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In vitro to *in vivo* extrapolation for decision-making

Katie Paul Friedman, PhD

October 29, 2021

Presented to the SETAC 2021 Continuing Education Course: Toxicokinetic New Approach Methodologies (NAMs)

Overview

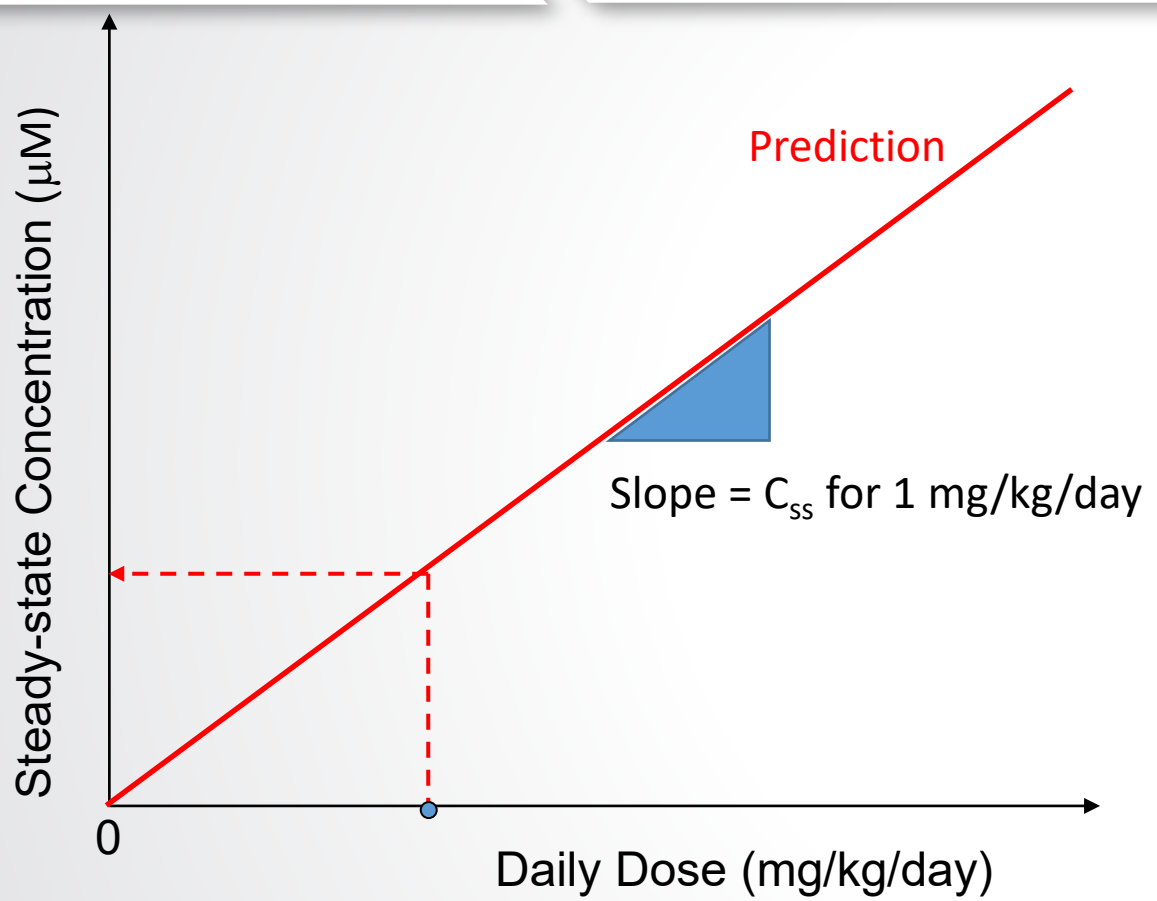
- Reverse dosimetry for in vitro to in vivo extrapolation (IVIVE)
  - Key assumptions
  - Operationalizing library(httk)

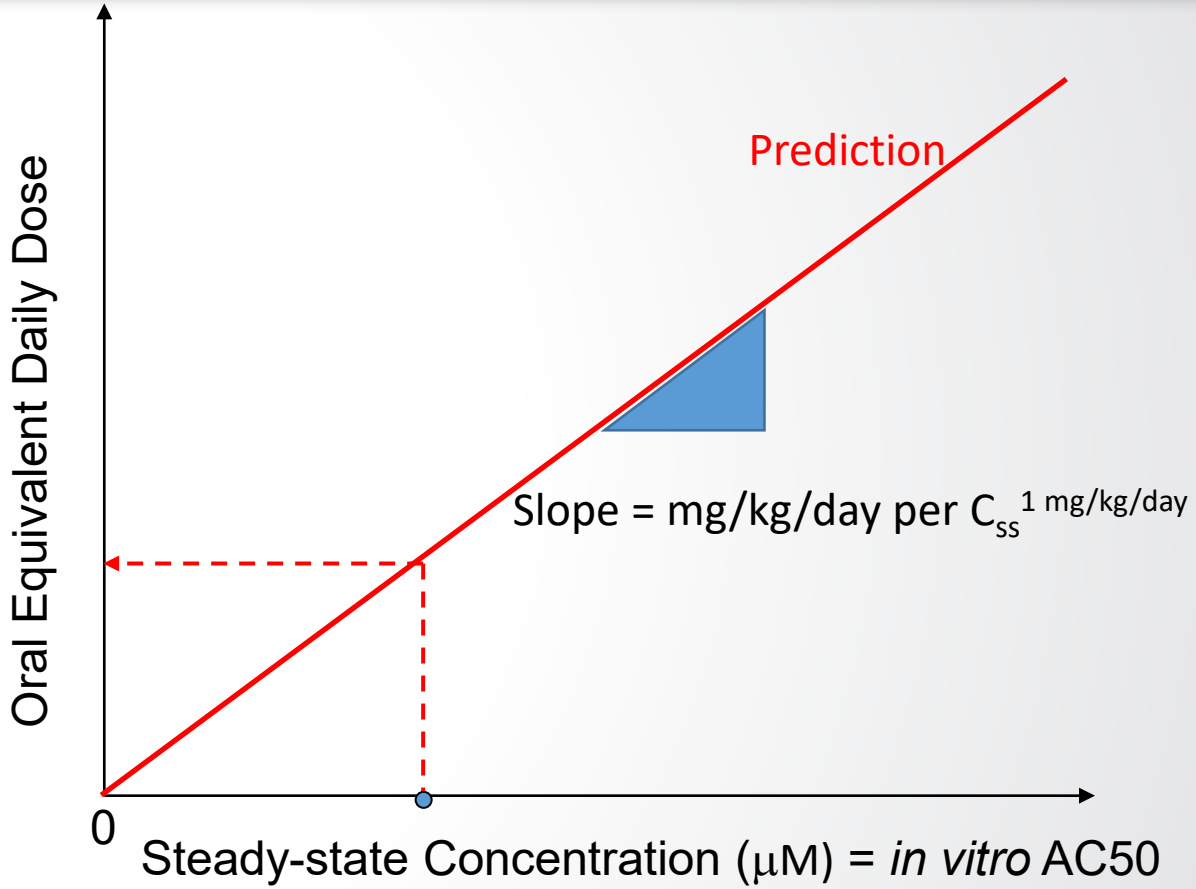
- Impacts of choices made in IVIVE on a NAM-based point of departure (POD_{NAM})
  - What are the key choices to be made in using library(httk)
  - Continuing uncertainties

