High-Throughput Behavioral Effects of Multiple PFAS Chemicals in Larval *Pimephales promelas*

J.X. Hoang[1], M. Le[1], B. Blackwell[2], K. Bush[1], M. Ellman[1], K. Flynn[2], M. Hazemi[1], E. Stacy[2], D.L. Villeneuve[2]

1. Oak Ridge Institute of Science Education, Great Lakes Toxicology & Ecology Division 2. US EPA, ORD, Center for Computational Toxicology & Exposure, Great Lakes Toxicology & Ecology Division

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John Hoang | jhoan073@ucr.edu | 714-299-1619

Introduction

- Per- and polyfluoroalkyl substances (PFAS) are a class of man-made chemicals, used in various industries and consumer products, that are of public interest due to their prevalence in the environment and potential for human exposure.
- A vast amount of PFAS lack toxicity data, so a 1-day high-throughput assay was conducted with larval *Pimephales promelas* (Fathead Minnow; FHM) and 22 different PFAS to evaluate mortality-, behavioral-, and transcriptomic-based endpoints for these select chemicals. This poster will be focused on the behavioral results of the assays.
- Using video-based tracking, the locomotor behavior of FHM larvae was assessed. Specifically, the photomotor response (PMR), a reactive movement due to changes in light, was examined and determined to be of interest.

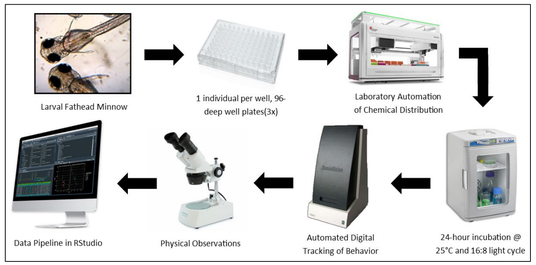
Objectives

- To determine whether any of the PFAS tested altered photomotor or other behavioral responses in larval *Pimephales promelas*.
- To compare mortality- and behavior-based endpoints of the 22 PFAS tested and establish whether a behavioral response could be a precursor to mortality.

Methods

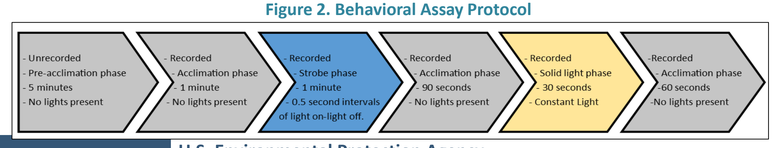
- Five-day-old FHM larvae were exposed to individual PFAS at 0.03, 0.1, 0.32, 1, 3.17, 10, 31.67, or 100 µM (nominal) for 24 hours in deep well, 96-well microplates at 25°C and 16:8 light cycle (Figure 1 – Exposure Protocol).
  - Potassium perfluorooctanesulfonate
  - Perfluorooctanoic acid
  - 1H,1H,8H,8H-Perfluoro-3,6-dioxaoctane-1,8-diol
  - Potassium perfluorohexanesulfonate
  - 6:1 Fluorotelomer alcohol
  - Perfluorohexanesulfonamide
  - Perfluoro-(2,5,8-trimethyl-3,6,9-trioxadodecanoic)acid
  - Perfluoro-3-methoxypropanoic acid
  - Perfluoro-4-isopropoxybutanoic acid
  - N-Ethylperfluorooctanesulfonamide
  - 3H-Perfluoro-2,2,4,4-tetrahydroxypentane
  - 4:2 Fluorotelomer sulfonic acid
  - 6:2 Fluorotelomer sulfonic acid
  - 8:2 Fluorotelomer sulfonic acid
  - Perfluorotetradecanoic acid
  - Perfluoroundecanoic acid
  - Perfluorotridecanoic acid
  - Perfluorononanoic acid
  - Perfluoroheptanoic acid
  - Perfluoropentanoic acid
  - 1H,1H,8H,8H-Perfluorooctane-1,8-diol
  - 1H,1H,10H,10H-Perfluorodecane-1,10-diol
- FHM larvae were also exposed to CuSO4 (30 µM) as a transcriptomic reference.
- Following 24 hours, FHM larvae were placed in a video tracking observation chamber(Daniovision, Noldus) and subject to a 10-minute light on-off protocol to test locomotor behavior and photomotor response (Figure 2 – behavioral assay protocol).

Figure 1. Exposure Protocol



- This process was then repeated with caffeine (21 mg/L) and ethanol (1%) as a positive control test.
- Data collected as percent activity (PA) and distance traveled (DT) via EthoVision XT 15 was then processed through a custom data analysis pipeline in R to extract the following endpoints:
  - Acclimation 1 average DT and PA
  - Strobe Phase average DT and PA
  - Strobe Phase overall peak DT and PA
  - Strobe Phase first peak DT and PA
  - Solid Light Phase average DT and PA
  - Normalized Strobe Phase and Solid Light Phase endpoints
  - Normalized endpoints calculated using preceding acclimation phase to determine strobe/solid light impact
- Caffeine and Ethanol PA and DT were analyzed through a similar pipeline but with an additional bootstrap step to estimate the positivity rate of the assay (Figure 3 – Bootstrap Analysis Protocol).

