

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

Developmental Neurotoxicity Journal Club

April 8th 2020, 12PM Room B109



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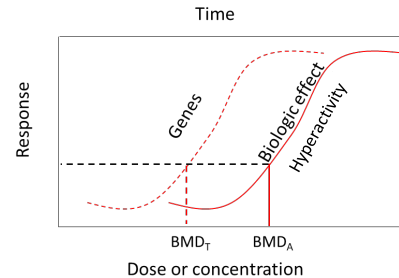
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Ecotoxicology Department, Leipzig, Germany; ³US EPA/ORD/CPHEA/PHITD, RTP, NC; ⁴US EPA/ORD/CCTE/CCED/ETTB, RTP, NC

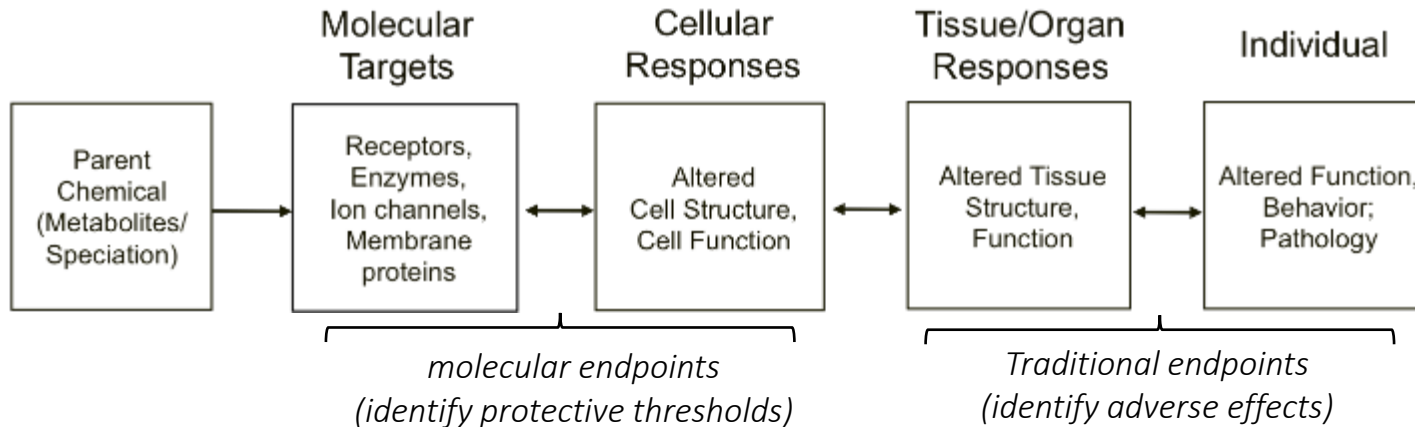
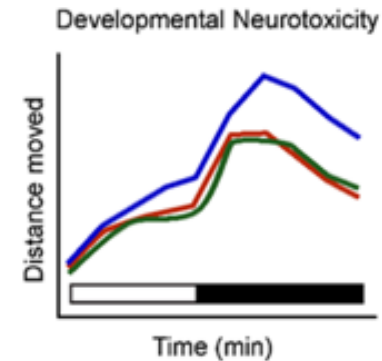
The data presented do not necessarily reflect EPA policy.

Changes in gene response from chemical exposure, which precede adverse behavioral effects, can help us understand developmental neurotoxicity

Gene expression

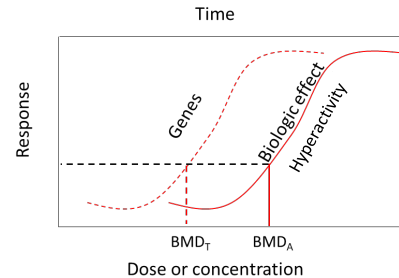


BMD: Benchmark dose or concentration resulting in 10% change in gene response (BMD_T) or traditional adverse effect (BMD_A) compared to matched controls

