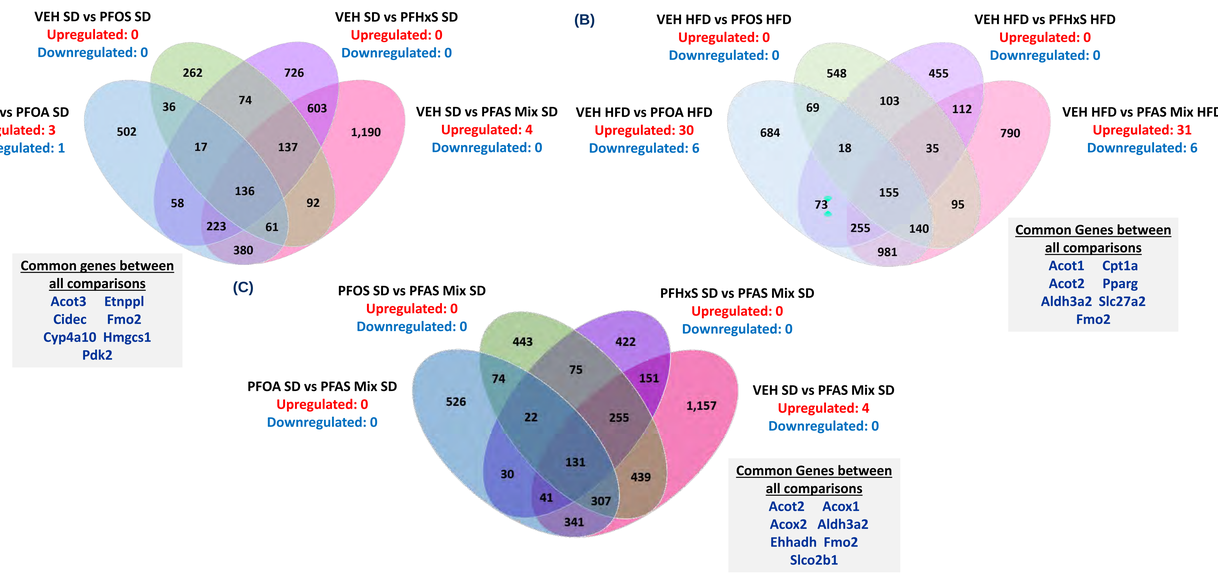


Figure 3. Individual and commonly observed genes within treatment comparisons - focusing on diet and PFAS-based effects, the top commonly observed genes between all comparisons are highlighted. Venn diagrams were created with significant (p <0.05) Log2FC values. (A) VEH SD vs *PFAS treatment* SD*, (B) VEH HFD vs *PFAS treatment* HFD*, and (C) *PFAS treatment* SD* vs PFAS Mix SD. Up/downregulated genes highlighted have Log2FC ≥ 2.
**PFAS Treatments* include: PFOA, PFOS, PFHxS, and PFAS Mixture

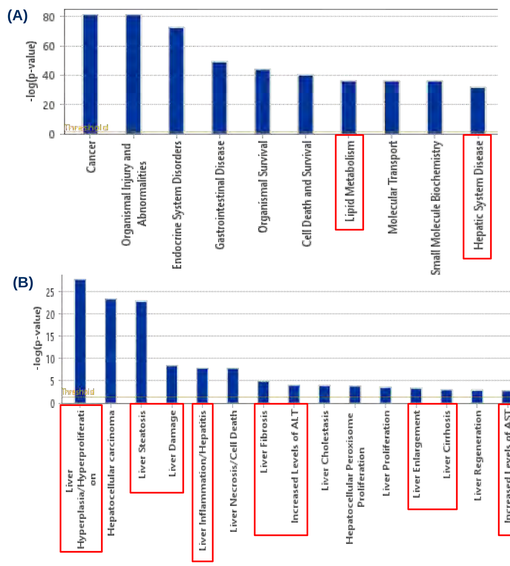


Figure 4. The SD PFAS mixture activated pathways involved in liver damage and disease in the neonatal transcriptome. (A) IPA Diseases and Biological Functions Analysis. (B) IPA Toxicological Functions Analysis. Liver specific pathways highlighted.

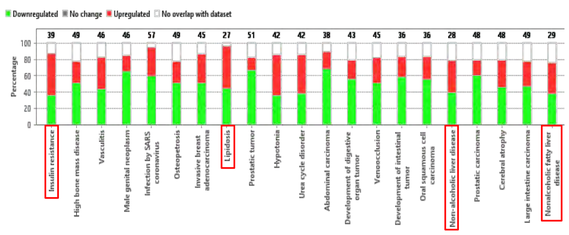


Figure 6. IPA machine learning predicted dysregulated disease pathways using key molecules within the dataset that may be causally connected to the disease/phenotype. Liver specific pathways highlighted. all the relationships in the machine learning disease pathways are supported by findings from the Knowledge Base, though the implicit associations of some of the genes and functions with the disease may be inferred.

