Utility of New Approach Methodologies in Deriving Points-of-Departure

Katie Paul Friedman

November 17, 2020

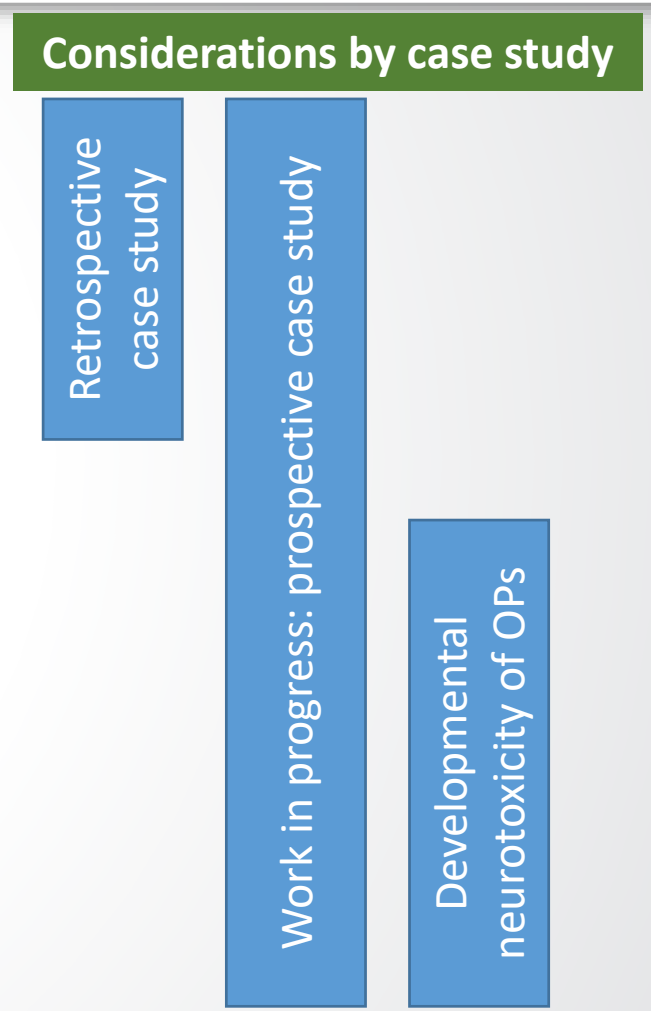
Presented to the OECD Working Party on Hazard Assessment

Critical questions for NAMs in safety assessment

- Background
- Part 1: A retrospective case study with the Accelerating the Pace of Chemical Risk Assessment (APCRA)
  - Can NAMs for hazard and toxicokinetics be used to derive a point-of-departure (POD) that is protective of traditional PODs?
  - Can NAMs for hazard, toxicokinetics, and exposure be used to prioritize substances for further consideration?
- Part 2: Work-in-progress for a prospective case study with APCRA using NAMs as they develop in real-time
  - How can NAMs for hazard from Tier 1 (broad-based NAMs) and Tier 2 (targeted high-throughput screening) be combined with toxicokinetics and exposure for prioritization of substances?
- Part 3: Application of hazard-specific NAMs to specific questions about the potential developmental neurotoxicity
  - Can NAMs that recapitulate important aspects of developmental neurobiology be applied for specific hazard and risk questions?

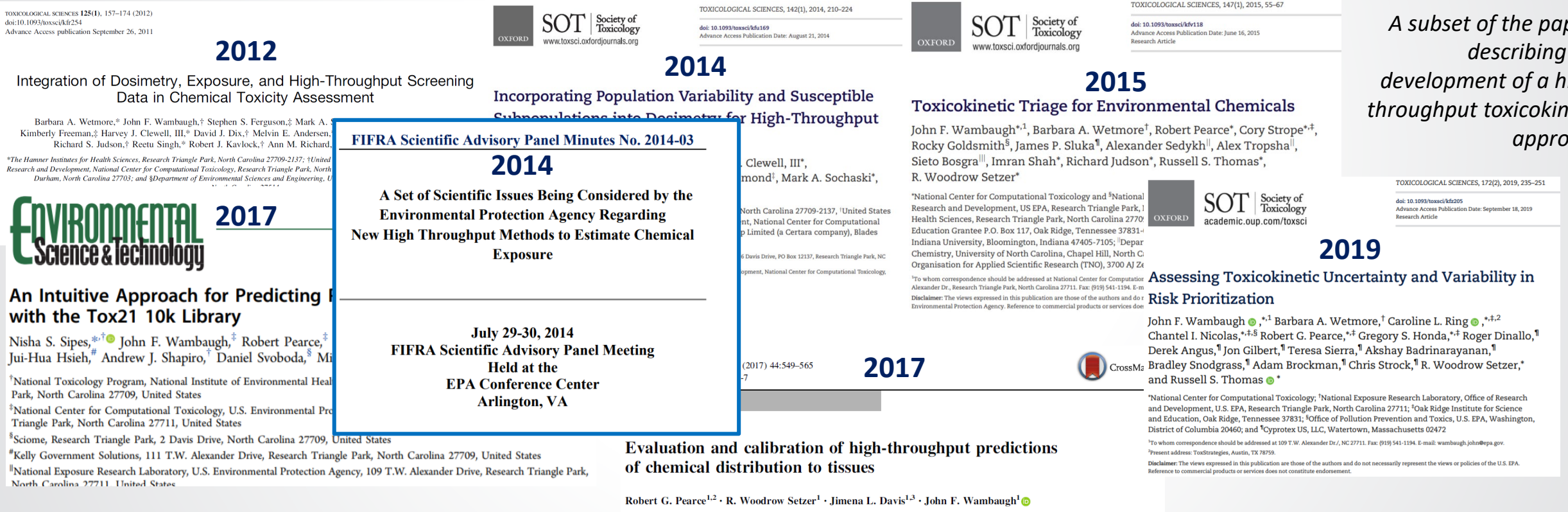
Fit-for-purpose considerations for NAMs in derivation of PODs

- Is the task one that is risk-informed?
  - *Use of a threshold for any bioactivity may be useful.*
  - *Mimics identification of animal-based POD, i.e., a threshold dose at which no effects are anticipated in the animal models employed.*
- Is the task one where specific hazards need to be considered?
  - *Identification of NAMs that are fit-for-purpose regarding the specific hazard may be needed. (how much uncertainty can be tolerated?)*
  - *Consideration of how to identify "selective" bioactivity from specific NAMs, i.e. a "lead" bioactivity that precedes other bioactivity types.*



Derivation of PODs from NAMs: IVIVE that employs toxicokinetic extrapolation of dose

High-throughput toxicokinetic (HTTK) approaches make it possible to predict doses corresponding to *in vitro* bioactivity for thousands of chemicals.



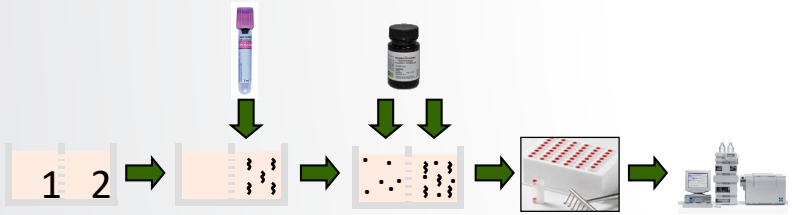
A subset of the papers describing the development of a high-throughput toxicokinetic approach

Reverse dosimetry can be leveraged in IVIVE to estimate the exposure that would produce the plasma concentration corresponding to bioactivity

High throughput toxicokinetics (HTTK)

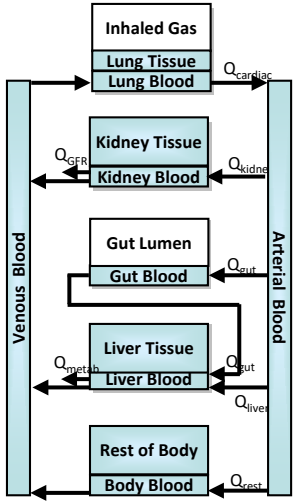
in vitro data

Hepatic clearance from suspended hepatocytes



Plasma protein binding

Generic toxicokinetic models



= *httk*

Some high-level assumptions:

(1) bioactive nominal *in vitro* assay concentration ~ *in vivo* plasma concentration that would correspond to a similar effect;

(2) external exposures (in mg/kg/day units) that may have resulted in that plasma concentration can be constructed using estimates of species-specific physiology and Phase I and Phase II enzyme-driven hepatic clearance; and,

(3) Often, we expect that plasma concentration can be approximated by steady-state kinetics (unless we have enough information to use other dose metrics).

Led by John Wambaugh, Barbara Wetmore, and colleagues

Many works apply HTTK to prioritization and assessment case studies

Chemical Research in Toxicology — PERSPECTIVE — pubs.acs.org/crt — **2011**

Estimating Toxicity-Related Biological Pathway Altering Doses for High-Throughput Chemical Risk Assessment

Richard S. Judson,*,† Robert J. Kavlock,† R. Woodrow Setzer,† Elaine A. Cohen Hubal,† Matthew T. Martin,† Thomas B. Knudsen,† Keith A. Houck,† Russell S. Thomas,‡ Barbara A. Wetmore,‡ and David J. Dix†

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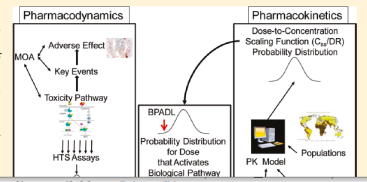
‡The Hamner Institutes for Health Sciences, Research Triangle Park, North Carolina 27709, United States

ABSTRACT: We describe a framework for estimating the human dose at which a chemical significantly alters a biological pathway *in vivo*, making use of *in vitro* assay data and an *in vitro*-derived pharmacokinetic model, coupled with estimates of population variability and uncertainty. The quantity we calculate, the biological pathway altering dose (BPAD), is analogous to current risk assessment metrics in that it combines dose−response data with analysis of uncertainty and population variability to arrive at conservative exposure limits. The analogy is closest when perturbation of a pathway is a key event in the mode of action (MOA) leading to a specified adverse outcome.



OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org — **2015**

TOXICOLOGICAL SCIENCES, 148(1), 2015, 121–136

doi: 10.1093/toxsci/kfv171
Advance Access Publication Date: August 6, 2015
Research Article

Incorporating High-Throughput Exposure Predictions With Dosimetry-Adjusted *In Vitro* Bioactivity to Inform Chemical Toxicity Testing

Barbara A. Wetmore,*,1 John F. Wambaugh,† Brittany Allen,* Stephen S. Ferguson,†,2 Mark A. Sochaski,* R. Woodrow Setzer,† Keith A. Houck,† Cory L. Strope,* Katherine Cantwell,* Richard S. Judson,† Edward LeCluyse,* Harvey J. Clewell,* Russell S. Thomas,*,†,3 and Melvin E. Andersen*

*The Hamner Institutes for Health Sciences, Institute for Chemical Safety Sciences, Research Triangle Park, North Carolina 27709-2137; †United States Environmental Protection Agency, Office of Research and Development, National Center for Computational Toxicology, Research Triangle Park, North Carolina 27711; and ‡Life Technologies, ADME/Tox Division of the Primary and Stem Cell Systems Business Unit, Durham, North Carolina 27703

Toxicology in Vitro 47 (2018) 213–227

Contents lists available at ScienceDirect

Toxicology in Vitro

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

ELSEVIER — **2018**

Review

In vitro to in vivo extrapolation for high throughput prioritization and decision making

Shannon M. Bell[a], Xiaoqing Chang[a], John F. Wambaugh[b], David G. Allen[a], Mike Bartels[c,1], Kim L.R. Brouwer[d], Warren M. Casey[e], Neepa Choksi[a], Stephen S. Ferguson[f], Grazyna Fraczkiewicz[g], Annie M. Jarabek[b], Alice Ke[h], Annie Lumen[i], Scott G. Lynn[j], Alicia Paini[k], Paul S. Price[b], Caroline Ring[l,2], Ted W. Simon[m], Nisha S. Sipes[f], Catherine S. Sprankle[a], Judy Strickland[a], John Troutman[n], Barbara A. Wetmore[o,3], Nicole C. Kleinstreuer[e,*]

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

ELSEVIER — **2019**

Profiling 58 compounds including cosmetic-relevant chemicals using ToxRefDB and ToxCast

Ly L. Pham[a,b], Lisa Truong[a,b,c], Gladys Ouedraogo[d], Sophie Loisel-Joubert[e], Matthew T. Martin[a,f], Katie Paul Friedman[a,*]

[a] National Cent
[b] ORISE Postdo
[c] Currently at C
[d] L'Oréal Safety
[e] L'Oréal Safety
[f] Currently at G

Environment International 137 (2020) 105470

Contents lists available at ScienceDirect

Environment International

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

ELSEVIER — **2020**

High-throughput screening tools facilitate calculation of a combined exposure-bioactivity index for chemicals with endocrine activity

Susanna H. Wegner[a,b,*], Caroline L. Pinto[a,b], Caroline L. Ring[a,c], John F. Wambaugh[c]

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OXFORD SOT Society of Toxicology academic.oup.com/toxsci — **2019**

TOXICOLOGICAL SCIENCES, 2019, 1–24

doi: 10.1093/toxsci/kfz201
Advance Access Publication Date: September 18, 2019
Research Article

Utility of *In Vitro* Bioactivity as a Lower Bound Estimate of *In Vivo* Adverse Effect Levels and in Risk-Based Prioritization

