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Background

- Tens of thousands of chemicals are currently in use and hundreds more are introduced to the market every year.
- Only a small fraction has been thoroughly evaluated for potential risks to human health and the environment.
- US EPA implementation HTP screening (ex.ToxCast and Tox21) focused on human health
- Coverage of pathways not conserved with mammals is lacking
- Need HTP assays that address pathways/physiology unique to aquatic organisms representing different trophic levels: https://doi.org/10.1093/toxsci/kfz058

Objectives

- Modify standard protocols and methods to allow rapid toxicity tests with small aquatic organisms in 96-well plates
- Conduct exposures with diverse chemicals (ex. metals, neonics, pharmaceuticals)
- Compare traditionally derived LC50 values to LC50 values calculated from 96-well plate-based exposures
- Use RNA-seq data to calculate transcriptomic-based point-of-departure (PODs) that can be anchored to apical responses

Accomplishments

- Assays developed in 4 species, representing diverse taxa, including *Daphnia magna, Pimephales promelas, Chironomus dilutus, Raphidocelis subcapitata*
- Exposures with diverse chemicals: Cu, Zn, Ni, clothianidin, imidacloprid, thiacloprid, fluoxetine, sertraline, paroxetine
- LC50s from 96-well plate exposures similar to published LC50s
- Transcriptomic-PODs (tPODs) calculated following NTP guidelines https://ntp.niehs.nih.gov/publications/reports/rr/rr05/index.html
- tPODs from 24-hour exposures more sensitive than 96-hour LC50s

Future Directions

- Identify appropriate replication level and # of concentrations through statistical analyses
- Determine within species transcriptomic variability across multiple tests
- Continue development of assays (ex. measure water quality parameters, exposure chemical kinetics)
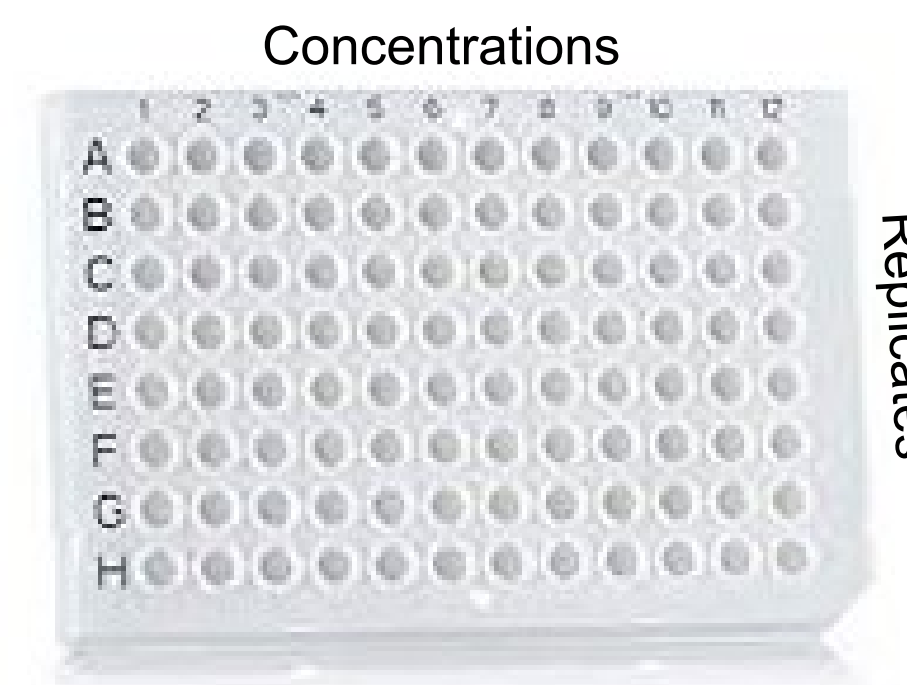- Exposures across diverse chemical classes (ex., PFOS, herbicides, phthalates, etc)

Assay Descriptions

Species	Guideline Test Method	Age at Start	Temp
Daphnia magna	850.1010 Aquatic Invert Acute Toxicity	72-hour old	20° C
Pimephales promelas	850.1075 Fish Acute Toxicity	24-hour post-hatch	25° C
Chironomus dilutus	850.1790 Chronomid Sediment Toxicity	3rd instar	20° C
Raphidocelis subcapitata	850.4500 Algal Toxicity	Log-phase growth	24° C

- Each HTP test species linked to an EPA Guideline Test Method to enable phenotypic anchoring
- Age of organism a compromise between water quality, sufficient tissue, observable behavior, etc
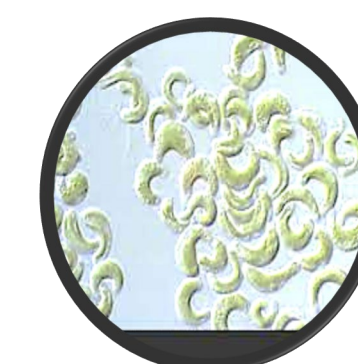- Exposures at guideline temperatures

Exposures Design

Concentrations

- 1 ml deep 96-well plates
- 12 concentration – 8 replicates per concentration
- 1 individual per well (algae ~5 x 10^4 cells/ml)
- 24-hour static exposures
- phenotypic endpoints assessed
  - animals: mortality and behavior
  - algae: cell viability & division, photopigments
- then after homogenization, RNA extracted for transcriptomics

