Transcriptomics-Based Points of Departure for Fish
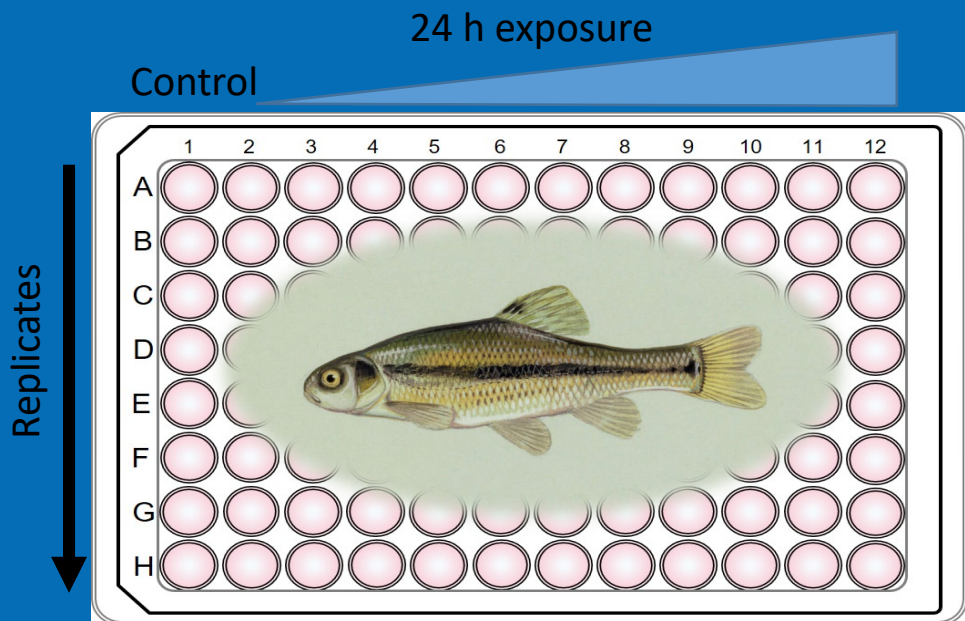
Use of In Silico Sub-Sampling to Inform High Throughput Assay Design

Monique Hazemi[1], Kevin Flynn[2], Kelsey Vitense[3], Michelle Le[1], Kendra Bush[1], Dan Villeneuve[2]
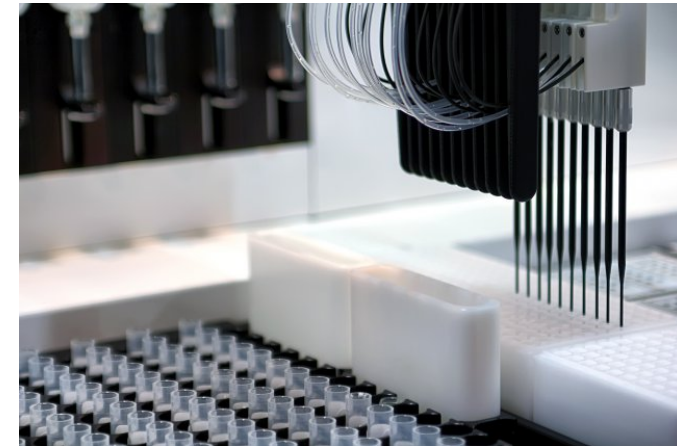
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<u>Summary</u>: *A minimum of 10,000 transcript features and 5 biological replicates in fathead minnow high throughput assays are needed to generate reliable transcriptomic-based points of departure for regulatory hazard characterization. Quantifying additional criteria is anticipated, pending more chemical data.*

Control

24 h exposure

Replicates

Image credits: Duane Raver (fish); Dan Villeneuve (plate)

This presentation neither constitutes nor necessarily reflects official US EPA policy.

Background

- Traditional animal toxicity testing is time and resource intensive

- High-throughput transcriptomics as an alternative

  - Uses gene expression profiling as an endpoint for rapidly assessing the effects of chemicals
  - Can provide potency estimates for the concentrations of chemicals that produce perturbations
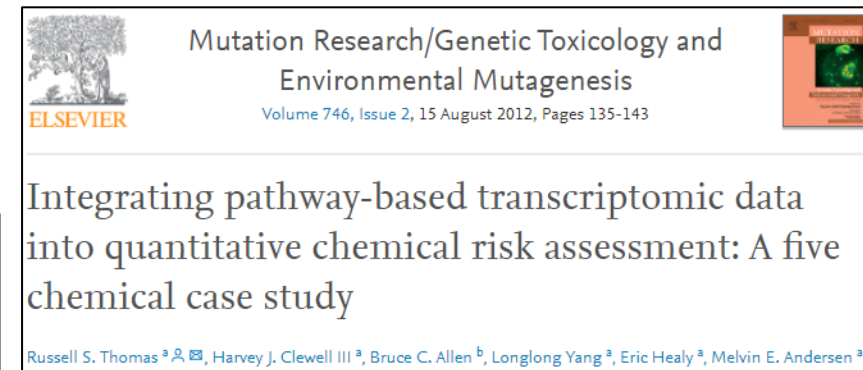
Objective