Katie Paul Friedman ,*,1 Matthew Gagne,† Lit-Hsin Loo,‡ Panagiotis Karamertzanis,§ Tatiana Netzeva,§ Tomasz Sobanski,§ Jill A. Francoeur,¶ Ann M. Richa: Angrish, Bahadori Rasenbe

PLOS ONE — **2020**

Toxicology and Applied Pharmacology 387 (2020) 114774

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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

ELSEVIER — **2020**

The role of fit-for-purpose assays within tiered testing approaches: A case study evaluating prioritized estrogen-active compounds in an in vitro human uterotrophic assay

Tyler Beames[a,*,1], Marjory Moreau[a,1], L. Avery Roberts[b], Kamel Mansouri[b], Saad Haider[a], Marci Smeltz[a], Chantel I. Nicolas[b], Daniel Doheny[b], Martin B. Phillips[a], Miyoung Yoon[b,2], Richard A. Becker[c], Patrick D. McMullen[a], Melvin E. Andersen[a], Rebecca A. Clewell[b,3], Jessica K. Hartman[a,*]

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[c] American Chemistry Council (ACC), Washington, DC 20002, USA

RESEARCH ARTICLE

Using the concordance of *in vitro* and *in vivo* data to evaluate extrapolation assumptions

Gregory S. Honda[1,2], Robert G. Pearce[1,2], Ly L. Pham[1,2], R. W. Setzer[1], Barbara A. Wetmore[3], Nisha S. Sipes[4], Jon Gilbert[5], Briana Franz[5], Russell S. Thomas[1], John F. Wambaugh[1]*

1 National Center for Computational Toxicology, U.S. EPA, Research Triangle Park, North Carolina, United States of America, 2 Oak Ridge Institute for Science and Education, Oak Ridge, Tennessee, United States of America, 3 National Exposure Research Laboratory, U.S. EPA, Research Triangle Park, North Carolina, United States of America, 4 Division of the National Toxicology Program, NIEHS, Research Triangle Park, North Carolina, United States of America, 5 Cyprotex, Watertown, MA, United States of America

A subset of the papers describing the application of a high-throughput toxicokinetic approach – too many to fit

Part 1: A retrospective case study with the Accelerating the Pace of Chemical Risk Assessment (APCRA)

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

TOXICOLOGICAL SCIENCES, 2019, 1–24

doi: 10.1093/toxsci/kfz201
Advance Access Publication Date: September 18, 2019
Research Article

Utility of *In Vitro* Bioactivity as a Lower Bound Estimate of *In Vivo* Adverse Effect Levels and in Risk-Based Prioritization

Katie Paul Friedman ,*,[1] Matthew Gagne,[†] Lit-Hsin Loo,[‡] Panagiotis Karamertzanis,[§] Tatiana Netzeva,[§] Tomasz Sobanski,[§] Jill A. Franzosa,[¶] Ann M. Richard,[*] Ryan R. Lougee,*,[||] Andrea Gissi,[§] Jia-Ying Joey Lee,[‡] Michelle Angrish,[|||] Jean Lou Dorne,[||||] Stiven Foster,[#] Kathleen Raffaele,[#] Tina Bahadori,[||] Maureen R. Gwinn,[*] Jason Lambert,[*] Maurice Whelan,[**] Mike Rasenberg,[§] Tara Barton-Maclaren,[†] and Russell S. Thomas *

(APCRA partners for these two case studies)

Why is the retrospective case study important?

- Clear need to demonstrate in practical terms, for as many chemicals as possible, how preliminary screening level risk assessment using a new approach methodologies (NAM) based approach would perform when compared to traditional approaches to deriving points-of-departure (PODs).
- Illustrate the current state-of-the-science.
- Evaluate the specific strengths and weaknesses of rapid, screening level risk assessment using NAMs.
- Approach: Take a retrospective look at the traditional and NAM data for as many chemicals as possible (448 at the time).

See the forest for the trees

The big question:

Can *in vitro* bioactivity be used to derive a conservative point-of-departure (POD) for prioritization and screening level risk assessment?

Case study workflow

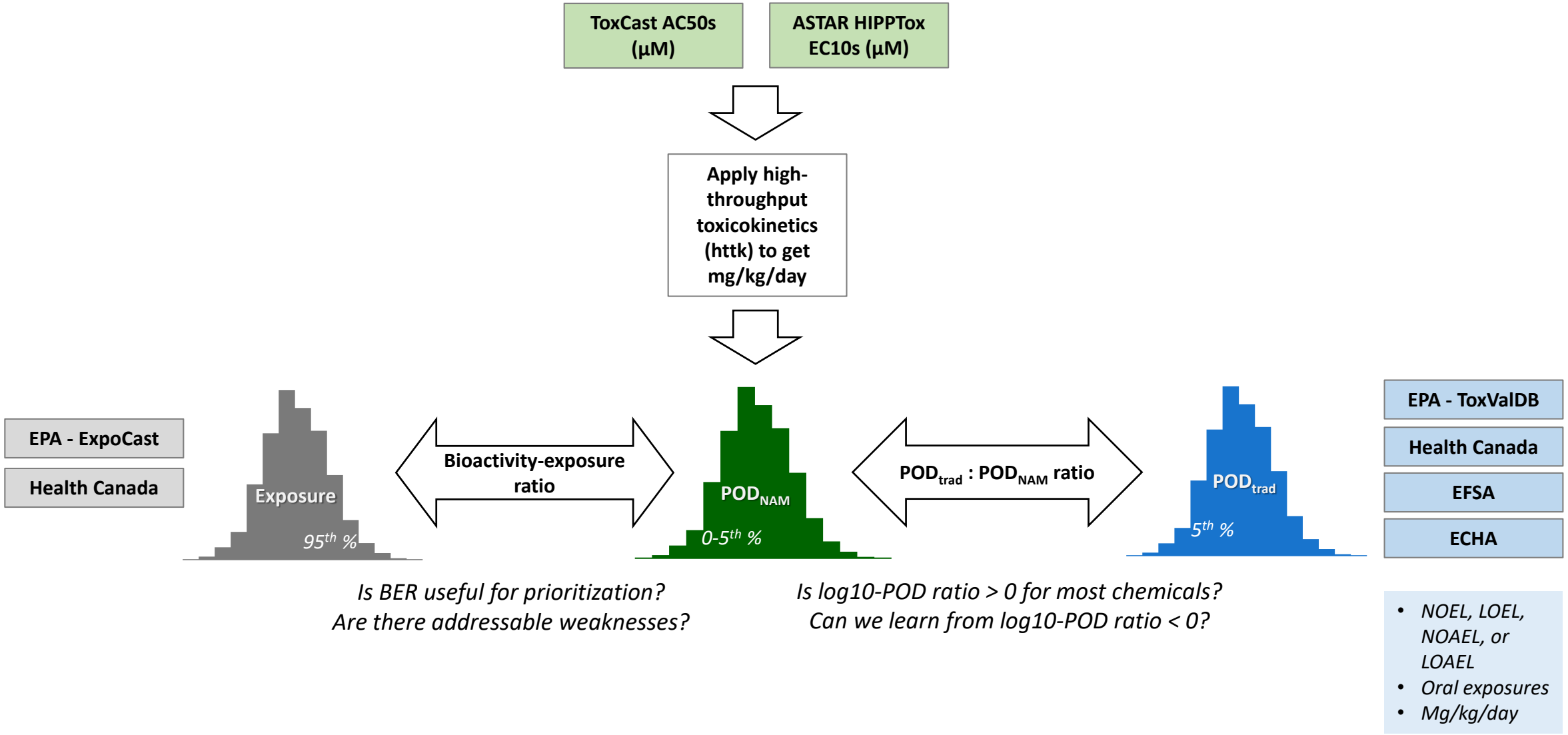
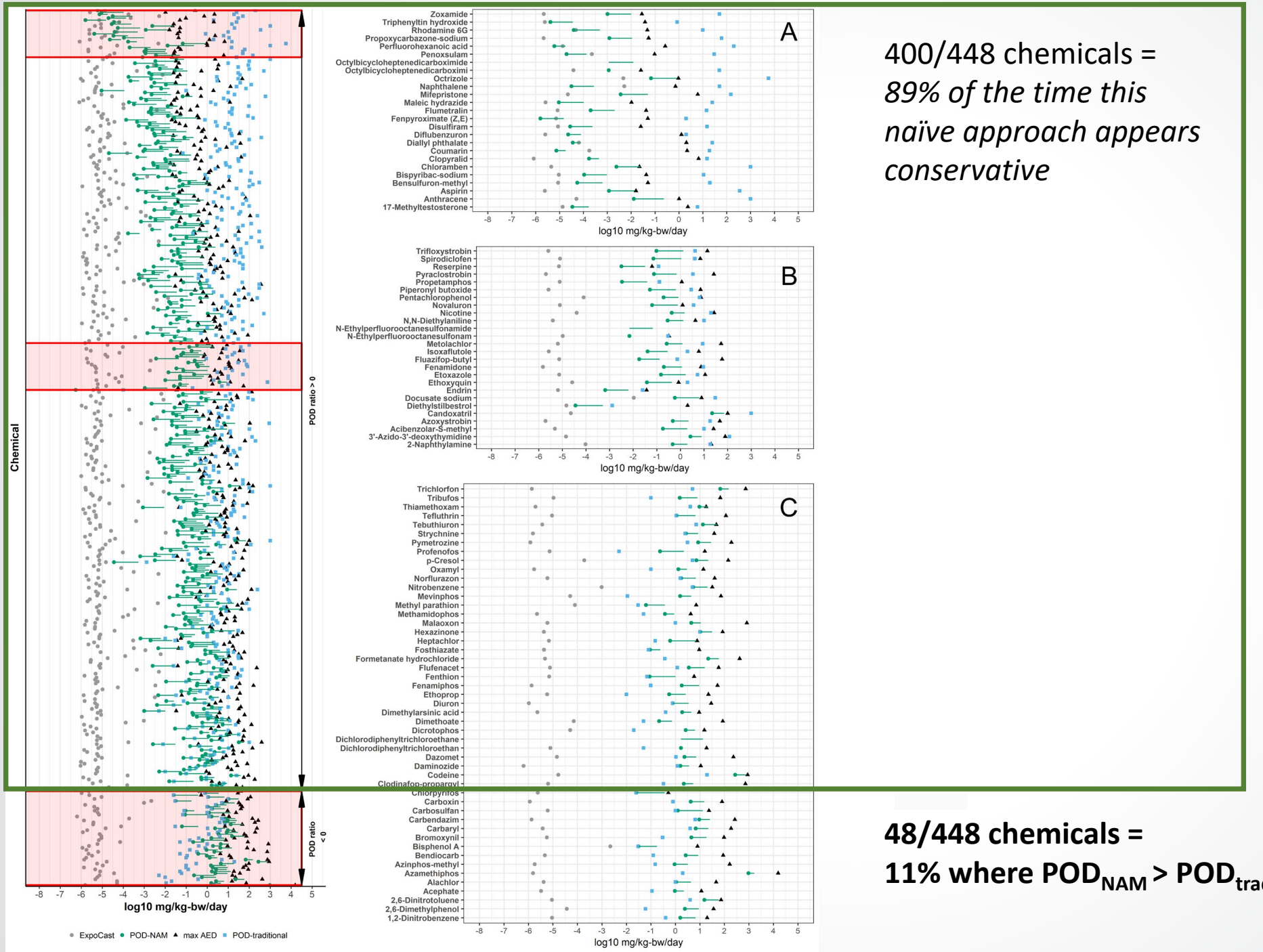


