In Vitro Transcriptomic Points of Departure (tPODs) for 42 diverse chemicals in a human (Caco-2) and fish (RT-gill-W1) cell line

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Transcriptomic Points of Departure (tPOD)

- transcriptomics provide lots of information efficiently [1]
- in recent years growing interest in benchmark dose (BMD) modeling of transcriptomic data to identify an exposure level associated with a significant, concerted change in gene expression = tPOD[2]
- 2024: US EPA published Transcriptomic Assessment Product (ETAP) to yield transcriptomic-based reference values
  - tPODs from 5-day animal study associate with chronic animal toxicity data
- so, what about tPODs from in vitro data? Might they inform of chronic toxicity estimates?

1: Head, J.A., et al. *Environmental Toxicology and Chemistry,* 5954.
2: Johnson, K.J., et al *Toxicological Sciences*, *190*(2), 127-132.

Objectives

- to determine if tPOD data from in vitro studies of rainbow trout (*Oncorhynchus mykiss*) gill (RTgill-W1) cells and human intestinal epithelial cells (Caco-2) exposed to chemicals of varying properties associate with chronic toxicity data for each respective compound

Secondary objectives:

- test and refine the TPD-Seq workflow[3], from in vitro exposures to sequencing to bioinformatics to IVIVE calculations to chronic data
- assess this approach's chemical domains of applicability

3: Mittal, K., et al. (2025). TPD-seq: A high throughput RNA-seq method to derive transcriptomic points of departure from cell lines. *Toxicology in Vitro*, *104*, 106001.

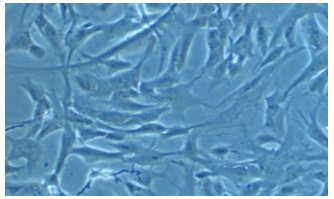
Methods (TPD-Seq Workflow[3])

- expose cells (RTgill-W1, Caco-2) to 42 chemicals
  - blinded to chemicals tested, and so an in vitro screening strategy developed with all chemicals assessed at 100uM and then binned into 4 potency groups
- cytotoxic and transcriptomic responses measured and compared
  - 24 hour exposures; 3-fold dilution series over 8 concentrations
  - each microplate exposure had a solvent and positive control
- resulting data compared to available measures of chronic toxicity for each compound in both fish and mammalian models
- focus today on RTgill-W1 results i.e., ‘omics-enhanced OECD249

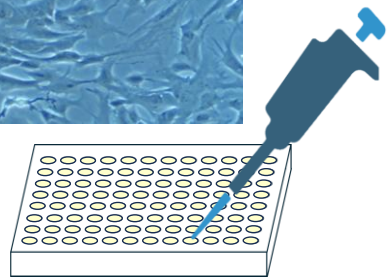
3: Mittal, K., et al. (2025). TPD-seq: A high throughput RNA-seq method to derive transcriptomic points of departure from cell lines. *Toxicology in Vitro*, *104*, 106001.

Exposure, library preparation & RNA-sequencing

RTgill-W1 cells



42 Chemicals

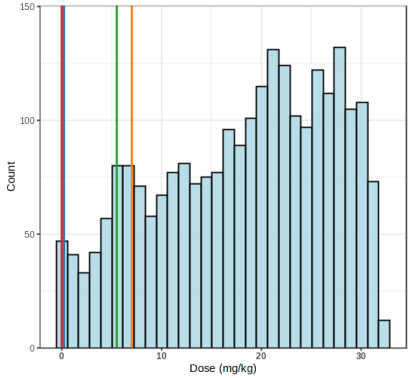


UPXome library preparation

tPOD calculation & modeling

In vitro - Mass Balance Model (IV-MBM) predicted tPODs

Nominal transcriptomic point of departure (tPODs)



versus

versus

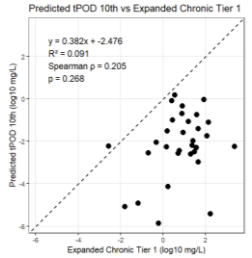
Comparison with *in vitro* studies & chronic animal studies

