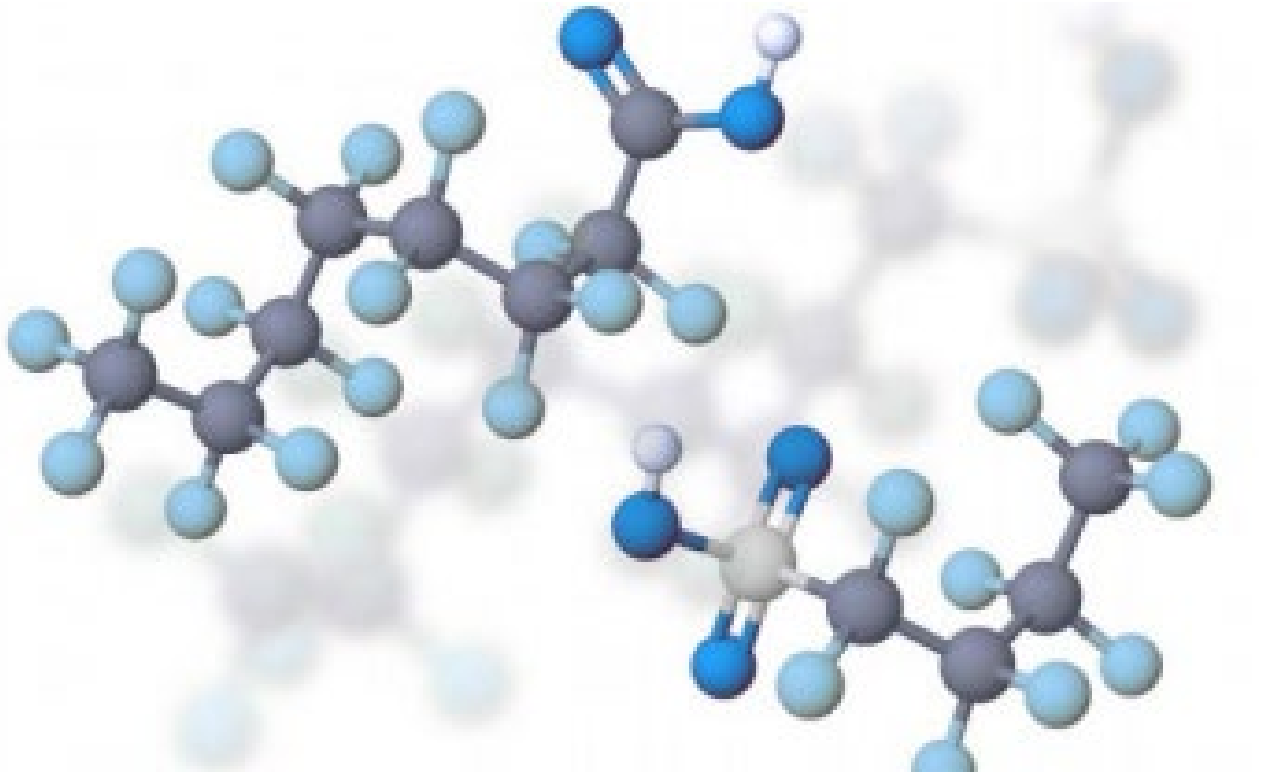
An Approach for Development of Provisional Protective Benchmarks for Ecological Effects of Data Poor PFAS



Dan Villeneuve[1], Kevin Flynn[1], Adam Biales[2]

[1]Great Lakes Toxicology and Ecology Division, Systems Toxicology Branch, Duluth, MN

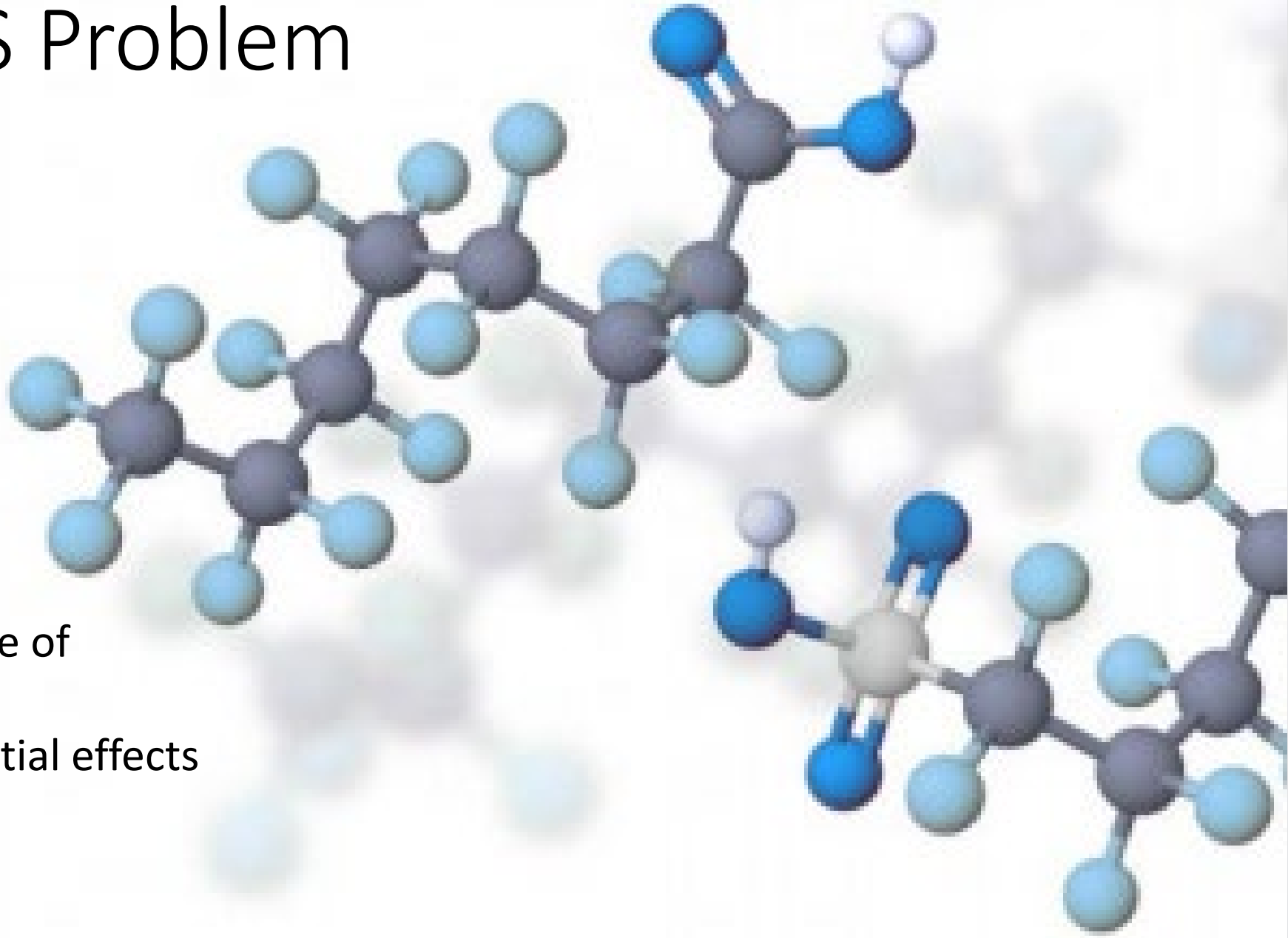
[2]Great Lakes Toxicology and Ecology Division, Molecular Indicators Branch, Cincinnati, OH

