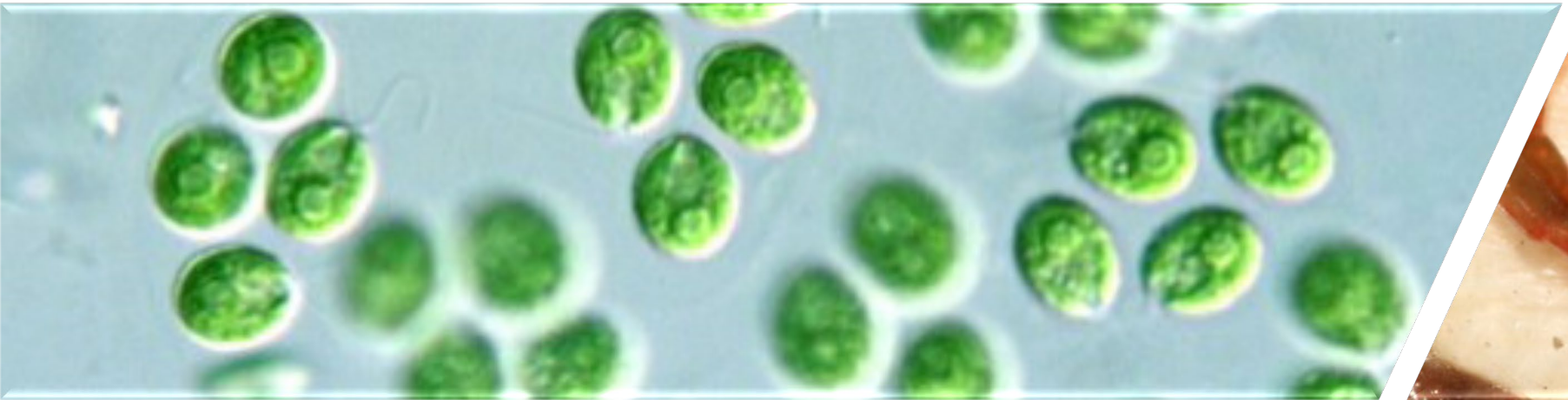
Transcriptomics-based points of departure for ecotoxicology – an update



Disclaimer:

Results contained herein are preliminary and should be regarded as “work in progress”. Specific values may change as analysis methods are refined.

Please contact Villeneuve.dan@epa.gov for updates before using or citing any results described below.

APCRA Case study: Transcriptomics-based PODs for Ecotoxicology

Hypothesis: 24 h whole body transcriptomics can provide a protective, but not overly protective, point of departure for ecological toxicity.

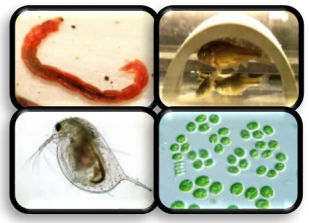
Approach:

1. Generate transcriptomic PODs for ≈ 20 chemicals
   - Initial focus on fathead minnow

2. Compare tPODs with available acute and chronic toxicity data

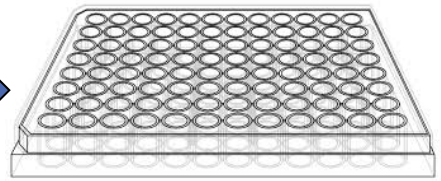
3. Compare tPODs with in vitro-derived PODs