ECOTOX Knowledgebase

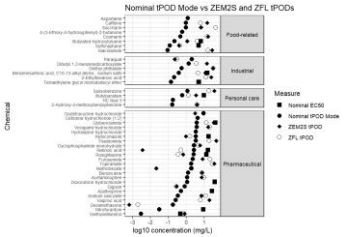
Zebrafish cell lines and high-throughput transcriptomics: advancing in vitro and bioinformatics methods for supporting environmental risk assessment

Peter G Schumann [1], Joseph Bundy [2], Derik E Haggard [3], Logan Everett [4], Joshua A Harrill [5], Felix Harris [6], David Ryoo [7], Jacob Collins [8], Claudia Rivetti [9], Bruno Campos [10,ffff], Geoff Hodges [11], Carlie A LaLone [12,ffff]

In vivo chronic mortality and non-mortality PODs



In vitro tPODs from ZEM2S and ZFL cell lines



Data analysis pipeline

Raw Data (Fastq)

QIAGEN

- Demultiplexing
- Mapping & aligning to the reference

Count tables

- Differential expression analysis
- tPOD calculation

- tPODs
- External database comparisons

Demultiplexing: separate samples by barcode/index
Mapping: Locate reads on the reference genome
Alignment: base-by-base compare, record mismatches

Demultiplexing, mapping & alignment → QC & normalization → Differential expression analysis & tPOD

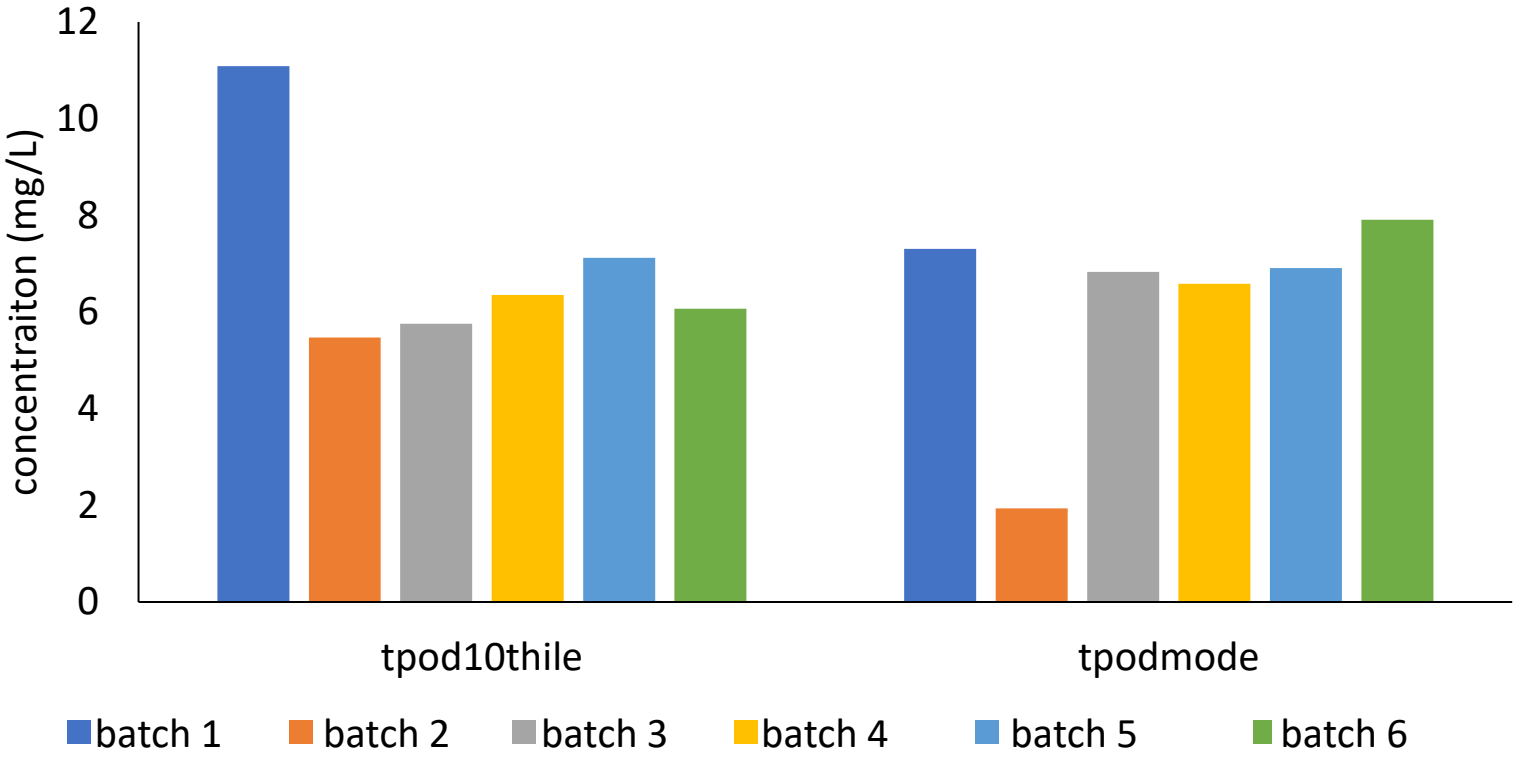
1. Can we screen chemicals “blinded”?

