High Throughput Transcriptomics: A Multi-Species Approach

Presented by Kevin Flynn

to

US EPA BOSC

Chemical Safety Subcommittee Meeting

Office of Research and Development
Center for Computational Toxicology and Exposure

February 2, 2021

EPA
United States
Environmental Protection
Agency

ORD Strategic Research Action Plan

CSS.1.7:

Develop, evaluate, and apply non-mammalian high-throughput toxicity tests for priority endpoints and pathways in ecological species for ecological risk assessment

CSS.4.4:

Develop rationale and case studies that apply AOPs and HTT data to inform test-order decisions and establish scientific support for waiving testing requirements for pesticides

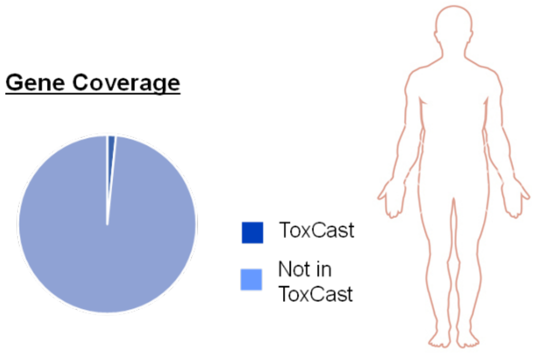
A Chemical Numbers Problem

U.S. EPA Strategic Plan (2018-2022), Objective 1.4, Ensure Safety of Chemicals in the Marketplace

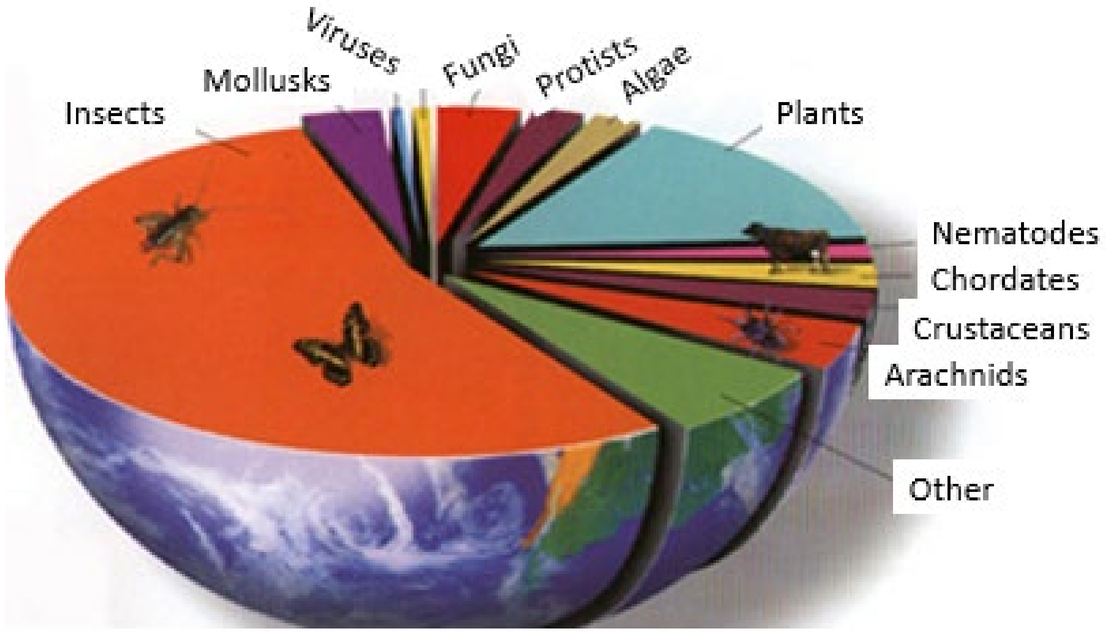
Problem Statement:
Tens of thousands of chemicals are currently in use and hundreds more are introduced to the market every year. Only a small fraction has been thoroughly evaluated for potential risks to human health and the environment.