Figure 1, Paul Friedman et al. *2019*



400/448 chemicals =
89% of the time this naïve approach appears conservative

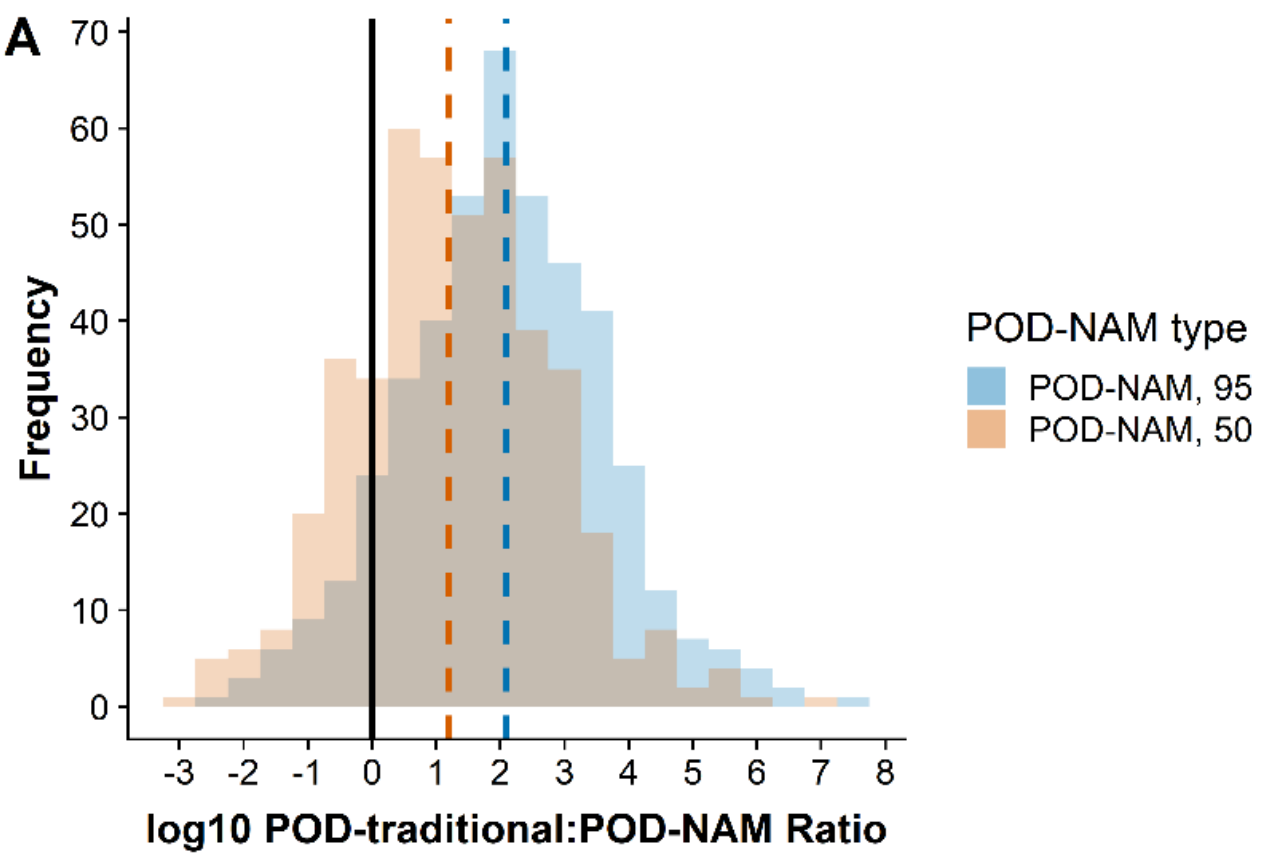
48/448 chemicals =
11% where $POD_{NAM} > POD_{traditional}$

$POD_{NAM} < POD_{traditional}$

(most of the time)

Figure 3, Paul Friedman et al. *2019*

The log10-POD ratio distribution shows POD_{NAM} is generally conservative *and adjustable.*

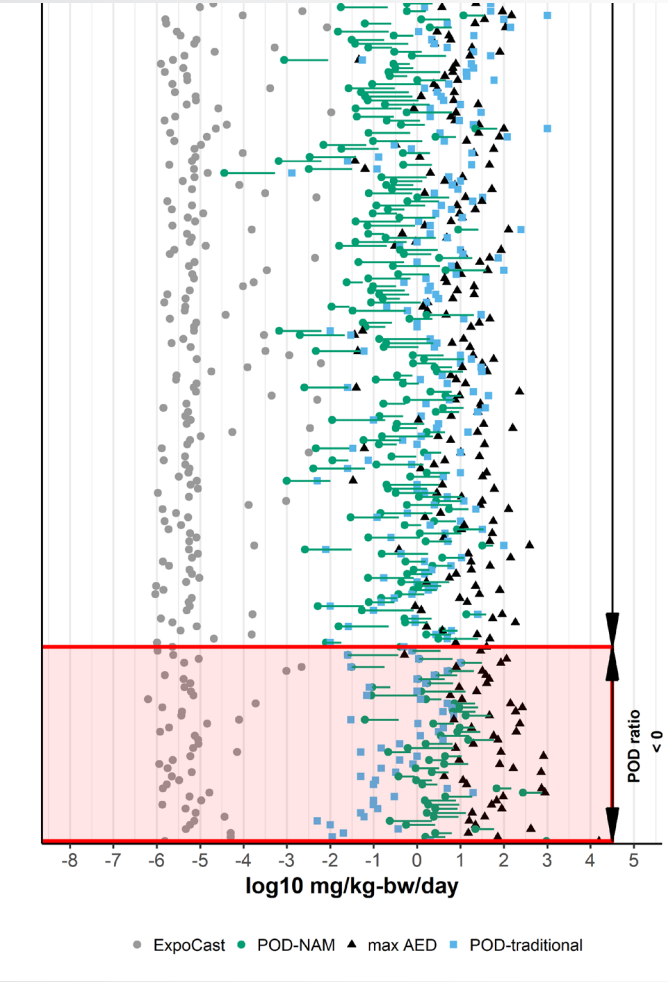


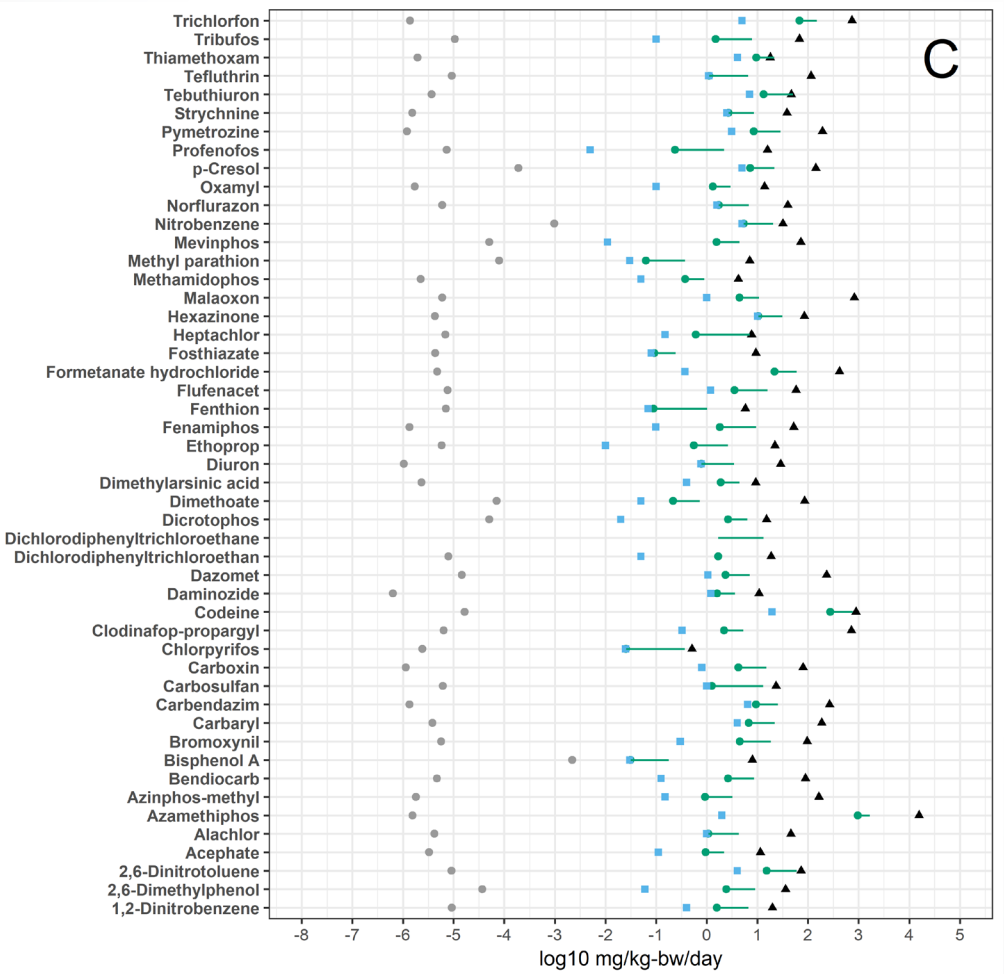
$POD_{NAM,95}$ includes interindividual variability in the in vitro to in vivo extrapolation process to a greater extent and is more often a conservative estimate of $POD_{traditional}$.

This should trigger thinking regarding uncertainty and uncertainty factors/safety factors. In the NAM-based process, we have quantitatively informed uncertainty that can be included explicitly at multiple steps in the screening assessment process.

- log_{10}POD ratio is illustrated for the $POD_{NAM,95}$ and the $POD_{NAM,\ 50}$.
- Using the more conservative (i.e., lower) $POD_{NAM,95}$, 48 of the 448 substances (10.7%) demonstrated a log_{10}POD ratio < 0 (to the left of the solid vertical line), whereas 92 of the 448 substances (20.5%) demonstrated a log10-POD ratio < 0 using the $POD_{NAM,50}$.
- The medians of the log10-POD ratio distributions are indicated by dashed lines for $POD_{NAM,\ 95}$ and $POD_{NAM,\ 50}$ as 2 and 1.2, respectively.

Are there key drivers of examples where POD ratio ≤ 0?





POD_{NAM} : $POD_{traditional}$ ≤ 0

- Are some *in vivo* toxicity types poorly captured by ToxCast?
- Are some study types enriched in this space, and difficult to predict from bioactivity?

When the log_{10}POD ratio < 0, was it driven by a specific study type (as a surrogate for phenotypes)?

Condition	Dev/Repro is min POD	Dev/Repro is not min POD
log10-POD ratio,95 < 0	3	45
log10-POD ratio,95 > 0	41	359

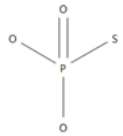
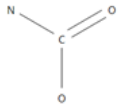
Condition	Chronic is min POD	Chronic is not min POD
log10-POD ratio,95 < 0	28	20
log10-POD ratio,95 > 0	244	156

- No.
- *Based on a Fisher's exact test, when log_{10}POD ratio <0. it was not driven by a specific study type.*

Hypothesis	Fisher's exact test results	Caveats
Reproductive and/or developmental studies over-represented when POD ratio ≤ 0?	• No • p-value = 0.98; • odds-ratio = 0.26	Some ambiguity or error expected in assigning study classes; preference given to: DNT, neuro, dev/repro, acute, repeat, chronic (in that order) in the event of a min POD tie
Carcinogenicity or chronic studies over-represented when POD ratio ≤ 0?	• No • p-value = 0.25; • odds-ratio=1.4	

When the log_{10}POD ratio < 0, was it driven by a specific chemical features?

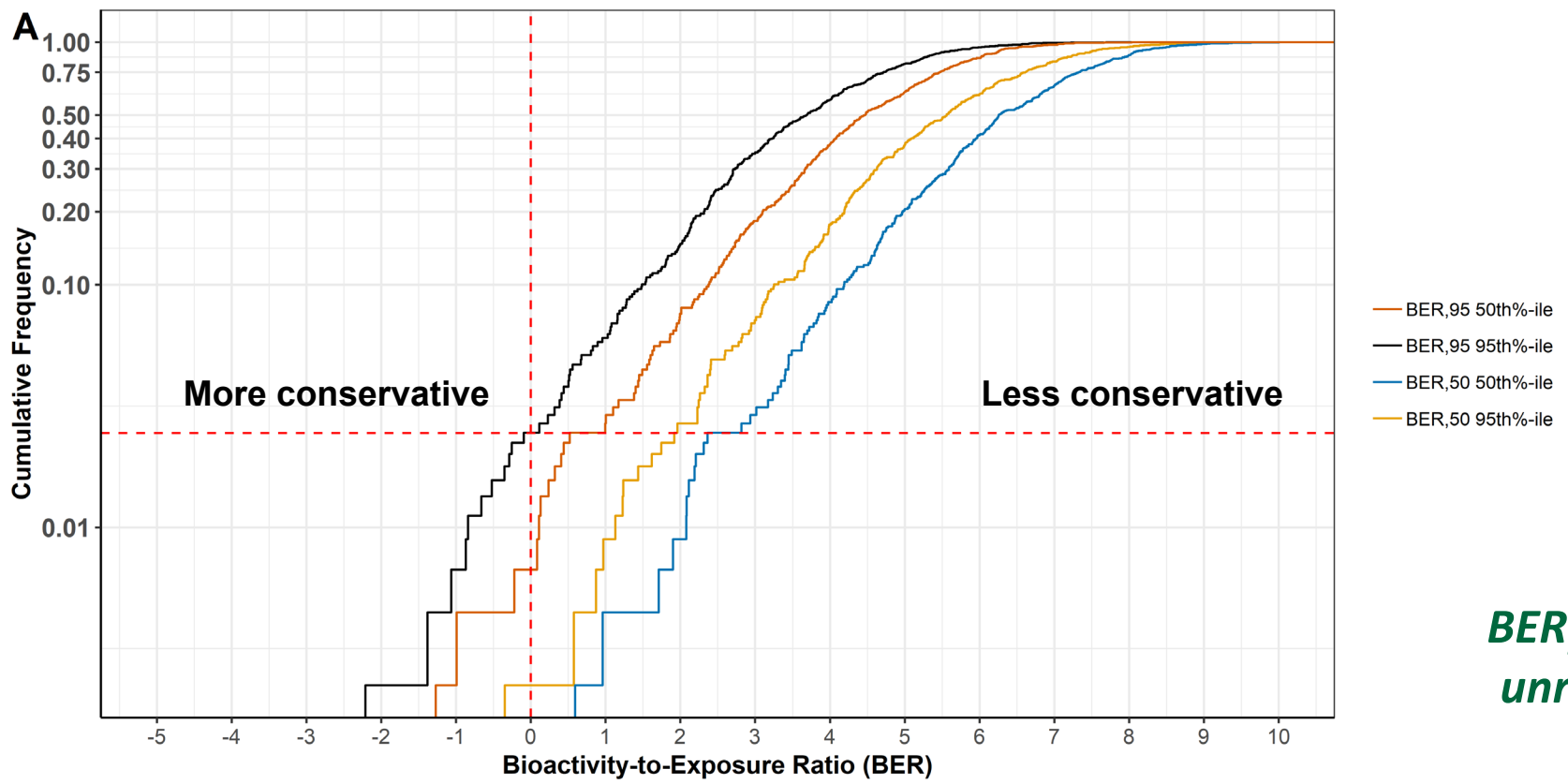
- Yes
- *Based on a Fisher's exact test, chemical features associated with organophosphate pesticides and carbamates are more likely to drive a log_{10}POD ratio < 0.*

ChemoType Information		Appearance of the ToxPrint			Metrics			ChemoType Information		Appearance of the ToxPrint			Metrics		
Label	ToxPrint	Total	POD ratio ≤ 0	POD ratio > 0	BA	OR	p-value	Label	ToxPrint	Total	POD ratio ≤ 0	POD ratio > 0	BA	OR	p-value
bond:P=O_ phosphorus_oxo		18	12	6	0.62	22	7.4E-09	bond:P~N_ generic		5	4	1	0.54	36	0.00055
bond:P=O_ phosphate_thio		3	3	0	0.53	NA	0.0012	bond:C(=O)N_ carbamate		20	6	14	0.54	3.9	0.014
bond:P~S_ generic		27	13	14	0.62	10	3.5E-7	bond:CS_sulfide		53	15	38	0.61	4.3	0.00011

using the ChemoType Enrichment beta workflow,
Ann Richard and Ryan Lougee, EPA-ORD-NCCT

So, we have a sense that a NAM-based POD can be protective of an *in vivo* POD, especially in concert with structure-based strategies like threshold of toxicological concern (TTC). How would prioritization work?