- Case studies using the bioactivity:exposure ratio (BER)

Reverse dosimetry for in vitro to in vivo extrapolation (IVIVE)

Steady state in vitro-in vivo extrapolation assumption: blood::tissue partitioning ≈ cells::medium partitioning





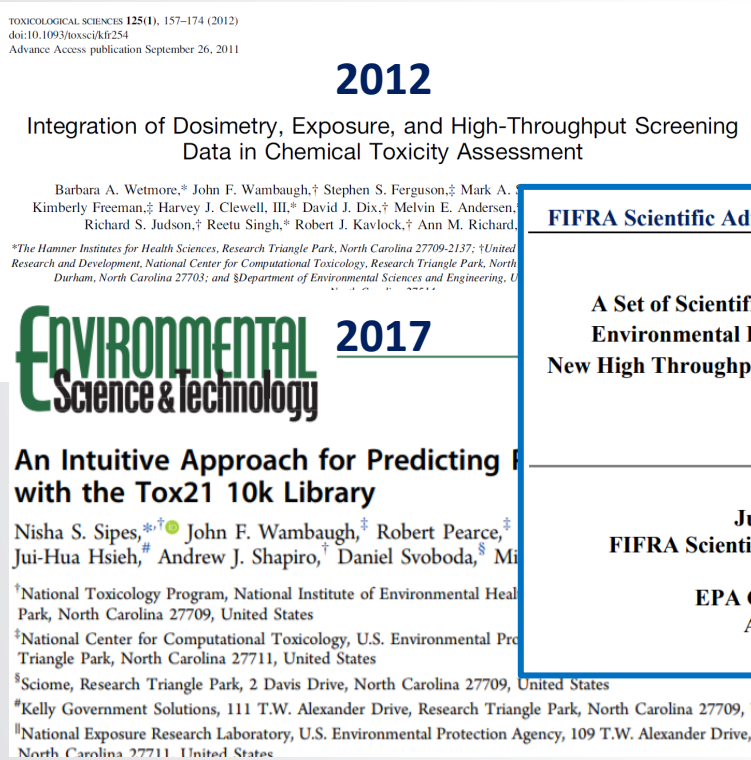
$$C_{ss} = \frac{\text{oral dose rate}}{(GFR * F_{ub}) + \left(Q_l * F_{ub} * \frac{Cl_{int}}{Q_l + F_{ub} * Cl_{int}}\right)}$$

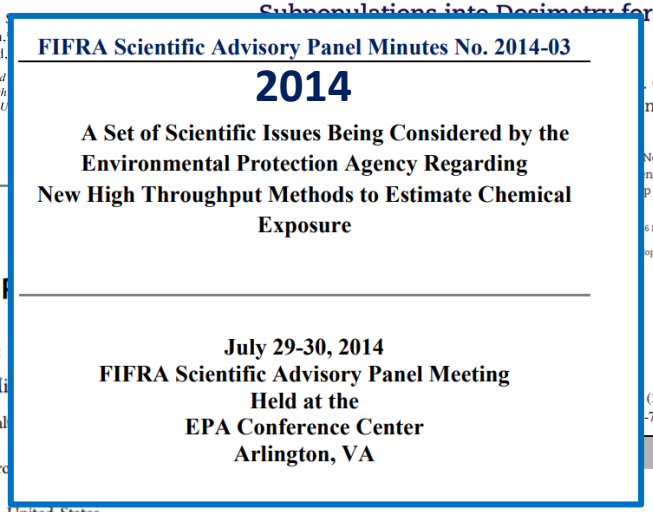
Wetmore *et al*. (2012)

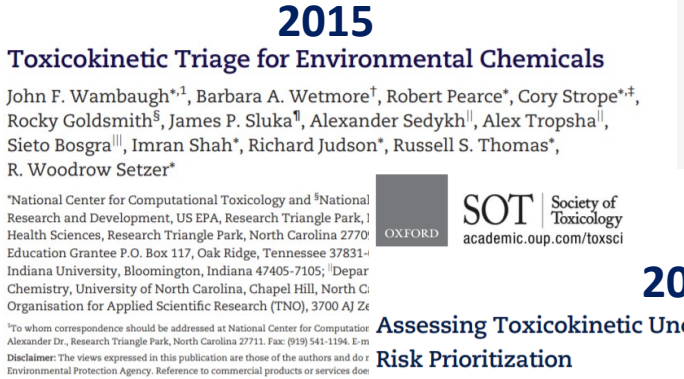
- Swap the axes (this is the "reverse" part of reverse dosimetry)
- Can divide bioactive concentration by C_{ss} for for a 1 mg/kg/day dose to get oral equivalent dose

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.







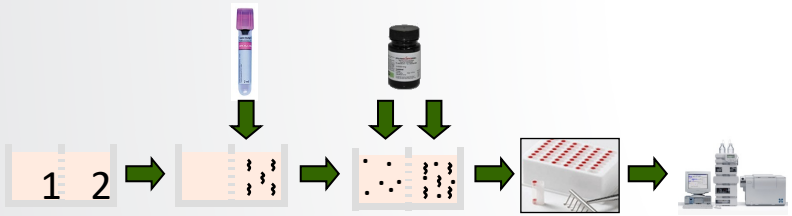
A subset of the papers describing the development of a high-throughput toxicokinetic approach

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

High throughput toxicokinetics (HTTK)

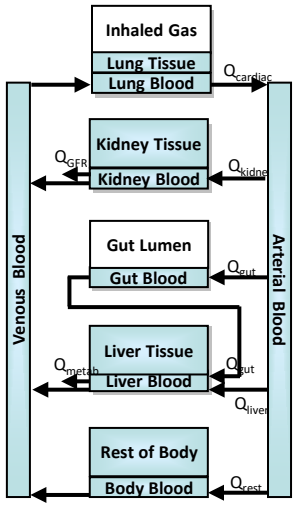
in vitro toxicokinetic data

Hepatic clearance from suspended hepatocytes



Plasma protein binding

Generic toxicokinetic models



httk

Some high-level assumptions commonly employed:

(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, Caroline Ring, and colleagues

Simplifying assumptions for a steady-state model

- 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:

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

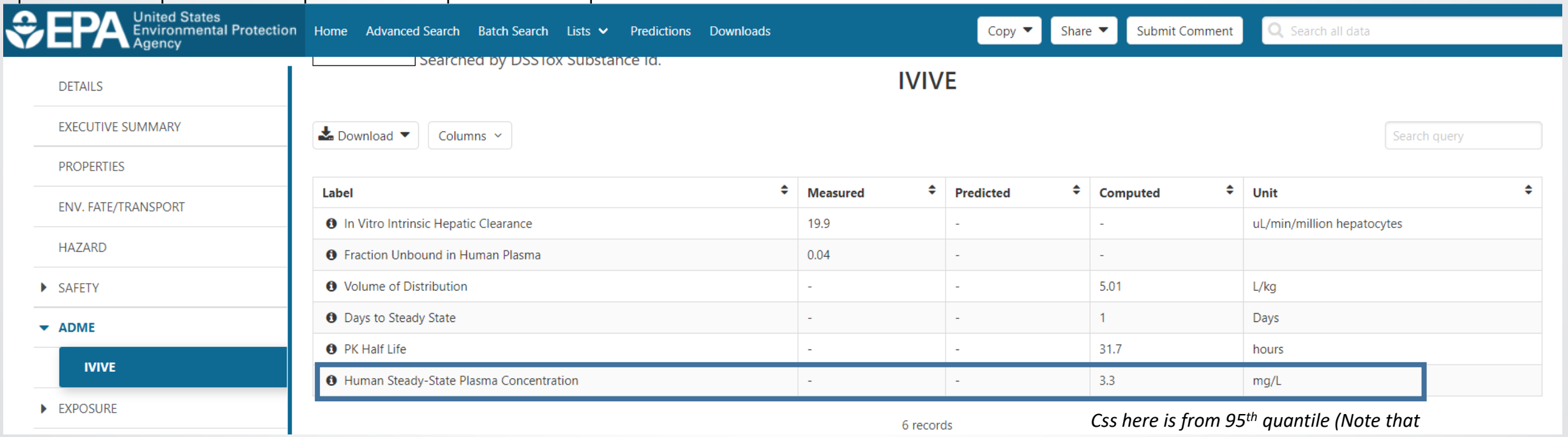
Where the Css (steady-state concentration) values for the median individual based on Monte Carlo simulation of species-specific physiological parameters (Css_{50}) (Pearce et al. 2017) were generated using the 3-compartment steady state model.

A simple approach for using the CompTox Chemicals Dashboard to estimate a POD_{NAM}

- Operationally, the httk R package (v 2.0.4) can be downloaded from CRAN or GitHub for reproducible generation of administered equivalent doses (AEDs).
- AC50 or LEC (micromolar) * (1 mg/kg/day/Css (micromolar)) = AED prediction
- Httk package optionally implements multiple models that can have increasing complexity based on data available (e.g., using pbtk model or including interindividual toxicokinetic variability).

$$\frac{3.3\ \cancel{mg}}{L} \times \frac{\cancel{g}}{1000\ \cancel{mg}} \times \frac{\cancel{mol}}{228.291\ \cancel{g}} \times \frac{1e6\ \mu mol}{\cancel{mol}} = 14.45523\ \mu mol/L = \mu M$$

$$\frac{0.1\ \cancel{\mu M}}{} \times \frac{1\ mg/kg/day}{14.45523\ \cancel{\mu M}} = \mathbf{0.007\ mg/kg/day = AED95}$$



Css here is from 95th quantile (Note that 95th concentration quantile is the same population as the 5th dose quantile).

A simple operational use of library(httk)

Default micromolar concentration; this is the in vitro point of departure you want to use

Which quantile from Monte Carlo steady-state simulation (for Css). 95th concentration quantile produces the 5th dose quantile.

Which generic toxicokinetic model to use?

```
> set.seed(12345)
> library(httk)
> calc_mc_oral_equiv(0.1, dtxsid='DTXSID7020182', species = 'Human', which.quantile = c(0.5), output.units = 'mgpkgpday', restrictive.clearance = TRUE, model = '3compartmentss')
uM concentration converted to mgpkgpday dose for 0.5 quantile.
    50%
0.04836
```

'Rat', 'Rabbit', 'Dog', 'Mouse' or default 'Human'

Restrictive clearance indicates that chemical bound to protein is relatively unavailable for hepatic metabolism or renal excretion (whereas non-restrictive clearance assumes that chemical bound to protein rapidly disassociates from that protein for metabolism and excretion).

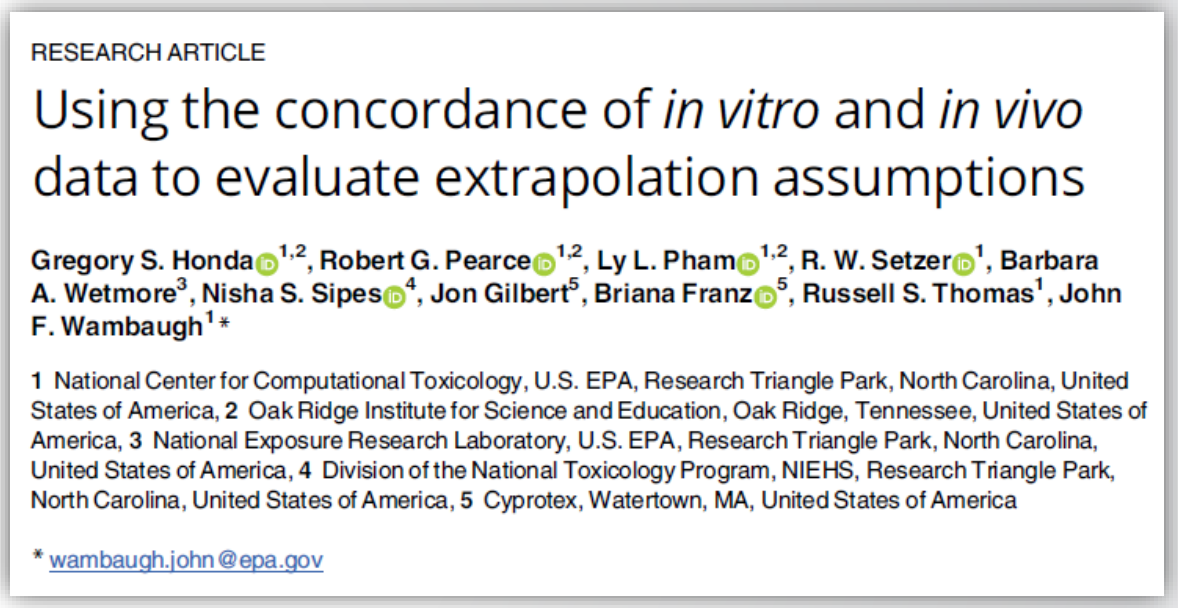
Impacts of choices in the IVIVE approach to POD_{NAM}

Some key choices

- What species physiology should be considered for the application?
- Which generic HTTK model is fit-for-purpose?
- How should interindividual variability be considered?
- What assumptions should be made about restrictive clearance and bioavailability of a chemical for bioactivity?
- To what extent will our predictions of POD be inaccurate because of differential *in vitro* partitioning of the chemical?

On selection of the species for the physiology

- Does the application require comparison to animal-based PODs or human exposure predictions or both?
- How much *in vitro* toxicokinetic data is available for the species in question/how many chemicals can IVIVE be performed?
- Another approach: is allometric scaling (based on body surface area) useful for converting human administered equivalent doses to other species?