The contents of this presentation neither constitute, nor necessarily reflect US EPA policy.

PFAS Problem

1000s of structures

Relatively little toxicity data for most



Ecotoxicology

- Need to protect wide range of organisms
- Cover wide range of potential effects (lethal and sublethal)

Chemical Risk Assessment

Exposure:

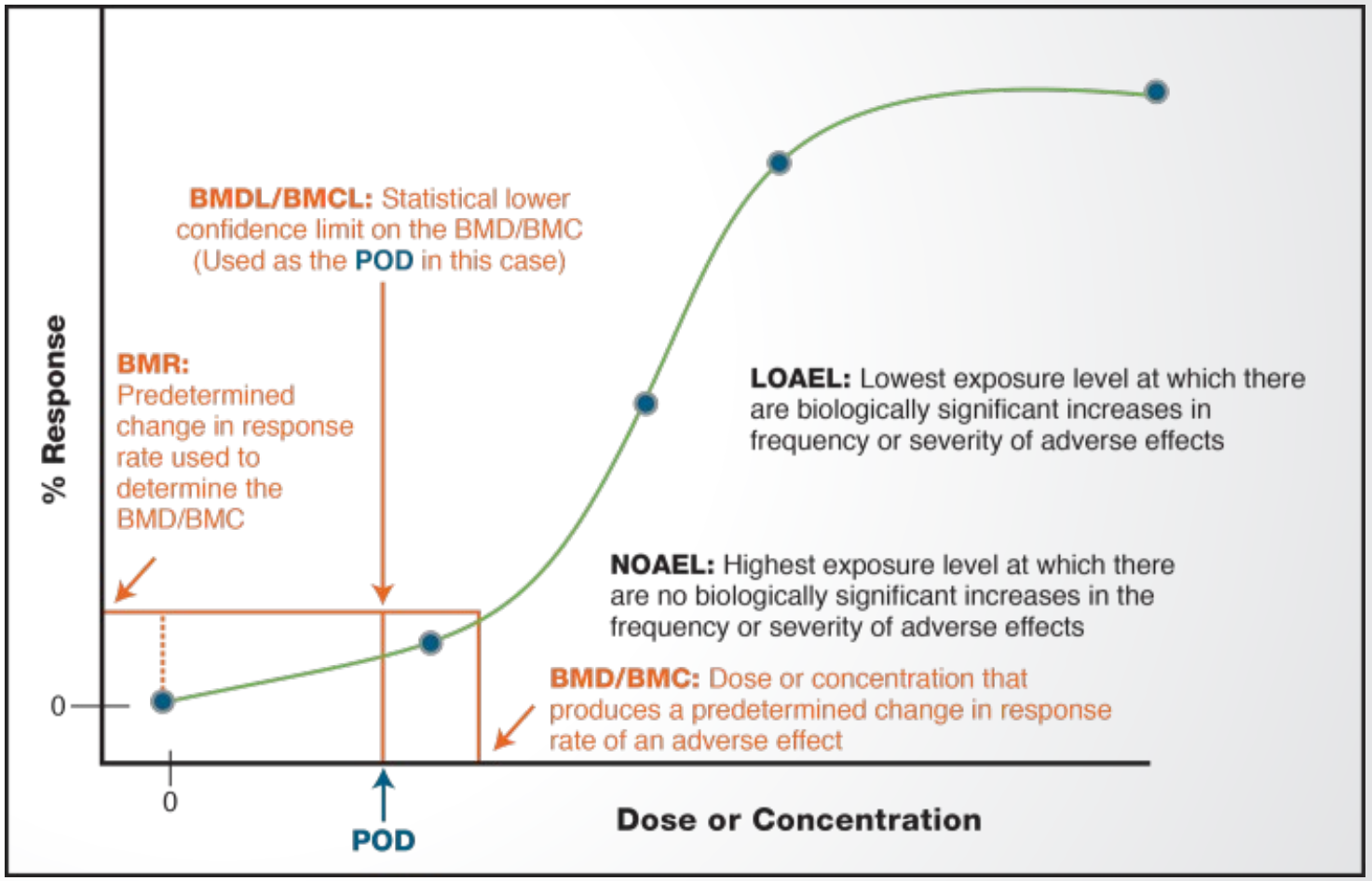
What concentrations occur in organisms or the environment?

Hazard/Effect:

What concentrations cause adverse effects to exposed organisms?

Safety:

At what concentration is there likely to be little or no hazard (adverse effects unlikely)?