The bioactivity:exposure ratio (BER) provides a way of prioritizing substances for further review.



- Make choices based on tolerable uncertainty (i.e., based on use case).
- BER_{95} used 95th percentile from the credible interval to predict median total US population exposure (ExpoCast SEEM2);BER_{50} the 50th percentile.
- BER_{95} and BER_{50} values were calculated as the "95th%-ile" and "50th%-ile," using the $POD_{NAM,95}$ and $POD_{NAM,50}$, respectively.

BER_{95} , 95th percentile did not prioritize an unreasonable number of substances; the BER selected reflects the level of conservatism and uncertainty considered within a screening assessment.

Conclusions and limitations

- An approach to using *in vitro* bioactivity data as a POD appears to be a conservative estimate ~ 90% of the time for 448 chemicals.
- POD_{NAM} estimates appear conservative with a margin of ~100-fold.
- POD_{NAM} may provide a refinement of a TTC approach.
- When combined with high-throughput exposure estimates, this approach provides a reasonable basis for risk-based prioritization and screening level risk assessments.

- Specific types of chemicals may be currently outside the domain of applicability due to assay limitations, e.g., organophosphate insecticides: how do we identify these in the future?
- This is the largest retrospective look at this to-date; but what if new chemicals perform differently? What will be the prospective approach?
- Additional research to include expanded and improved high-throughput toxicokinetics and *in vitro* disposition kinetics may help improve POD_{NAM} estimates.

Part 2: Work-in-progress for a prospective case study with APCRA using NAMs as they develop in real-time

(APCRA partners)

The following work is in progress and unpublished

NAMs available for hazard (and toxicokinetics and exposure) are evolving rapidly

Thomas et al. 2019 further evolves a tiered screening strategy that adds in broader biological coverage.

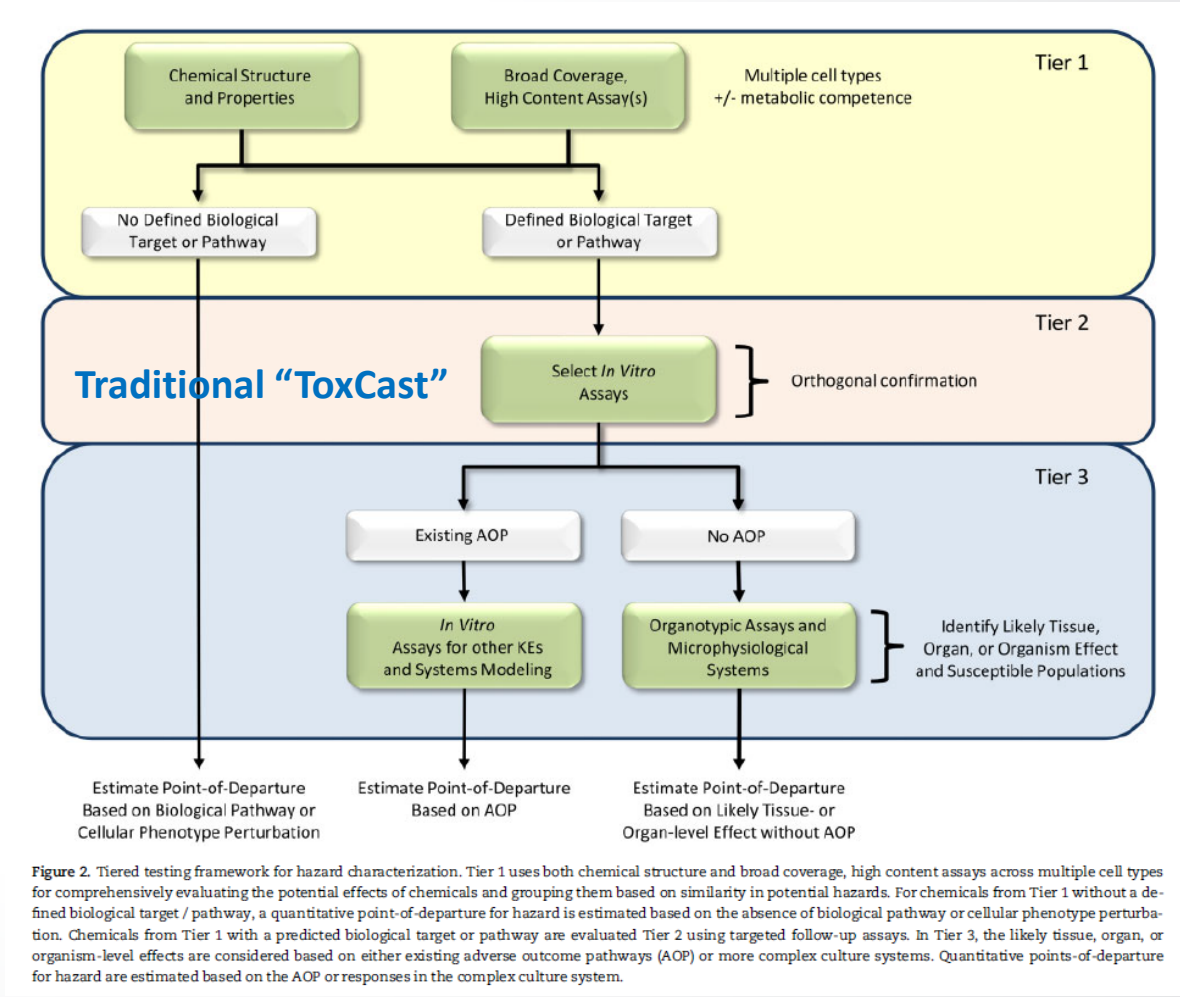


Figure 2. Tiered testing framework for hazard characterization. Tier 1 uses both chemical structure and broad coverage, high content assays across multiple cell types for comprehensively evaluating the potential effects of chemicals and grouping them based on similarity in potential hazards. For chemicals from Tier 1 without a defined biological target / pathway, a quantitative point-of-departure for hazard is estimated based on the absence of biological pathway or cellular phenotype perturbation. Chemicals from Tier 1 with a predicted biological target or pathway are evaluated Tier 2 using targeted follow-up assays. In Tier 3, the likely tissue, organ, or organism-level effects are considered based on either existing adverse outcome pathways (AOP) or more complex culture systems. Quantitative points-of-departure for hazard are estimated based on the AOP or responses in the complex culture system.

Tier 1 becomes a broad-based screening that segues to Tier 2 (targeted screening).

High-throughput phenotypic-profiling

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Toxicology and Applied Pharmacology

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

Bioactivity screening of environmental chemicals using imaging-based high-throughput phenotypic profiling

Johanna Nyffeler[a,b], Clinton Willis[a,c], Ryan Lougee[a,b], Ann Richard[a], Katie Paul-Friedman[a], Joshua A. Harrill[a,*]

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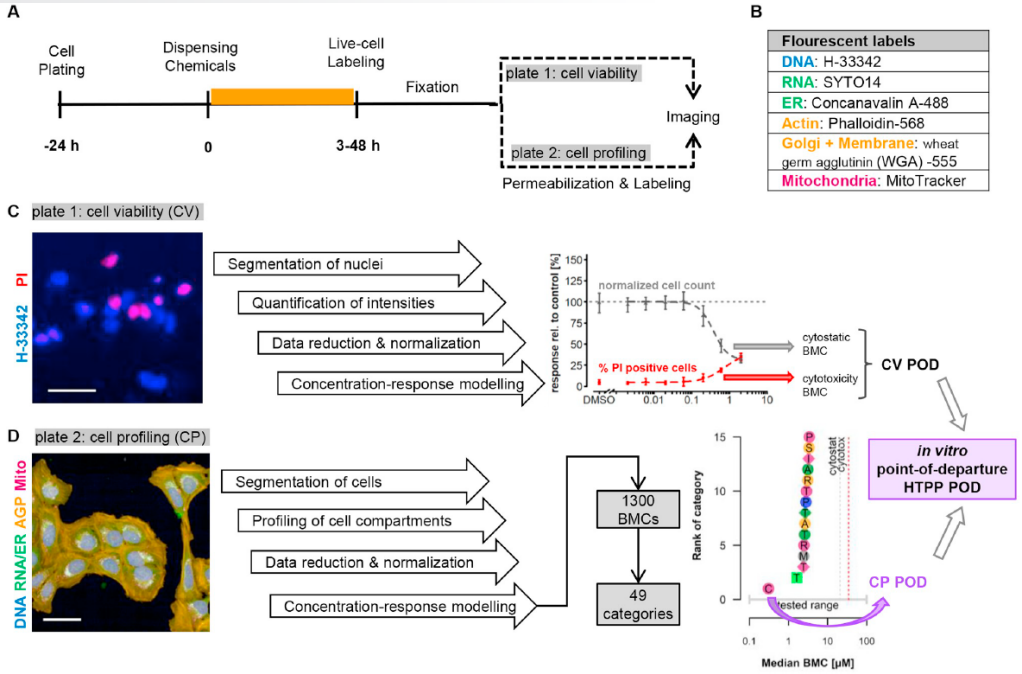
[b] Oak Ridge Institute for Science and Education (ORISE), Oak Ridge, TN 37831, United States of America
[c] Oak Ridge Associated Universities (ORAU) National Student Services Contractor, Oak Ridge, TN 37831, United States of America



High-throughput transcriptomics

Published in final edited form as:
Curr Opin Toxicol. 2019 ; 15: 64–75. doi:10.1016/j.cotox.2019.05.004.

Considerations for Strategic Use of High-Throughput Transcriptomics Chemical Screening Data in Regulatory Decisions

Joshua Harrill[1], Imran Shah[1], R. Woodrow Setzer[1], Derik Haggard[2], Scott Auerbach[3], Richard Judson[1], Russell S. Thomas[1]

- High-throughput phenotypic profiling and high-throughput transcriptomics will provide broad screening coverage
- Points-of-departure based on these techniques could then be augmented/refined using targeted screens (e.g., subsets of existing ToxCast assays and new assays to fill gaps)

Goals of the prospective case study

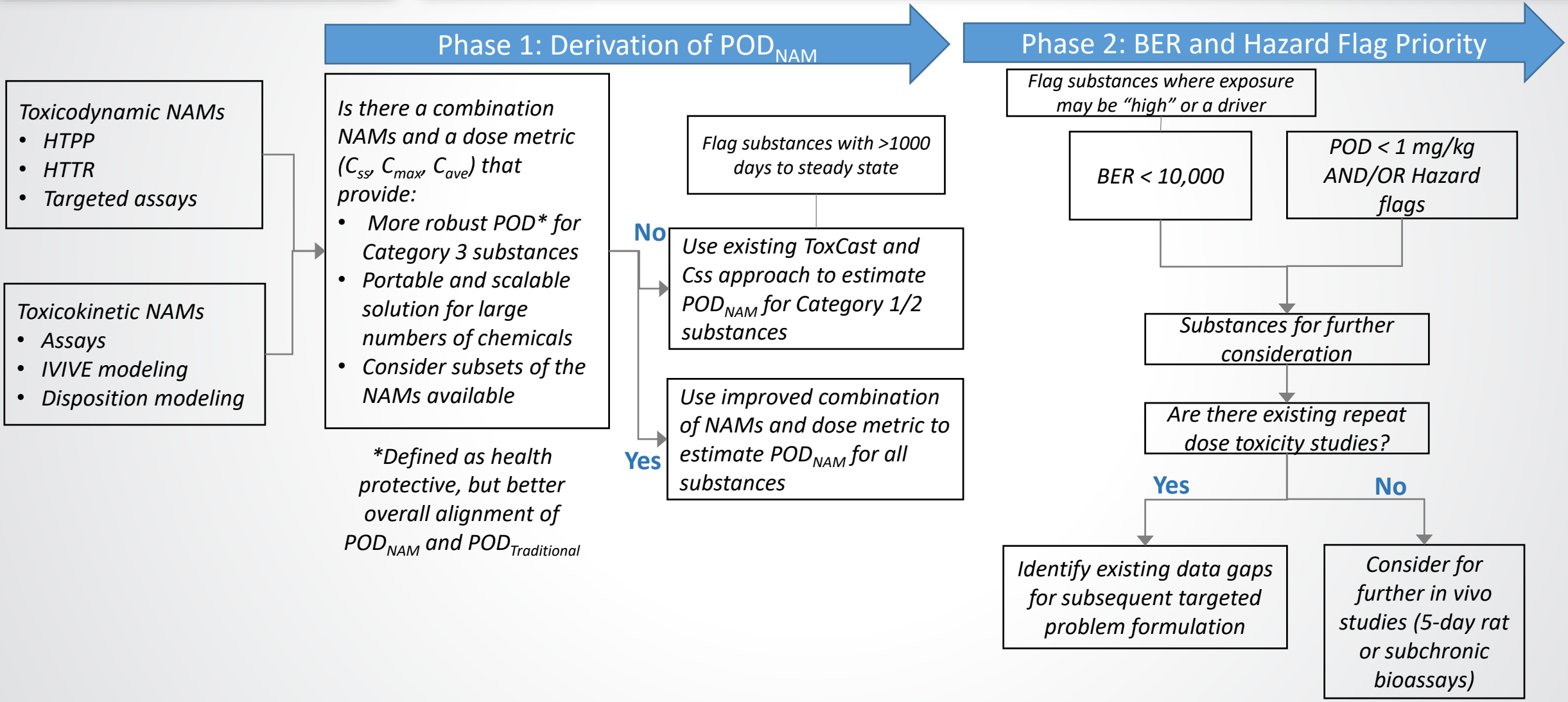
Question

Can an *in vitro* assay battery be used to derive a (health protective) point of departure (POD_{NAM}) and qualitative hazard flags comparable with the outcome from *in vivo* repeat dose toxicity (RDT) studies used in traditional hazard assessment?