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]*

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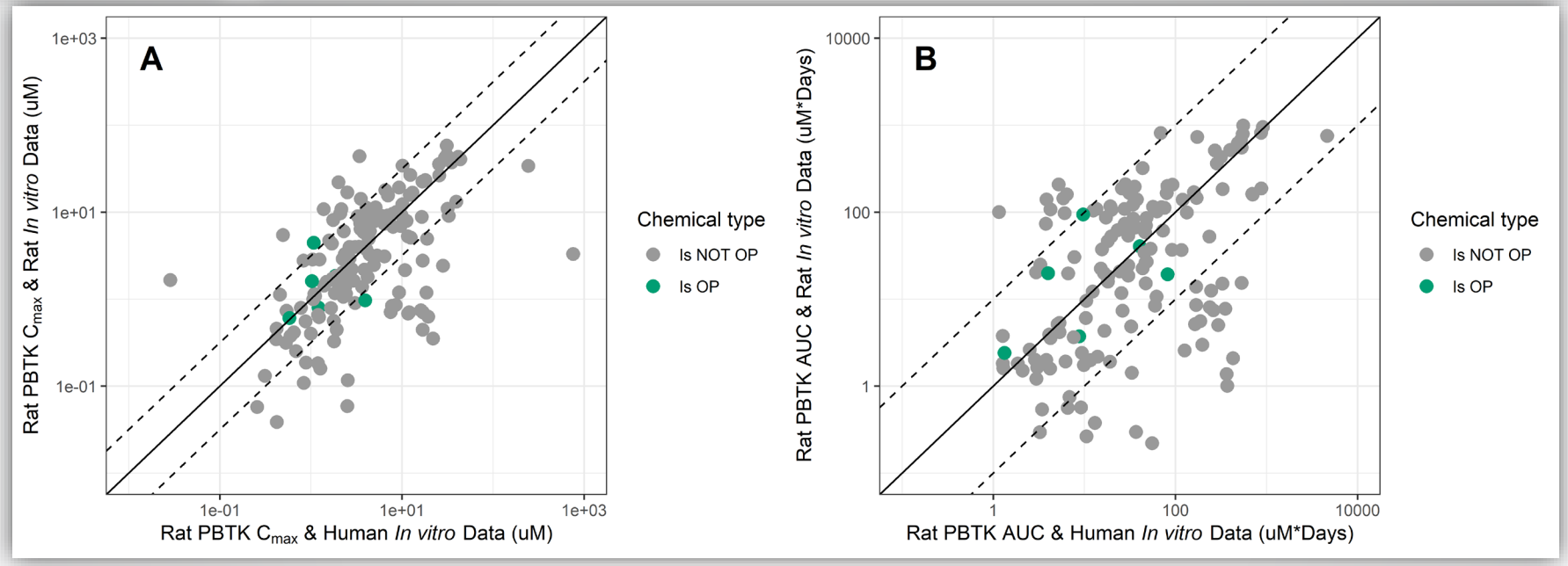
With this paper came the introduction of a larger set of rat intrinsic hepatic clearance and fraction unbound in plasma data, but there is still more data available for humans.

What to do when data is missing by species?



Supplemental Appendix Figure 2, https://www.regulations.gov/docket/EPA-HQ-OPP-2020-0263/document

- 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?

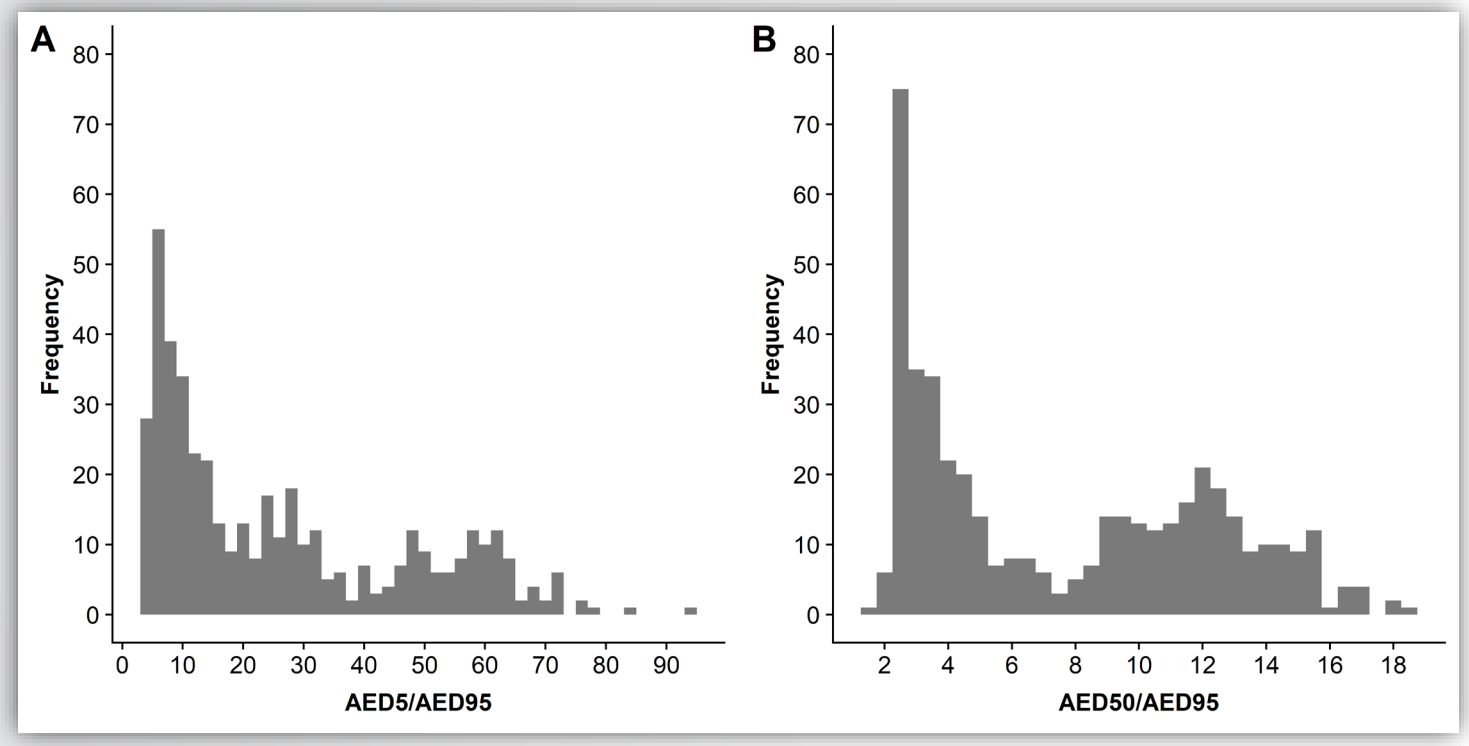
- The Cmax values obtained from the rat PBTK model, using either rat or human HTTK data for Fup and Clint, result in values that are similar (generally within ± 0.5 log10-µM) for the 151 substances compared. Similarly, the plasma AUC values that result from using rat or human HTTK data in a rat PBTK model generally were within ± 1 log10-µM.

On selection of a generic HTTK 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.	
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.	

- How many chemicals of interest have sufficient data for the model?
- Can *in silico* predictions of Fup or other parameters be used?
- Because the fraction unbound in plasma (Fup) assay fails for highly bound chemicals (Wambaugh et al., 2015), the steady state model can be used with the assumption that plasma protein binding is simply "small," i.e., typically 0.5% (Wetmore et al., 2012).

On consideration of population toxicokinetic variability



Paul Friedman et al., 2020 Supplemental Appendix; 10.1093/toxsci/kfz201

For the 448 chemicals in Paul Friedman et al., 2020, AED50 was typically 2-5 times larger than AED95, though in some cases the differences was much greater.