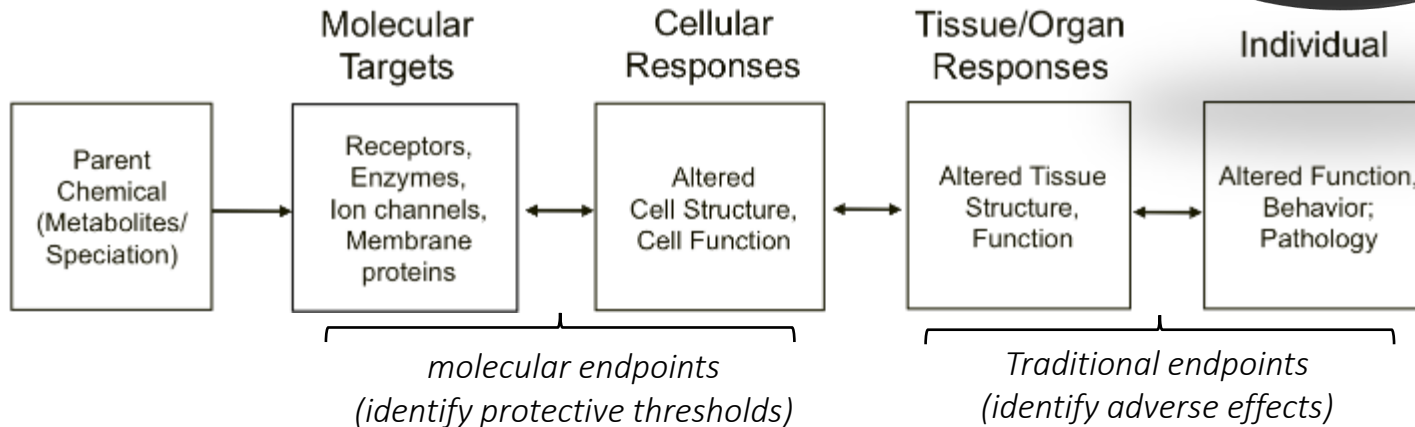
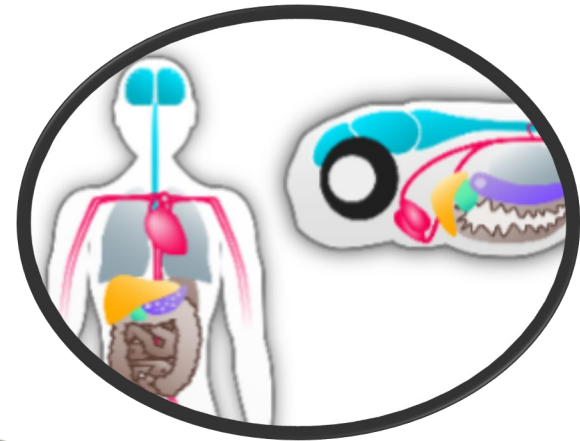


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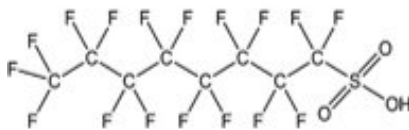


Measure impact effects of similar PFAS on zebrafish light/dark response

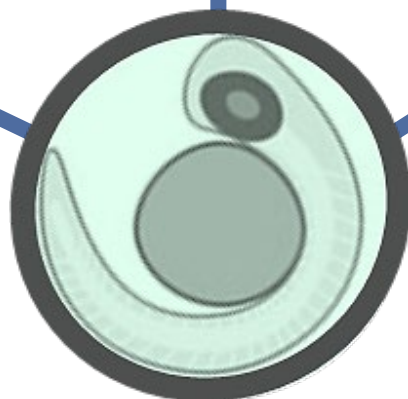
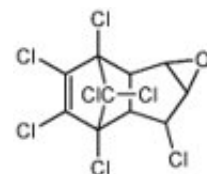
PFHxS