Goals

- Identify a portable and scalable combination of NAMs that provides a robust and health protective estimate of the POD for repeat dose toxicities studies and mechanistically-based hazard flags for important health endpoints
  - A number of chemicals overlap with the retrospective case study and can be used to evaluate the POD_{NAM}
- Using the NAM battery, assess a set of chemicals derived from multiple national inventories that have limited/unclear toxicological data and significant potential exposure.
- Inform the further development needs for NAMs:
  - For screening, prioritization, and first tier assessments
  - For conclusive hazard characterization/assessment and risk management
  - To assess chemicals in an international context

APCRA Prospective Case Study Tier 1 Outline

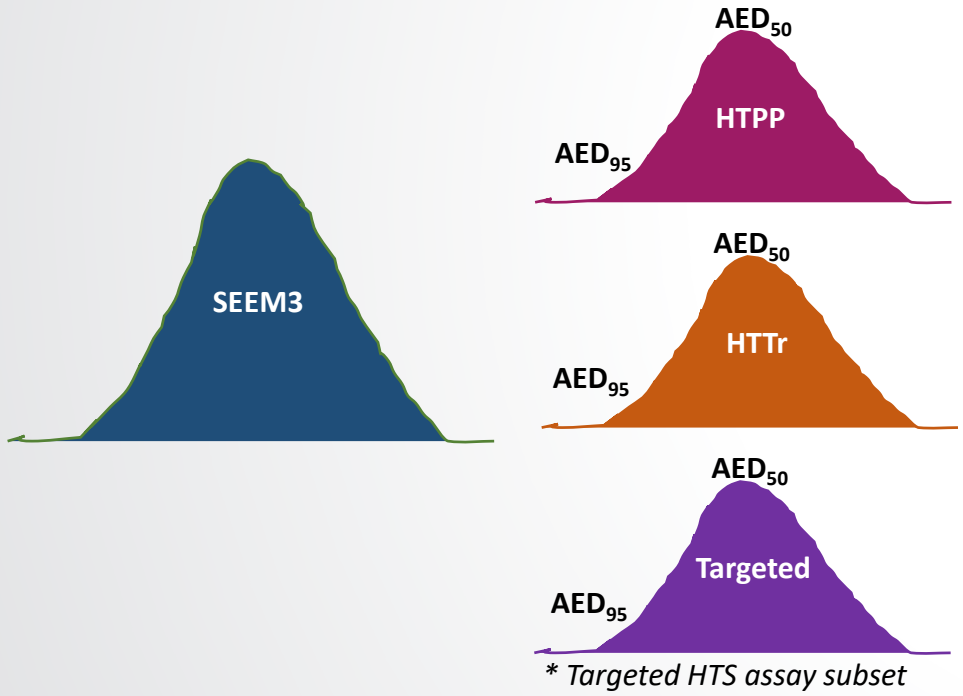


Global BER and hazard flags

Phase 2a: Global Bioactivity:Exposure Ratio and POD_{NAM} Cutoff

SEEM3 exposures estimates

Global POD_{NAM} estimates



** Targeted HTS assay subset*

At this point in the analysis, we will look for the potency of bioactivities relevant to typical 90-day studies and potency of bioactivities that may be more targeted (part of considering POD_{NAM} as an alternative for 90-day studies)

Phase 2b: Hazard flagging and selectivity

ER activity: in silico or in vitro

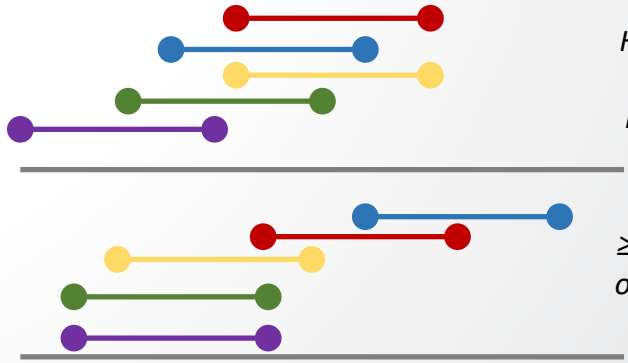
AR activity: in silico or in vitro

Developmental hazard flag

Immune response flag

Acute neurotoxicity flag

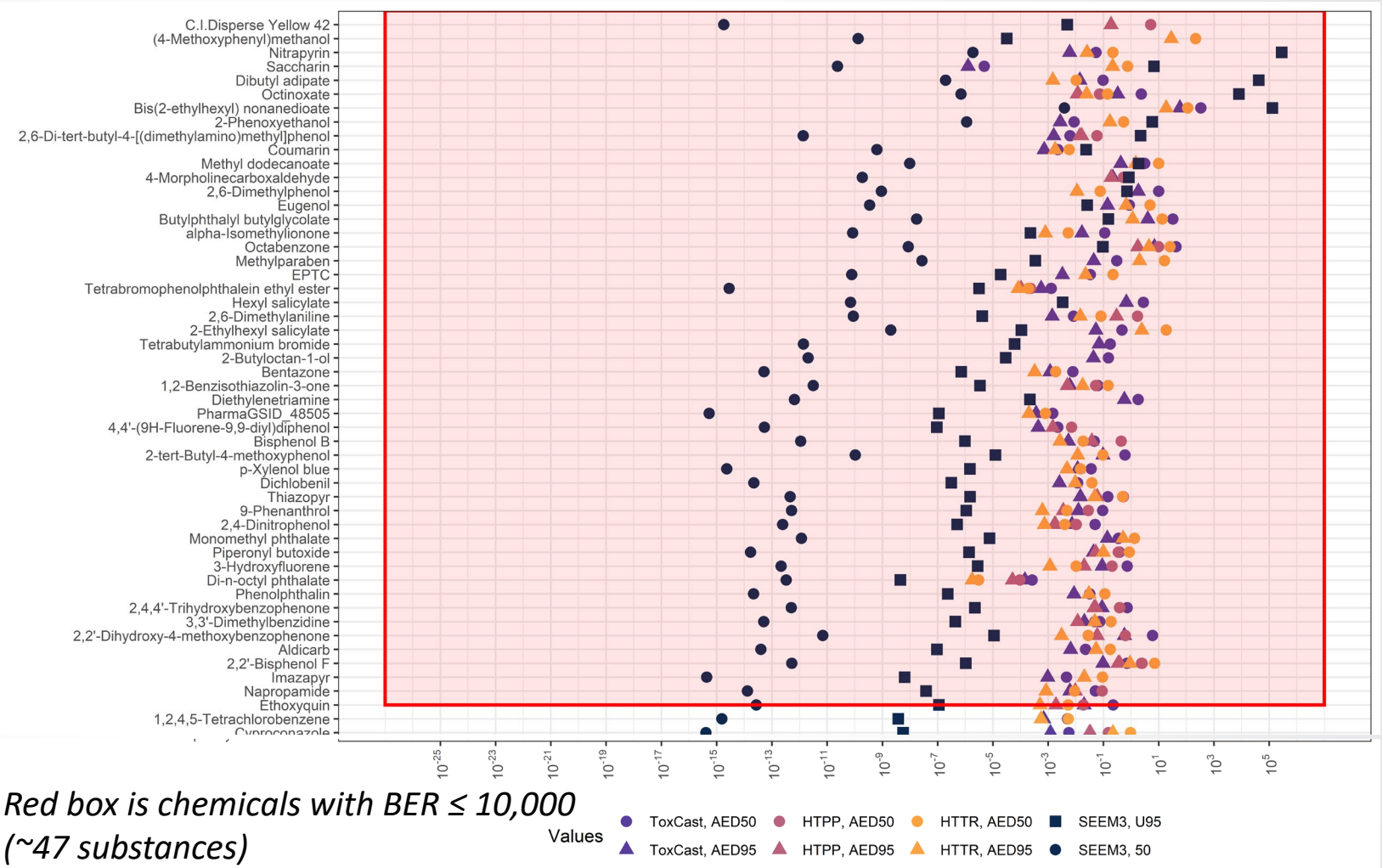
** Note that in silico consensus QSAR models are qualitative*



Hazards of interest may not drive minimum POD_{NAM}

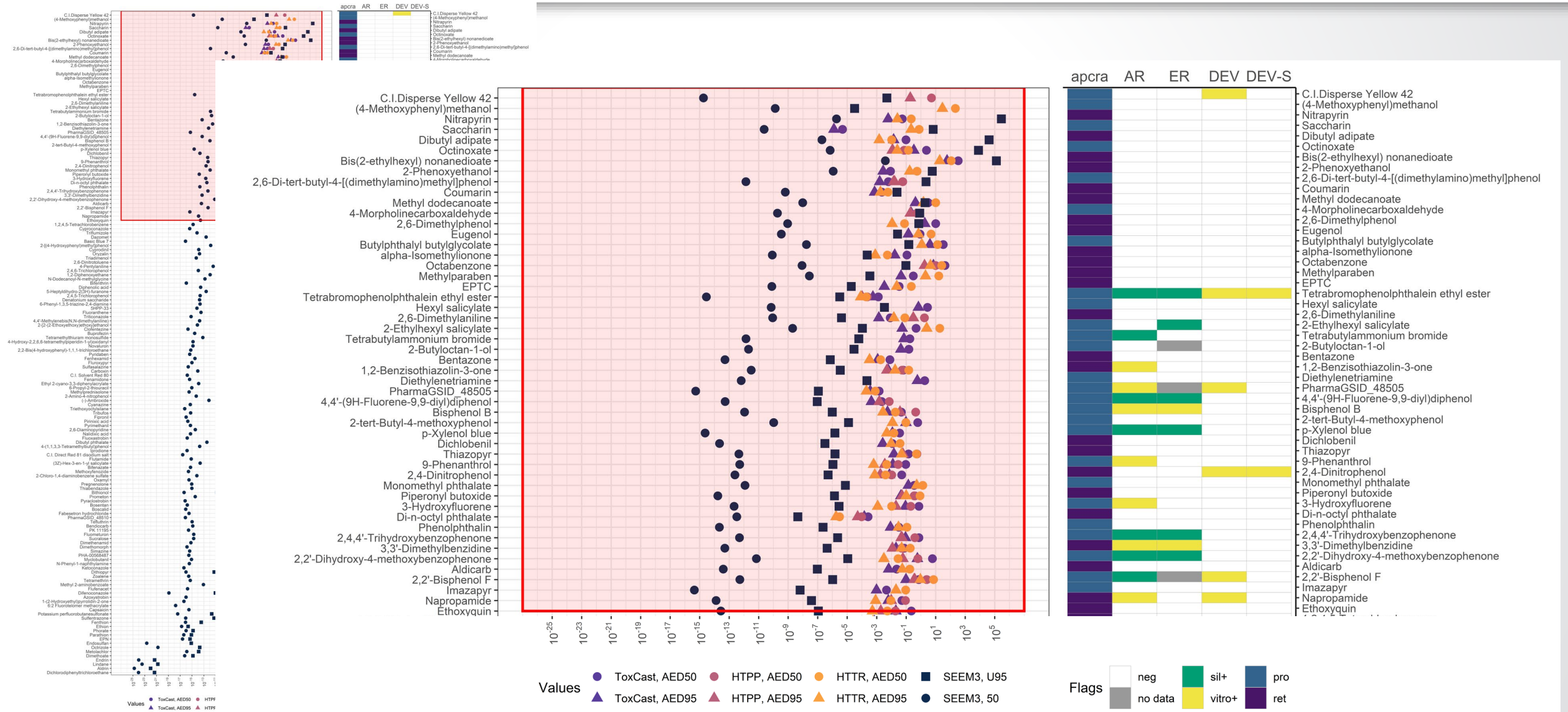
≥ 1 hazards of interest may overlap with a lower bound estimate of POD_{NAM}