Method Overview



Load organisms

24 h exposure

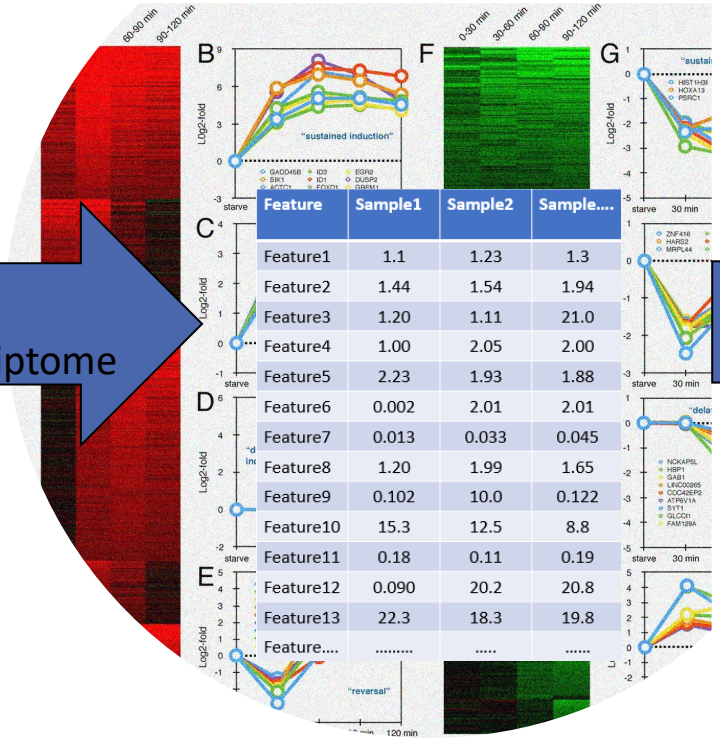


Phenotypic anchoring

Homogenate /extract

RNA

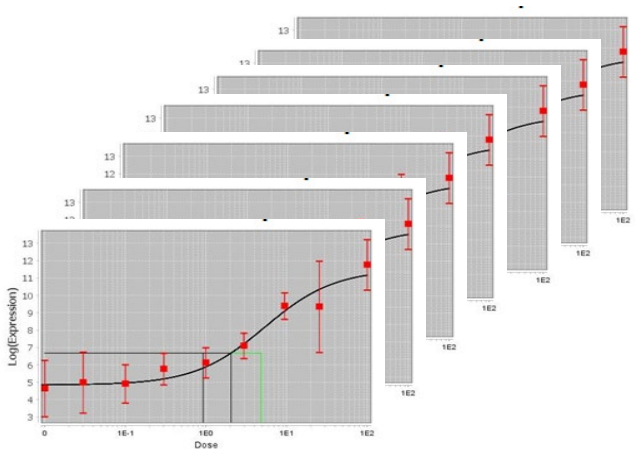
Whole transcriptome



Feature	Sample1	Sample2	Sample....
Feature1	1.1	1.23	1.3
Feature2	1.44	1.54	1.94
Feature3	1.20	1.11	21.0
Feature4	1.00	2.05	2.00
Feature5	2.23	1.93	1.88
Feature6	0.002	2.01	2.01
Feature7	0.013	0.033	0.045
Feature8	1.20	1.99	1.65
Feature9	0.102	10.0	0.122
Feature10	15.3	12.5	8.8
Feature11	0.18	0.11	0.19
Feature12	0.090	20.2	20.8
Feature13	22.3	18.3	19.8
Feature....	.....	.....	.....

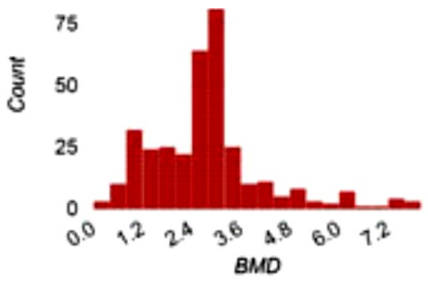
Curv

Curve fitting



BMD Express

Distribution of BMCs



Point of departure

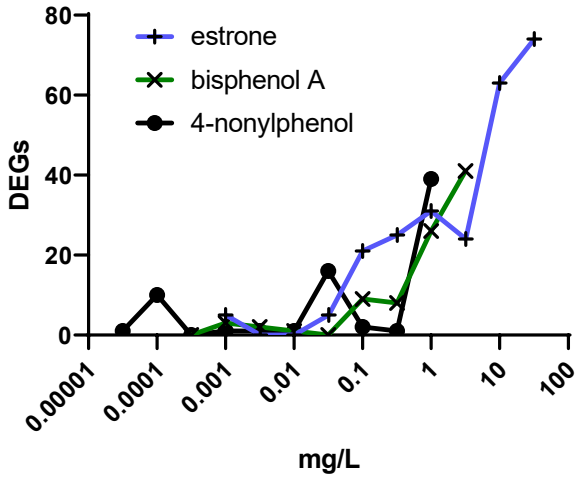
[tPOD]

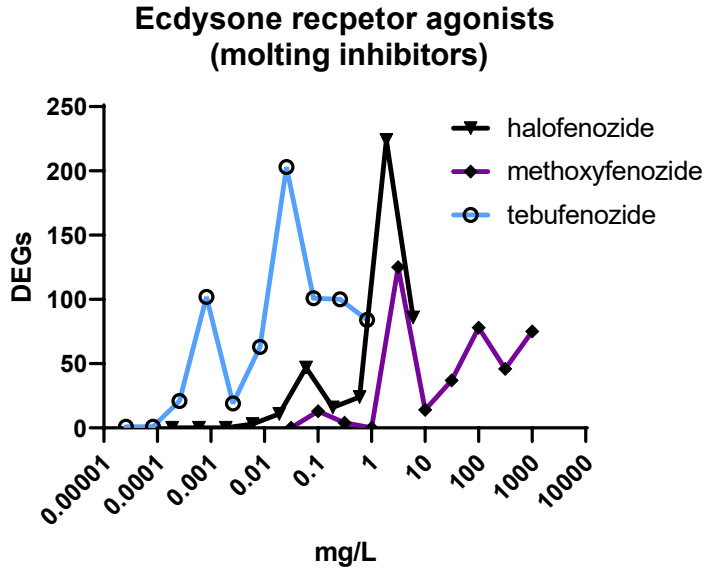
Case study Progress

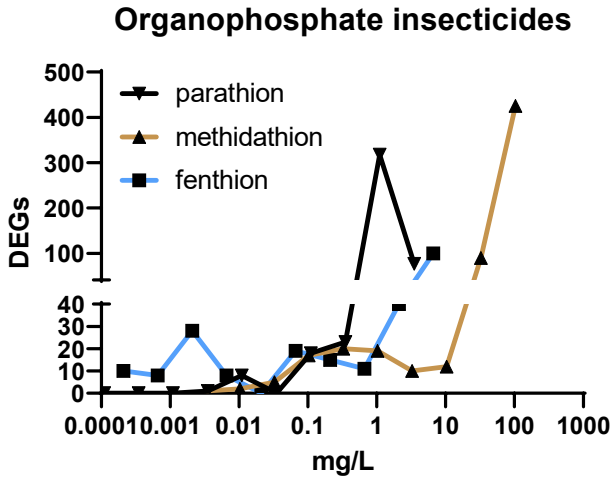
- Exposures completed with 25 chemicals.
  - 8 mode of action groupings
  - 3-4 chemicals per MoA group
- Sequencing complete for 22
  - 3 phthalates excluded due to low and/or variable exposure concentrations
- Results of the first 10 chemicals – revised MS in review
- **Preliminary analysis complete for second set of 12 chemicals**