"Too many chemicals, too little data"

A Biological Numbers Problem



"Throughout the development and execution of ToxCast and Tox21, key limitations of the current suite of HTS assays have been identified (Tice, et al., 2013). The limitations include ***inadequate coverage of biological targets and pathways***" Thomas et al. 2019



The Eco Data Gap:

- Humans are just a tiny fraction of the biological diversity we are charged to protect.
- Many genes/pathways are conserved
- Unique physiology in other kingdoms, phyla, classes…

HTP Eco Assay Development

Daphnia magna

Pimephales promelas

Chironomus dilutus

Raphidocelis subcapitata

- Modify standard protocols and methods to allow rapid toxicity tests with small aquatic organisms in 96-well plates – 4 species
- Conduct exposures with diverse chemicals (ex. metals, neonics, pharmaceuticals, PFAS)
- 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

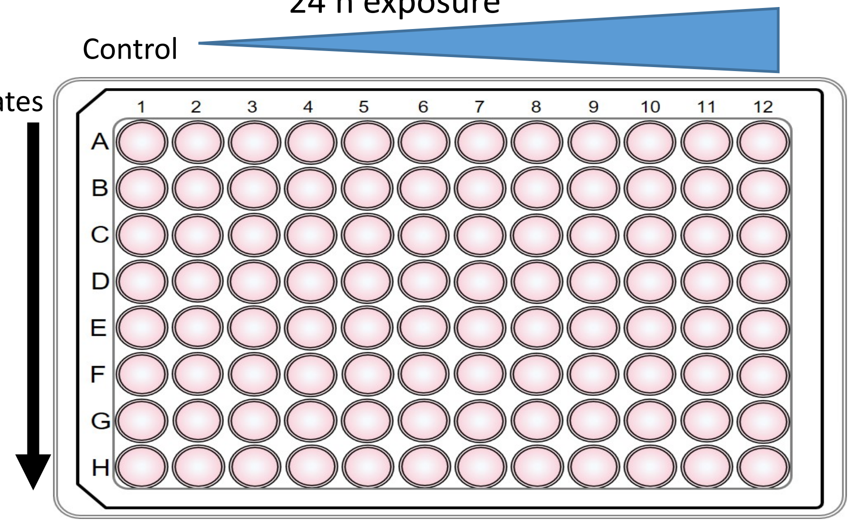
HTP Eco Assay Development

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

24 h exposure

Control

Replicates



Exposures Design

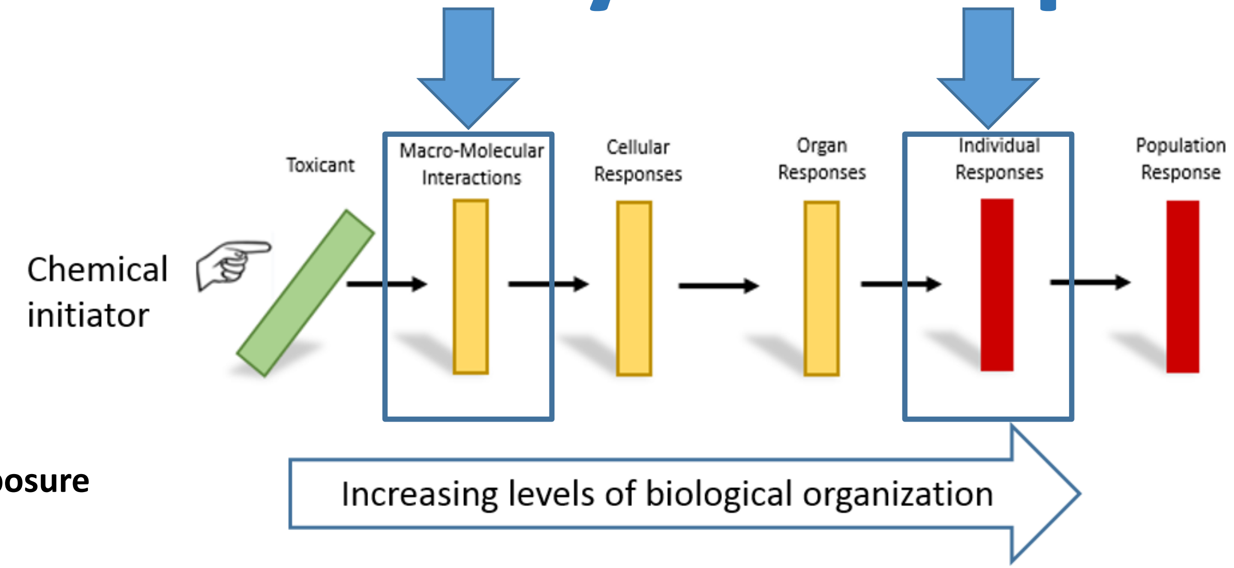
- 1 ml deep 96-well plates
- 12 concentration – 8 replicates per concentration
- 1 individual per well (algae ~5 x 10^4 cells/ml)
- 24-hour static exposures
- phenotypic endpoints assessed
  - animals: survival and behavior
  - algae: cell viability & division, photopigments
- then after homogenization,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