Zone #	# of chem.	Procedure
Zone 1	24	proceed directly to transcriptomics exposure at 100 µM
Zone 2	9	conduct rangefinder test at 100 µM on a semi-log scale over an eight-point dilution series to determine EC20.
Zone 3		
Zone 4	9	conduct rangefinder test starting at 100 µM over an eight-point 10-fold dilution series to determine EC20

Cytotoxicity QC, OECD249

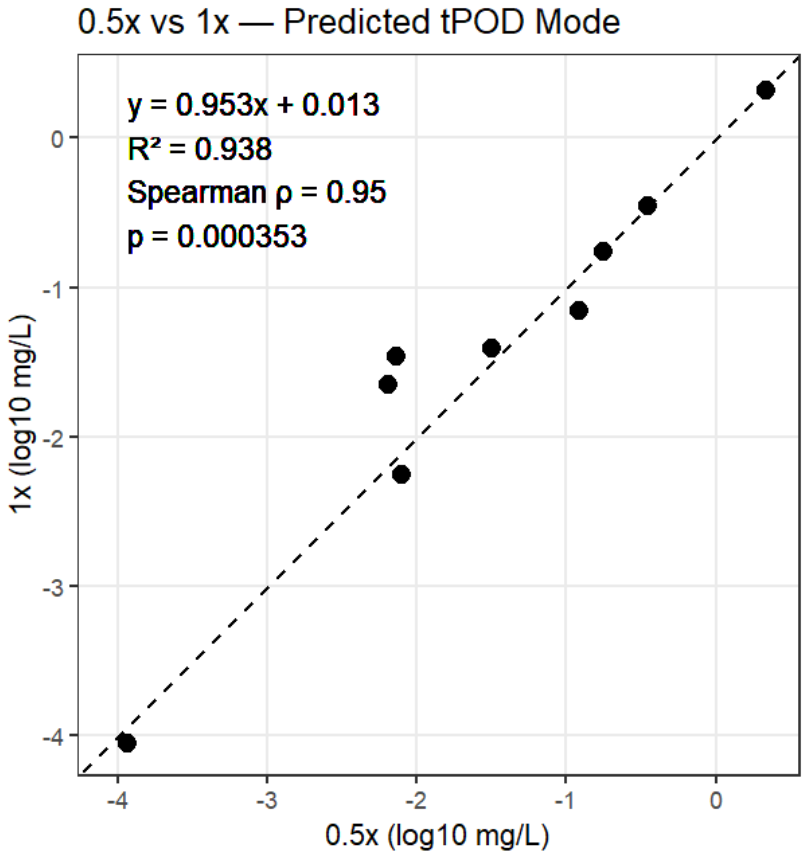
- Background no-cell control variation:
  - Across all plates difference between no-cell control wells < 20%
- % cell viability in solvent control
  - % cell viability >90% relative to unexposed cells; averaged 99%