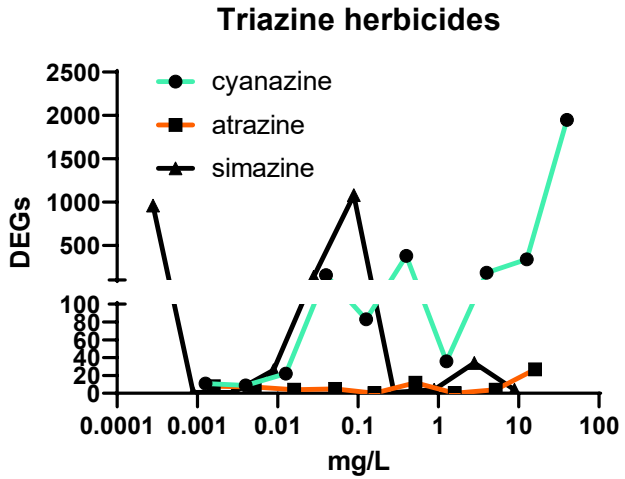
	Test Chemical	Assay Completed	Library Prep	tPOD	Free fraction measured?
1	$CuSO_4$	X	X	X	No
2	$ZnSO_4$	X	X	X	No
3	$NiSO_4$	X	X	X	No
4	Clothinidin	X	X	X	No
5	Flupyradifurone	X	X	X	No
6	Imidacloprid	X	X	X	No
7	Thiacloprid	X	X	X	No
8	Sertraline	X	X	X	No
9	Fluoxetine	X	X	X	No
10	Paroxetine	X	X	X	No
11	Dibutyl phthalate	X			Yes
12	DEHP	X			Yes
13	Benzyl butyl pthalate	X			Yes
14	Parathion	X	X	X	Yes
15	Fenthion	X	X	X	Yes
16	Methidathion	X	X	X	Yes
17	Bisphenol A	X	X	X	No
18	4-nonyl phenol	X	X	X	Yes
19	Estrone	X	X	X	Yes
20	Methoxyfenozide	X	X	X	Yes
21	Tebufenozide	X	X	X	No
22	Halofenozide	X	X	X	No
23	Atrazine	X	X	X	Yes
24	Simazine	X	X	X	No
25	Cyanazine	X	X	x	Yes

DEGs based on univariate statistical testing (DeSeq2)







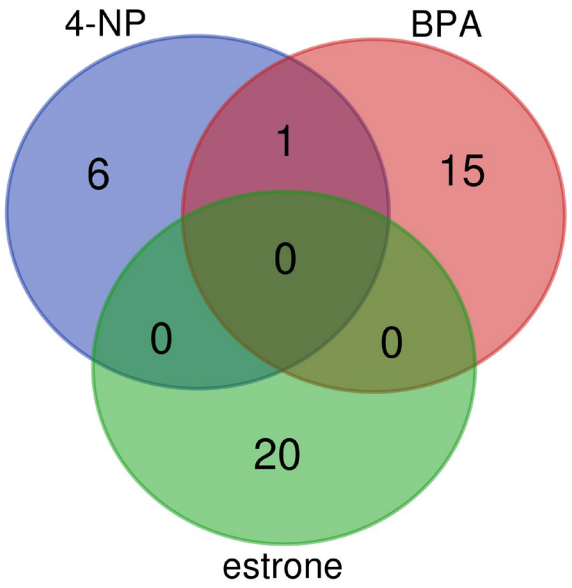


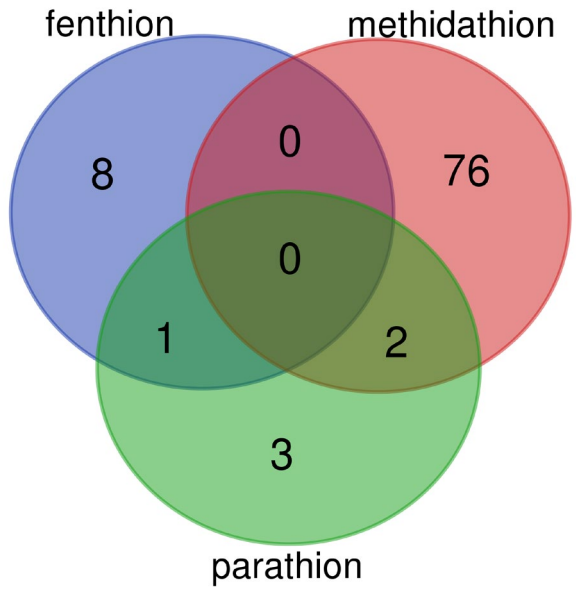
12 new chemicals tested represented 4 modes of action

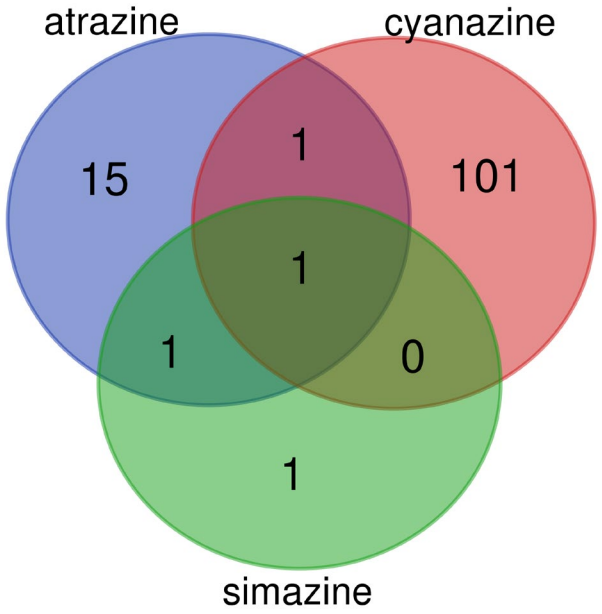
Response to the triazine herbicides was the most varied within class

