Identifying key events that drive neurotoxicity in larval zebrafish with transcriptomic concentration response modeling

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Background

- Gene expression data from short-term *in vivo* zebrafish studies shows efficacy in quantifying concentration-dependent effects that inform toxicity outcomes.
- We previously showed that potassium perfluorohexane-1-sulfonate (PFHxS) and perfluorooctane sulfonic acid (PFOS) elicit developmental neurotoxicity (DNT).
- We hypothesized that shared changes in gene expression could be identified in zebrafish exposed to PFHxS, PFOS, or heptachlor (i.e. positive control for DNT) that can serve as the basis for gene editing studies to solve mechanisms by which putative environmental chemicals cause hyperactivity *in vivo.*

1. Experimental design

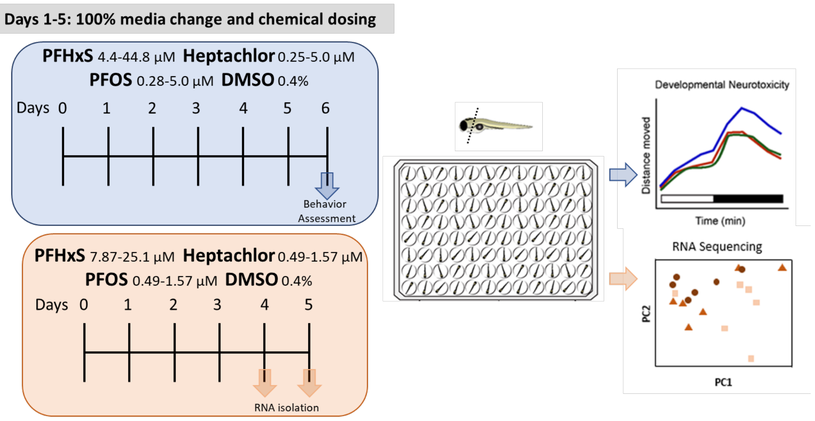


Figure 1. Automated behavioral assessments on 6 days post fertilization (dpf) revealed hyperactivity for all compounds at non-teratogenic concentrations. RNA was isolated from pooled head tissue collected at 4 and 5 dpf, before the onset of hyperactivity, for sequencing (NextSeq 500).

2. RNA-sequencing analysis methods

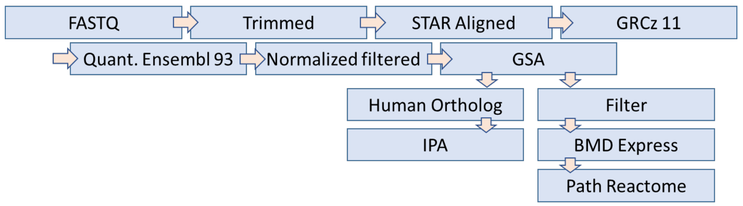


Figure 2. FASTQ files were uploaded into Partek Flow, adapters trimmed and reads aligned to GRCz11 (STAR v.2.6.4d). Reads were quantified to Ensembl GRCz11 v. 93 gene annotation model, low expression filtered (geometric read <1), quantile-normalized count data, and adjusted for batch effects. DEGs (differentially expressed genes) were identified using Gene Specific Analysis (Partek Flow). Significant DEGs (p-value <0.05 and ±1.5 fold change filter) were mapped to human orthologs for analyses in Ingenuity Pathway Analysis (IPA). Benchmark concentration modeling of gene expression data was completed in BMDExpress 2.2.

3. Exposure to PFAS compounds or heptachlor yielded distinct behavior phenotypes at 6 dpf and gene expression changes at 4 and 5 dpf

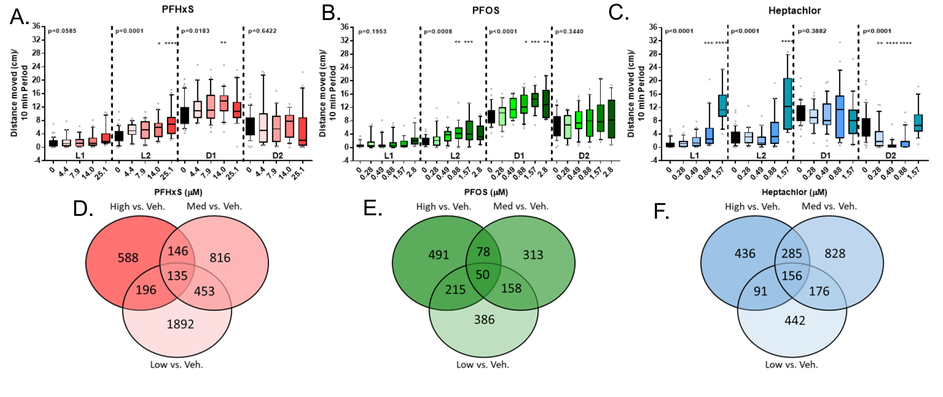


Figure 3. (A-C) Exposure to PFOS, PFHxS, caused hyperactivity in the light 1 (L1) and dark 1 (D1) period whereas Heptachlor caused hyperactivity in the L1 and L2 period and hypoactivity in the D2 period. Differentially expressed genes (DEGs) at 4dpf measured in head tissue obtained from zebrafish developmentally exposed to PFHxS, PFOS, and Heptachlor.