Hazard/Safety Data

Toxicity Testing

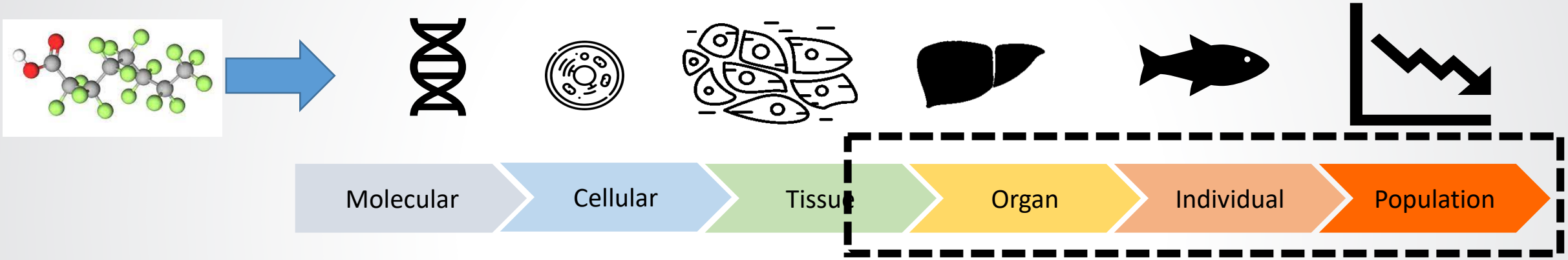
- Costly
- Time-consuming
- Animal intensive
- Lacking in mechanistic insight

Structure-based Prediction

- Requires understanding about what chemical properties/structural features are associated with toxicity.
- Understanding of mechanism(s) of toxicity relevant to different structural groups.
- Traditional models don’t work well for PFAS

Approach – NAMs

(New Approach Methodologies)



High throughput assays

- Smaller scale
- More rapid response
- Simplified systems
- Pathway coverage via batteries, multiplexing, or high content
- Dose-response more cost-effective

Direct observation of apical adverse effects

- Often slower, more latent response (especially when sub-lethal)
- Complex systems, integrate pathways
- Larger scales
- Dose response characterization is costly

Example – High Throughput Screening (Attagene)

Toxicology 457 (2021) 152789

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

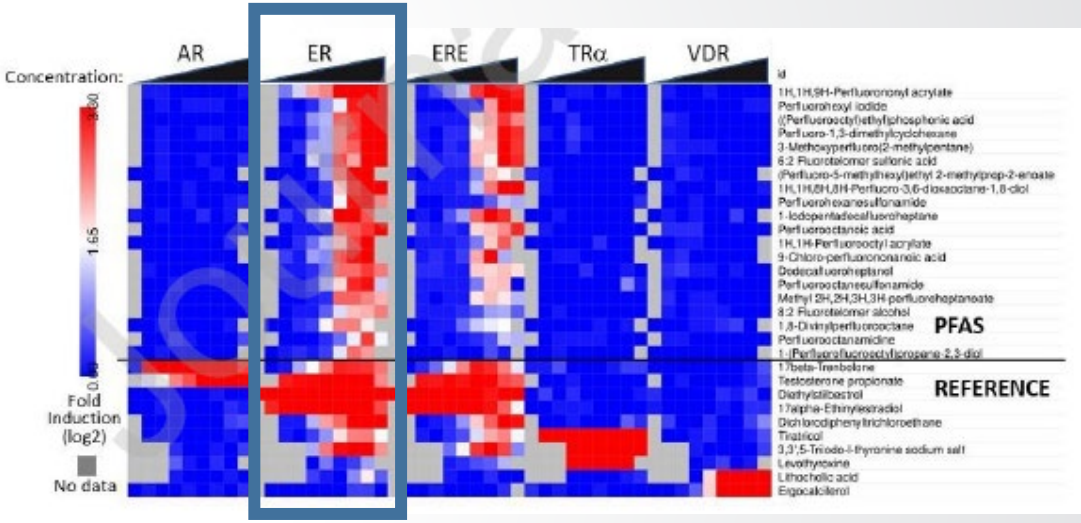
Bioactivity profiling of per- and polyfluoroalkyl substances (PFAS) identifies potential toxicity pathways related to molecular structure

Keith A. Houck [a,*], Grace Patlewicz [a], Ann M. Richard [a], Antony J. Williams [a], Mahmoud A. Shobair [a], Marci Smeltz [a], M. Scott Clifton [a], Barbara Wetmore [a], Alex Medvedev [b], Sergei Makarov [b]

[a] Center for Computational Toxicology and Exposure, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, 27711, USA

[b] Attagene, Inc., 7020 Kit Creek Rd, Morrisville, NC, 27560, USA

Fig. 3b



Houck et al. 2021. Toxicology. DOI: 10.1016/j.tox.2021.152789

- Screened 142 PFAS for activity against 25 human nuclear receptors (81 transcription factor activities overall)
- Detected multiple PFAS that activate the human estrogen receptor (ER)

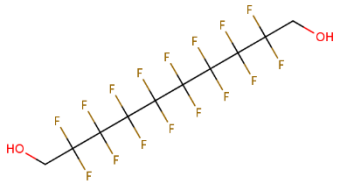
- In fish, activation of ER is associated with reproductive and developmental toxicity