2. Are in vitro tPODs reproducible?



Tested DCA 6x: tPOD Coefficient of variance ~30%

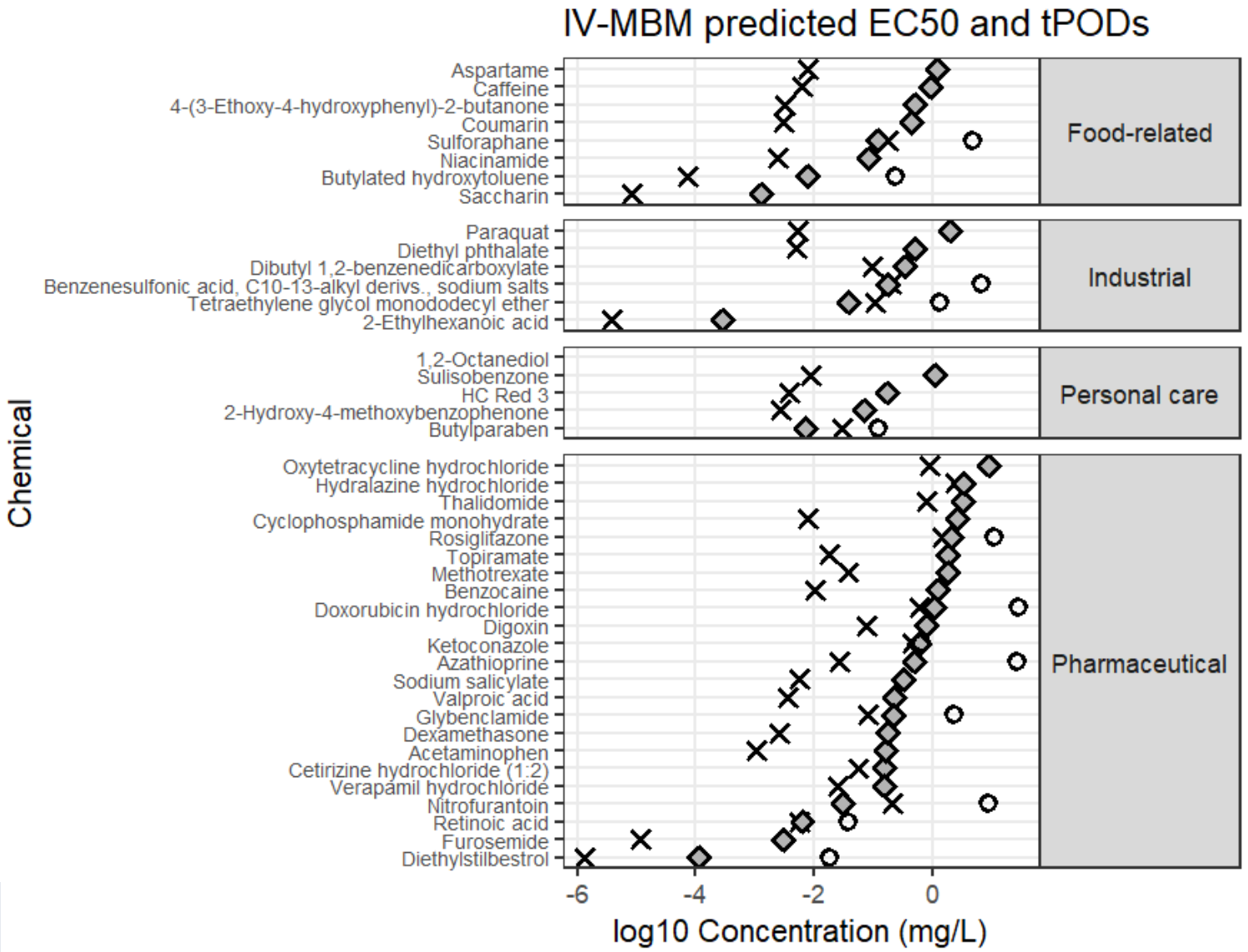
3. Can the workflow be more cost-effective?



-half-volume reactions successful

-free online bioinformatics tool

4. in vitro tPODs vs cytotoxicity EC50s?



