Comparison of Apical Points of Departure to Transcriptomic Points of Departure in Fathead Minnow Exposures

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Background

A core mission of the U.S. Environmental Protection Agency (USEPA) is to assess the effect of anthropogenic chemicals using data gathered from standardized tests across many biological taxa. These tests measure chemical induced mortality and vary in observable endpoints. With increasing amounts of chemicals available in the marketplace each year, there creates the need for short-term and cost effective high-throughput assays that can be used to evaluate chemical potency and infer potential hazards to human health and/or ecosystems.

- One common species used for acute testing is Pimephales promelas, also known as the Fathead Minnow (FHM). Traditionally, these tests employ long time frames and large amounts of chemical to conduct exposures.
- An alternative to these standardized tests involves a high-throughput format of testing to cost-effectively and efficiently collect large amounts of toxicological data.
- Over the past decade, a number of mammalian studies have indicated short-term transcriptomics-based points of departure (PODs) are predictive of apical potency, often providing a POD that is within a factor of 10 of those derived from much longer-term tests.[1]

We hypothesize that high-throughput transcriptomics assays with aquatic organisms may be a viable alternative to traditional aquatic toxicity tests for ecological safety evaluations.

Objective

To compare short-term transcriptomics-based points of departure (tPODs) against apical points of departures (aPODs) and determine:

1. whether tPODs are generally health protective relative to apical effects
2. how conservative they may be relative to traditional endpoints

Exposures

24-hour static exposures to FHM larvae (6 dpf) were conducted with 8 replicates of 12 concentrations of the following chemicals: CuSO4, NiSO4, ZnSO4; fluoxetine, sertraline, paroxetine; clothianidin, flupyradifurone, imidacloprid, and thiacloprid, using a ½ log dilution series

Whole body RNA was extracted and whole transcriptome gene expression (RNA Seq) was evaluated.

RNA Seq Data

RNA-Seq raw reads were assembled into transcript models, aligned with annotations, counted, normalized, and log2 transformed for each transcript

- Low count feature filtering: any given feature had to have a count of 10 or more in a minimum of 4 samples or that feature was filtered out

RNA-seq data was collected from all 12 concentrations of the 96WP

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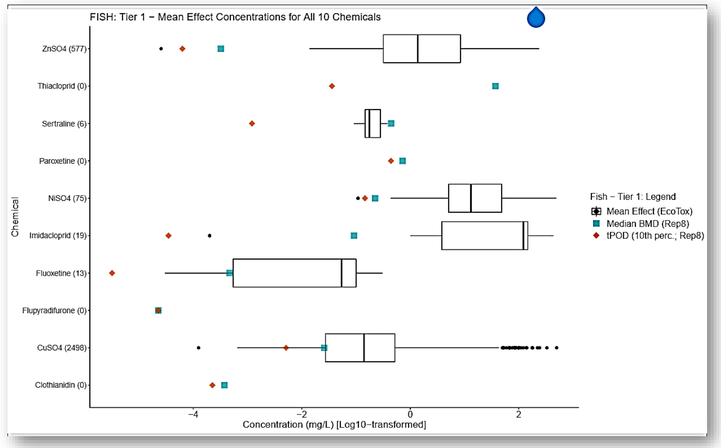
BMDExpress2

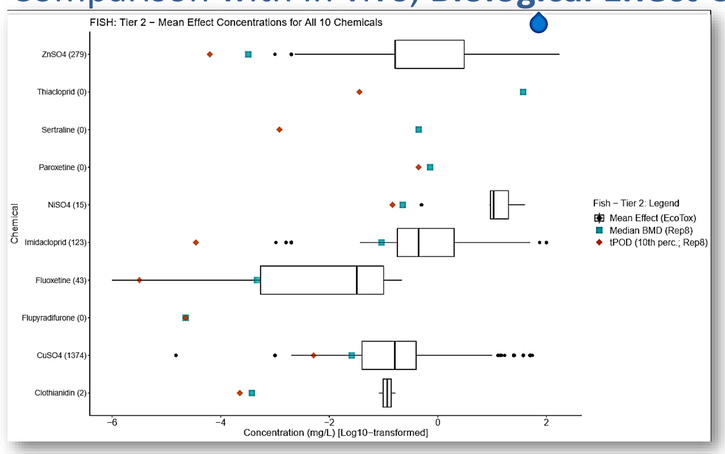
BMDExpress2[2] is a desktop application that enables analysis of dose/concentration-response data produced in differential gene expression experiments

- Transcripts that exhibited a concentration-dependent response were subsequently fit to concentration-response models using BMDExpress2 software to determine benchmark doses. A tPOD was then calculated based on the 10th centile of the distribution of benchmark doses.
- Transcriptomics-based PODs for three metals, three selective serotonin reuptake inhibitors, and four neonicotinoid(like) insecticides were compared against available apical effect data from the ECOTOX knowledgebase[3], focusing on impacts on survival, growth, and reproduction (Tier 1 endpoints) or biological effect concentrations considering impacts on endpoints like gene expression, hormone concentrations, enzyme activities, behaviors, etc. (Tier 2 – not necessarily adverse).

Comparison with In vivo, **Adverse Effect** Concentrations (Fish)



Comparison with In vivo, **Biological Effect** Concentrations (Fish)



96-well plate (96WP)

Results

FHM Tier 1 endpoints:

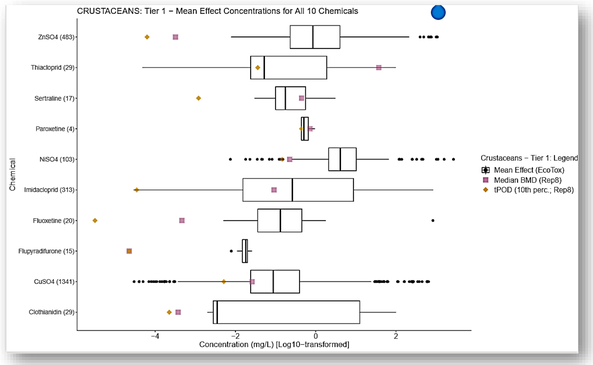
- tPODs (10th centile BMD) were uniformly lower than in vivo effect concentrations reported for fish
- up to 4 orders of magnitude lower

FHM Tier 2 endpoints:

- tPODs (10th centile BMD) were still generally more sensitive in vivo biological effect concentrations
- up to 2 order of magnitude lower
- in a few cases, tPODs overlapped the lower quartile in the interquartile range of that chemical

In contrast to the same study design using crustaceans, FHM tPODs were not always lower than apical effect concentrations reported for invertebrates, suggesting the need to include additional taxa in a high-throughput transcriptomics-based screening program.

Comparison with In vivo, **Adverse Effect** Concentrations (Crustaceans)



Next Steps

On-going research is extending these comparisons to additional chemicals and additional species to evaluate the hypothesis that high-throughput transcriptomics assays with aquatic organisms may be a viable alternative to traditional aquatic toxicity tests for ecological safety evaluations.

[1]Follows National Toxicology Program (NTP) Approach to Genomic Dose-Response Modeling, https://ntp.niehs.nih.gov/ntp/results/pubs/rr/reports/rr05_508.pdf?utm_source=direct&utm_medium=prod&utm_campaign=ntpgolinks&utm_term=rr05; Farmahin et al. 2017; Thomas et al. 2012

[2] BMDExpress2, https://www.sciome.com/bmdexpress/),

[3] ECOTOX Knowledgebase, https://cfpub.epa.gov/ecotox/index.cfm