PFOS



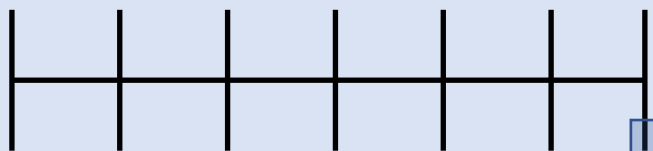
Heptachlor



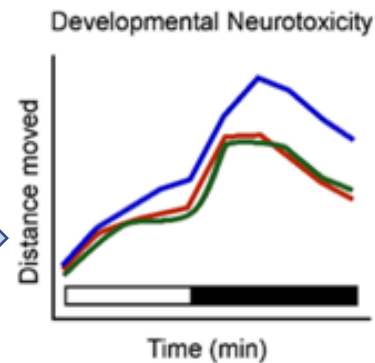
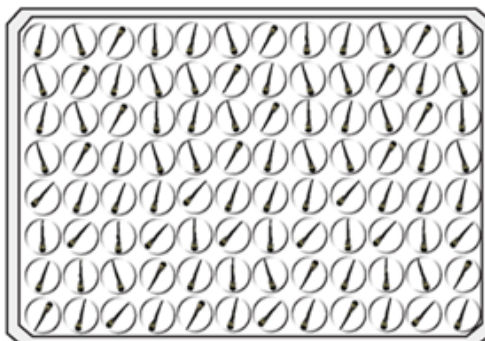
PFHxS 4.4-44.8 μ M **Heptachlor** 0.25-5.0 μ M

PFOS 0.28-5.0 μ M **DMSO** 0.4%

Days 0 1 2 3 4 5 6



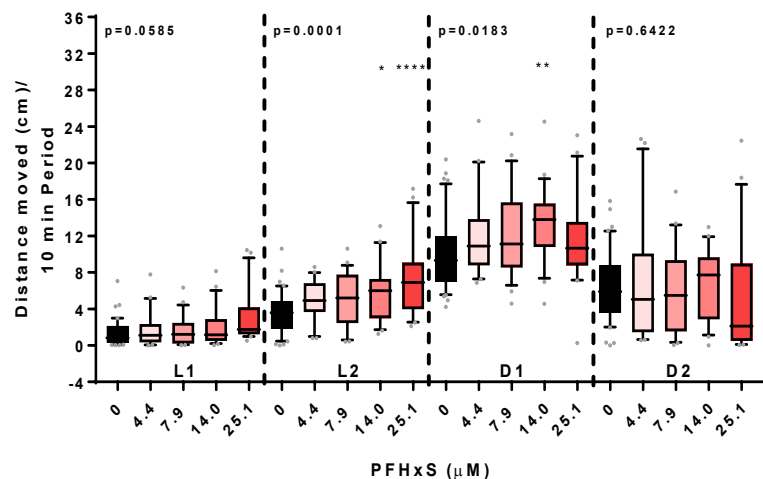
Behavior
Assessment



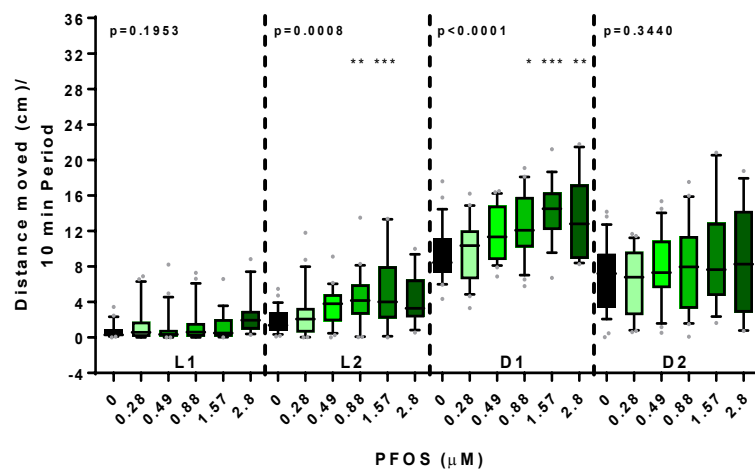
Days 1-5: 100% media change and chemical dosing