Replicates

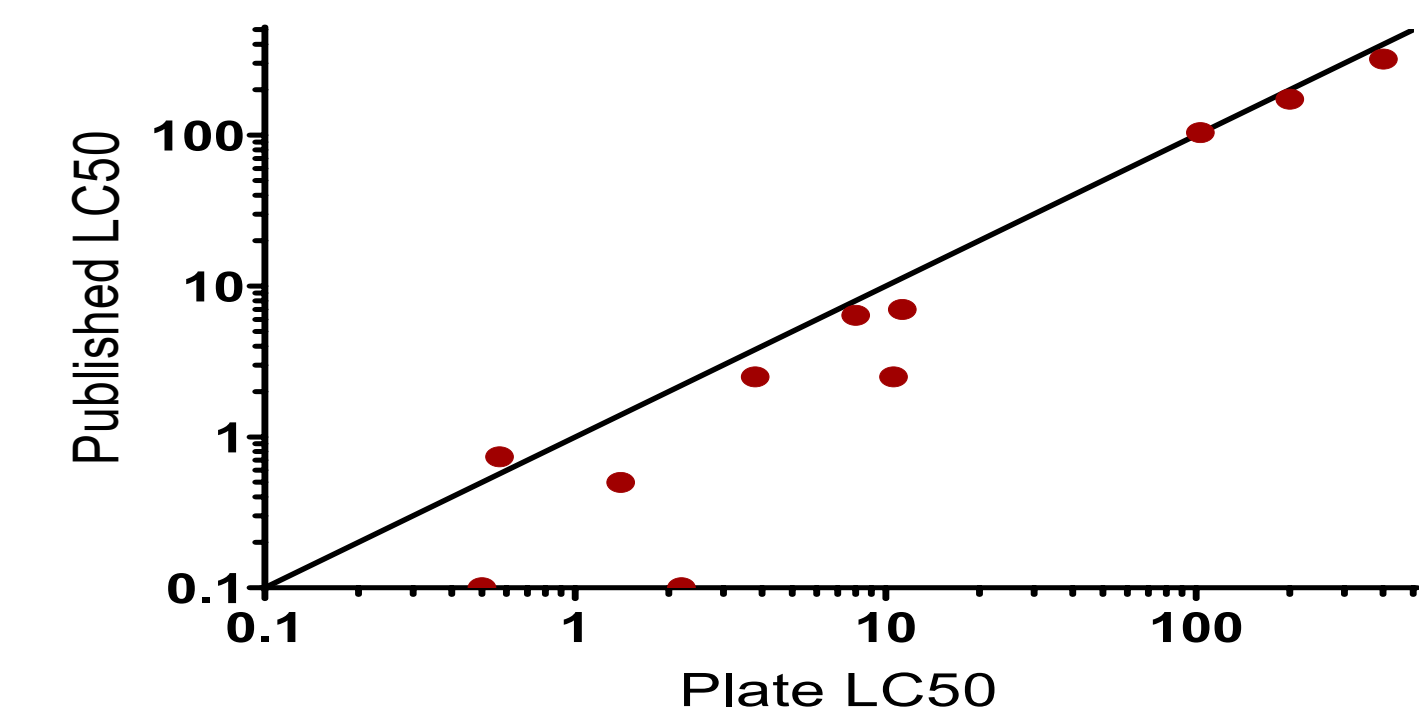### Assay Notes

Species	Time to Load Plate	Control 24-hour Survival	RNA Qty per Well
Daphnia magna	~45 minutes	>95%	~1000 ng
Pimephales promelas	~30 minutes	>95%	~1500 ng
Chironomus dilutus	~60 minutes	>85%	~ 900 ng
Raphidocelis subcapitata	~10 minutes	Increased biomass	~ 300 ng

- Each species can easily be loaded and appropriate exposure media delivered to each well
- High control survival in all assays
- Sufficient, high quality total RNA from each well allows well specific transcriptomic analysis

Data Analysis

LC50s: Published vs 96-well Exposure

- Comparison of published LC50s found in ECOTOX (https://cfpub.epa.gov/ecotox/) vs LC50s calculated from 96-well exposures
- Published 24-hour LC50 data not available for many chemical – species combinations
- Reference line is a 1:1 relationship

RNA-seq data was obtained from each well; all 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
- Differentially expressed genes (DEGs) determined by NTP guidelines and transcriptomic POD for a chemical defined as median POD of all (DEGs) (https://ntp.niehs.nih.gov/publications/reports/rr/rr05/index.html)

Evaluating the Approach

[tPOD] ≤ [Sensitive apical endpoint] 👍

[tPOD] > <<< [Sensitive apical endpoint] 👎

Chemical	DEGs	Transcriptomic POD	96-hour LC50	Mortality-based POD
CuSO4	208	0.025 mg/L	0.25 mg/L	0.16 mg/L
ZnSO4	206	0.00023 mg/L	2.2 mg/L	3.2 mg/L
NiSO4	445	0.33 mg/L	6.2 mg/L	3.9 mg/L

- Due to COVID-19, RNA-seq data from only above 3 chemicals in P promelas analyzed
- tPODs lower but not much lower than mortality endpoint suggesting approach is appropriate

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