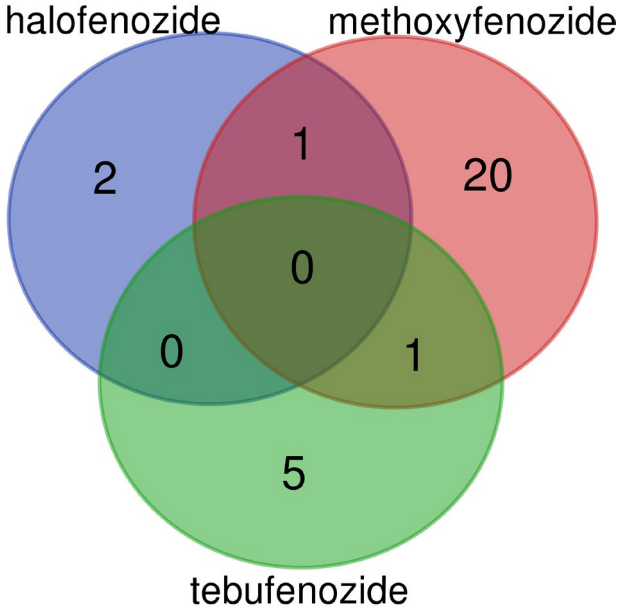
Identity of filtered DEGs (used to determine BMCs)

Very little consistency in the identity of the genes for which a BMC could be derived within MoA class.

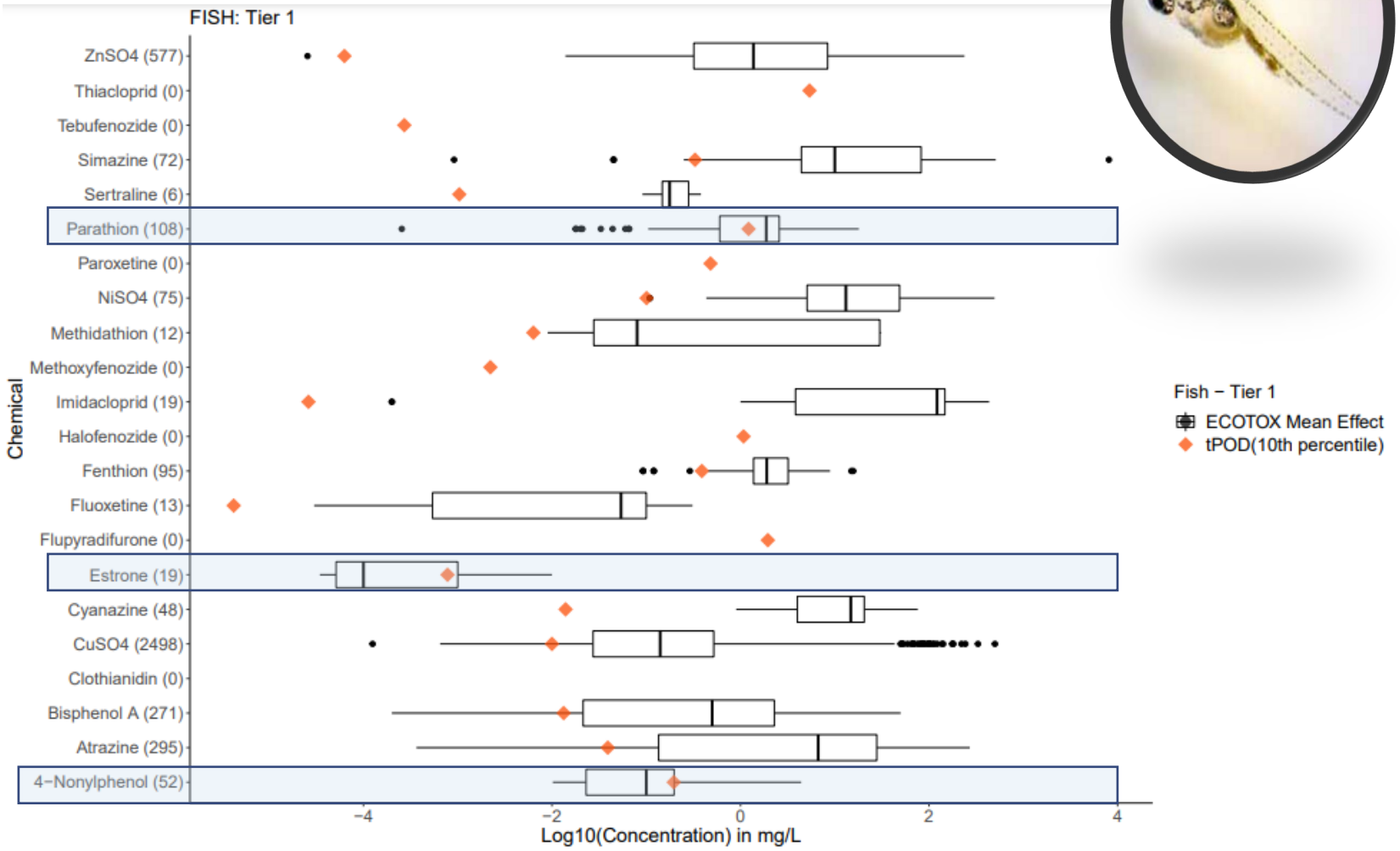








Comparison with In vivo, **Adverse Effect** Concentrations (Fish)