Phenotypic anchoring

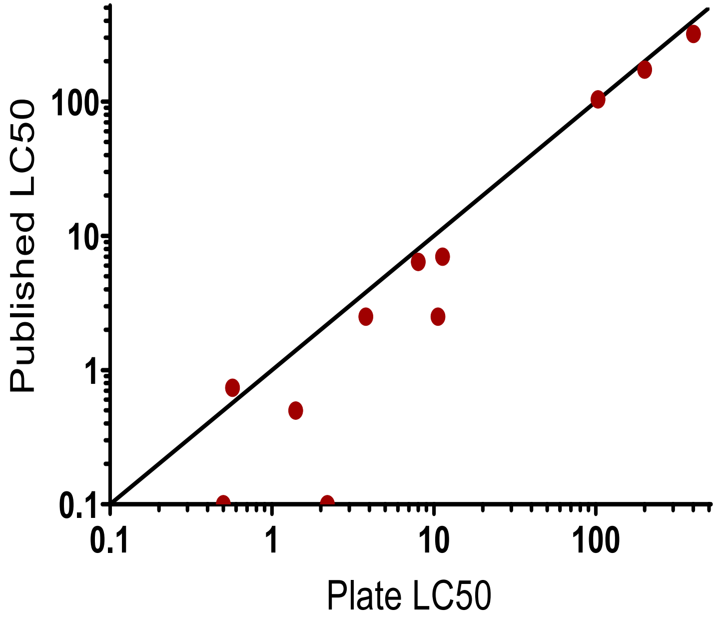
- survival
- behavior
- growth?

Species	Time to Load Plate	Control 24-hour Survival	RNA Qty per Well
Daphnia magna	~45 minutes	72-hour	~1000 ng
Pimephales promelas	~30 minutes	24-hour	~1500 ng
Chironomus dilutus	~60 minutes	3rd instar	~900 ng
Raphidocelis subcapitata	~10 minutes	Log-phase	~300 ng

HTP Eco Assay Development



LC50s:
Published vs 96-well Exposure



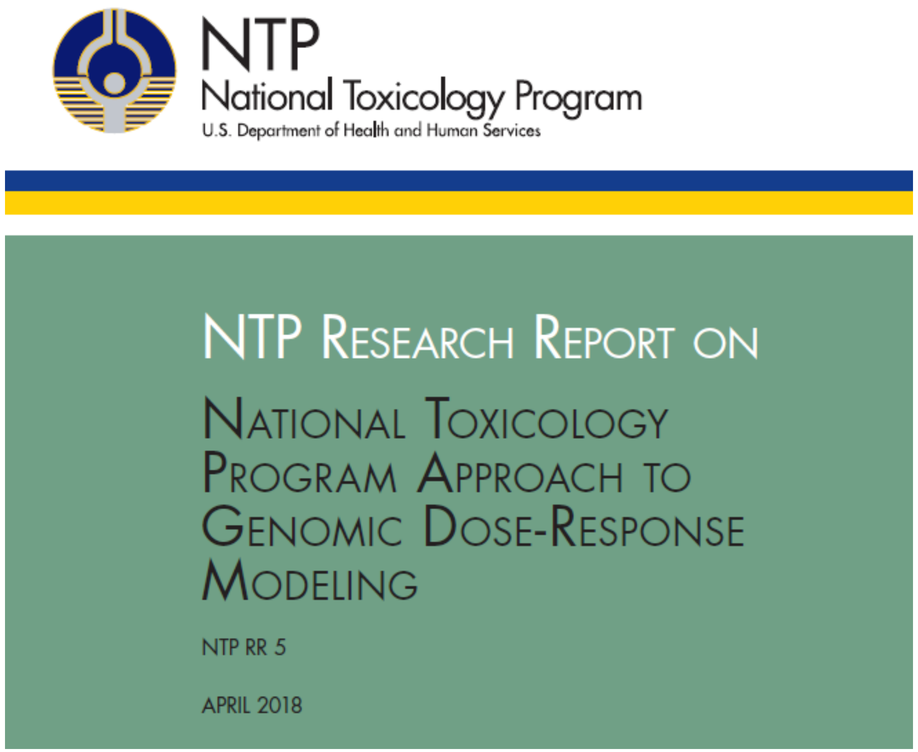
- In internal review process, linking to apical endpoints essential
- Apical Endpoints
  - Survival 👍
  - Reproduction 👎
  - Growth 👍 or 👎
- Behavior
- “Imageable” measurements