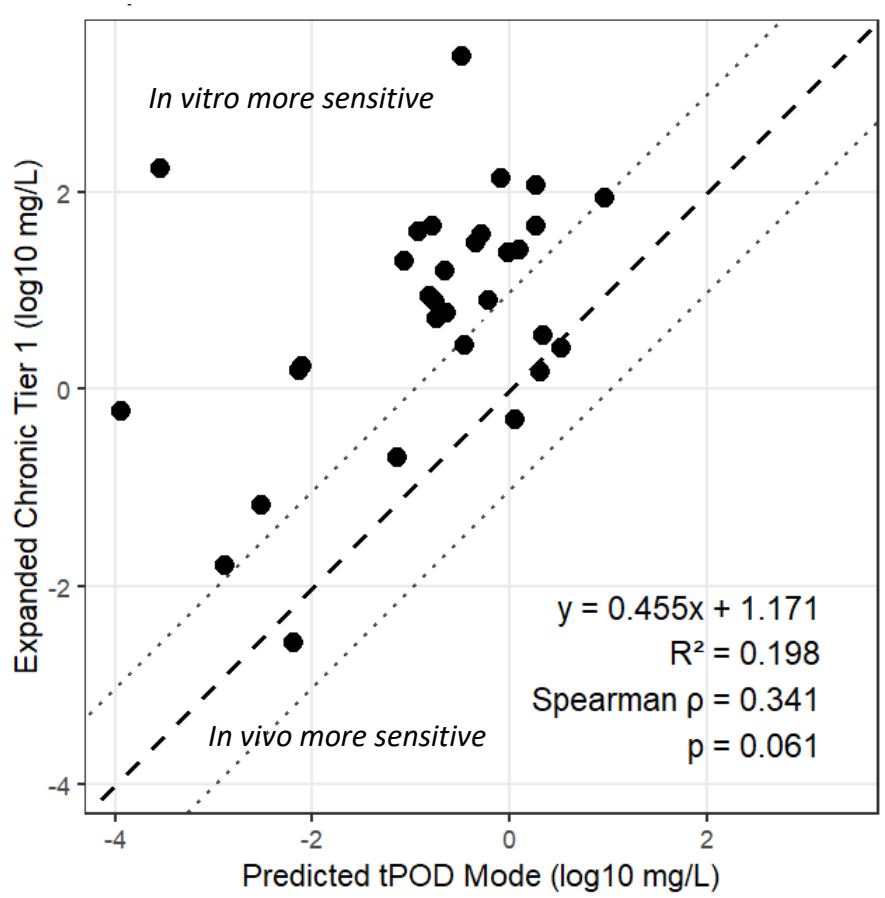
EC50: 13/44 chemicals
tPOD: 43/44 chemicals
-tPOD ~23x lower than EC50