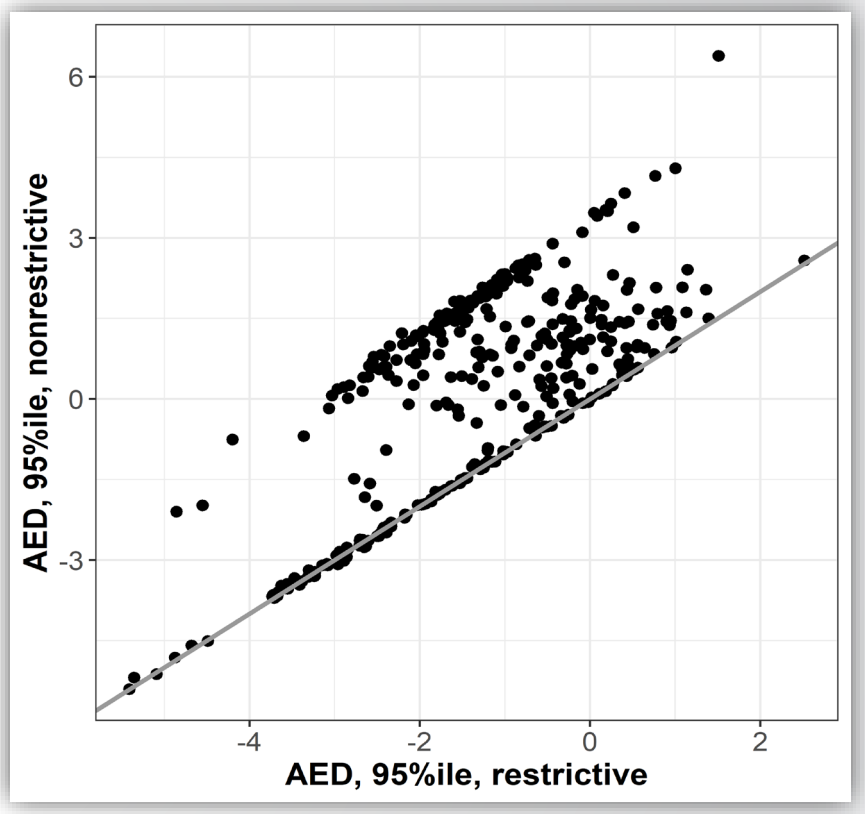
What is the application: screening or assessment?

On consideration of restrictive clearance

The degree to which a protein bound chemical is available for metabolism and excretion is likely chemical specific and a continuous function (i.e., not binary).

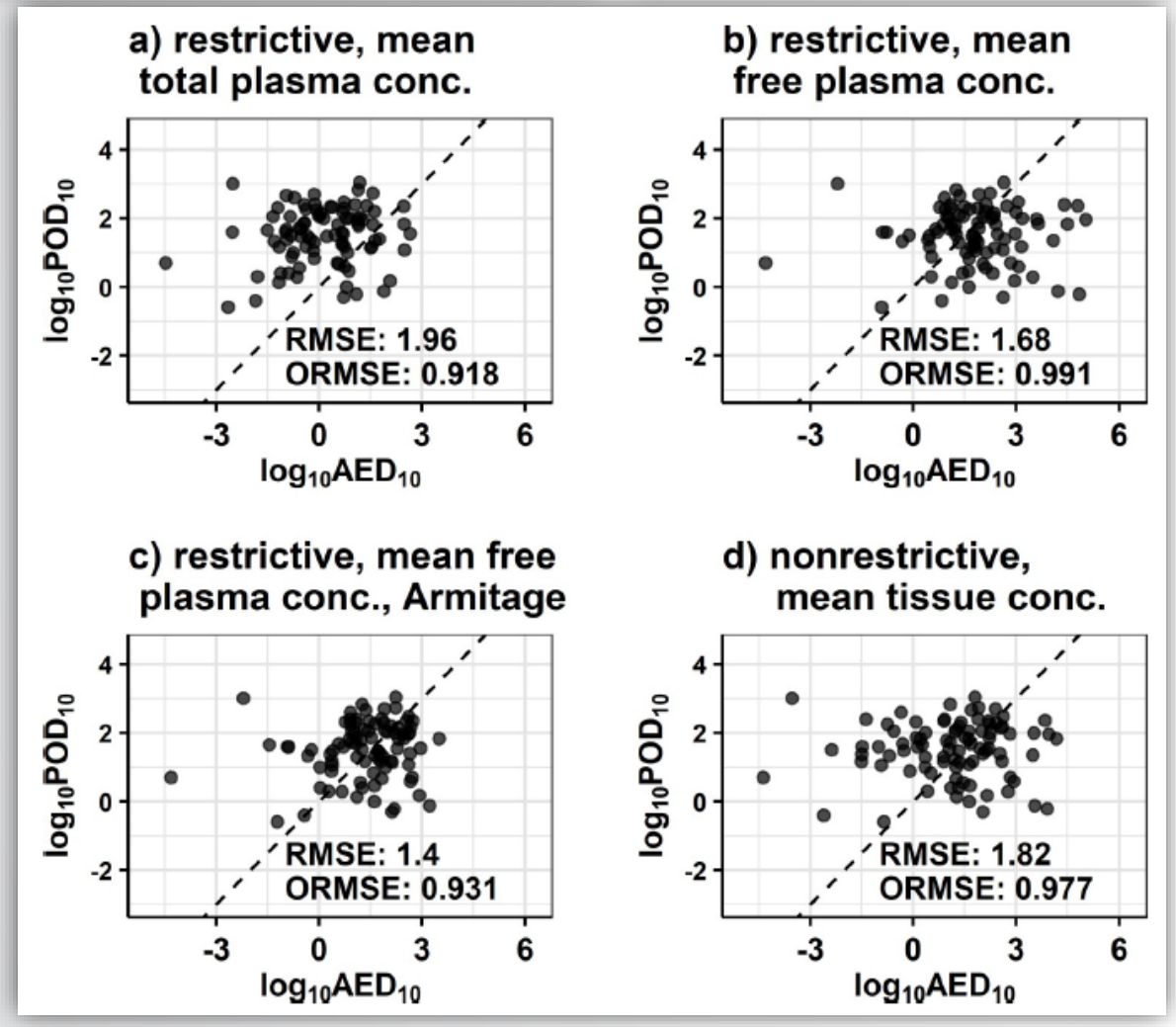
Currently, there is no way to predict or measure this property for a chemical. Restrictive clearance has been used as a conservative assumption.

Because the amount of chemical bound to protein can vary from 0-100%, the AEDs produced using a non-restrictive clearance assumption may be as much as two or three orders of magnitude greater than those produced using a restrictive clearance assumption (on a log10-mg/kg/day scale and based on current measurement ability). The amount of difference observed depends on how much of the chemical is thought to be protein-bound; the more highly protein-bound the chemical, the greater the shift observed.



Paul Friedman et al., 2020 Supplemental Appendix; 10.1093/toxsci/kfz201

Restrictive clearance with the free 'bioactive' fraction in the media may perform best



Honda et al. 2019, Figure 8; 10.1371/journal.pone.0217564

In predicting in vivo PODs, restrictive clearance with the modeled mean free (media) concentration may perform the better.

One would need good curated information and models for *in vitro* disposition of the chemical – here we have ongoing work to apply an existing model (Armitage model) to more data.

The Armitage 2014 model operationalized in Honda et al. 2019 is available in library(httk).

```
# Run the Armitage et al. (2014) model:
out <- armitage_eval(
  casrn.vector = "793-24-8",
  this.FBSf = 0.1,
  this.well_number = 384,
  nomconc = 10)

print(out)
```

What factors really influence in vitro partitioning?