Figure 2. Behavioral Assay Protocol



Results

Potassium Perfluorooctanesulfonate (DTXSID8037706)

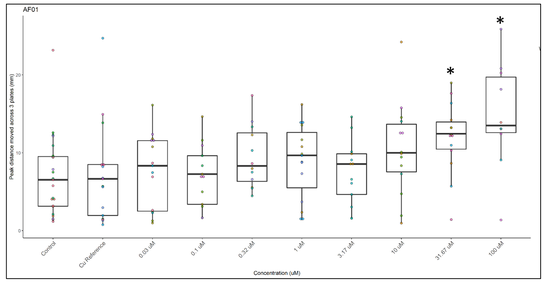


Figure 4. Strobe Phase – First Peak Distance Traveled

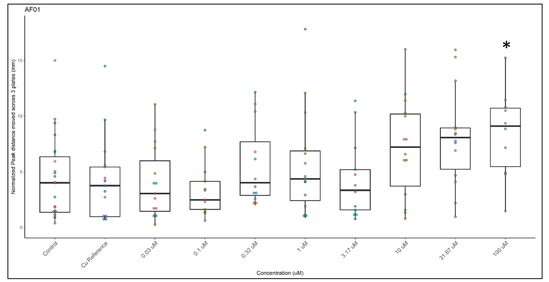


Figure 5. Strobe Phase -Normalized First Peak Distance Traveled

3H-Perfluoro-2,2,4,4-tetrahydroxypentane (DTXSID70379295)

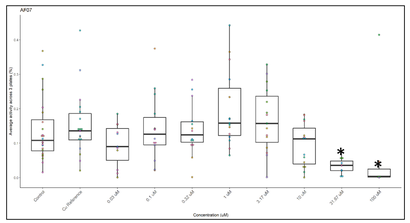


Figure 6. Light Phase – Average Percent Activity

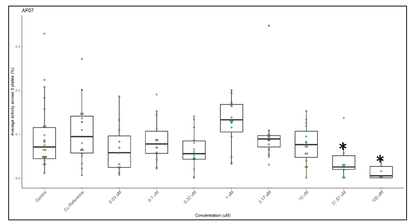


Figure 7. Strobe Phase – Average Percent Activity

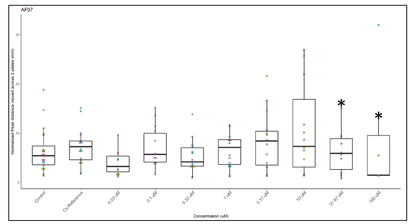


Figure 8. Strobe Phase – Normalized Overall Peak Distance Traveled

- Potassium Perfluorooctanesulfonate increased response of distance-based endpoints beginning at 31.67 µM nominal (Figures 4 & 5).
- 3H-Perfluoro-2,2,4,4-tetrahydroxypentane decreased response of both activity- and distance-based endpoints beginning at 31.67 µM nominal (Figures 6-8).
- Through an initial bootstrap analysis, caffeine elicited a positivity rate of 21.67-83.33% depending on the specific endpoint (Figure 9).

Figure 3. Bootstrap Analysis Protocol

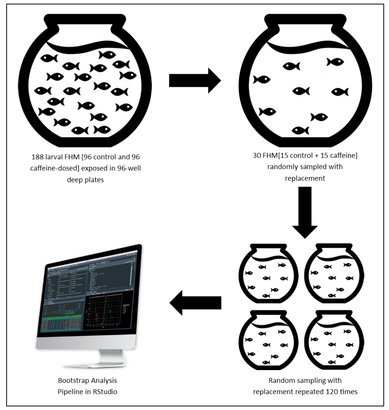
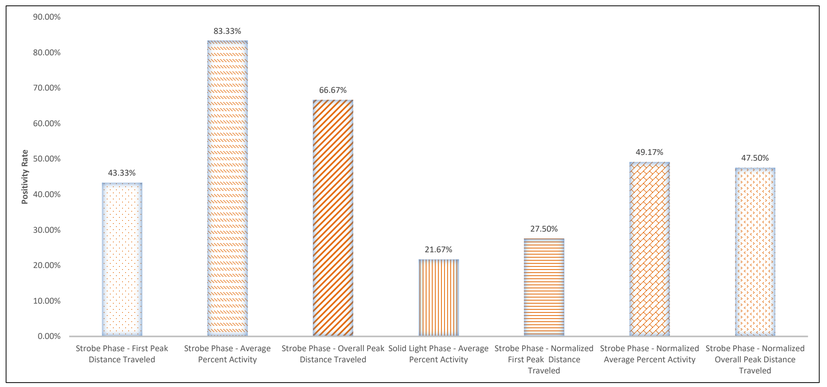


Figure 9. Caffeine - Bootstrap Analysis



Discussion

- The majority of PFAS chemicals did not alter locomotor behavior or the photomotor response in larval *Pimephales promelas*.
- Two of the PFAS induced dose-dependent alteration of photomotor responses. When the assay was repeated for the 2 PFAS that elicited altered photomotor responses, this altered behavior was not reproducible. Further investigation is required.
- When compared to the mortality endpoint, PFAS chemicals that elicited 100% mortality within the high concentration such as 1H,1H,8H,8H-Perfluorooctane-1,8-diol showed little to no altered behavioral effects in this assay.
- 3H-Perfluoro-2,2,4,4-tetrahydroxypentane decreased photomotor response beginning around 31.67 µM and elicited 13.33% mortality in 100 µM concentration after 24-hours.
- Bootstrap results suggest that unnormalized strobe phase endpoints particularly with caffeine as a hyperactive positive control are reliable. Ethanol remains inconclusive as a hypoactive positive control and requires further testing.

Conclusion & Next Steps

- These data suggest that while the majority of PFAS chemicals do not alter photomotor response and locomotor behavior of larval *Pimephales promelas,* some PFAS may elicit behavioral effects.
- As 3H-Perfluoro-2,2,4,4-tetrahydroxypentane may elicit both behavioral and mortality effects on larval fathead minnow, there may be interest in further research on this PFAS.
- Assay will need to repeated further for the 2 PFAS of interest to determine reproducibility.
- Caffeine is a strong hyperactive positive control; however, further positive control testing is needed to determine a hypoactive positive control.

Acknowledgement

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