HTP Eco Assay Development

Chemicals	Chemical Class	Rationale	Data Use
$CuSO_4$, $NiSO_4$, $ZnSO_4$	metal	OW; ease of exp.; mouse & RBT data	**APCRA case study**; 4 eco-species
Clothianidin, Thiacloprid, Imidacloprid	Neonicotinoid	OPP	**APCRA case study**; 4 eco-species; **Challenge**
Flupyradifurone	Butenolide	OPP	**APCRA case study**; 4 eco-species
Sertraline, Fluoxetine, Paroxetine	SSRI	Existing data at GLTED	**APCRA case study**; 4 eco-species
Atrazine and similar	Herbicide	Herbicide	**Challenge**; 4 eco-species
Methoxyfenozide and similar	Carbohydrazide	Insecticide	**Challenge**; 4 eco-species
Parathion, methidathion, fenthion	Organophosphate	mouse data	4 eco-species
Phthalate TBD	Phthalates	TSCA high priority	4 eco-species
~20 specific PFAS	PFAS	PFAS plus up; small # *in vivo*	4 eco-species
50 – 100 additional		StRAP	4 eco-species

HTP Eco Transcriptomics

EPA United States Environmental Protection Agency


NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP Research Report on

National Toxicology Program Approach to Genomic Dose-Response Modeling

NTP RR 5

April 2018

- Number of mammalian studies have shown short-term transcriptomics-based PODs are predictive of apical potency.
- Generally within ½ log.