- Armitage et al. (2014) suggest that in vitro partitioning relates strongly to **logK_{ow}** and concentration of **serum** in the medium

- Sorption to plastic played a smaller role in determining the cellular concentration

$$C_W = \frac{M_T}{K_{AW}V_A + V_W + K_{SaW}V_{Sa} + K_{SlW}V_{Sl} + K_{DW}V_D + K_{CW}V_C} \quad (1)$$

Mass-balance model

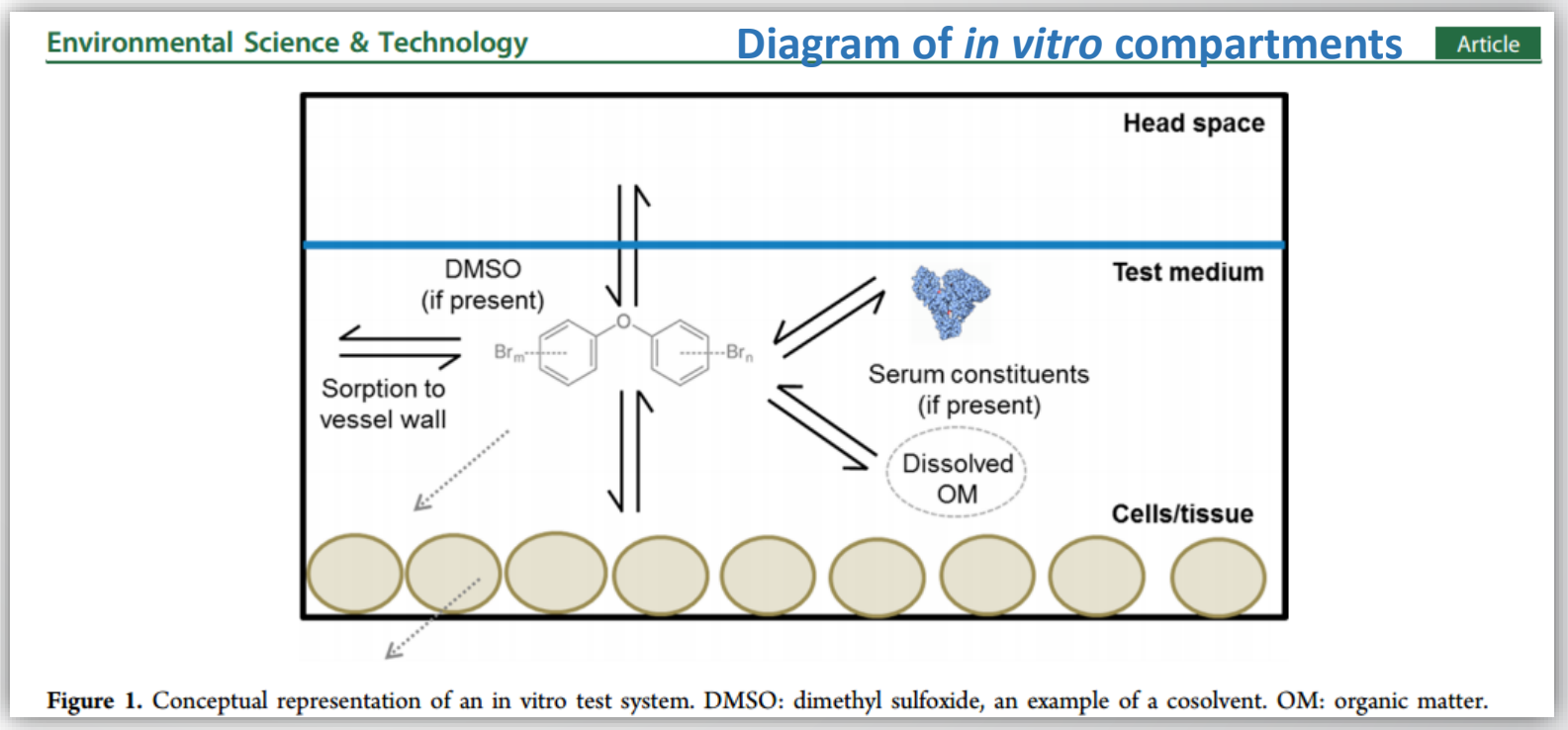


Figure 1. Conceptual representation of an in vitro test system. DMSO: dimethyl sulfoxide, an example of a cosolvent. OM: organic matter.

Armitage et al. 2014; 10.1021/es501955g

Others reinforce that lipid and protein content of media formulations may be an important determinant