In vivo verification - approach

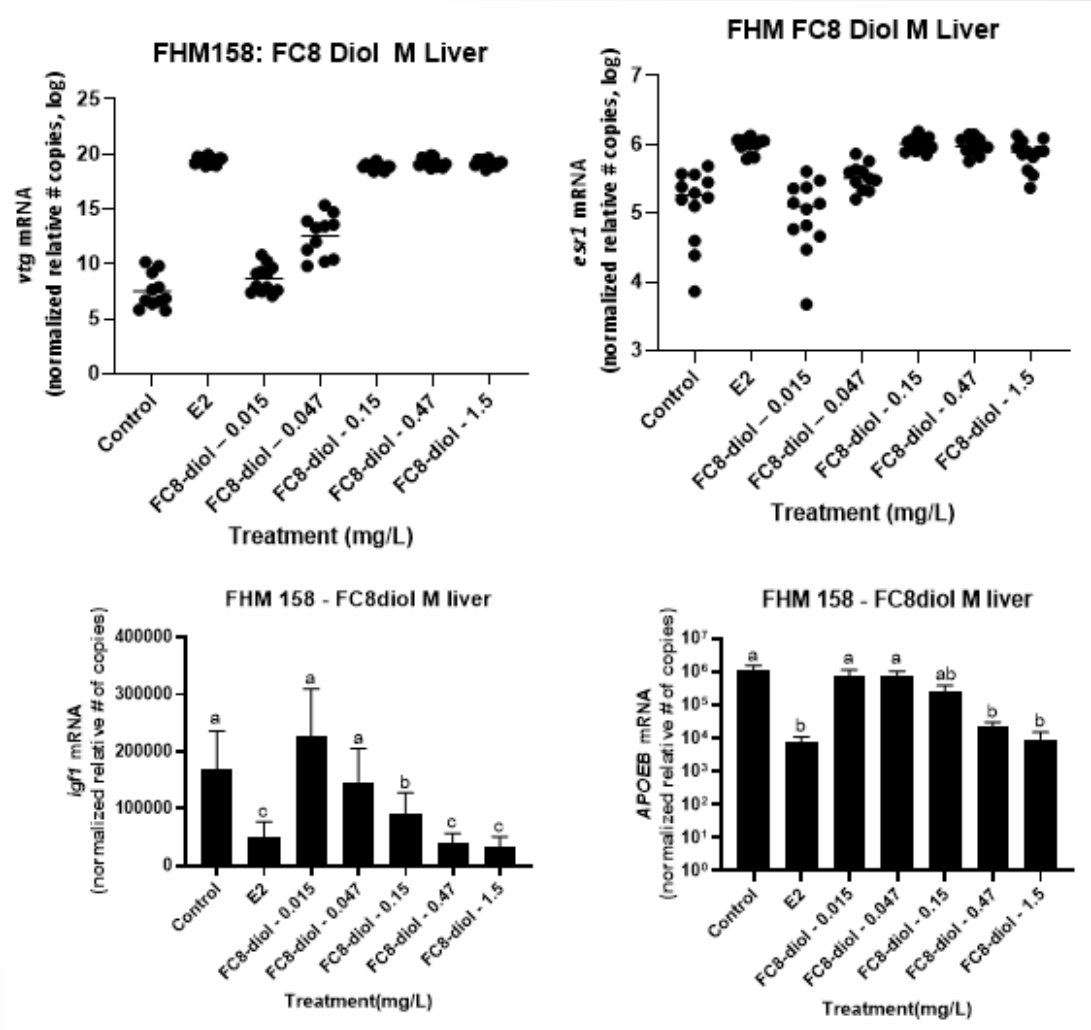
- Five in vivo experiments
  - Four ER-active PFAS of varying potency
  - One ER-negative PFAS (in progress)
- Adult male fathead minnows exposed to PFAS for 96 h
  - Includes E2 positive control
- Gene expression (QPCR)
  - Four orthogonal ER-regulated genes
  - Two – expected up-regulation
  - Two – expected down-regulation

In vivo verification - results

Preliminary Results

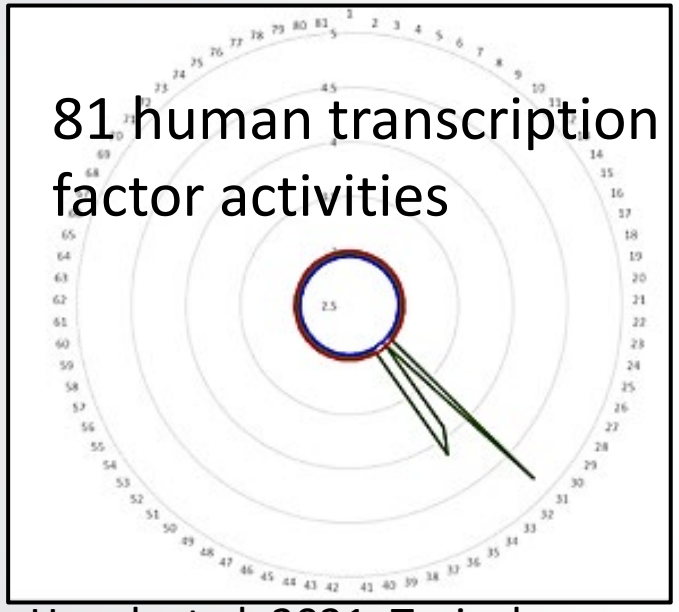


- Activity in molecular screening assays was effective in predicting longer-term in vivo responses.
- Observed in vivo gene expression responses consistent with fish estrogen receptor activation
- In vivo potency consistent with rank-order of in vitro potency
- In vivo effect concentrations are very high (e.g., ≥ 150 µg/L)



Results shown for 1H,1H,8H,8H-Perfluorooctane-1,8-diol; DTXSID 30396867

Limitations



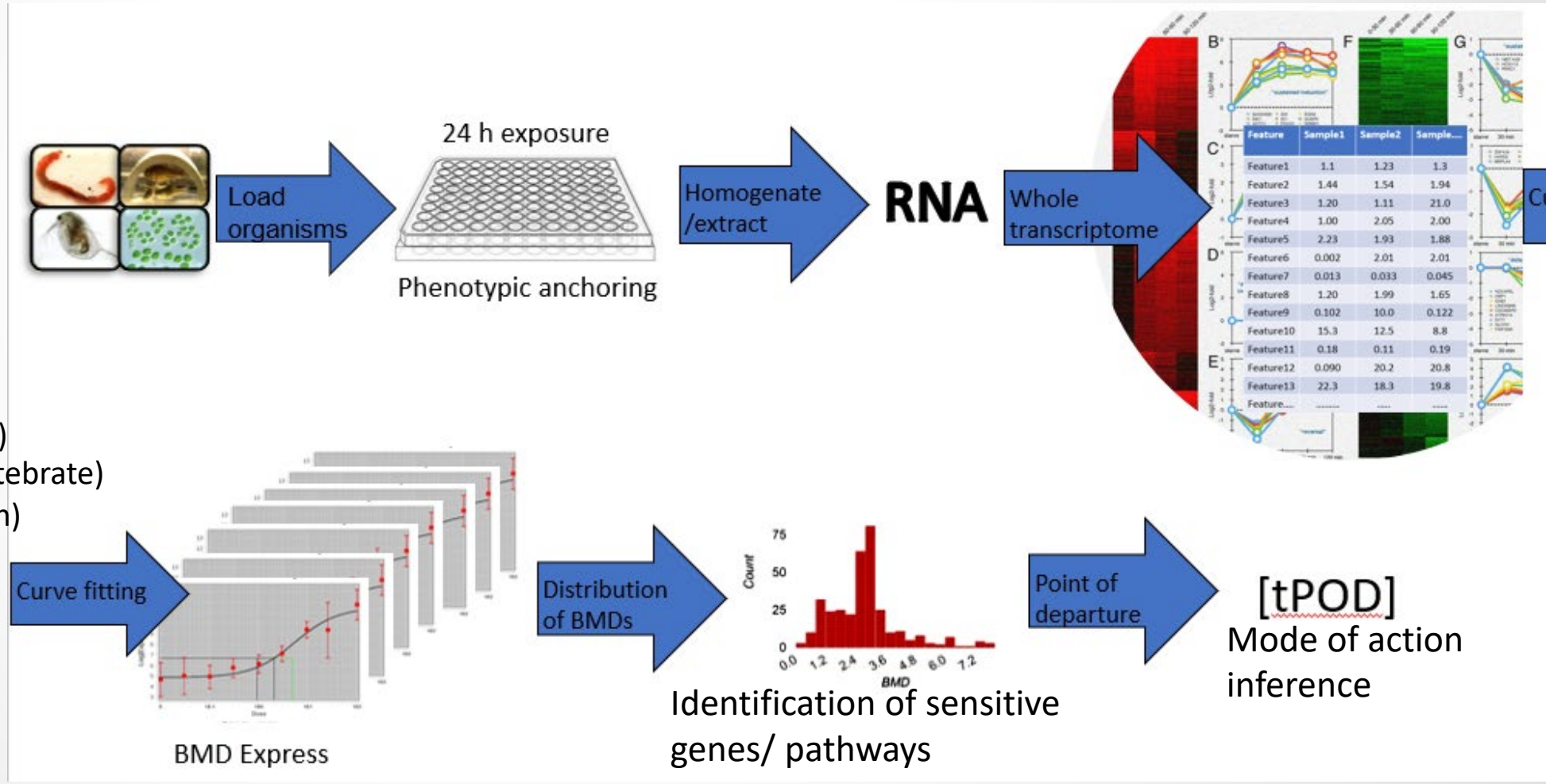
Houck et al. 2021. Toxicology.
DOI: 10.1016/j.tox.2021.152789

