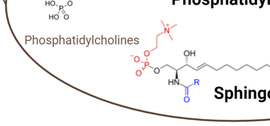
Brain Metabolomics of Fathead Minnows (*Pimephales promelas*) Exposed to Short-chain Perfluoroalkyl Substances

Cristina Henriques[1], Theresa Warriner[1], Denina Simmons[1]

Introduction

What are PFAS?

- Perfluoroalkyl substances (PFAS) are anthropogenic compounds found in non-stick cookware, water resistant clothing, hydraulic fluids, and fire fighting foams.[1]
- PFAS have a long half life, giving them "forever chemical" status. [1]
- Exposure to PFAS results in liver toxicity, cell membrane instability, thyroid hormone dysregulation, and neurological changes as PFAS are structurally similar to lipids. [2,3,4]

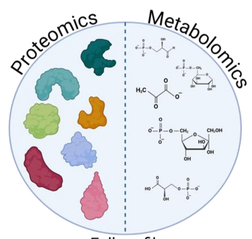


(Octanoic acid)

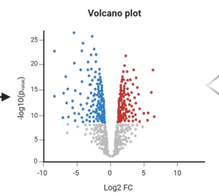


(PFOS)

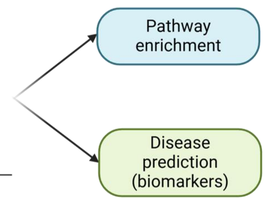
What is 'metabolomics' in toxicology?



Full profile
+
relative abundance



Change in protein/metabolite abundance



- The use of metabolites (small, biological molecules) for the assessment of an organisms' physiological response to a toxicant [5]
- Can be used to predict more severe outcomes without the need for high exposure levels or organismal suffering [5]

Study objective:

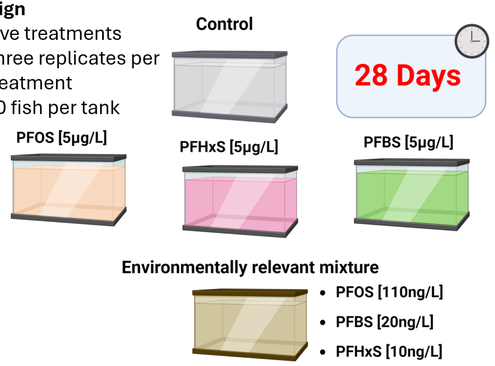
Determine the neurological impacts of short-chain PFAS (PFBS, PFHxS) exposure by assessing fine molecular changes in lipid and aqueous metabolite abundances.

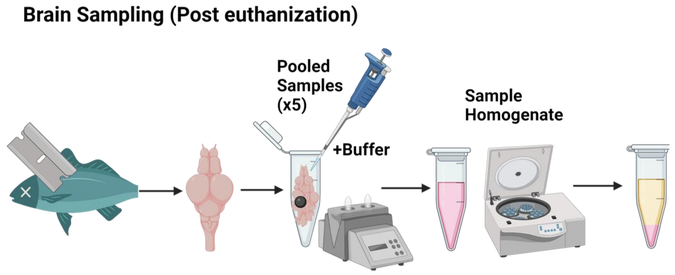
Methods

Lab Exposure and Sampling

Design

- Five treatments
- Three replicates per treatment
- 20 fish per tank

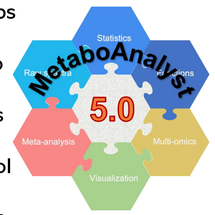


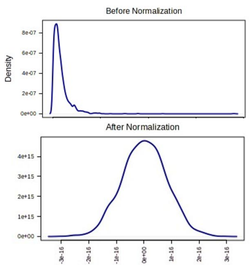


Sample Extraction & Instrument Analysis

Statistical Analysis

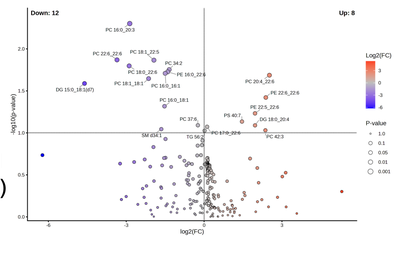
Experimental groups were selectively filtered out prior to data normalization and statistical tests to individually compare the control to a singular experimental group.