5. In vitro tPODs vs. in vivo fish chronic

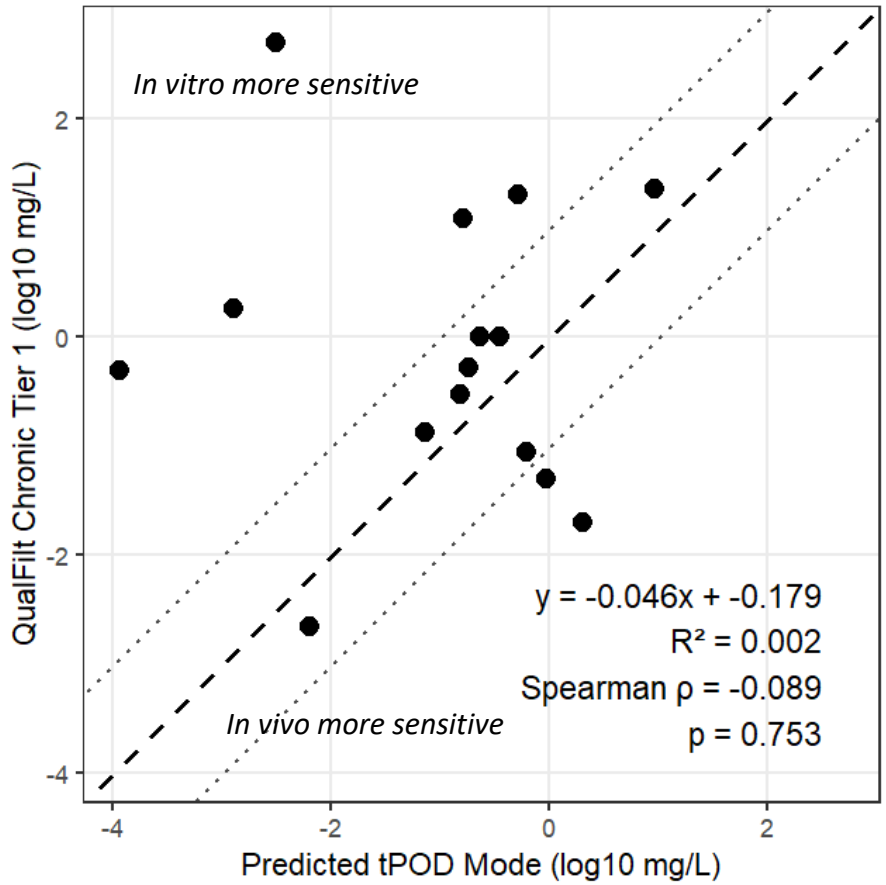
- Compared to the following datasets obtained from Schumann et al. as per next talk by Dr. Rivetti:
  - Tier 1 dataset – fish chronic apical endpoints, mortality
  - Tier 2 dataset – fish chronic, non-mortality endpoints (e.g., biochemical)
  - Expanded criteria dataset (Exp) – not quality filtered
  - Quality filtered dataset (QualFilt) – quality filtered (filtered for chemical purity < 85%, non-relevant exposure routes, chemical formulations rather than pure substances, non-statistically significant effects, non-standard endpoints, records with insufficient controls, records missing test duration information, mixture studies, studies lacking clear dose-response relationships, and records where NOEC were equal to highest tested concentration).
- Schumann et al. 2025. Zebrafish cell lines and high-throughput transcriptomics: advancing in vitro and bioinformatics methods for supporting environmental risk assessment. Toxicol Sci. 208(2):343-356. doi: 10.1093/toxsci/kfaf127

tPODs vs. in vivo fish chronic (Mortality endpoint)

Fish Chronic Toxicity (Mortality) all studies



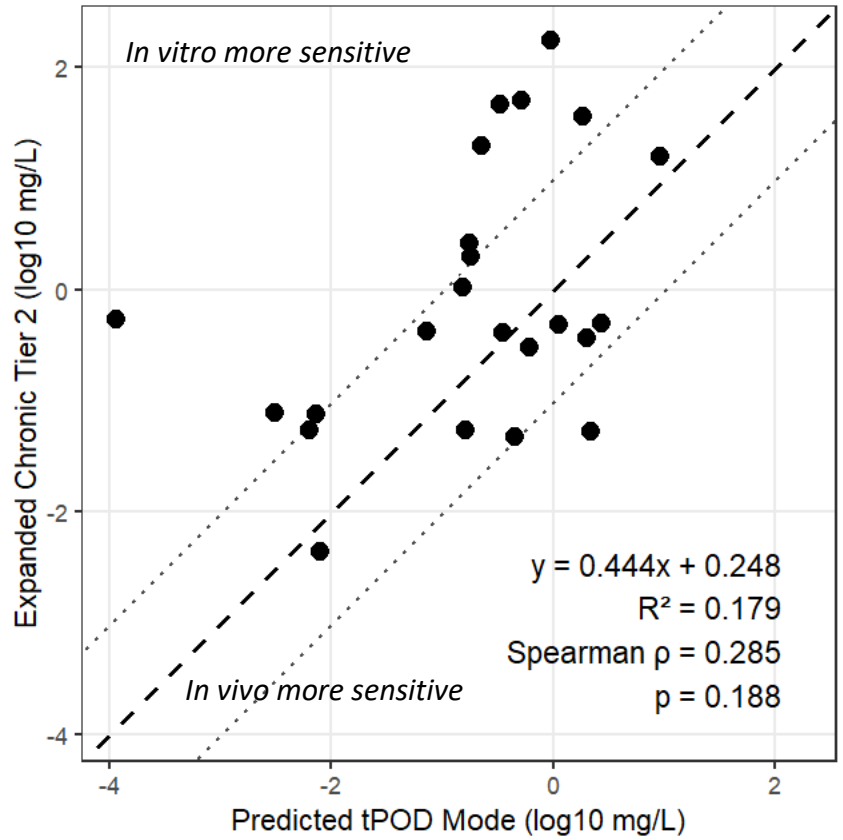
Fish Chronic Toxicity (Mortality) Higher quality studies