- Adequate to screen for certain hazards
- Not adequate to address “safety”
- Only captures a small fraction of pathways relevant to the human biology (genome)
- Only covers pathways/physiology conserved with humans

Approach – Eco-HTTr

(Ecological High Throughput Transcriptomics)

- Rapid (24 h)
- Small scale
  - 96 well plates
  - 700 µl/well
- Whole genome coverage
- Three major trophic levels
  - Primary producer (algae)
  - Primary consumer (invertebrate)
  - Secondary consumer (fish)
- Diversity of physiology



Scientific Foundation for approach

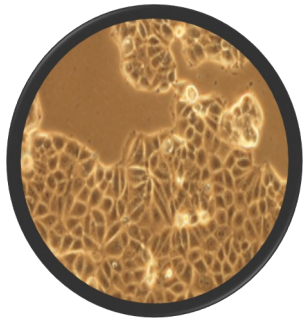
NTP
National Toxicology Program
U.S. Department of Health and Human Services

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.
- Health protective points of departure.



Whole human transcriptome

OXFORD SOT Society of Toxicology
academic.oup.com/toxsci

TOXICOLOGICAL SCIENCES, 181(1), 2021, 68–89

doi: 10.1093/toxsci/kfab009
Advance Access Publication Date: 4 February 2021
Research Article

High-Throughput Transcriptomics Platform for Screening Environmental Chemicals

Joshua A. Harrill,*,1 Logan J. Everett,* Derik E. Haggard,*,† Thomas Sheffield,*,† Joseph L. Bundy,* Clinton M. Willis,*,‡ Russell S. Thomas,* Imran Shah,* and Richard S. Judson*

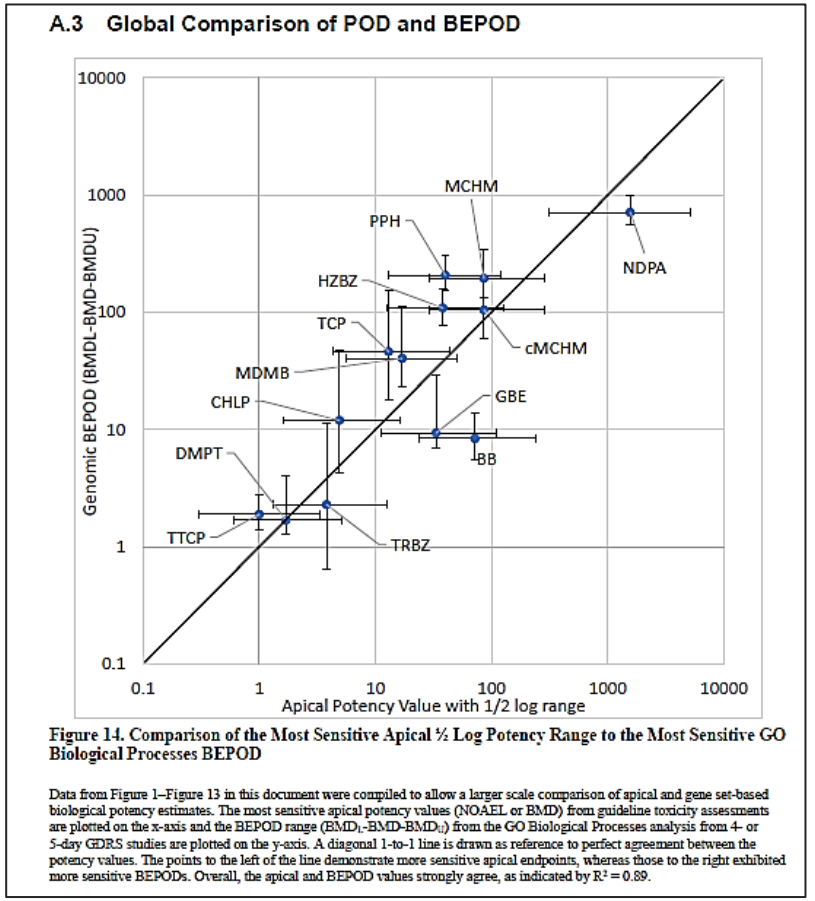
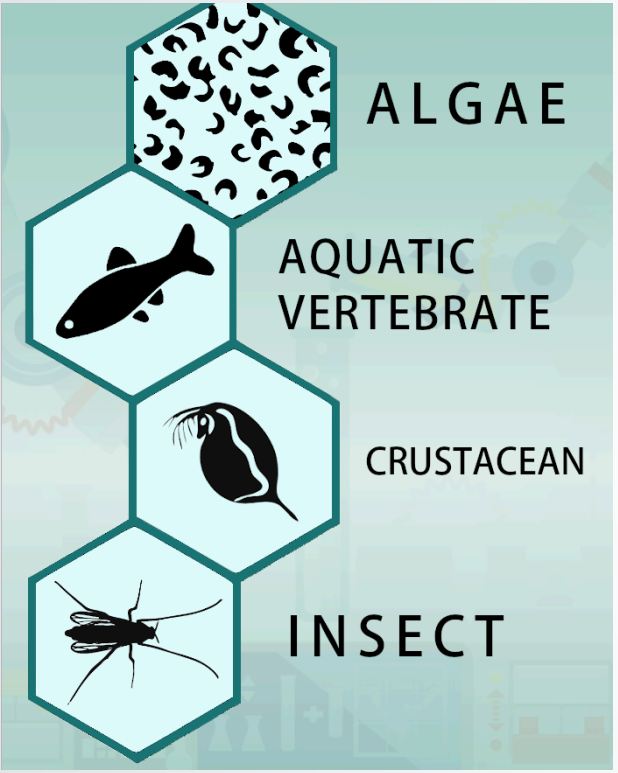