Exposure to PFOS, PFHxS, caused hyperactivity effects distinct from Heptachlor

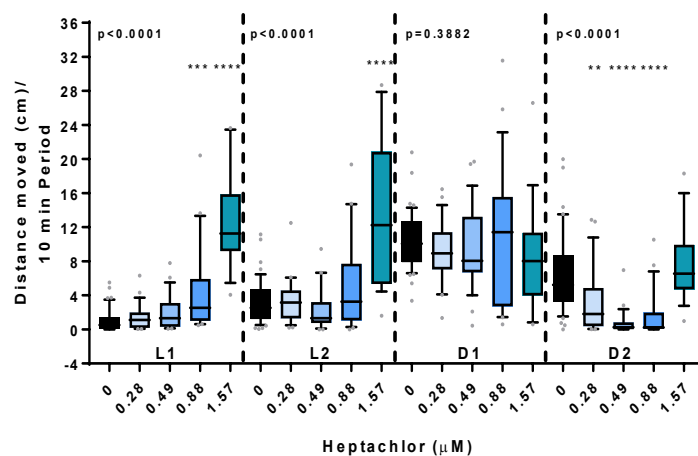
PFHxS



PFOS



Heptachlor

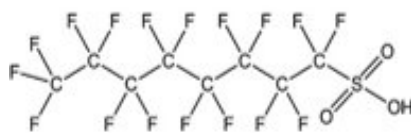


Assess gene response at doses that cause hyper or hypoactivity in zebrafish

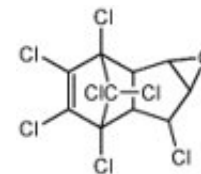
PFHxS



PFOS



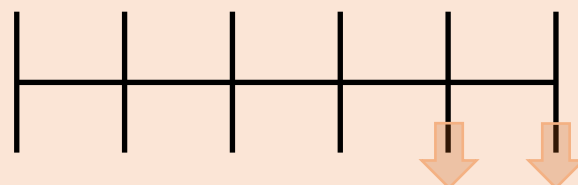
Heptachlor



PFHxS 7.87-25.1 μ M **Heptachlor** 0.49-1.57 μ M

PFOS 0.49-1.57 μ M **DMSO** 0.4%

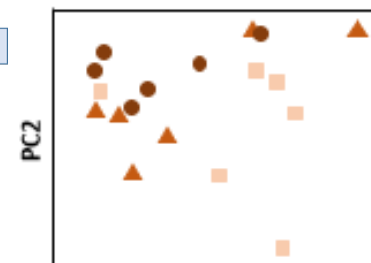
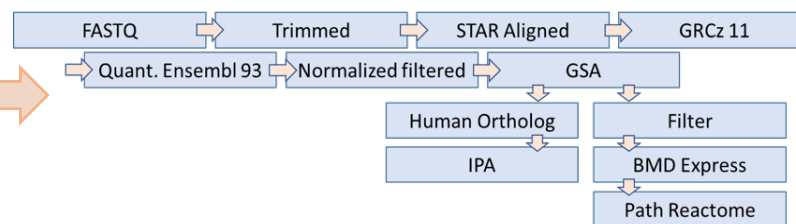
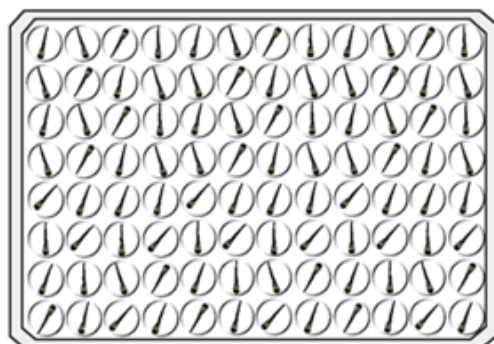
Days 0 1 2 3 4 5