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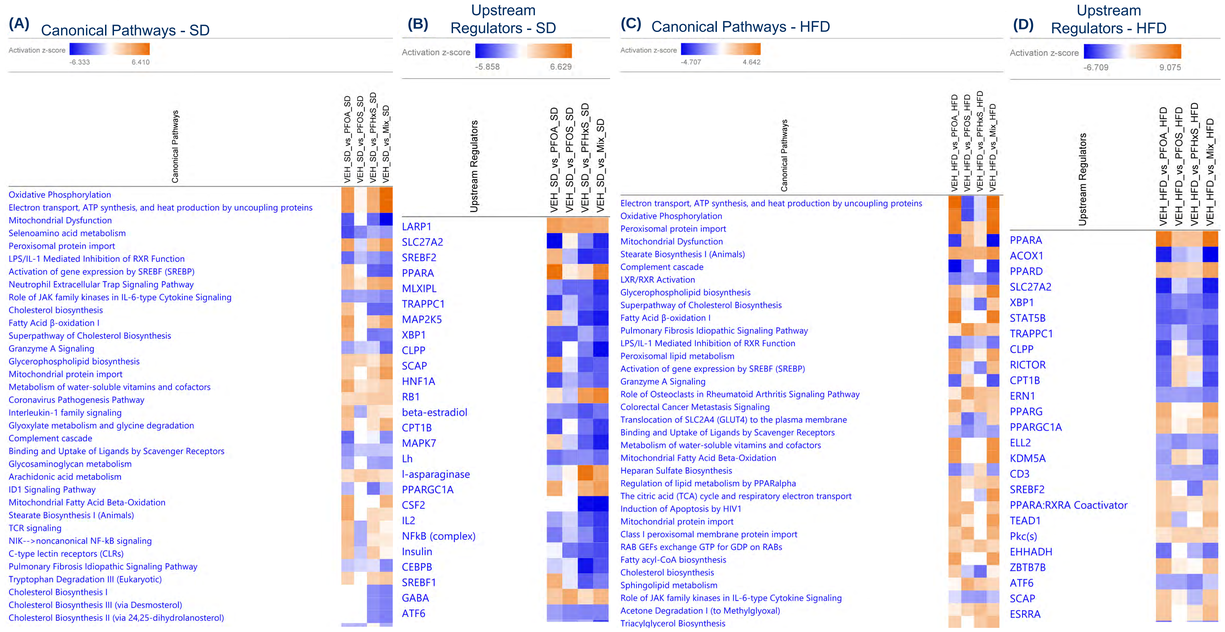

Figure 5. Canonical Pathways and Upstream Regulators involved in liver damage, lipid metabolism, and immune response in both SD and HFD comparisons are differentially activated with indirect PFAS exposure to the pup. (A) SD Comparisons – IPA Canonical Pathways Analysis. (B) SD Comparisons – IPA Upstream Regulator Analysis. (C) HFD Comparisons – IPA Canonical Pathways Analysis. (D) HFD Comparisons – IPA Upstream Regulator Analysis.

CONCLUSIONS

Indirect maternal exposure (gestational and lactational) to PFOA, PFOS, PFHxS and a 1:1:1 mixture significantly modulated the neonatal liver transcriptome in CD-1 pups. The PFAS Mix, both SD and HFD, altered the highest number of genes as compared to individual PFOA, PFOS, and PFHxS treatment independent of diet, as seen in **Figure 2**. The venn diagrams in **Figure 3 A-C** highlight the number of individual and commonly observed genes between treatments and diets, with commonly observed genes highlighted. Pathways involved in liver damage and disease, fatty acid metabolism, lipid accumulation, and immune dysfunction were activated as visualized in **Figure 4.** The PFOA SD and PFHxS SD treatments resemble the response of the PFAS mixture SD, with the mixture showing a more robust up/downregulation. The PFOS SD signature is weaker individually, suggesting PFOA and PFHxS potentiates PFOS within the mixture, as visualized in **Figure 5 A-D**. IPA machine learning predicted dysregulated insulin resistance, lipidosis, and NAFLD pathways using the most important genes causally connected to the disease/phenotypes and to one another, visualized in **Figure 6.**