Comparison of Zebrafish Toxicity Between Different Developmental Windows of Exposure to Three Environmentally Relevant PFAS Compounds

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Background

- The per- and polyfluoroalkyl substances (PFAS) are of significant global concern due to their highly ubiquitous and persistent nature, bioaccumulation in organisms, and potential toxicity.
- The aquatic environment is known as an important sink for PFAS resulting in high concentrations in aquatic organisms[1].
- Several studies have described the developmental effects of PFAS chemicals in aquatic organisms, including an uninflated swim bladder and altered swimming behavior, as well as defects to apical endpoints such as growth, traditionally considered relevant to environmental risk assessment[2,3].
- However, little is known about the developmental windows of sensitivity in which the PFAS chemicals are biologically active, in addition to the toxicity endpoints that best reflect chemical hazard.

Objective

To systematically evaluate developmental toxicity from exposure to PFOS, PFOA, and PFHxS for 14 exposure lengths between 6 and 144 hours post fertilization (hpf) in the developmental zebrafish model.

Methods

- Zebrafish (n=10/window split into two plates) were exposed to eight concentrations (0-100 µM) of PFOS, PFOA, or PFHxS (**Figure 1**), 1 µM chlorpyrifos (control for 120 hpf neurobehavior), and 0.33% DMSO (vehicle control).

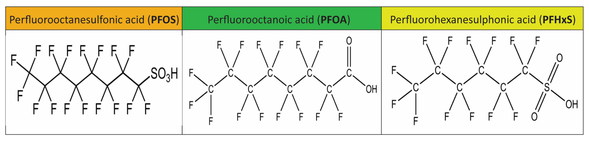

***Figure 1**. Three PFAS chemical structures with their names and abbreviations.*

- Exposure was conducted for varying lengths starting at 6, 24, 48, or 96 hpf, and ending at 24, 48, 96, 120, or 144 hpf (**Figure 2**).
- Solutions were renewed (50%) daily, and chemical water concentration was confirmed by liquid chromatography-mass spectrometry at the start and end of each window (data not shown).
- Mortality, hatch, length, and swim bladder inflation were evaluated at timepoints appropriate for each endpoint (**Figure 2**).
- Neurobehavioral responses to two alternating dark and light cycles of two minutes each were measured at 120 hpf.
- Individual zebrafish samples were collected at the end of exposure windows ending at 24, 48, and 120 hpf for RNA sequencing analyses and transcriptomic points of departure (TPOD) estimations.

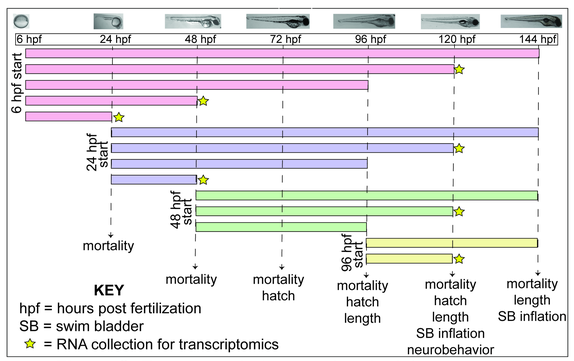

***Figure 2**. Exposure windows and endpoints measured.*

Preliminary results: Endpoint- and timepoint-specific differences between exposure start times were detected

Mortality

PFOS: Earlier exposure start times led to higher percent mortality, as well as mortality occurring at lower concentrations. Percent morality at 144 hpf was greater than at 120 hpf.
PFOA: No significant mortality.
PFHxS: No significant mortality.