A.3 Global Comparison of POD and BEPOD

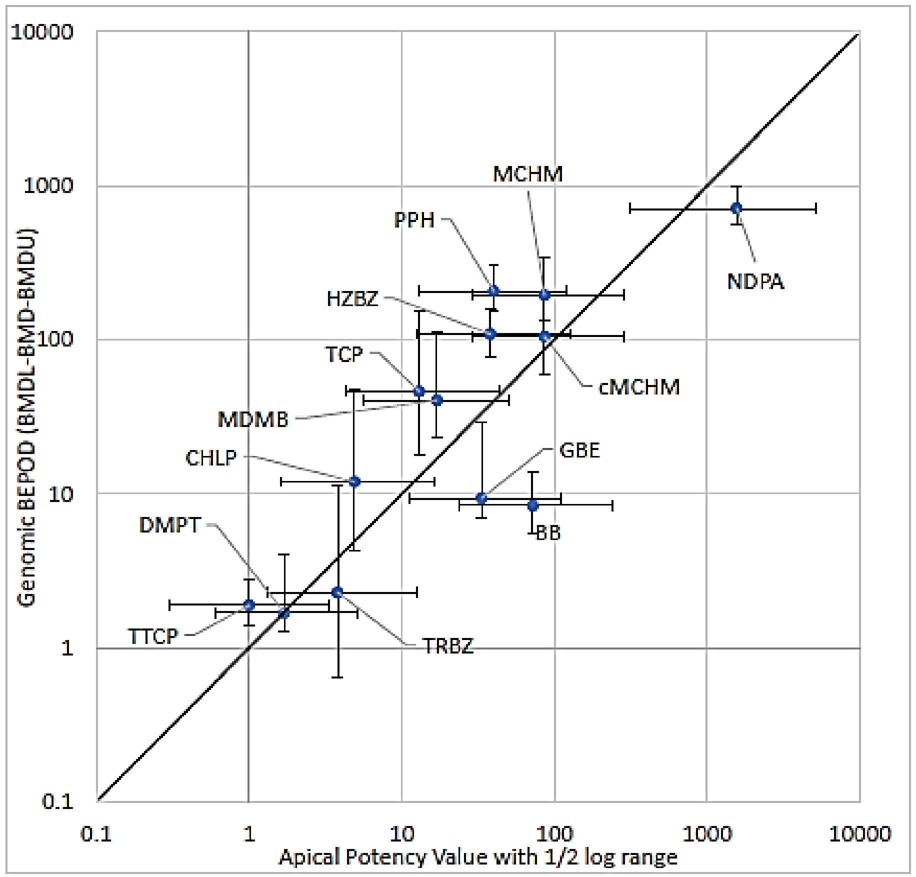
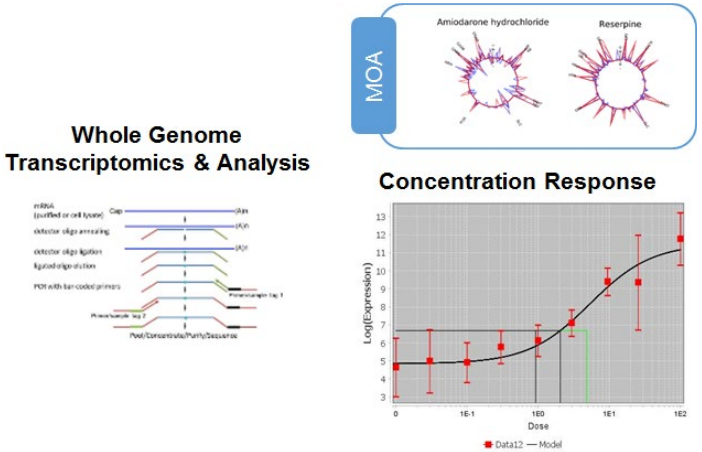
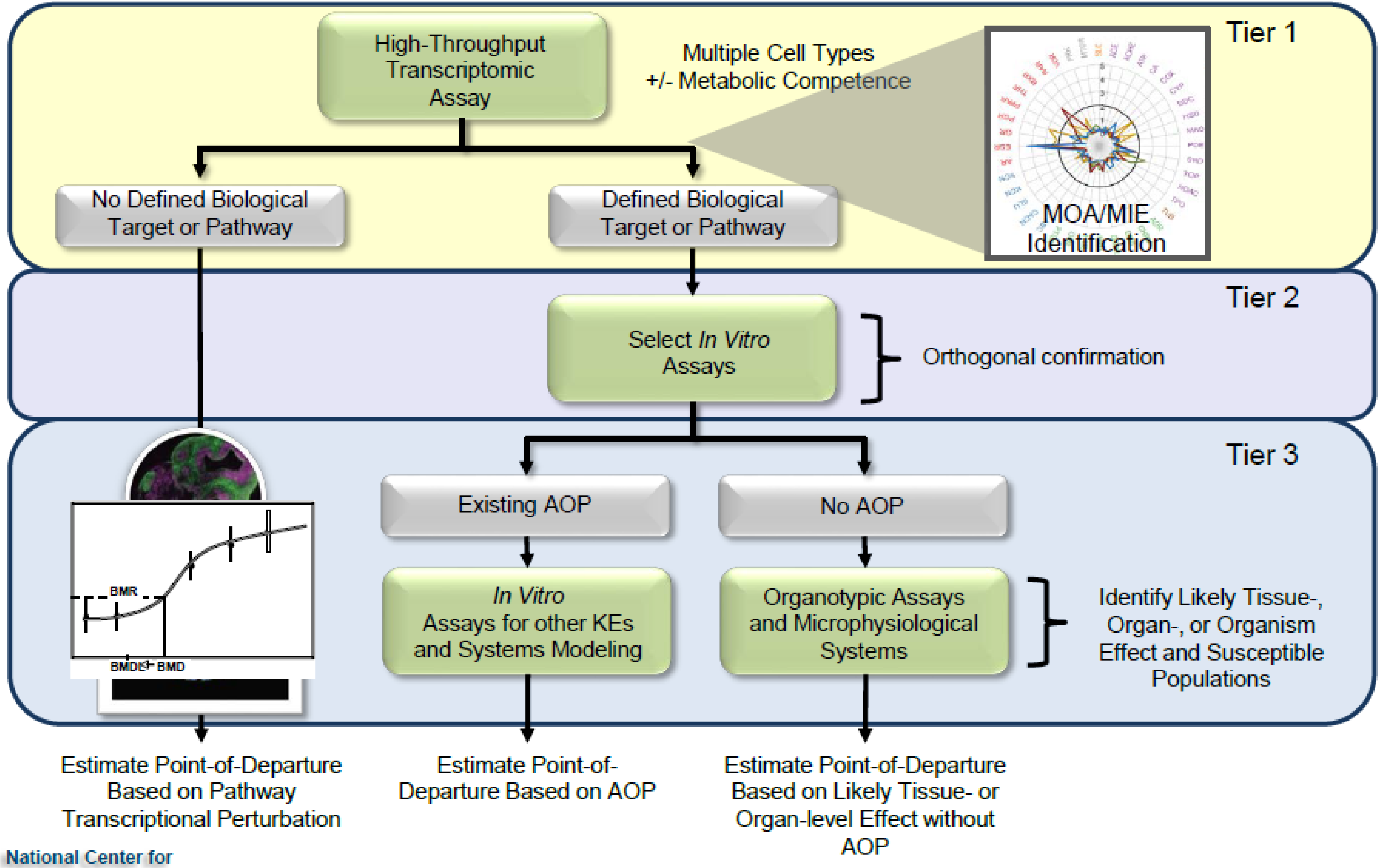