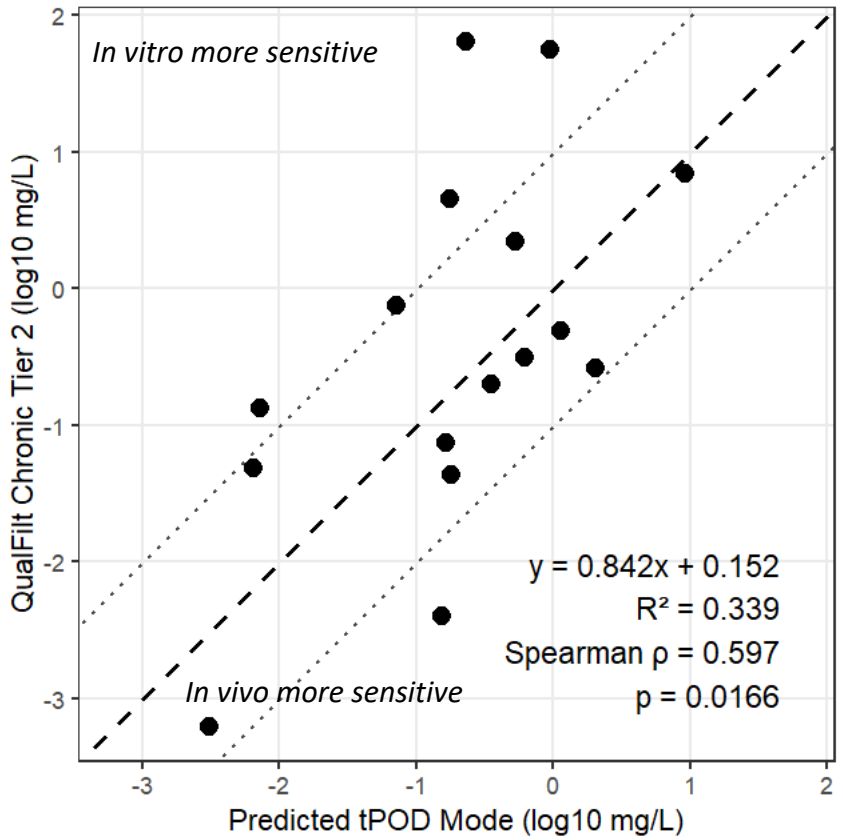
In Vitro tPOD (RTgill-W1) – Current Study

tPODs vs. in vivo fish chronic (Non-Mortality)