4. Ingenuity Pathway Analysis (IPA) of PFAS and Heptachlor DEGs revealed enrichment in neurologic related pathways and peroxisome proliferation at 4 dpf

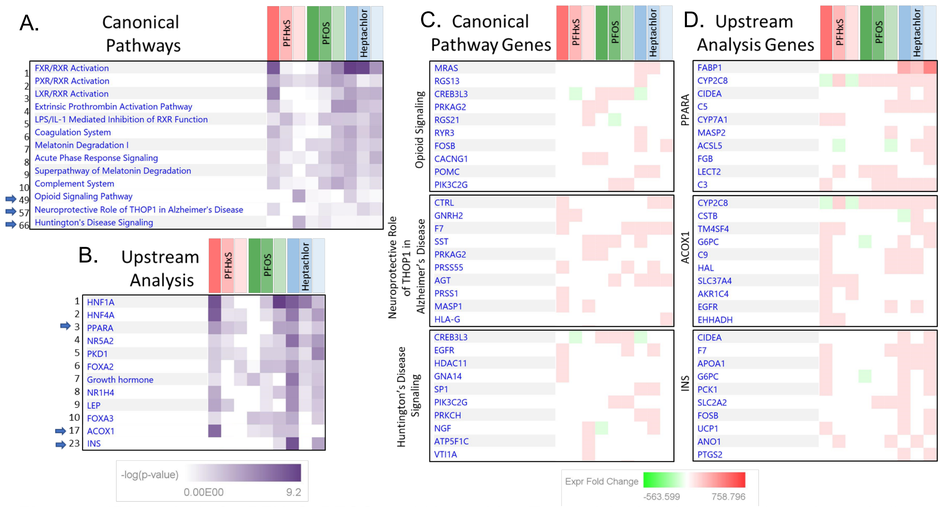
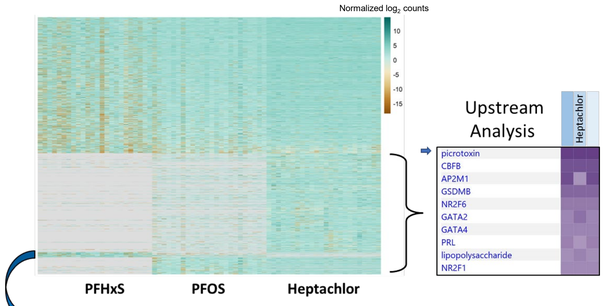


Figure 4. IPA analysis for PFHxS, PFOS, and Heptachlor. Top enriched pathways and regulators indicate a lot of similarity across the three chemicals. Some Heptachlor specific pathways and regulators (MRAS and INS) may help explain differences in behavior response. PFOS enrichment was not always dose-dependent.

5. DEGs enriched solely by Heptachlor implicate $GABA_A$ receptor as a possible mediator of the behavioral differences observed between PFAS and Heptachlor



PFAS compounds yielded 42 distinct genes predicted to be involved in regulating DNA binding activity, G protein coupled receptors, nucleosomes, and nucleolar RNA

Figure 5. DEG enrichment differences across tested chemicals. DEGs impacted by Heptachlor and not PFOS and PFHxS and vice versa may help explain behavioral differences in mode of action. Heptachlor specific DEGs were similar to a known $GABA_A$ antagonist and convulsant, picrotoxin. PFAS specific DEGs suggest possible epigenetic effects.

6. Transcriptomic benchmark concentration response modeling exhibits concordance with *in vivo* behavioral response

	LOEC hyperactivity µM	Median BMD_T values 4dpf µM (Median BMDL)	Median BMD_T values 5dpf µM (Median BMDL)
PFHxS	14	18 (10)	10 (5)
PFOS	0.88	2 (1)	1 (1)
Heptachlor	0.88	1 (1)	1 (1)

Figure 6. Concentration estimates from transcriptomic benchmark concentration modeling were comparable to *in vivo* LOEC values for hyperactivity.

Summary

- These data show that transcriptomic points of departure can be linked to hyperactivity (i.e. a functional DNT toxicity outcome) in larval zebrafish.
- This can inform mode of action delineation and enhance chemical risk assessments.
- Future work will evaluate the essentiality of predicted upstream regulators using gene editing coupled with automated behavior testing.

The data presented do not necessarily reflect EPA policy.