Figure 14. Comparison of the Most Sensitive Apical ½ Log Potency Range to the Most Sensitive GO Biological Processes BEPOD

Data from Figure 1–Figure 13 in this document were compiled to allow a larger scale comparison of apical and gene set-based biological potency estimates. The most sensitive apical potency values (NOAEL or BMD) from guideline toxicity assessments are plotted on the x-axis and the BEPOD range (BMD_L-BMD-BMD_U) from the GO Biological Processes analysis from 4- or 5-day GDRS studies are plotted on the y-axis. A diagonal 1-to-1 line is drawn as reference to perfect agreement between the potency values. The points to the left of the line demonstrate more sensitive apical endpoints, whereas those to the right exhibited more sensitive BEPODs. Overall, the apical and BEPOD values strongly agree, as indicated by $R^2 = 0.89$.

HTP Eco Transcriptomics





National Center for Computational Toxicology

HTP Eco Transcriptomics

Challenge.gov
Government Challenges, Your Solutions

EcoTox TARGET Challenge

Develop high quality, low-cost tools that assess global gene expression in common aquatic toxicity test organisms

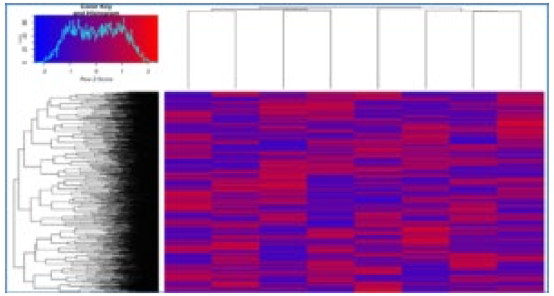
CHALLENGE DETAILS

- TOTAL CASH PRIZES OFFERED: $300,000
- TYPE OF CHALLENGE: Scientific
- PARTNER AGENCIES | FEDERAL: U.S. Army Engineer Research and Development Center
- PARTNER AGENCIES | NON-FEDERAL: DoW, Environment and Climate Change Canada, European Commission Joint Research Centre, Syngenta
- SUBMISSION START: 03/19/2020 12:00 PM ET
- SUBMISSION END: 06/15/2021 11:59 PM ET

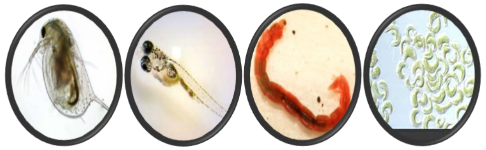
Commercial Development

4 "Solvers"