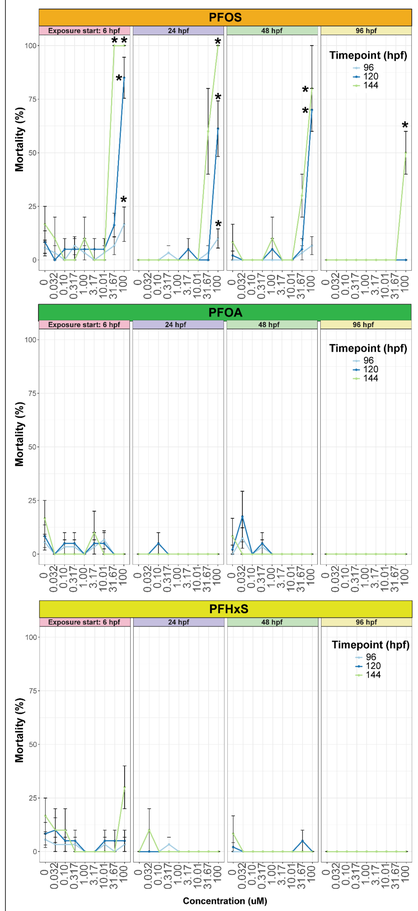

***Figure 3**. Percent mortality of zebrafish exposed from different exposure start times measured at 96, 120, and 144 hpf. Error bars represent mean ± SEM of 2-9 plate replicates. * indicates statistical significance ($p < 0.05$) compared to respective exposure start and timepoint controls using a Kruskal-Wallis with a post-hoc Dunn's test.*

Length

PFOS: Trends towards decrease in length at higher concentrations.
PFOA: Significant increase in length only in 96-120 hpf window.
PFHxS: Significant increase in length primarily at 96 and 120 hpf detected in all exposure start times, at concentrations greater than 1.00 µM.

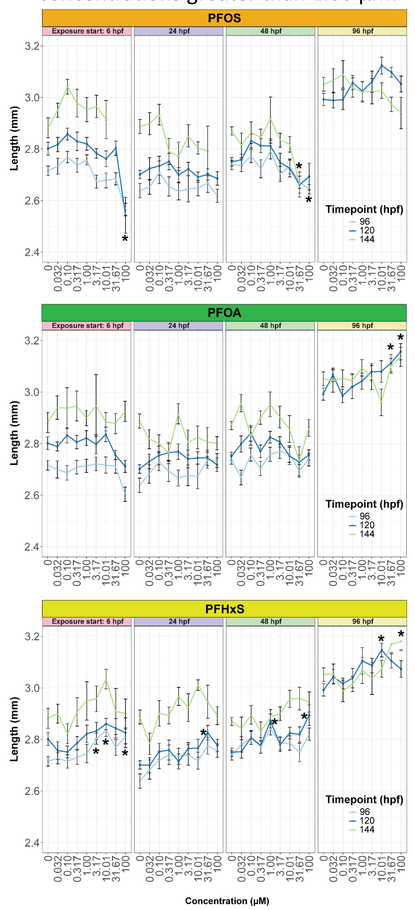

***Figure 4**. Length of zebrafish exposed from different exposure start times measured at 96, 120, and 144 hpf. Error bars represent mean ± SEM of 1-72 fish replicates. * indicates statistical significance ($p < 0.05$) compared to respective exposure start and timepoint controls using a Kruskal-Wallis with a post-hoc Dunn's test.*

SB inflation

PFOS: Earlier exposure start time led to lower percent SB inflation at 120 hpf at the higher exposure concentrations.
PFOA: No significant SB inflation differences.
PFHxS: No significant SB inflation differences.