Fish Chronic Toxicity (Sub-Lethal) all studies

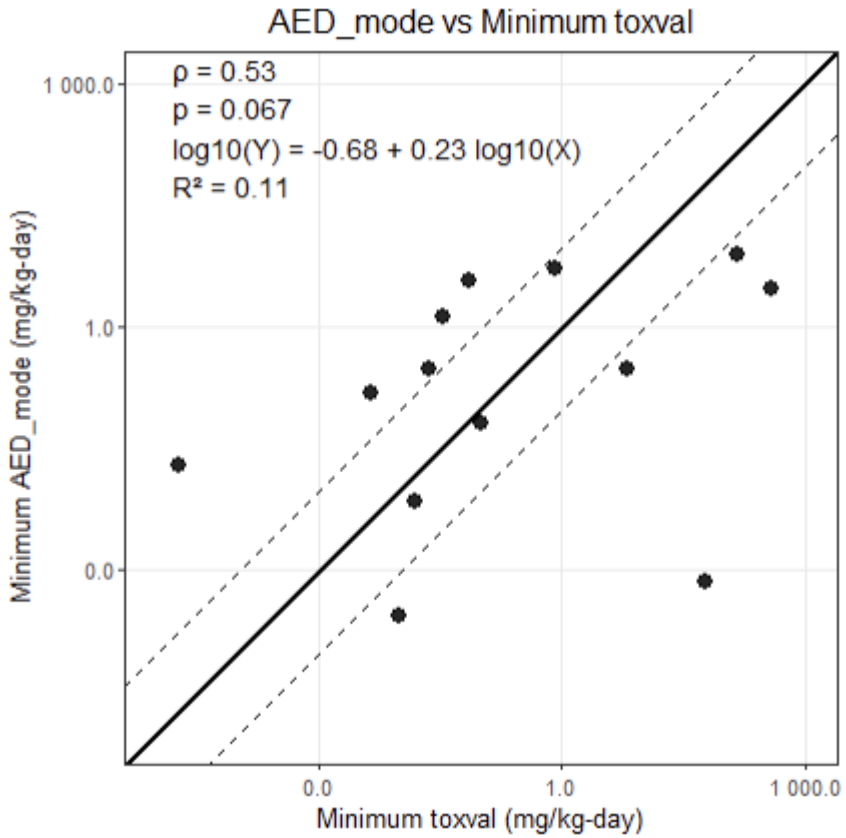


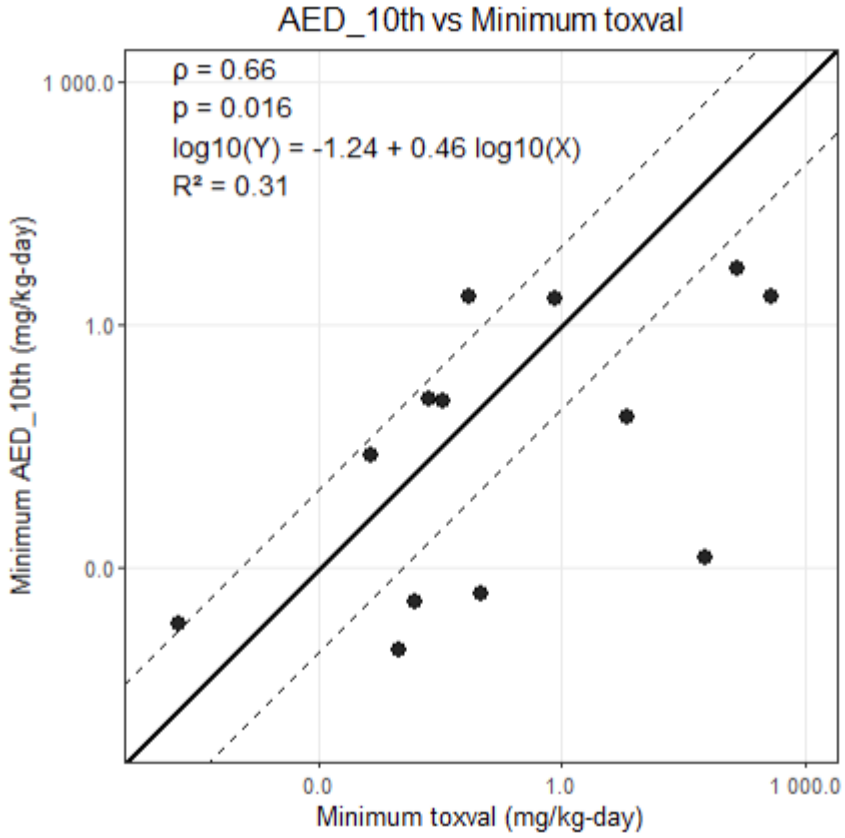
Fish Chronic Toxicity (Sub-Lethal) Higher quality studies



In Vitro tPOD (RTgill-W1) – Current Study

Aside: In vitro tPOD (Caco-2 cells) vs. animal (rodent) chronic toxicity





Minimum AED (of 4 cell lines) vs minimum chronic animal studies

Concluding remarks

- results (to date) suggest in vitro tPODs yield data that are comparable to fish chronic toxicity data → 'omics enhanced OECD249
- findings align with our past studies in vitro (e.g., 18 pesticides in RTgill-W1 cells), and also in vivo (US EPA ETAP), among others
- results presented are comparable to those from Caco-2 cells
- next steps:
  - deeper bioinformatics (e.g., biological pathways) and tPODs (e.g., sensitivity tests) analyses, and further examination of fish chronic data; both tPODs and fish chronic data can vary a lot
  - embrace the outlier! To understand domains of applicability, examine outliers (e.g., physico-chem properties, mechanisms of action, etc.)

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