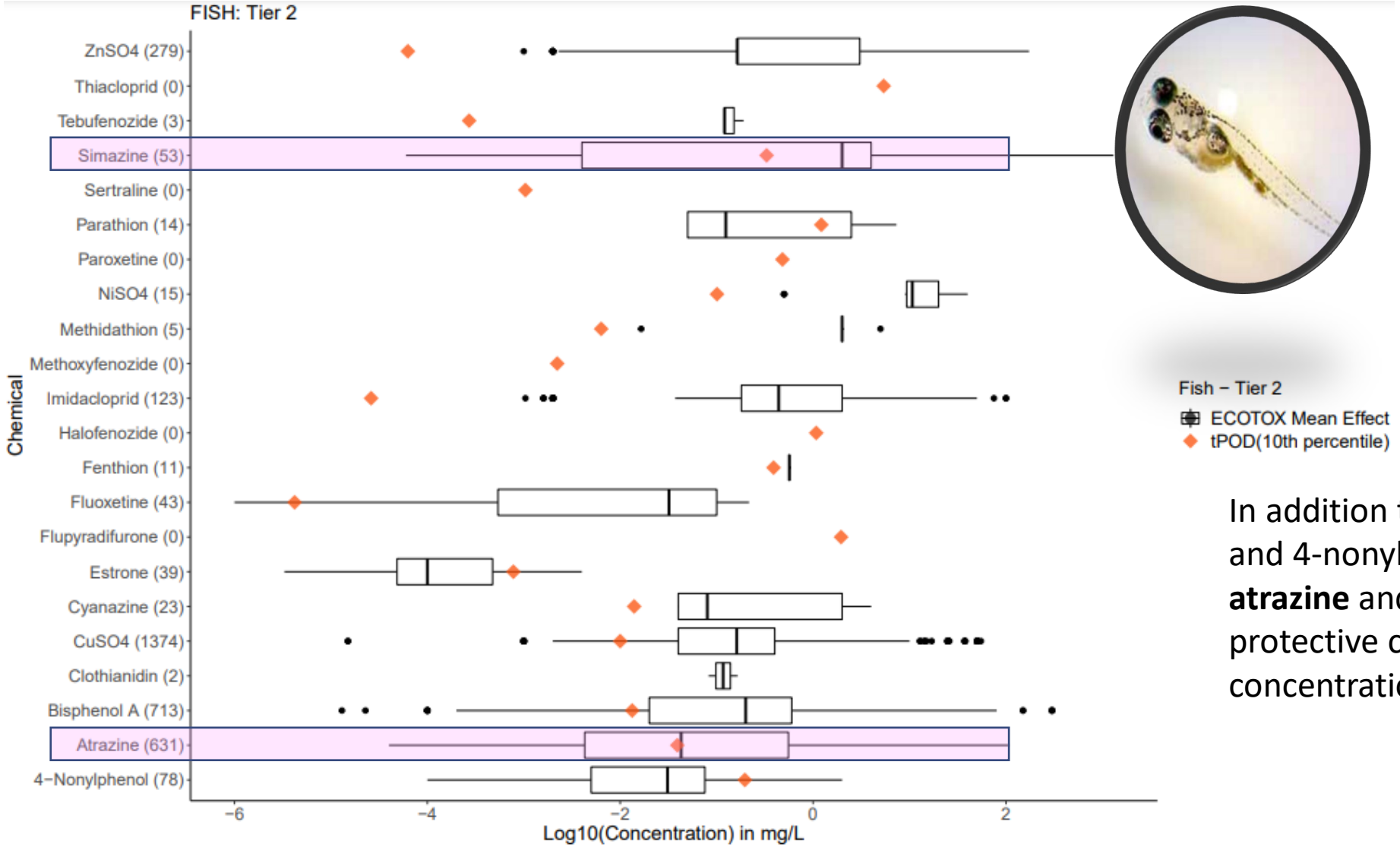
First 10 chemicals:

- tPODs were more sensitive than apical adverse effect concentrations.

Next 12 chemicals:

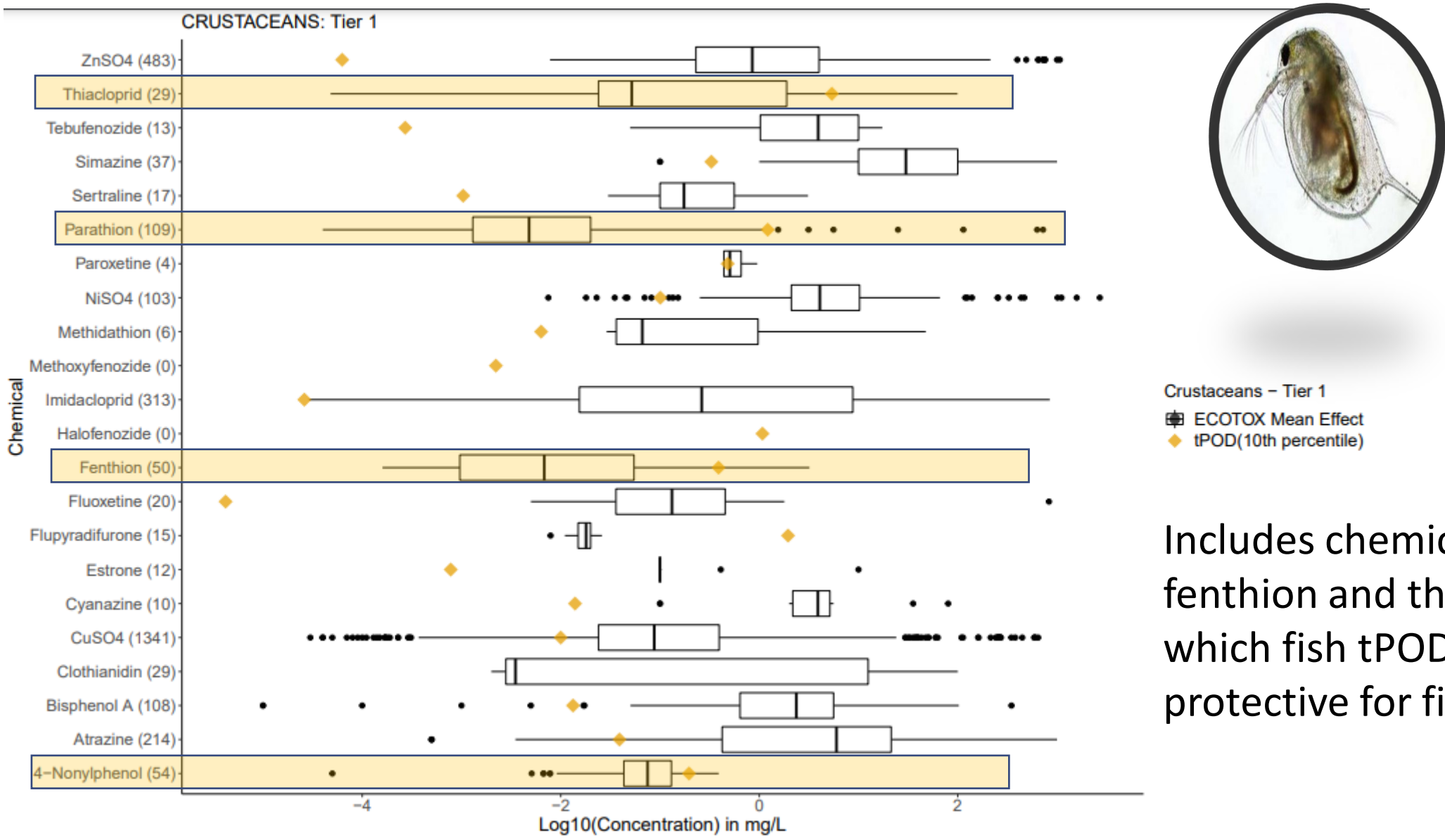
- tPODs for **parathion**, **estrone**, and **4-nonylphenol** exceeded the 25th percentile of apical effect concentrations from ECOTOX. (i.e., not protective)