Figure 14. Comparison of the Most Sensitive Apical ½ Log Potency Range to the Most Sensitive GO Biological Processes BEPOD

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 $R^2 = 0.89$.

Toxicological Sciences, Volume 181, Issue 1, May 2021, Pages 68–89, https://doi.org/10.1093/toxsci/kfab009

Eco-HTTr Research at EPA



Assay Optimization

- How many replicate wells (animals)?
- How much genome coverage?
- Assay acceptance criteria?

} Reliable point of departure [tPOD] with defined uncertainty range

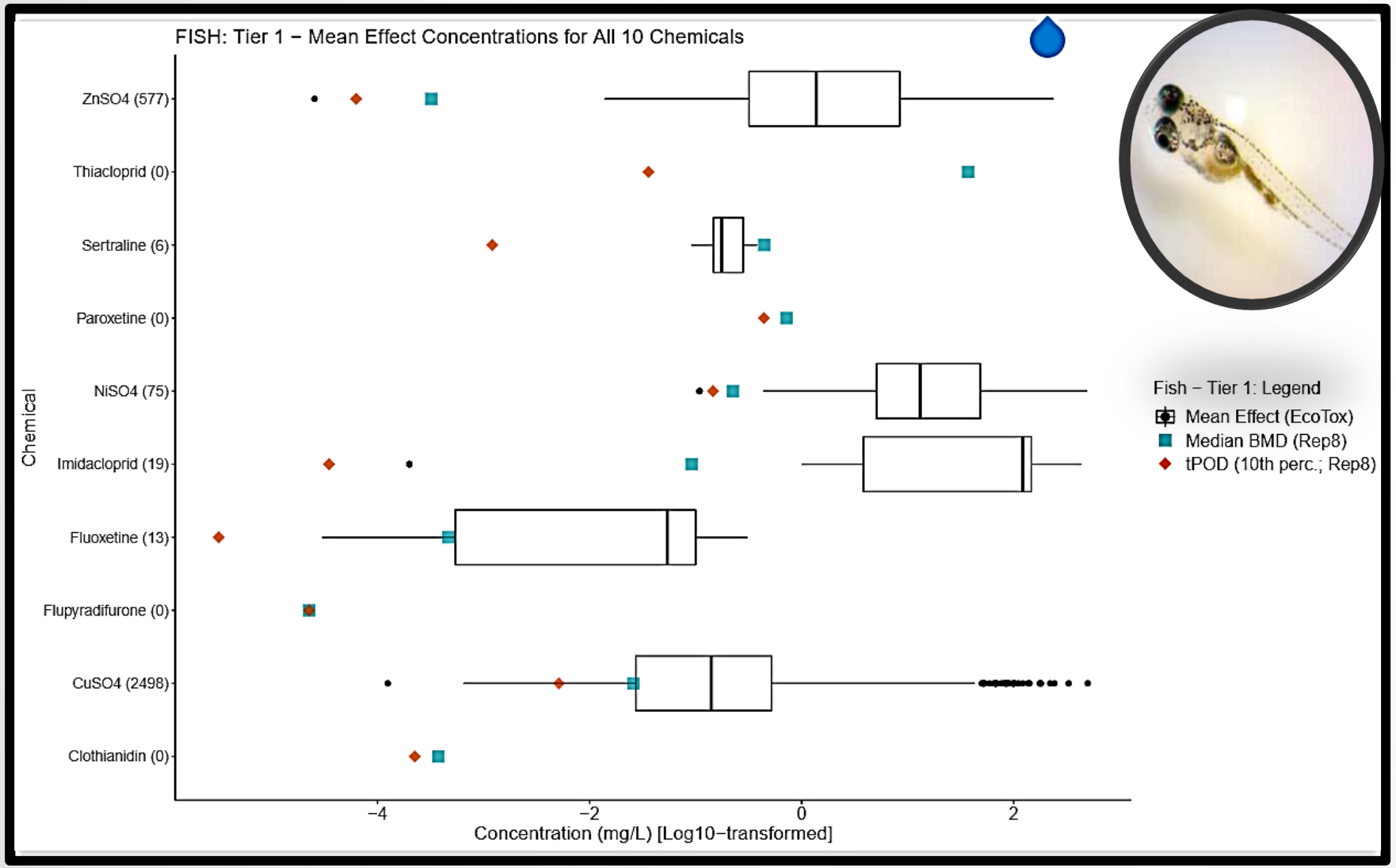
Assay Evaluation

[tPOD] ≤ [Most sensitive chronic endpoint]
Effective provisional, protective value

[tPOD] <<< [Most sensitive chronic endpoint]
Overly conservative

[tPOD] > [Most sensitive chronic endpoint]
Not protective

Results – Assay Evaluation



Approach appears promising

tPODs were generally more sensitive than apical adverse effect concentrations.