RNA isolation



RNA sequencing

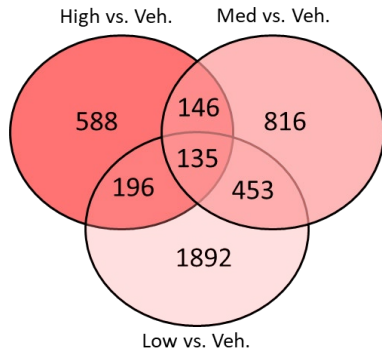
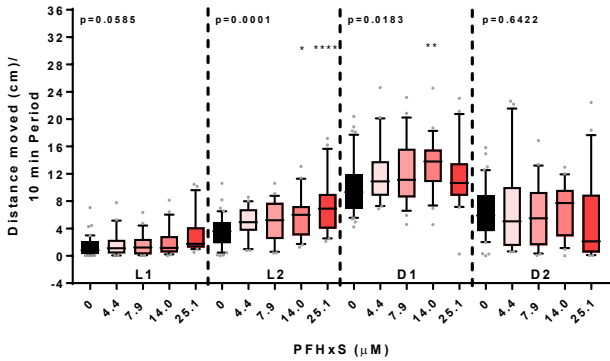


PC1

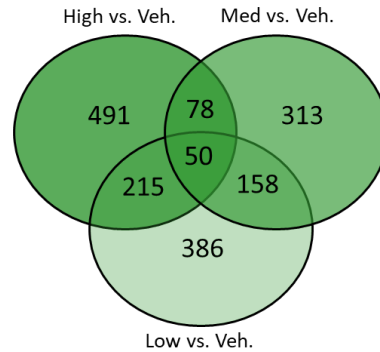
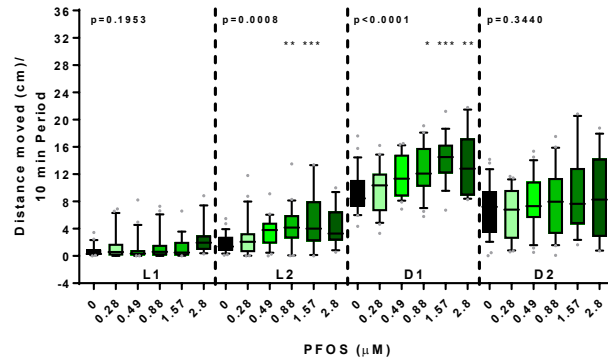
Days 1-5: 100% media change and chemical dosing

Concentrations causing behavioral effects caused distinct but similar gene expression changes at 4 and 5 dpf

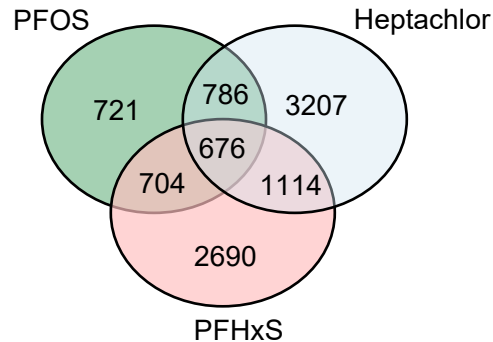
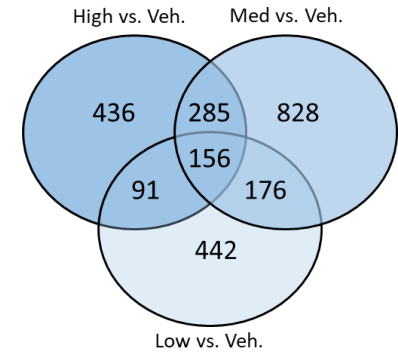
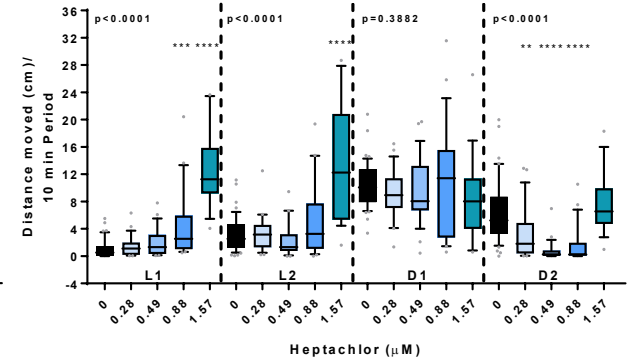
PFHxS