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



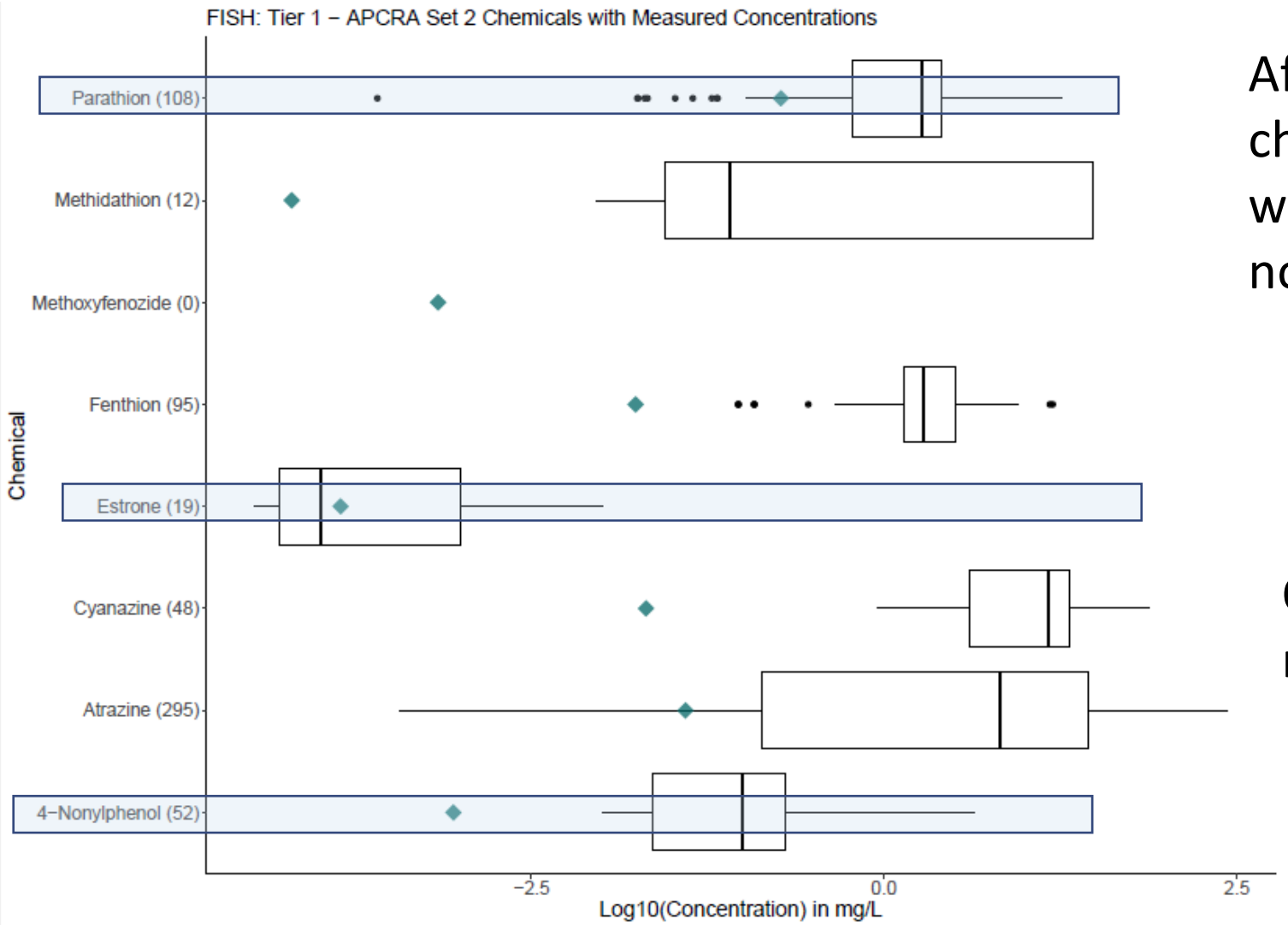
In addition to paroxetine, estrone, and 4-nonylphenol, tPODs for **atrazine** and **simazine** were not protective compared to tier 2 effect concentrations from ECOTOX

Fish tPODs are not universally protective of other taxonomic groups of aquatic organisms



Includes chemicals like fenthion and thiacloprid for which fish tPODs were protective for fish.