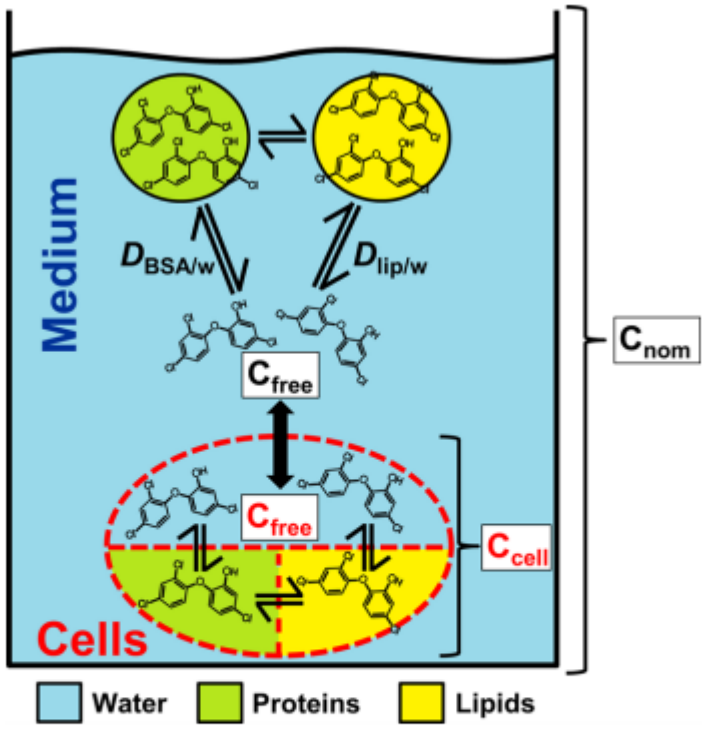


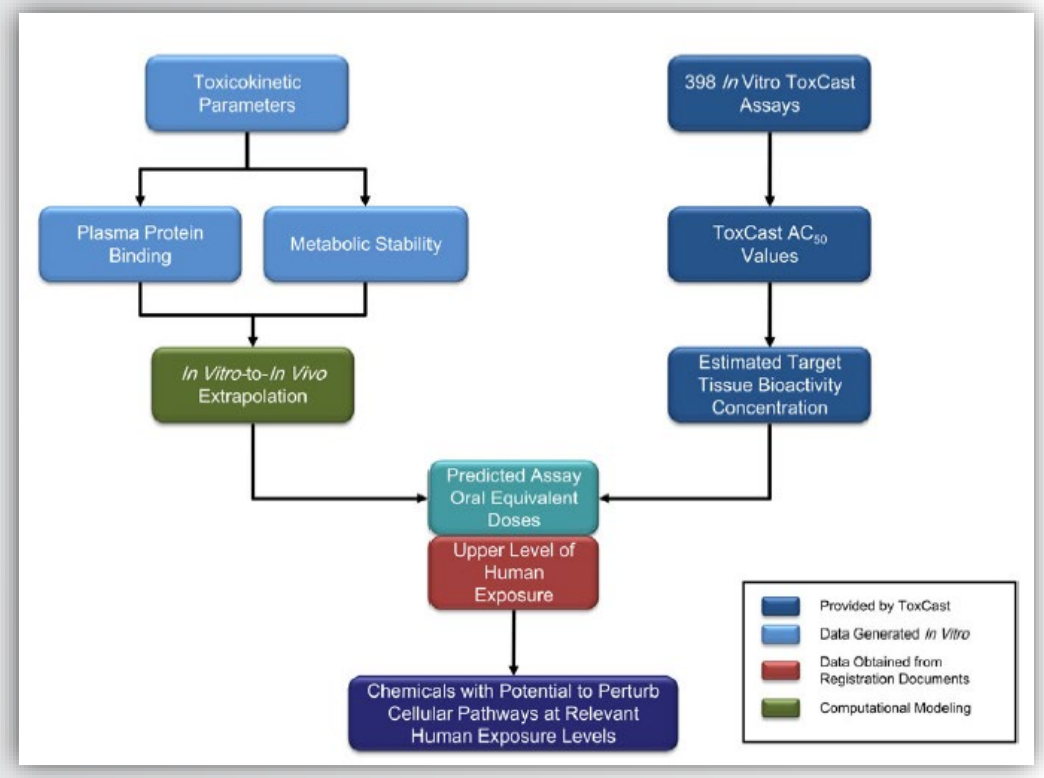
Figure 1. Mass balance model used for this study. The chemical partitioning was calculated from the distribution ratios between medium and cells at a medium pH of 7.4. Both compartments are composed of water, proteins, and lipids. Proteins and lipids are represented by BSA and lip.

- Fischer et al. (2017) suggest that in vitro partitioning relates strongly to medium formulation (lipid and protein content)
- Time may play a role; perhaps equilibrium is not always reached rapidly?
- *What we really need are some additional empirical measures and refinements to models to understand the extent to which differential partitioning is leading to large differences in cellular and media concentrations for the chemical space.*

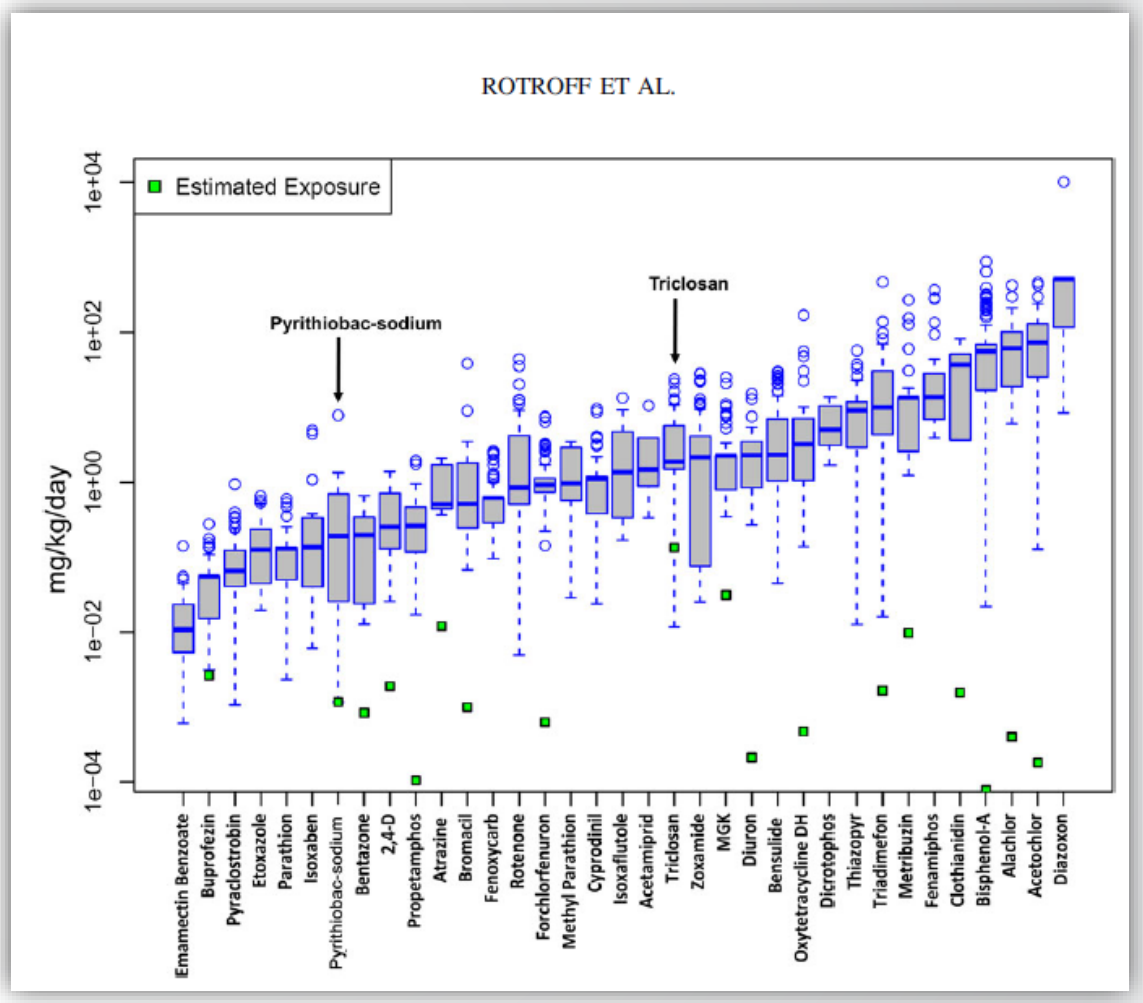
Fischer et al. 2017; Modeling Exposure in the Tox21 in Vitro Bioassays | Chemical Research in Toxicology (acs.org)

Bioactivity:exposure ratios

Bioactivity:exposure ratios are not new



Rotroff et al., 2010 10.1093/toxsci/kfq220



Many works apply HTTK to prioritization and assessment case studies

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

2011

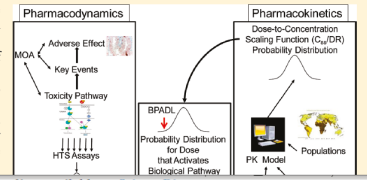
Estimating Toxicity-Related Biological Pathway Altering Doses for High-Throughput Chemical Risk Assessment

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†

†National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, United States

‡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

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[c], 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[1,2], 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[c,*]

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

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 O
[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

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]

[a] Oak Ridge Institute for Science and Education (ORISE), Oak Ridge, TN, United States
[b] Office of Science Coordination and Policy, Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, DC, United States
[c] Center for Computational Toxicology and Exposure, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, United States