In some cases 2 orders of magnitude more protective

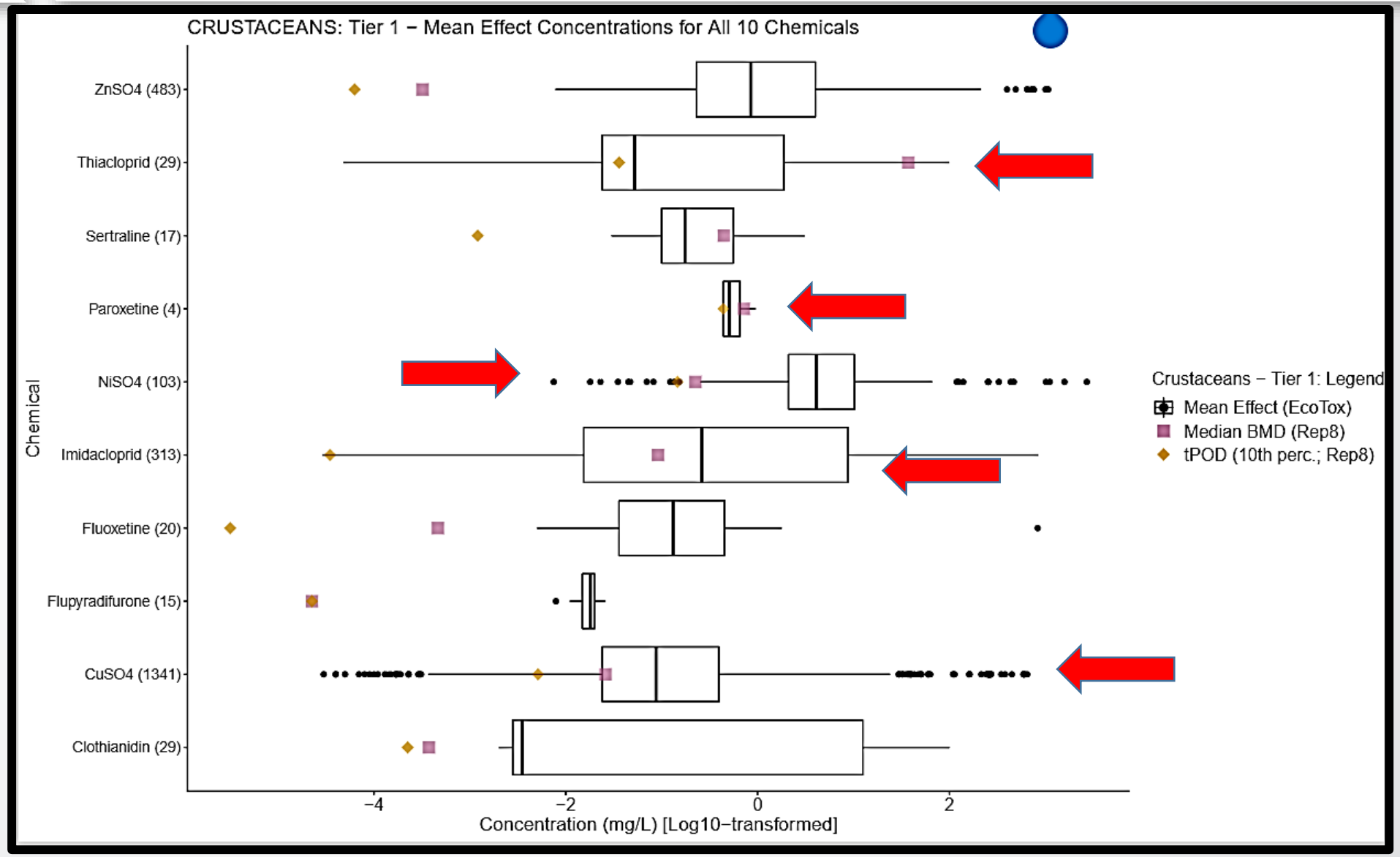
In the process of testing more "data rich" chemicals to evaluate the approach

- >20 for four species by end of FY22

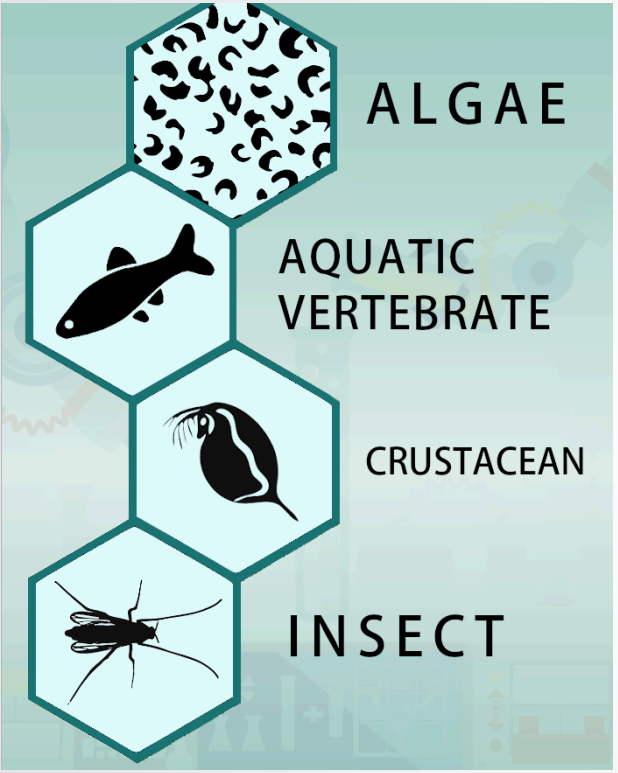
Results – Assay Evaluation

Including representative from diverse taxonomic groups appear necessary

Fish-based tPODs are not protective of all aquatic organisms



PFAS: Eco-HTTr Research at EPA



- Generate transcriptomics-based PODs for 12-20 PFAS; ≥ 3 species including an invertebrate and algae
- Compare Eco-tPODs with PODs derived from other mammalian NAMs (e.g., tPODs for mammalian cell lines, rodent-derived tPODs, ToxCast-based PODs, etc.)
- If algae, invertebrates, or fish appear more sensitive to select PFAS:
  - Identify the pathways that respond most sensitively (mode of action inference)

EPA Program Interests

Office*	Interest
OLEM	PODs for setting clean-up targets at contaminated sites
R10	Benchmarks for biological evaluations supporting consultations under the Endangered Species Act
R5, GLNPO	Benchmarks for understanding risks of contaminants to Great Lakes fish and wildlife
OW	Provisional protective values as guidance until more stringent criteria derivation is feasible.
OCSPP	Data to support chemical grouping of PFAS and development of predictive models of PFAS toxicity

OLEM: Office of Land and Emergency Management; R10: Region 10; R5: Region 5; GLNPO: Great Lakes National Program Office; OW: Office of Water; OCSPP: Office of Chemical Safety and Pollution Prevention

**Program interests outlined in this table neither constitute, nor necessarily reflect policy of the US EPA or program offices listed.*

How will this research help you?