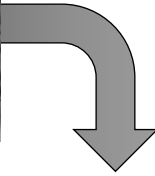- Identify high-throughput transcriptomic assay parameters that can produce reliable points of departure estimates while minimizing time and resource use

Toxicology and Applied Pharmacology
Volume 378, 1 September 2019, 114634

Transcriptomic points-of-departure from short-term exposure studies are protective of chronic effects for fish exposed to estrogenic chemicals

Florence Pagé-Larivière, Doug Crump, Jason M. O'Brien

Temporal Concordance Between Apical and Transcriptional Points of Departure for Chemical Risk Assessment FREE

Russell S. Thomas, Scott C. Wesselkamper, Nina Ching Y. Wang, Q. Jay Zhao, Dan D. Petersen, Jason C. Lambert, Ila Cote, Longlong Yang, Eric Healy, Michael B. Black, Harvey J. Clewell, III, Bruce C. Allen, Melvin E. Andersen

Toxicological Sciences, Volume 134, Issue 1, July 2013, Pages 180–194,
https://doi.org/10.1093/toxsci/kft094

Mutation Research/Genetic Toxicology and Environmental Mutagenesis
Volume 746, Issue 2, 15 August 2012, Pages 135-143

Integrating pathway-based transcriptomic data into quantitative chemical risk assessment: A five chemical case study

Russell S. Thomas[a], Harvey J. Clewell III[a], Bruce C. Allen[b], Longlong Yang[a], Eric Healy[a], Melvin E. Andersen[a]

Methods – Assay Design

Species	Age at Start	Temp	Time to Load Plate	Control 24-hr Survival	RNA Qty per Well
Pimephales promelas	24-hour	25° C	~30 minutes	24-hour	~1500 ng

24 h exposure

Control

Replicates

Exposure Design

- 1 ml deep 96-well plates
- 12 concentrations – 8 replicates per concentration
- Half-log dilution series
- 1 individual per well
- 24-hour static exposures
- Phenotypic endpoints assessed
  - Survival and behavior
- After homogenization, RNA extracted for transcriptomics

See Michelle Le's poster for more details on assay design

Image credit: Dan Villeneuve

Chemicals

Metals	Neonicotinoids	Pharmaceuticals
$CuSO_4$	Clothianidin	Fluoxetine
$NiSO_4$	Flupyradifurone*	Paroxetine
$ZnSO_4$	Imidacloprid	Sertraline
	Thiacloprid	

*Belongs to butenolide class of insecticides, but has similar mode of action to neonicotinoid insecticides

Methods – Transcriptomic Analysis

Modified from Richard et al. 2018.

Methods – *In silico* Subsampling

Sample ID

Transcript ID

Dose used in that sample

10th percentile(BMD) = tPOD

Methods – *In silico* Subsampling

LEGEND

– – – – – Transition to next replicate set

☐ Sampled data

Original expression matrix

Full dataset:
- 31,158 transcripts
- 12 doses, 8 reps per dose
- 96 samples total

Transcript(m), m=100 - 30,000 at random intervals

"Transcript(100) example"

Transcript 100 dataset:
- **100 transcripts**
- 12 doses, 8 reps per dose
- 96 samples total

Methods – *In silico* Subsampling

Variable Transcript Set Sizes: DEGs

Metals

Pharms

Neonics

Variable Replicate (12x) Sizes: DEGs

Metals

Pharms

Neonics

Variable Transcript Set Sizes: tPOD

Metals

Pharms

Neonics

Variable Replicate (12x) Sizes: tPOD

Metals

Pharms

Neonics

tPOD Variability

Copper sulfate (100-150 DEGs)

Zinc sulfate (20-60 DEGs)

Linear scale, 3-4 fold variability

Log 10 scale, ≈ 1 log variability

tPOD Variability

Paroxetine (500-600 DEGs)

Linear scale, 2-3 fold variability

Fluoxetine (25-60 DEGs)

Log 10 scale, 1-1.5 log variability

Conclusions

Minimum Assay Acceptance Criteria

• 10,000 transcript features

• 5 biological replicates

• Minimum number of differentially expressed genes **[TBD]**

• BMD distribution constraint **[TBD]**

tPODs that have reasonable, and quantifiable, levels of uncertainty is expected to aid the adoption of high-throughput transcriptomics in regulatory hazard characterization

Conclusions are preliminary and subject to change as additional data are obtained.

Contributors

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The research presented here neither constitutes nor necessarily reflects official U.S. EPA policy.

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