Statistical test

Volcano plot (combined t-test and fold-change analysis.)



Results & Discussion

Lipids

Changes to phosphatidylethanol amines (PE's)

- Assists in membrane protein folding (related to protein function)[6]
- Stimulates oxidative phosphorylation (cellular energy production)[6]
- Autophagosome formation (cell environment cleaning and defense)[6]
- PE 16:0_22:6 is associated with membrane stability in fish brain[7]

Changes to phosphatidylcholines (PC's)

- Acetylcholine precursor (neurotransmitter)[7]
- Protection from inflammatory stress[8]
- Brain cell membrane structure and fluidity - synaptic membrane integrity[8]

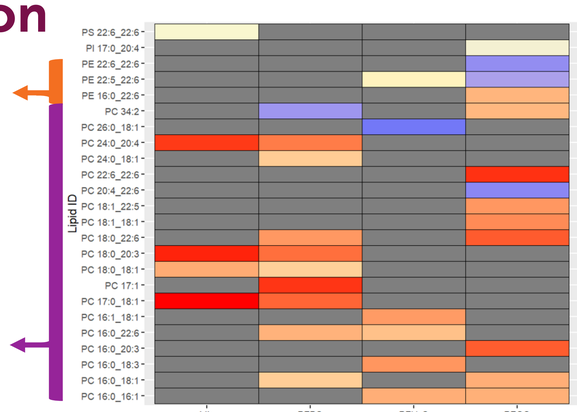
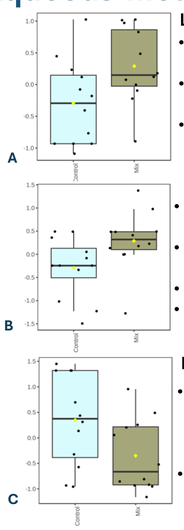


Figure 1. Heat map depicting significantly impacted lipids, by sample type ($p<0.05$ and $\log2(FC) > |1|$).

Aqueous metabolites



L-alanine ($p<0.05$)

- Glial Cell- Neuron coupling[9]
- Modulates glutamate transmission[9]
- Helps sustain memory formation[9]

L-arginine ($p<0.05$)

- Precursor to several metabolites, including nitric oxide (NO) [10]
- Reduces oxidative damage in the brain[10]
- Cerebral blood flow[10]
- Long-term potentiation (memory formation) [11]

L-cystine ($p<0.05$)

- Glutathione production and oxidative stress response[12]
- Lipid peroxidation (metabolism) [13]

Figure 2. Box plots depicting the normalized concentration for L-alanine **(A)**, L-Arginine **(B)** and L-cystine **(C)** in brain samples obtained from the mixture group (right), contrasted to the control group (left).

What do we think is going on?

And what are the downstream implications?

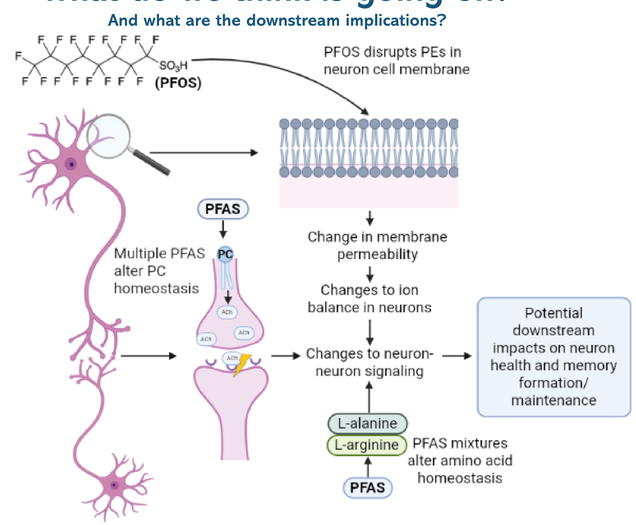


Figure 3. Graphical summary illustrating the effects of PFAS exposure on neurons. Each interaction converges on changes to neuron-neuron signaling.

Acknowledgements

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