Near term

- Ecological effect benchmarks for 20 PFAS (mostly data poor structures)
- Concentrations below which we do not expect adverse effects of PFAS exposure
- Increased understanding of most sensitive species
- Improved understanding of what pathways/targets PFAS may interact with at the molecular level

Longer Term

- New approach methods for ecological toxicity testing that can be applied to other data poor compounds (e.g., 6ppd-quinone)
- Lower bound toxicity estimates (tPODs) for additional PFAS structures
- Identification of structures that are associated with greatest biological potency/activity
- Improved understanding to aid the development of structure-based predictive models

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- Kevin Flynn
- Adam Biales
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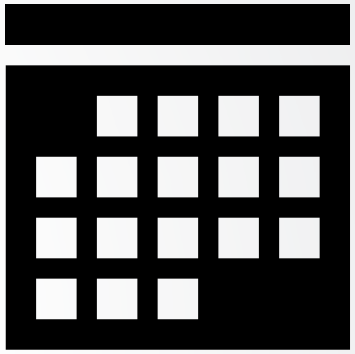
- Kathy Jensen
- Jenna Cavallin
- David Murphy
- Brett Blackwell
- Michelle Le
- Kendra Bush
- Kelvin Santana Rodriguez
- Mackenzie Morshead
- John Hoang

Estimated Time-Line

- **Summer 2021 – Additional Optimization**
  - Phenotypic anchors
    - Survival
    - Malformations
    - Heart-rate
    - Behaviors / movement
    - Photosynthetic pigments
  - Analytical verification workflows (measured rather than nominal conc.)



- **Fall 2021 – High throughput Exposures**

- **Winter 2021 – RNA sequencing and data analysis**

- **Spring 2022 - Results**

Chemical Selection

Considerations:

1. Overlap with CCTE 150 PFAS
2. Overlap with In vivo rodent tPODs
3. Positive for bioactivity in the Attagene assays; range of different activities and potencies.
4. Integration with other CSS/CCTE research objectives.
5. Detection in Great Lakes
6. Stability and DMSO solubility notes
7. Feedback from OLEM and OW

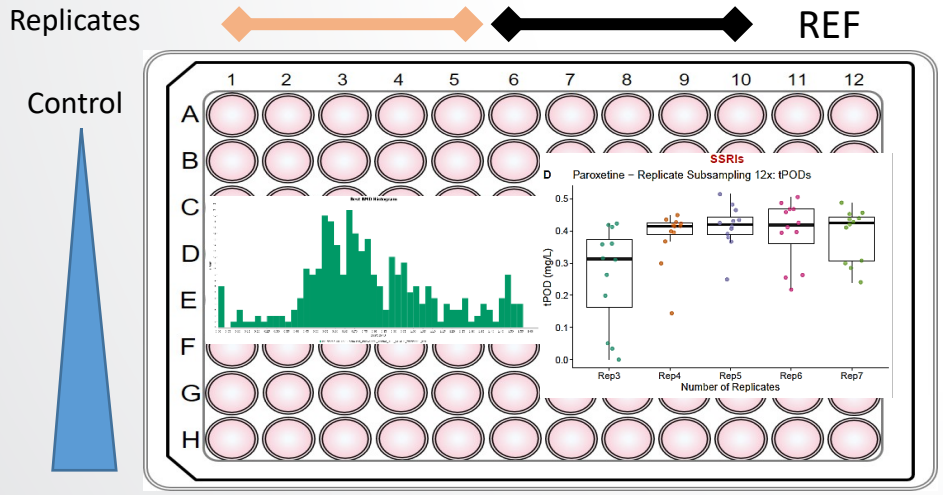
DTXSID	Substance_Name
DTXSID8037706	Potassium perfluorooctanesulfonate
DTXSID8031865	Perfluorooctanoic acid
DTXSID70381090	1H,1H,8H,8H-Perfluoro-3,6-dioxaoctane-1,8-diol
DTXSID3037709	Potassium perfluorohexanesulfonate
DTXSID00190950	6:1 Fluorotelomer alcohol
DTXSID50469320	Perfluorohexanesulfonamide
DTXSID70276659	Perfluoro-(2,5,8-trimethyl-3,6,9-trioxadodecanoic)acid
DTXSID70191136	Perfluoro-3-methoxypropanoic acid
DTXSID60663110	Perfluoro-4-isopropoxybutanoic acid
DTXSID1032646	N-Ethylperfluorooctanesulfonamide
DTXSID70379295	3H-Perfluoro-2,2,4,4-tetrahydroxypentane
DTXSID30891564	4:2 Fluorotelomer sulfonic acid
DTXSID6067331	6:2 Fluorotelomer sulfonic acid
DTXSID00192353	8:2 Fluorotelomer sulfonic acid
DTXSID3059921	Perfluorotetradecanoic acid
DTXSID8047553	Perfluoroundecanoic acid
DTXSID90868151	Perfluorotridecanoic acid
DTXSID8031863	Perfluorononanoic acid
DTXSID1037303	Perfluroheptanoic acid
DTXSID6062599	Perfluoropentanoic acid

Results - Assay Optimization

Pilot assays: 12 concentrations, 8 reps per conc., whole genome

Used in silico sub-sampling approach to optimize design and estimate uncertainty

- Minimum gene set size ≈ 10,000
- Minimum biological replication n=4; include n=5 to allow in silico sub-sampling
  - In silico sub-sampling facilitates approximation of the variability/uncertainty in the tPOD
- Minimum number of Differentially Expressed Genes as an assay acceptance criteria



Conceptual illustration – actual layout will be randomized

Revised Design:

- n= 5 biological replicates
- n= 8 concentrations
- Reference samples included on each plate