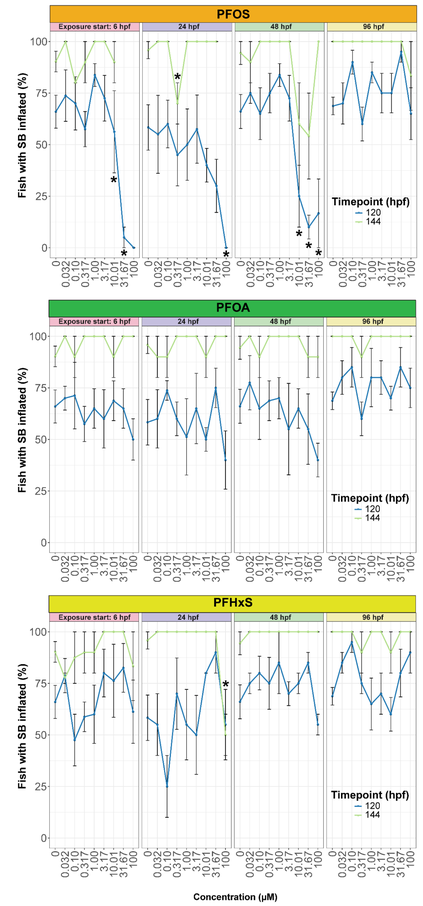

***Figure 5**. Percent zebrafish with swim bladder inflated for animals exposed from different exposure start times measured at 120 and 144 hpf. Error bars represent mean ± SEM of 1-6 plate replicates. * indicates statistical significance ($p < 0.05$) compared to respective exposure start and timepoint controls using a one-way ANOVA with a post-hoc Dunnett's test.*

Neurobehavior (6-120 hpf)

PFOS: Hyperactive at 3.17 and 10.01 µM. 31.67 µM animals are non-responsive and die by 144 hpf.
PFOA: Trend for hyperactivity at 10.01 and 31.67 µM, but not at 100 µM.
PFHxS: Hyperactivity detected at 31.67 and 100 µM.

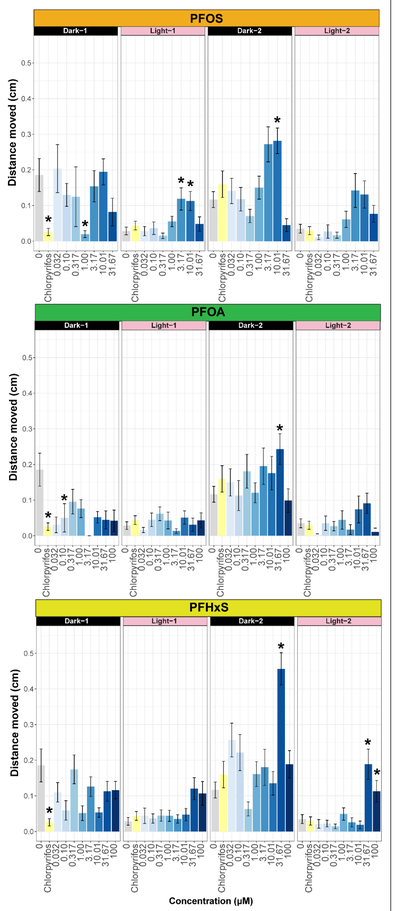

***Figure 6**. Distance moved by 120-hpf zebrafish (n=5-17) during 2-min alternating dark and light cycles. Zebrafish were exposed from 6-120 hpf to DMSO, 1 µM chlorpyrifos, or a concentration range of PFOS, PFOA, or PFHxS. Error bars represent mean ± SEM. * indicates statistical significance ($p < 0.05$) compared to respective exposure start and timepoint controls using a Kruskal-Wallis with a post-hoc Dunn's test.*

Conclusions

- PFOS is the most developmentally toxic of the three PFAS chemicals tested.
  - Longer exposure duration resulted in higher mortality.
- Neurobehavior assay is more sensitive to PFAS toxicity, particularly for PFOA and PFHxS.
- Alterations in length and swim bladder inflation due to PFAS exposure could be related to changes in hypothalamic-pituitary-thyroid axis dysfunction, which could also result in altered swimming behavior of zebrafish. No significant changes in hatch percent were detected (data not shown) for any of the PFAS compounds.

Current and future work

- Analyzing 120-hpf neurobehavior data for other exposure start times: 24, 48, and 96 hpf, and conducting time-to-event statistical analyses for all endpoints.
- RNA extraction and library prep of samples collected at 24, 48, and 120 hpf.
- Future work includes conducting RNA-sequencing, investigating the uncertainties in TPOD estimates, and analyzing correlations between developmental toxicity and transcriptomic data.

References

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