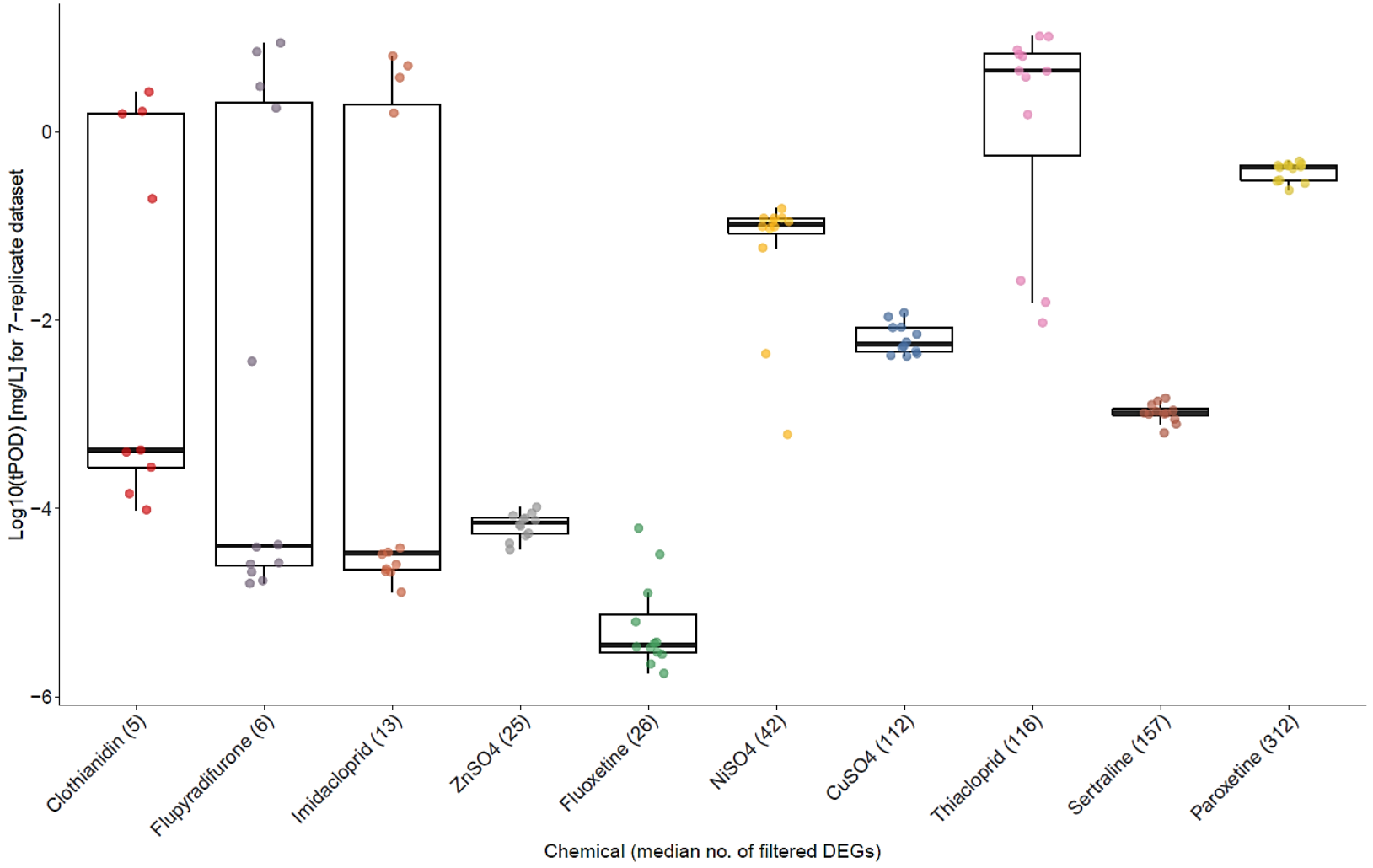
Use of **nominal concentrations** in deriving tPODs is a **source of error**



After correction for measured chemical concentration in the test well, tPODs for parathion and 4-nonylphenol were both protective.

Only the tPOD for estrone remained non-protective, after correction

Assay acceptance considerations – based on 1st 10 chemicals



tPODs based on < 15 fDEGs were highly variable

- 12 replicates of in silico sub-sampling

Based on 30 iterations of randomly assigning controls to treatment groups, 95% of time the number BMCs derived from false discovery is less than 15

≥15 BMCs was provisionally recommended as an assay acceptance criterion.

Next 12 chemicals – Assay acceptance

Chemical	Sequenced features	DEGs	fDEGs	tPOD (mg/L)
Estrone	22218	72	20	0.7793942
4-Nonylphenol	22305	40	7	0.1959934
Bisphenol A	22228	61	16	0.013298184
Fenthion	22554	48	9	0.38902023
Methidathion	22130	195	78	6.345331
Parathion	22044	45	6	1.217177
Atrazine	22276	117	18	0.0390717
Cyanazine	21891	447	103	0.01393464
Halofenozide	21313	39	3	1.0772216
Methoxyfenozide	21611	59	22	2.220094
Simazine	21515	65	3	0.329272
Tebufenozide	21357	48	6	0.00027112

50% of the chemicals tested did not meet our provisional assay acceptance criteria (≥ 15 fDEGs)