Detection/analysis technology



Commercially available
Low cost (<$50/sample)
High quality
Maximal coverage

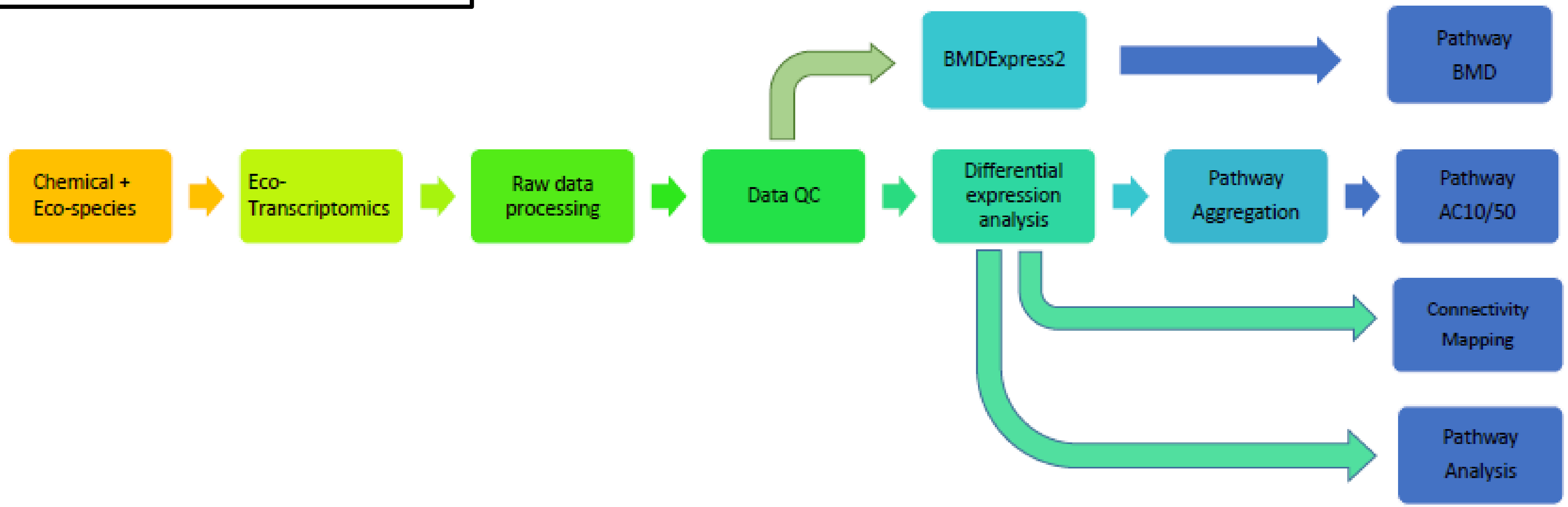


TARGET
EcoTox Challenge

Eco Transcriptomics Data Analysis

Transcriptomics Analysis Workflow

- not re-inventing the wheel
- mirror ToxCast data analysis



Current Status

- Derive transcriptomics-based points of departure for 20 chemicals
- Testing with fathead minnow only
- Compare with traditional apical PODs
- Evaluate hypothesis that tPODs are protective relative to apical
- Includes chemicals of direct interest to Program Offices and partners

Workflow in Brief

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)

Current Status

[tPOD] ≤ [Sensitive apical endpoint]

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

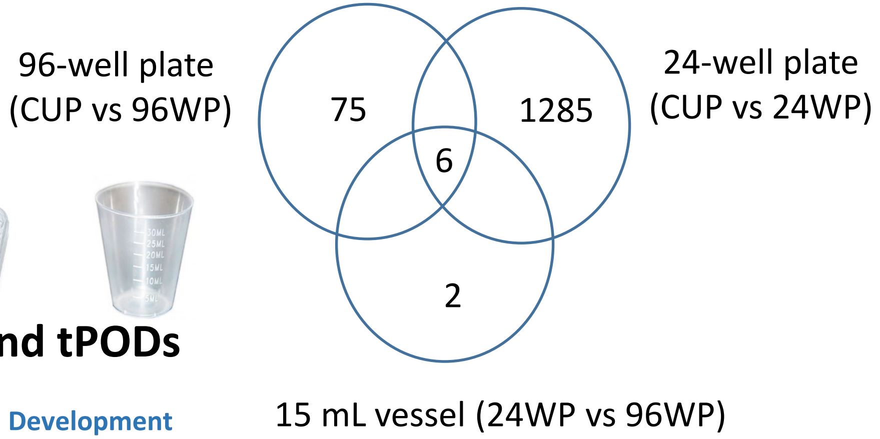
Chemical	Transcriptomic POD	96-hour LC50	Mortality-based POD
CuSO4	0.03 mg/L	0.3 mg/L	0.2 mg/L
ZnSO4	0.00023 mg/L	2.2 mg/L	3.2 mg/L
NiSO4	0.33 mg/L	6.2 mg/L	3.9 mg/L
Imidacloprid	8.8 mg/L	173 mg/L	> 10 mg/L
Flupyradifurone	1.3 mg/L	Not in ECOTOX	> 10 mg/L
Clothianidin	8.1 mg/L	0.5 (104) mg/L	> 10 mg/L
Thiacloprid	57.2 mg/L	104 mg/L	85 mg/L
Sertraline	0.6 mg/L	0.1 mg/L	0.9 mg/L
Fluoxetine	0.02 mg/L	0.2 mg/L	0.8 mg/L
Paroxetine	1.0 mg/L	3.5 mg/L	1.1 mg/L