Draft: using SEEM3 exposure predictions and ToxCast, HTPP, and HTTr PODs

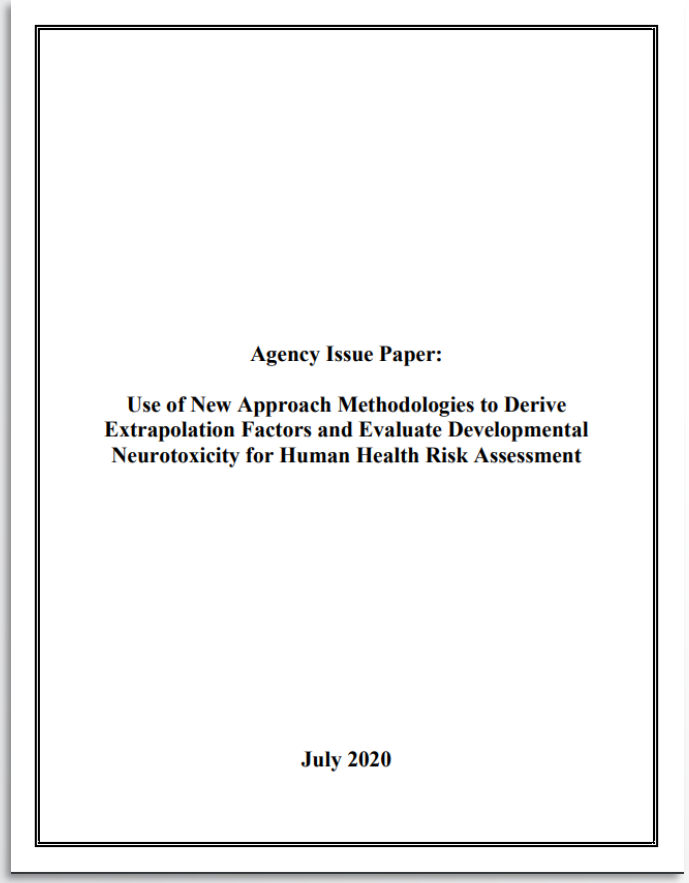


Red box is chemicals with BER ≤ 10,000 (~47 substances)

Draft look at subset of hazard flags and BER calculations



Part 3: Application of hazard-specific NAMs to specific questions about the potential developmental neurotoxicity


Agency Issue Paper:

Use of New Approach Methodologies to Derive Extrapolation Factors and Evaluate Developmental Neurotoxicity for Human Health Risk Assessment

July 2020

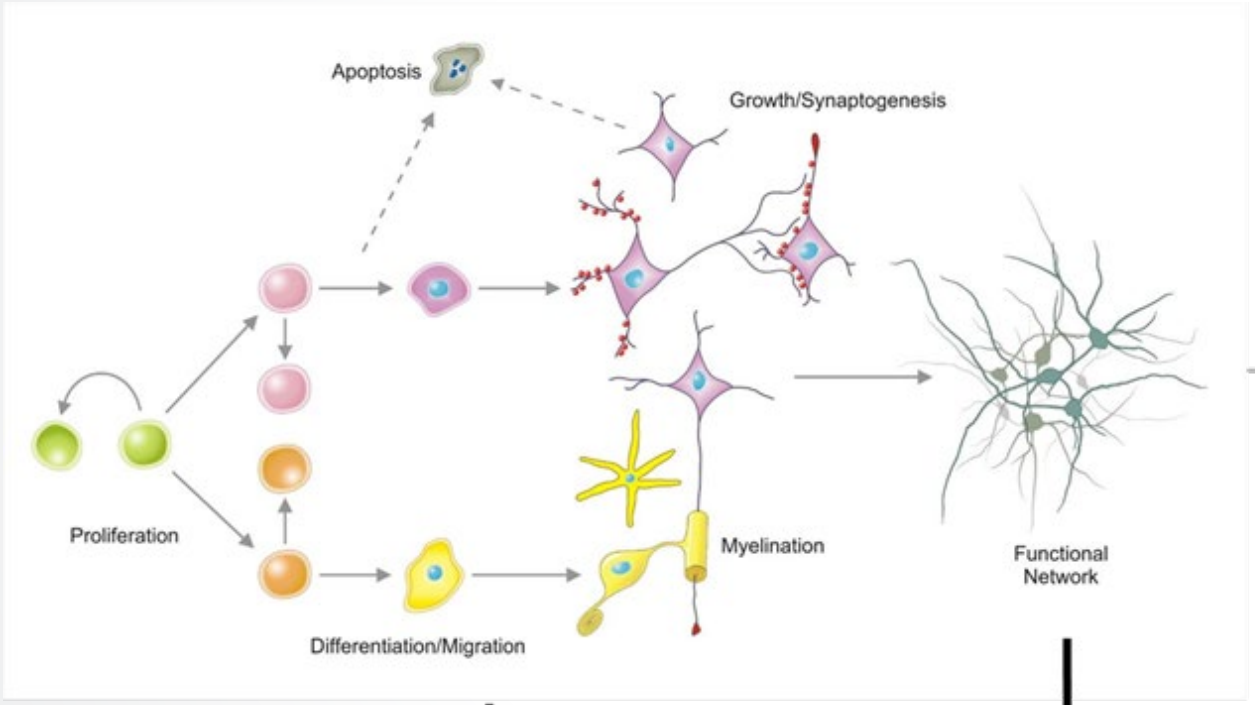
ORD DNT NAMs Team: Josh Harrill, Tim Shafer, Katie Paul Friedman

September 15-18, 2020 Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel met to review this Issue Paper and presentations

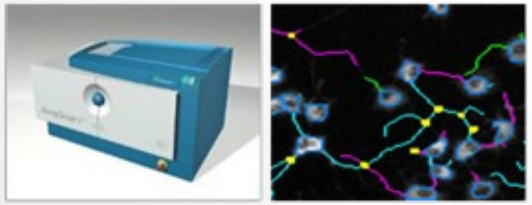
https://beta.regulations.gov/document/EPA-HQ-OPP-2020-0263-0006

Phenotypic Screening for DNT Hazard

Assays should allow quantitative measurements of key neurodevelopmental events *in vitro*



Key Neurodevelopmental Events (High Content Imaging)



Network Formation (Multi-Electrode Array)

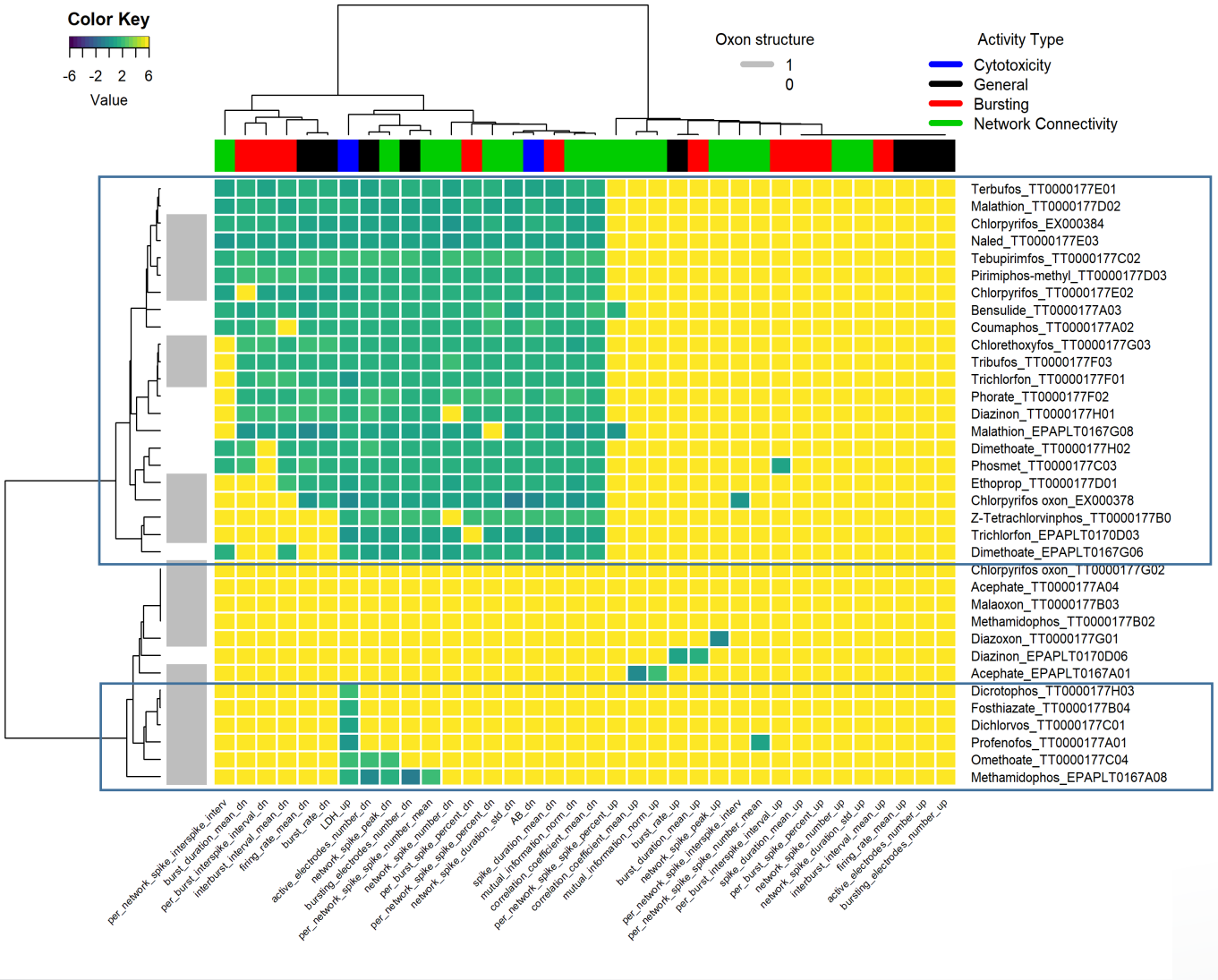
ZF behavior coming soon but not in this work

One of several charge questions addressed derivation of PODs

"In order to compare the relative sensitivity of the MEA NFA and HCI assay results to doses that inhibit acetylcholinesterase in laboratory animals, in vitro to in vivo extrapolation (or IVIVE) approaches were used to approximate NAM administered equivalent doses for a subset of organophosphate pesticides. ***Please comment on the strengths and limitations of this comparison and whether there are alternative approaches for this evaluation****."*

- Underscore the reproducibility of the DNT NAM assays.
- Describe the differential performance of OPs in the DNT NAM assays that are currently available.
- Demonstrate an IVIVE approach to derive doses for comparison to BMD and BMDL values based on rat AChE inhibition.

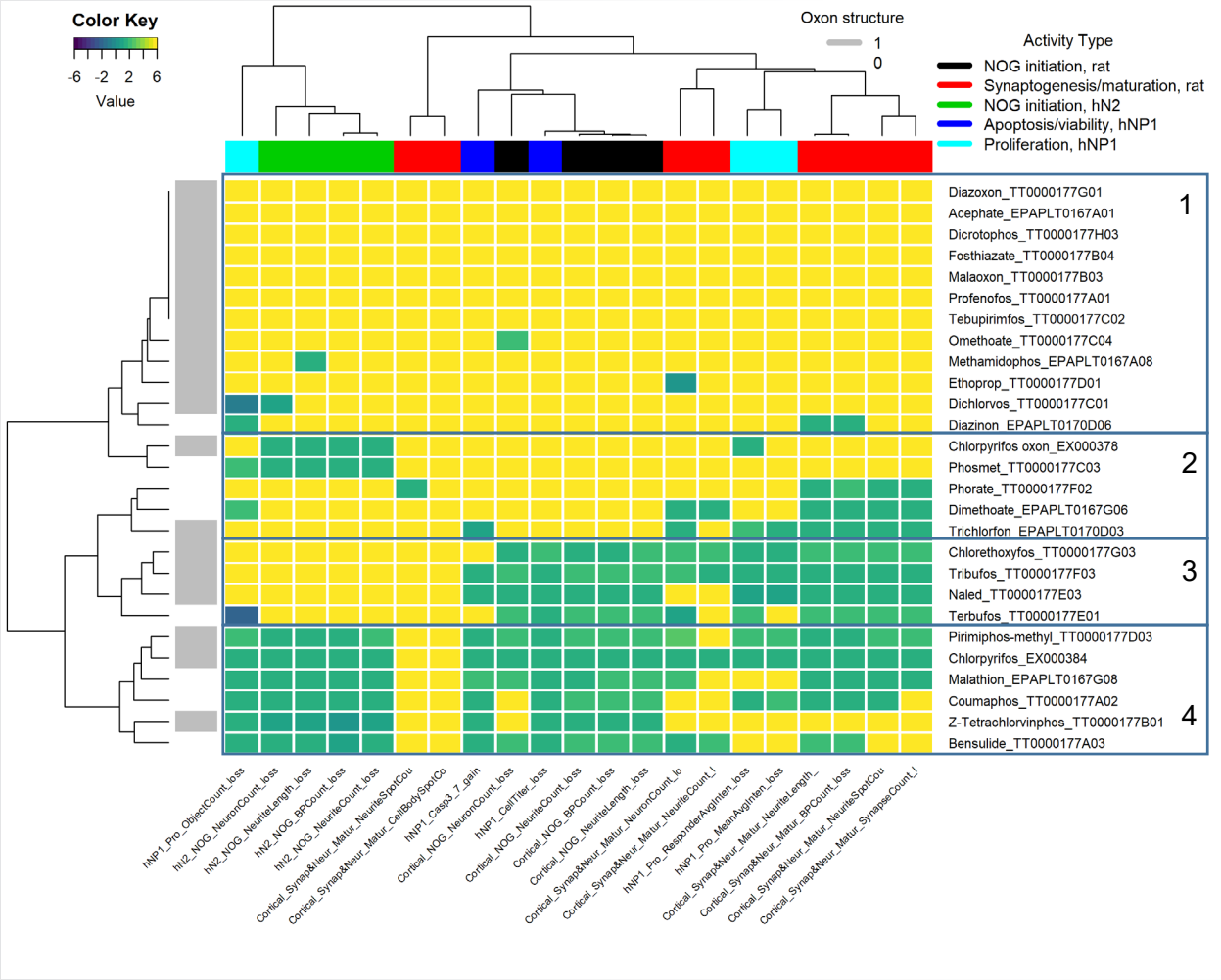
Like the assay controls, some OPs decrease MEA NFA activity types



- Top active cluster of OPs contains oxon and non-oxon structures.
- These OPs, like the assay performance controls, appear to generally decrease all activity types and most assay endpoints.
- Cytotoxicity and activity occur within a narrow concentration range.
- Bottom cluster with minimal actives appears somewhat driven by cytotoxicity in the LDH assay.