PFOS

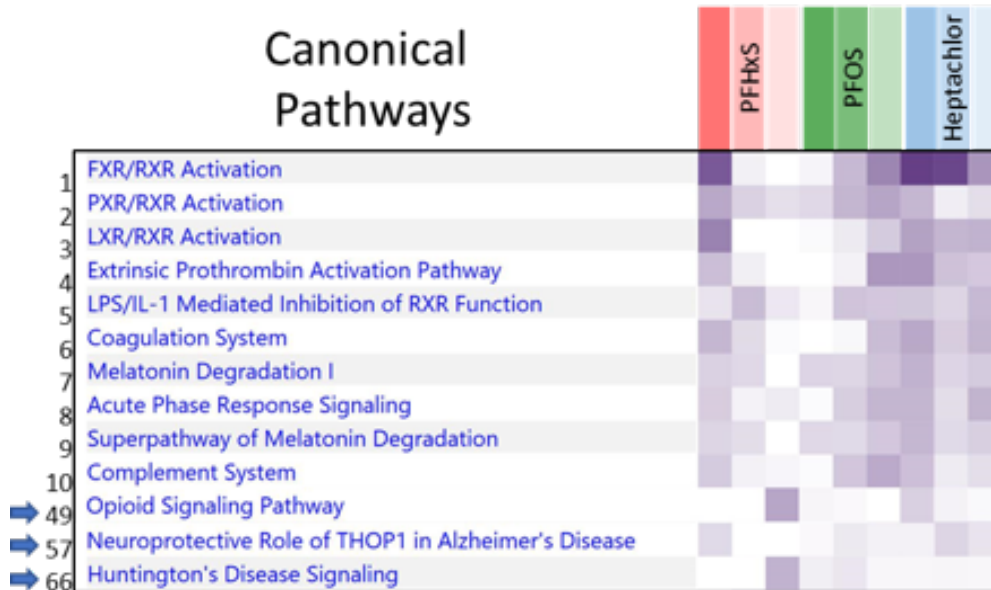


Heptachlor

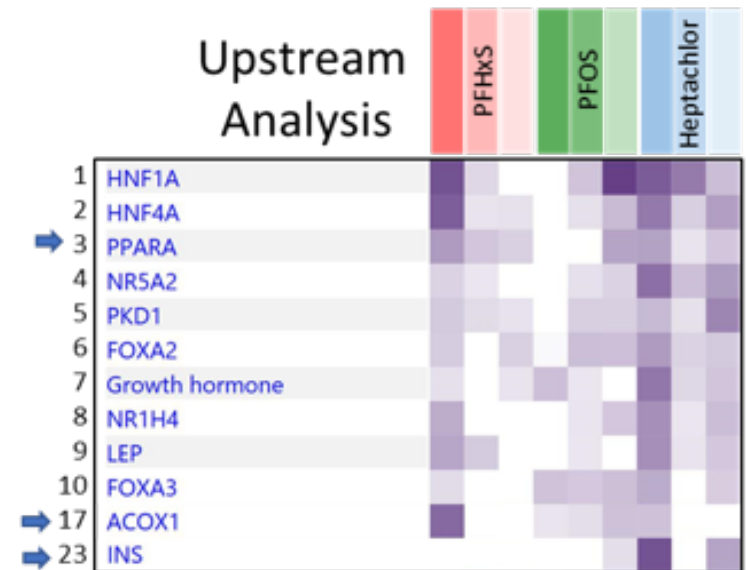


Pathway analysis revealed similar enrichment including effects on neurologic related pathways and peroxisome proliferation at 4 dpf