Upcoming Work - Validation

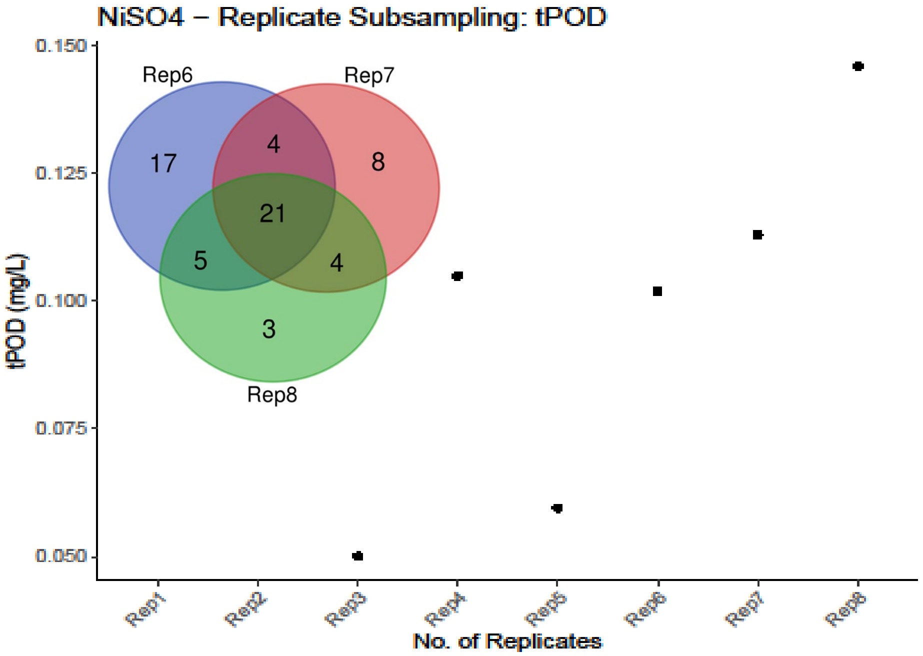
Assay Development
- Verify water quality parameters
  - dissolved oxygen
  - pH
  - ammonia
- Chemical bioavailability

Transcriptomics
- Complete Challenge
  - platform development
  - genome annotation
- Definition/Implementation of analysis pipeline
- Assess variability focused on tPODs
  - intra/inter exposure plate
  - between exposure plates
  - appropriate replication

POD Calculation for $CuSo_4$ in each Volume			
BMDExpress2 Results	Volume Format		
	CUP	24WP	96WP
#DEGs passing NTP filters[1]	128/369	52/159	108/208
Median POD (mg/L)	0.0445	0.045201	0.025



Exposure Volume and tPODs



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Contributors

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United States Environmental Protection Agency

References

- U.S. EPA Strategic Plan (2018-2022), Objective 1.4, Ensure Safety of Chemicals in the Marketplace
- Thomas, R. S., Bahadori, T., Buckley, T. J., Cowden, J., Deisenroth, C., Dionisio, K. L., ... & Williams, A. J. (2019). The next generation blueprint of computational toxicology at the US Environmental Protection Agency. Toxicological Sciences, 169(2), 317-332.
- EcoTox TARGET Challenge: https://www.challenge.gov/challenge/ecotox-challenge/
- National Toxicology Program Approach to Genomic Dose-Response Modeling: https://ntp.niehs.nih.gov/publications/reports/rr/rr05/index.html