Conclusion: while not all OPs are active in the MEA NFA, those that are active appear to behave much like the assay performance controls that inhibit NOG and/or synaptogenesis.

OPs demonstrate differential responses in the HCI assays.



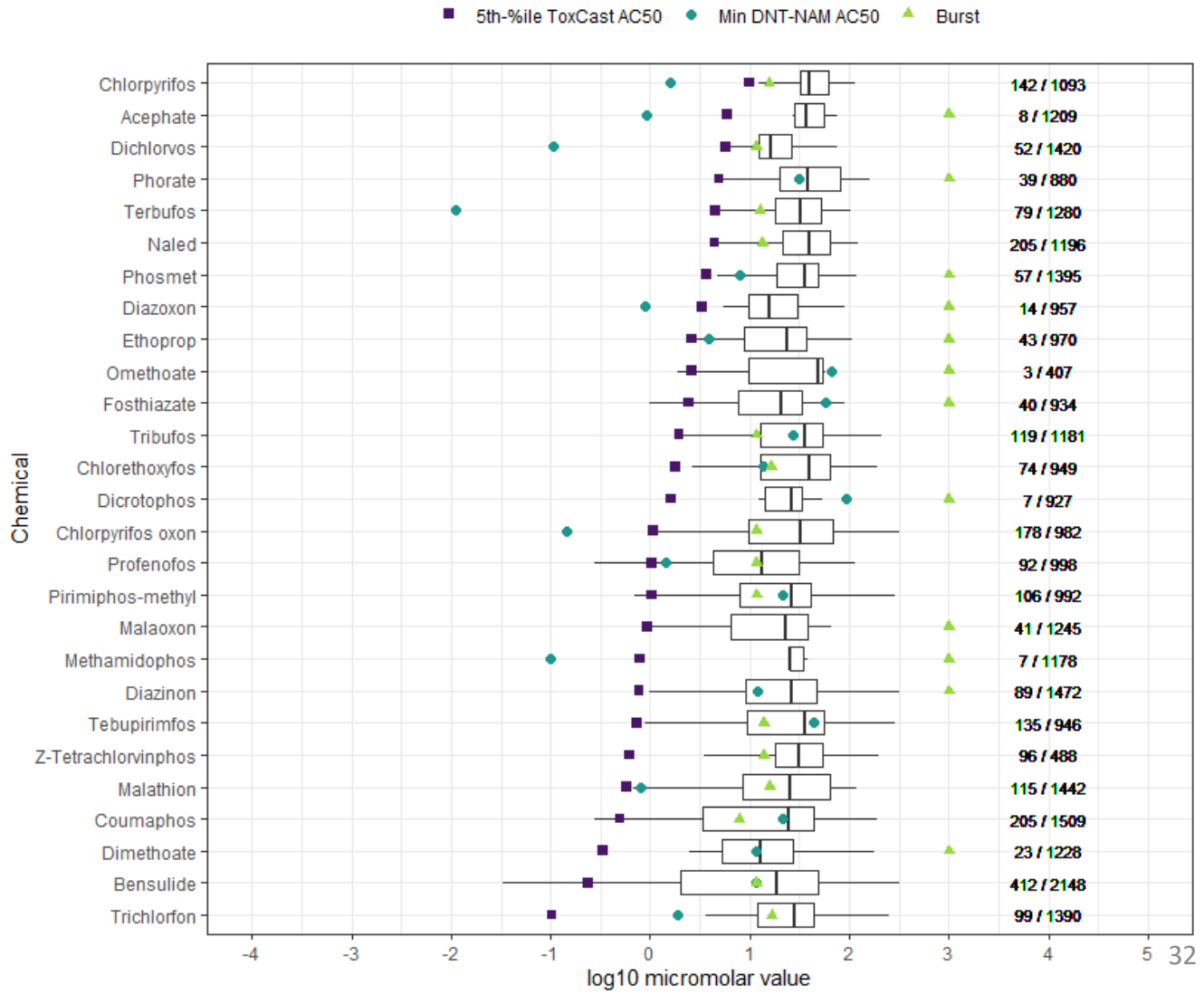
- Cluster 1: negative or with effects in 1-3 endpoints.
- Cluster 2: effects on five or more assay endpoints
- Cluster 3: OP samples with effects on all HCI assay activity types except for NOG initiation in hN2 cells
- Cluster 4: widespread effects across activity types

For some OPs, the minimum DNT-NAM AC50 < an estimate of bioactivity from the rest of ToxCast.

DNT-NAM battery may provide a more potent estimate of bioactivity for substances with minimum DNT-NAM AC50 < 5th percentile of filtered ToxCast AC50 values:

- Chlorpyrifos and chlorpyrifos oxon
- Acephate
- Dichlorvos
- Terbufos
- Diazoxon
- Methamidophos

Suggests that the DNT-NAM battery, in covering some new biology not previously in ToxCast, may yield bioactivity threshold concentrations lower than what is already available for some neuroactive substances in ToxCast.



Simplifying assumptions for the HTTK approach employed here using httk R package

- 100% bioavailability (all of an oral dose is received by the liver through the portal vein);
- No extrahepatic metabolism: the liver is the only source of chemical clearance from the body by metabolism;
- Hepatic metabolism is first order (proportional to concentration) and does not saturate;
- Renal clearance is proportional to fraction unbound in plasma and glomerular filtration rate (i.e., no active transport); and,
- No biliary excretion or enterohepatic recirculation occurs.

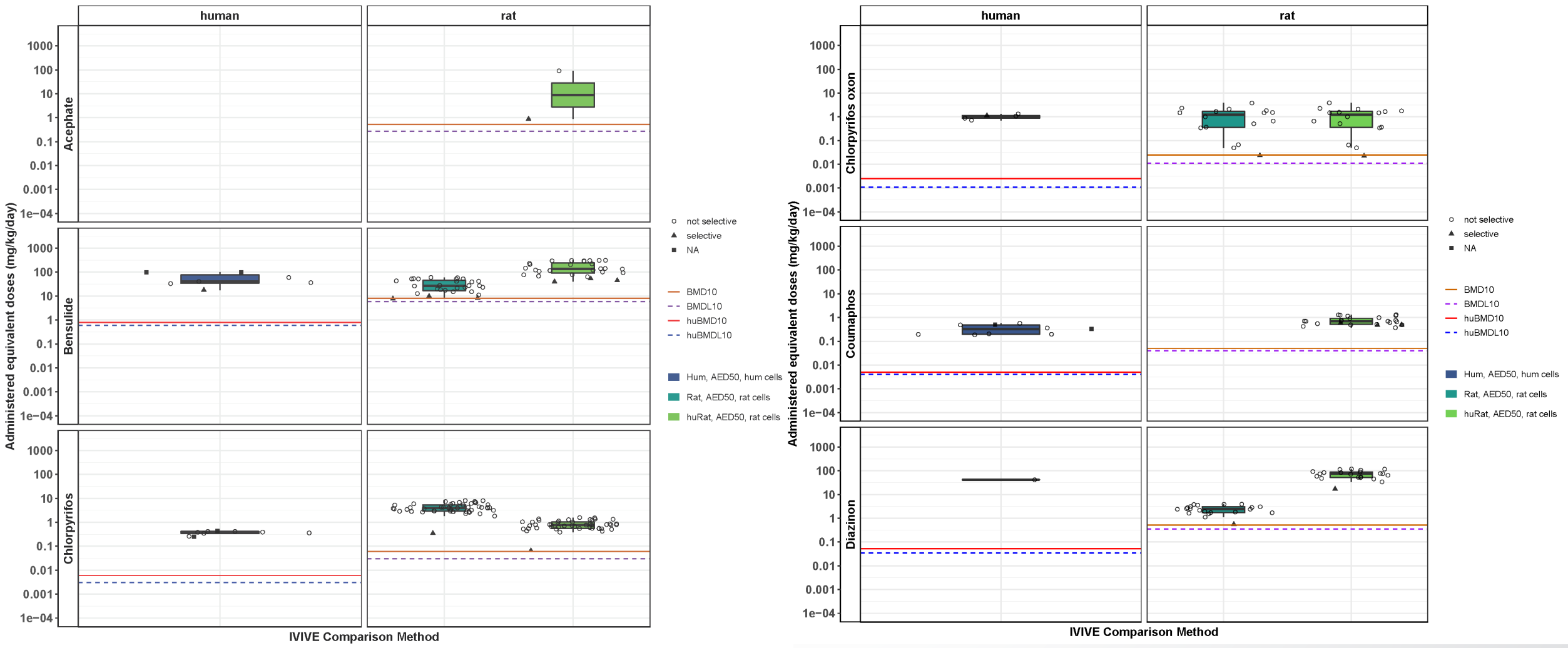
With these assumptions, HTTK models have demonstrated reasonable accuracy in predicting relevant TK endpoints, for example plasma concentrations over time (AUC) (R^2 = 0.62) and maximum plasma concentrations (Cmax) (R^2 = 0.48) (Wambaugh et al., 2018).

AED values in mg/kg/day units were calculated using the following equation (Eq. 2):

$$Eq.2: \quad AED_{50}\left(\frac{\frac{mg}{kg}}{day}\right) = AC_{50}(\mu M) * \frac{1\frac{\frac{mg}{kg}}{day}}{Css_{50}}$$

Where the Css (steady-state concentration) values for the median individual based on Monte Carlo simulation of species-specific physiological parameters (Css_{50}) (Pearce et al. 2017) were generated using the 3-compartment steady state model.

Example: AED50 to BMD/BMDL10 comparisons



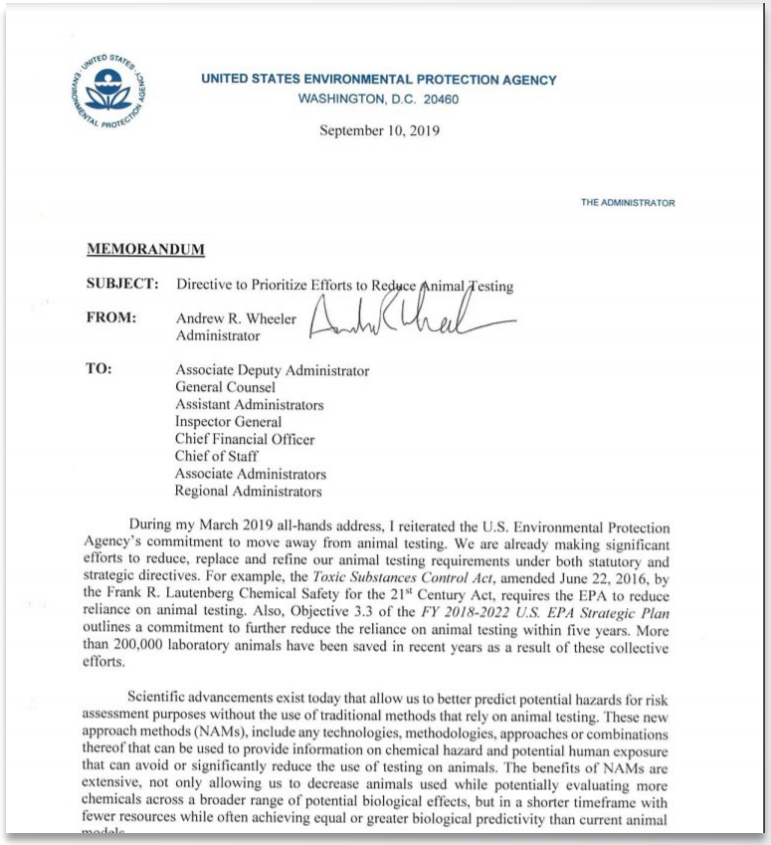
Overarching conclusions for application of DNT-NAMs

- MEA NFA and HCI assay suite recapitulates key cellular events and processes relevant to DNT, as demonstrated through the use of appropriate assay performance controls;
- the DNT-NAMs presented here represent a major milestone for *in vitro* fit-for-purpose identification of putative DNT-related hazard, though additional methods may be available in the future;
- the MEA NFA and HCI assay suite demonstrates reproducibility in terms of positive responses and potency of these responses;
- the 27 OP chemicals in this set are differentially active in the MEA NFA and HCI assay suite; and,
- application of IVIVE approaches for the *in vitro* bioactivity observed in these DNT-NAMs results in AED_{50} values that are greater than or in some cases approximate the doses that inhibit AChE *in vivo.*

Employing NAMs for derivation of PODs

EPA New Approach Methods Work Plan: Reducing Use of Animals in Chemical Testing

https://www.epa.gov/chemical-research/epa-new-approach-methods-work-plan-reducing-use-animals-chemical-testing



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

September 10, 2019

THE ADMINISTRATOR

MEMORANDUM

SUBJECT: Directive to Prioritize Efforts to Reduce Animal Testing

FROM: Andrew R. Wheeler
Administrator

TO: Associate Deputy Administrator
General Counsel
Assistant Administrators
Inspector General
Chief Financial Officer
Chief of Staff
Associate Administrators
Regional Administrators

During my March 2019 all-hands address, I reiterated the U.S. Environmental Protection Agency's commitment to move away from animal testing. We are already making significant efforts to reduce, replace and refine our animal testing requirements under both statutory and strategic directives. For example, the *Toxic Substances Control Act*, amended June 22, 2016, by the Frank R. Lautenberg Chemical Safety for the 21st Century Act, requires the EPA to reduce reliance on animal testing. Also, Objective 3.3 of the *FY 2018-2022 U.S. EPA Strategic Plan* outlines a commitment to further reduce the reliance on animal testing within five years. More than 200,000 laboratory animals have been saved in recent years as a result of these collective efforts.