Canonical Pathways

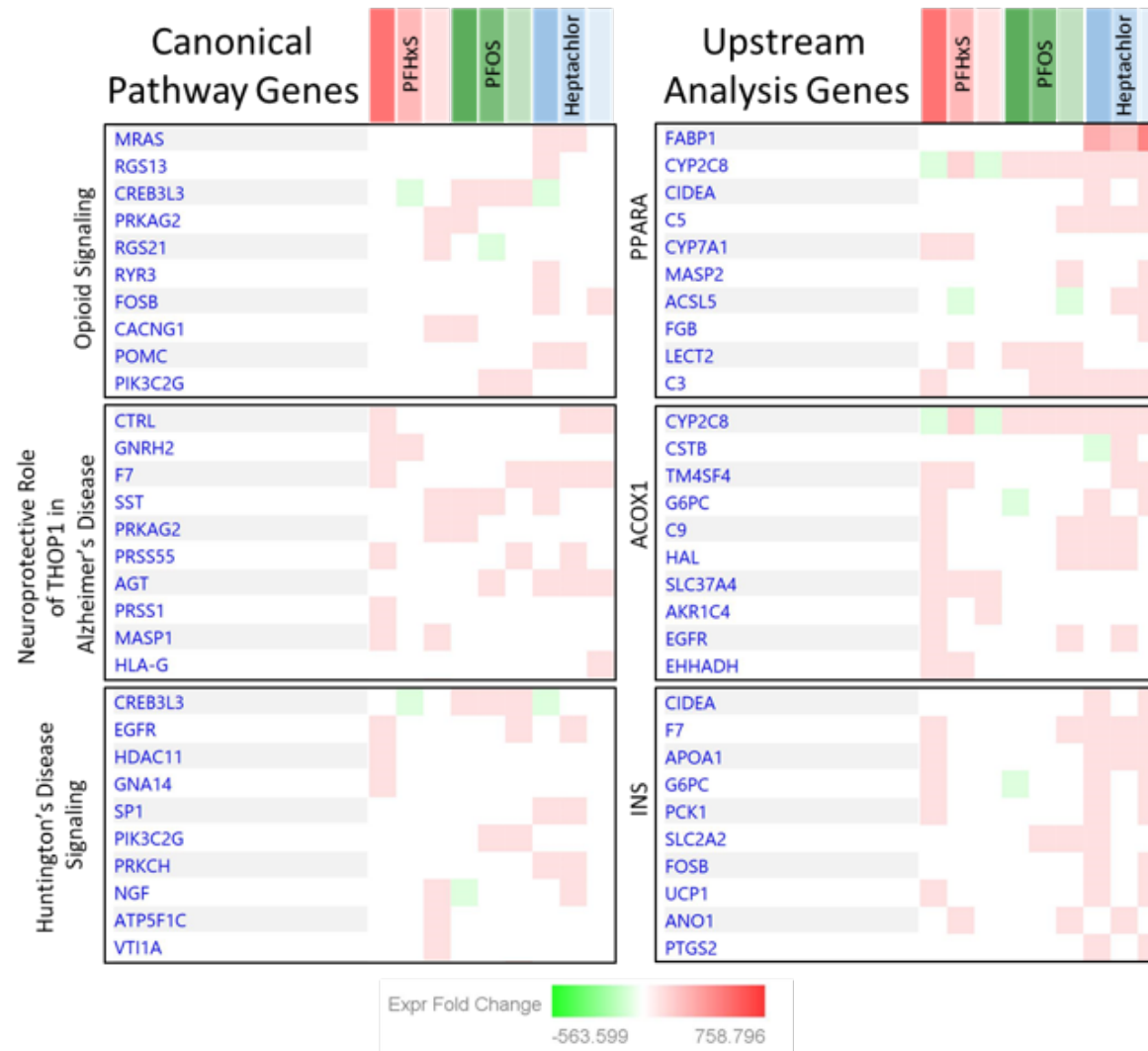


Upstream Analysis

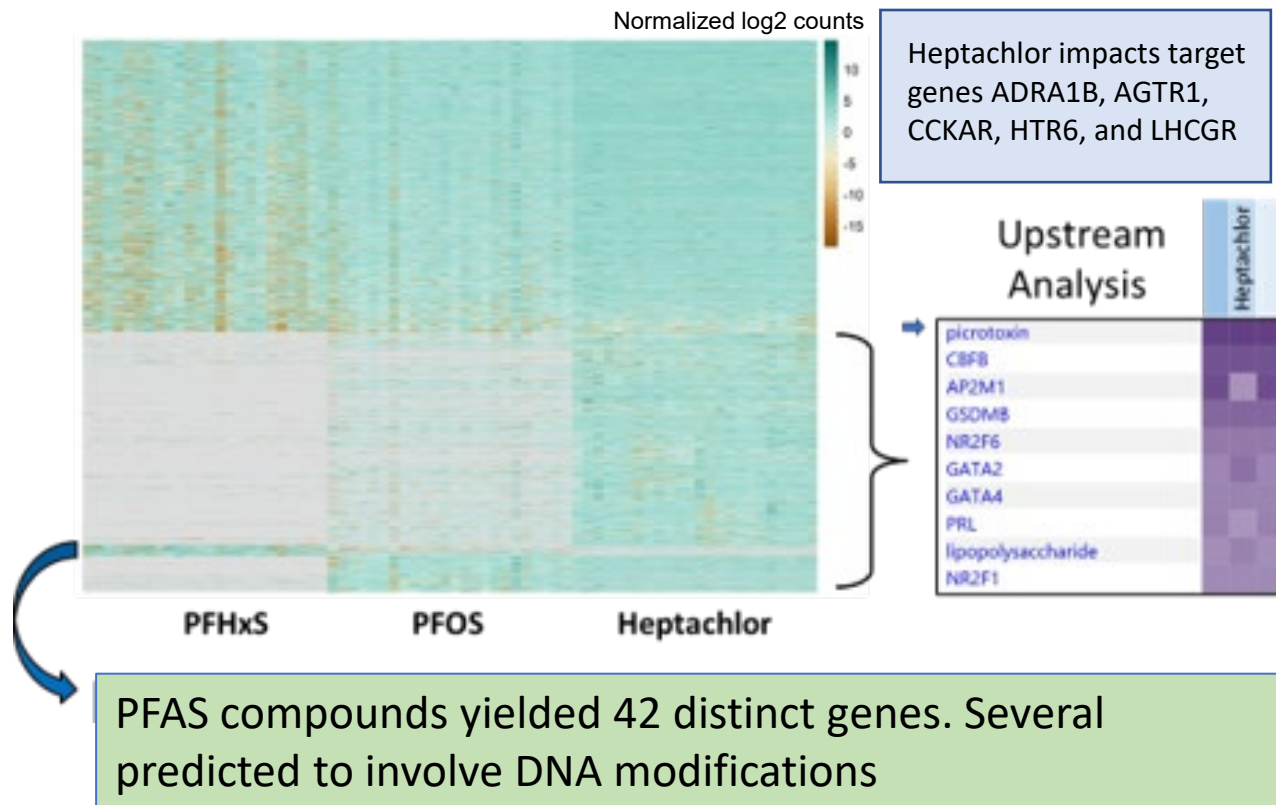


$-\log(p\text{-value})$
0.00E00 9.2

Genes belonging to specific pathways or upstream regulators reveal targets for gene editing to explain DNT mechanisms



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



Transcriptomic benchmark concentration response modeling exhibits concordance with larval zebrafish behavioral response

	LOEC hyperactivity μM	Median BMC values 4dpf μM (Median BMCL)	Median BMC _T values 5dpf μM (Median BMCL)
PFHxS	14	18 (10)	10 (5)
PFOS	0.88	2 (1)	1 (1)
Heptachlor	0.88	1 (1)	1 (1)

- Concentration estimates from transcriptomic benchmark concentration modeling were comparable to *in vivo* LOEC values for hyperactivity.
- The relevance of the zebrafish behavioral model for DNT is still being evaluated.

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.