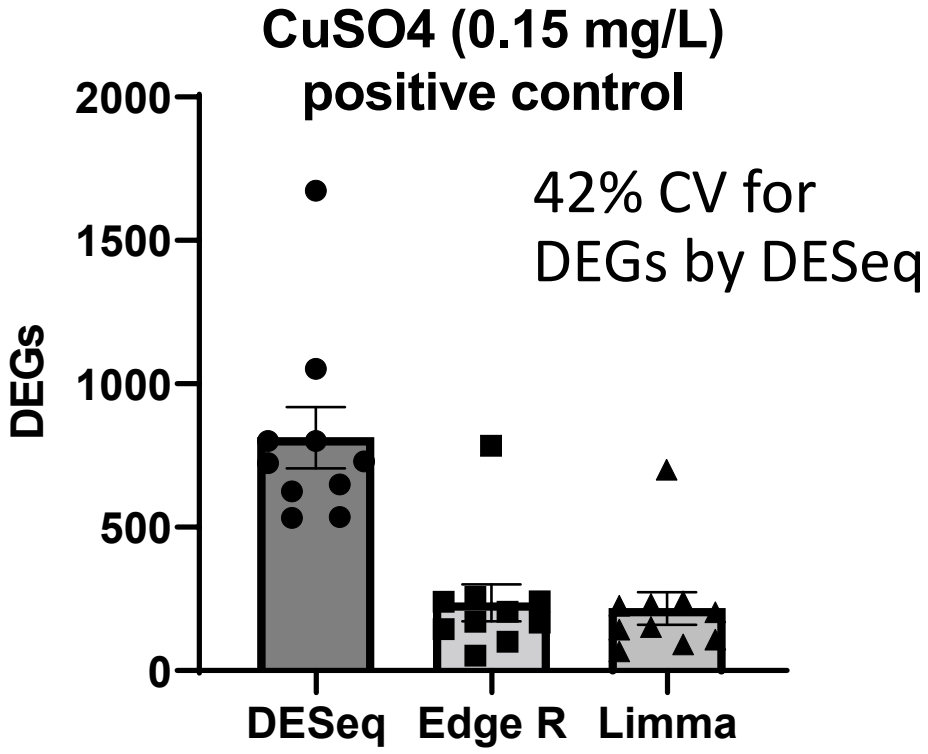
Unacceptable tests (low numbers of DEGs) was not grouped by mode of action.

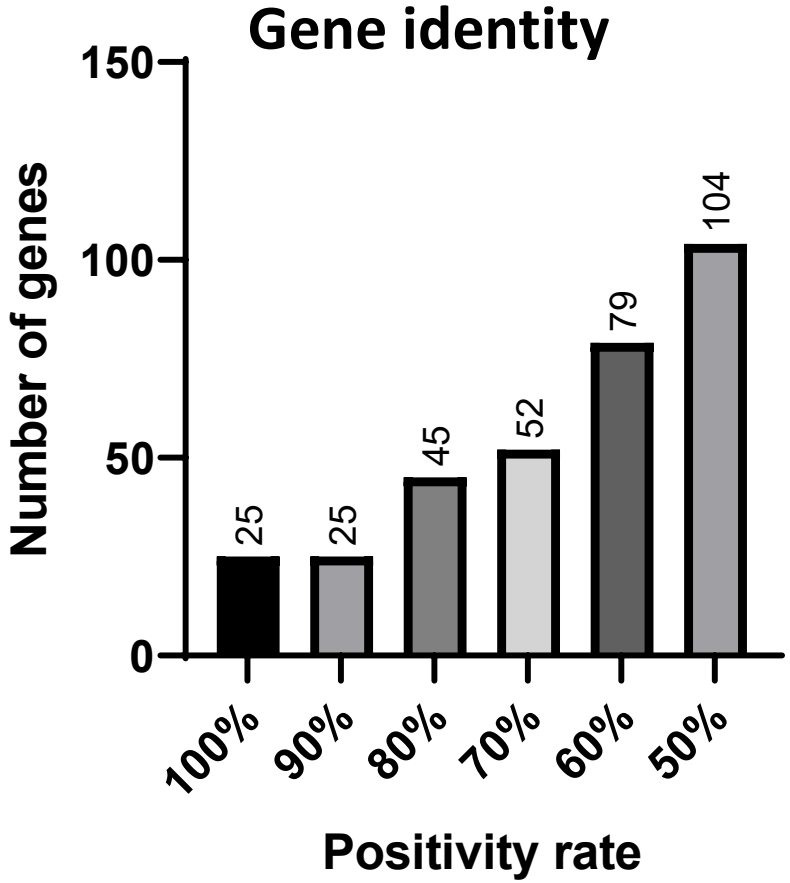
Likely driven by inter-individual variability – larval, whole body transcriptomics

More optimization needed – assay design and data analysis

Reproducibility of transcriptomic response

- 0.15 mg/L $CuSO_4$ tested n=10 independent assays





Out of 4271 unique genes identified as DEG in at least one experiment

330 DEGs (7.7%) observed at least 50% of experiments

Conclusions

- In its current form, larval fathead minnow HTTr assay does not appear reliable as a protective tier 1 screen for ecological hazard.
  - Potential issues with inter-individual variability
  - Whole-body dilution of tissue-specific responses

- There is opportunity for further optimization to improve performance
  - Correction of tPODs based on free fraction in the test well – measured or modeled
  - Testing strategy with increased pooling of individuals is being piloted

- Further optimization and testing is required before ready for regulatory / decision-making applications

Contributors

ORD CCTE GLTED-MIB: Adam Biales, David Bencic, Robert Flick, John Martinson

ORD CCTE GLTED-STB: Kevin Flynn, Dan Villeneuve, Kathy Jensen, Jenna Cavallin

ORD CCTE BCTD/CCED: Josh Harrill, Logan Everett, Leah Wehmas

ORISE FELLOWS: Michelle Le, Kelvin Santana-Rodriguez, Kendra Bush, Mackenzie Morshead, John Hoang, Monique Hazemi, Jacob Collins

SPEC-PRO PROFESSIONAL SERVICES: Kelsey Vitense

Environment and Climate Change Canada: Christina Inglis, John Prindiville, Andy Nong, Jason O'Brien, Florence Pagé-Larivière

The authors have no conflicts of interest to declare.

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