Scientific advancements exist today that allow us to better predict potential hazards for risk assessment purposes without the use of traditional methods that rely on animal testing. These new approach methods (NAMs), include any technologies, methodologies, approaches or combinations thereof that can be used to provide information on chemical hazard and potential human exposure that can avoid or significantly reduce the use of testing on animals. The benefits of NAMs are extensive, not only allowing us to decrease animals used while potentially evaluating more chemicals across a broader range of potential biological effects, but in a shorter timeframe with fewer resources while often achieving equal or greater biological predictivity than current animal

https://www.epa.gov/research/administrator-memo-prioritizing-efforts-reduce-animal-testing-september-10-2019

- How much uncertainty can be tolerated?
- Can BER be informative for the problem?
- Are there specific hazards of interest?
- How should toxicokinetic modeling be tuned?

There is a lot more work to do, and case studies will help build confidence and identify gaps to fill.

Acknowledgements

Many CCTE collaborators

Many OPP collaborators

Many APCRA partners

EPA
Office of Research and Development
Center for Computational Toxicology & Exposure (CCTE)

Appendix slides

Summary of the AED50 to BMD/BMDL comparison

	Chemicals with AED50 values >>> BMD/BMDL comparator	Chemicals with lowest AED50 within 1 log10 order of magnitude of BMD/BMDL comparator	Chemicals with lowest AED50 approaching BMD/BMDL comparator	Missing in vitro data for comparison
Rat/HuRat	Coumaphos, diazoxon, dicrotophos, ethoprop, fosthiazate, omethoate	acephate, bensulide, chlorpyrifos, chlorpyrifos oxon, diazinon, dimethoate, malathion, methamidophos, and phorate	lower quartile of huRat AED_{50} values for dimethoate and methamidophos (these AED_{50} values appear to have included selective assay endpoints). The huRat AED_{50} value for dichlorvos (only one positive rat assay endpoint) overlaps with the BMDL10 value, and it was not based on selective bioactivity in the DNT-NAM battery. The lowest huRat AED_{50} values (selective) for malathion also approach the BMD/BMDL10 values.	Malaoxon was negative in all assays.
Human	bensulide, chlorpyrifos, chlorpyrifos oxon, coumaphos, diazinon, dimethoate, malathion, methamidophos, phosmet, pirimiphos-methyl, tribufos, and trichlorfon		For dichlorvos, only two AED_{50} values are available for comparison, and these values are centered around the BMD10/10 and BMDL10/10 values. Neither of these AED_{50} values appear selective because the bioactivity was observed in assay endpoints relevant to cell viability. Similarly, for terbufos, only 3 human AED_{50} values are available for comparison, and the lowest one of these values approaches the BMD10/10 value. This lowest AED_{50} value for terbufos does not appear selective because it is derived from a cell viability related assay endpoint (object count in the HCI hNP1 proliferation assay endpoint).	Acephate, diazoxon, dicrotophos, ethoprop, fosthiazate, omethoate, phorate, profenofos, and tebupirimfos had positive rat assay data but lacked positive responses in the human cell-based assays. Malaoxon was negative in all assays.

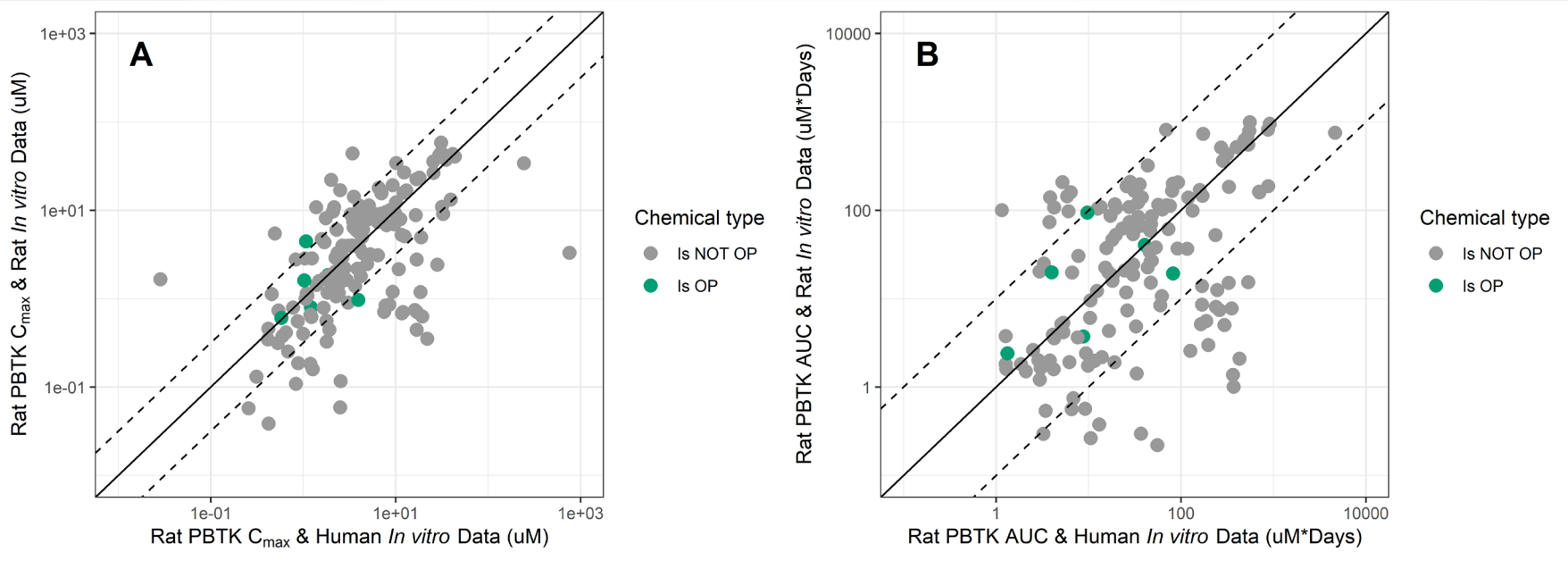
Selecting an HTTK model: 3 compartment steady state model

Models:	3-compartment steady state (3compss)	PBTK
Chemical-specific parameters	Clint only	Clint, Fup, logP, pKa
Model inputs	A single oral dose	A single oral dose
Model outputs	Steady-state blood concentrations	Time course of blood concentrations; estimate Cmax, AUC (24 hr), Cmean (AUC/time) from time course simulations
Human interindividual variability	Human physiological parameters (first order hepatic metabolic clearance; plasma protein binding; liver volume, blood flow, and cell density; and glomerular filtration rate) can be varied in a Monte Carlo simulation to estimate the dose required to achieve equivalent blood concentrations for the most to least sensitive individuals. In this Issue Paper, the median individual is used.	
Rat interindividual variability	Rat physiological parameters (rat liver volume and glomerular filtration rate) can be varied in a Monte Carlo simulation to estimate the dose required to achieve equivalent blood concentrations for the most to least sensitive individuals. In this Issue Paper, the median individual is used.	

- Only 6/27 OP insecticides discussed in this Issue Paper have sufficient rat-specific (fraction unbound in plasma and hepatic intrinsic clearance) to inform HTTK PBTK models
- Because the fraction unbound in plasma (Fup) assay fails for highly bound chemicals (Wambaugh et al., 2015), the steady state model is advantageous because it can be used with the assumption that plasma protein binding is simply "small," i.e., typically 0.5% (Wetmore et al., 2012)

To provide the most complete view of a potency comparison between AEDs based on DNT-NAMs and BMD10 and BMDL10 values based on observations of in vivo rat AChE inhibition, and to present an approach that would require the minimum amount of data using the simplest modeling approach, AED values in this Issue Paper were calculated using the 3-compartment steady state model.

To address more of the OPs, we used the "huRat"



Supplemental Appendix Figure 2

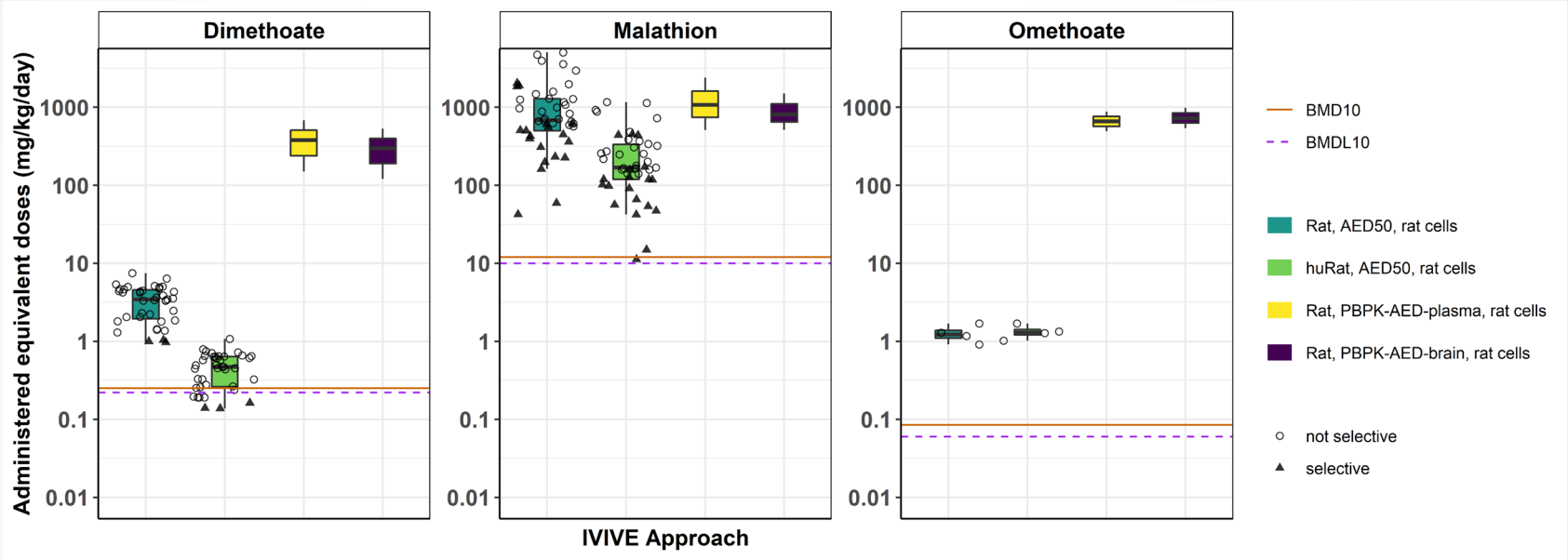
- In the absence of hepatic clearance values from rat hepatocytes, rat liver microsomes, or rat liver Phase I enzymes, would the use of human hepatocyte-derived hepatic clearance values be a reasonable substitute?

- In addition to comparing rat-derived AED_{50} values to BMD10 and BMDL10 values from rat studies, we also compared AED values from the "humanized-rat" or the huRat, which used human HTTK data in a model parameterized with rat physiology, to BMD10 and BMDL10 values from rat studies.

Comparing HTTK to PBPK-PD models

- Physiologically-based pharmacokinetic (PBPK)-pharmacodynamic (PD) models were available for: dimethoate, omethoate, and malathion *based on a chlorpyrifos model that is no longer available*.
- Though the HTTK model employed and the PBPK-PD models all assumed 100% bioavailability, the HTTK model accounts for hepatic Clint whereas PBPK-PD models incorporate additional metabolism sites in plasma, brain, and kidneys.

HTTK may provide more rapid results that are similar to or more conservative than PBPK-PD models



- Dimethoate and omethoate: PBPK-AED values using plasma and brain AUC were more than two orders of magnitude greater than the HTTK-derived AEDs
- Malathion, the PBPK-AED values were similar to the range of HTTK-derived AED_{50} values for rat