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

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

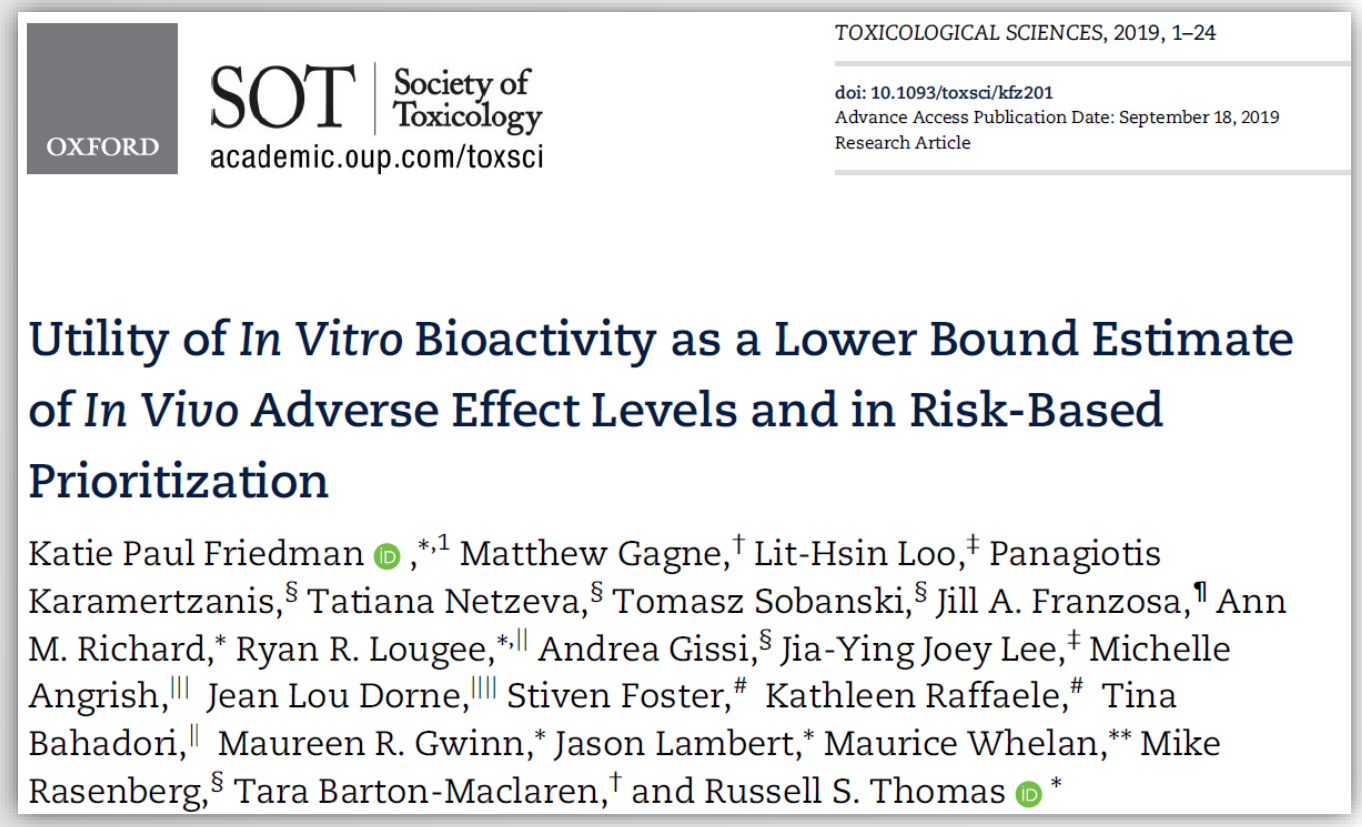
Using the concordance of *in vitro* and *in vivo* data to evaluate extrapolation assumptions

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

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



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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

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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

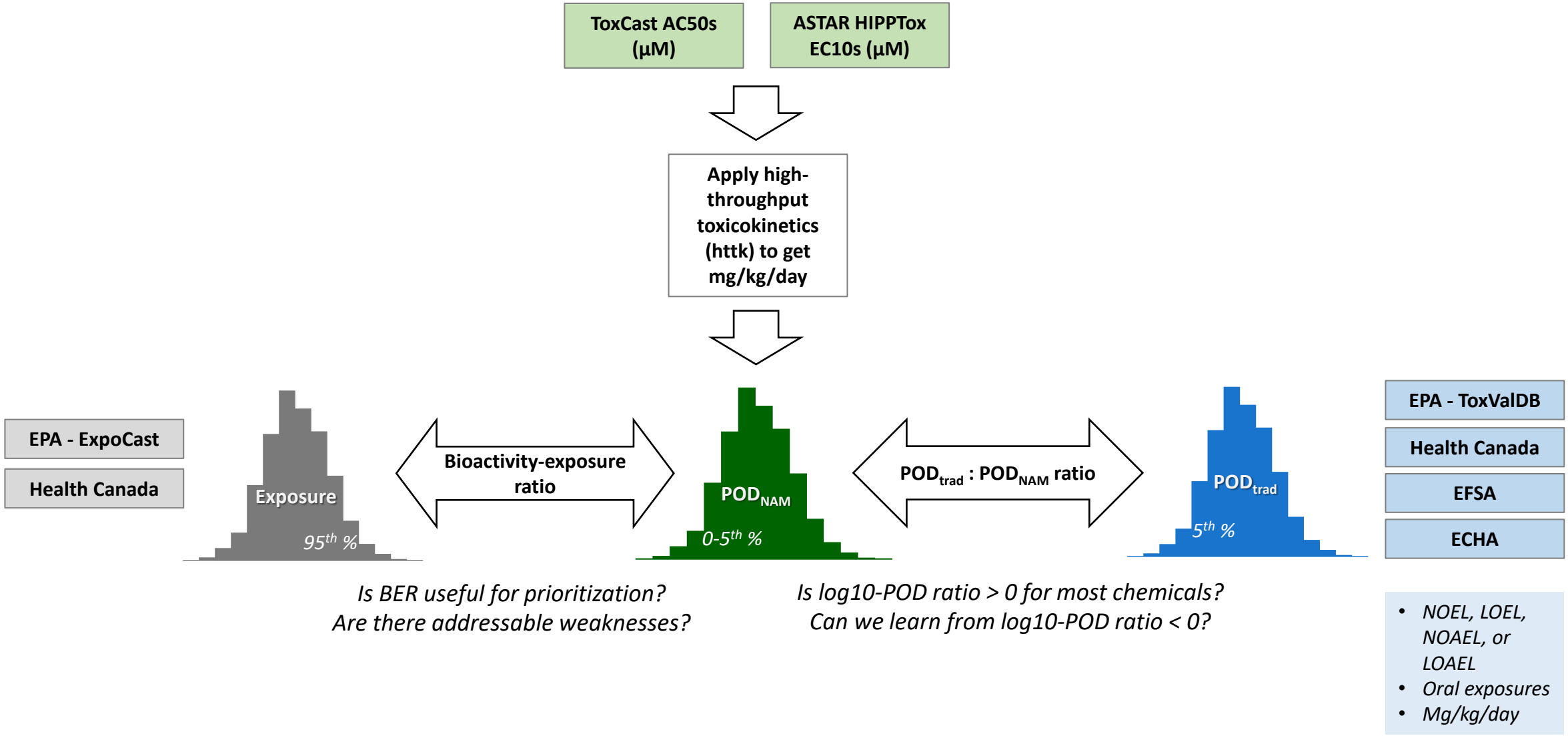
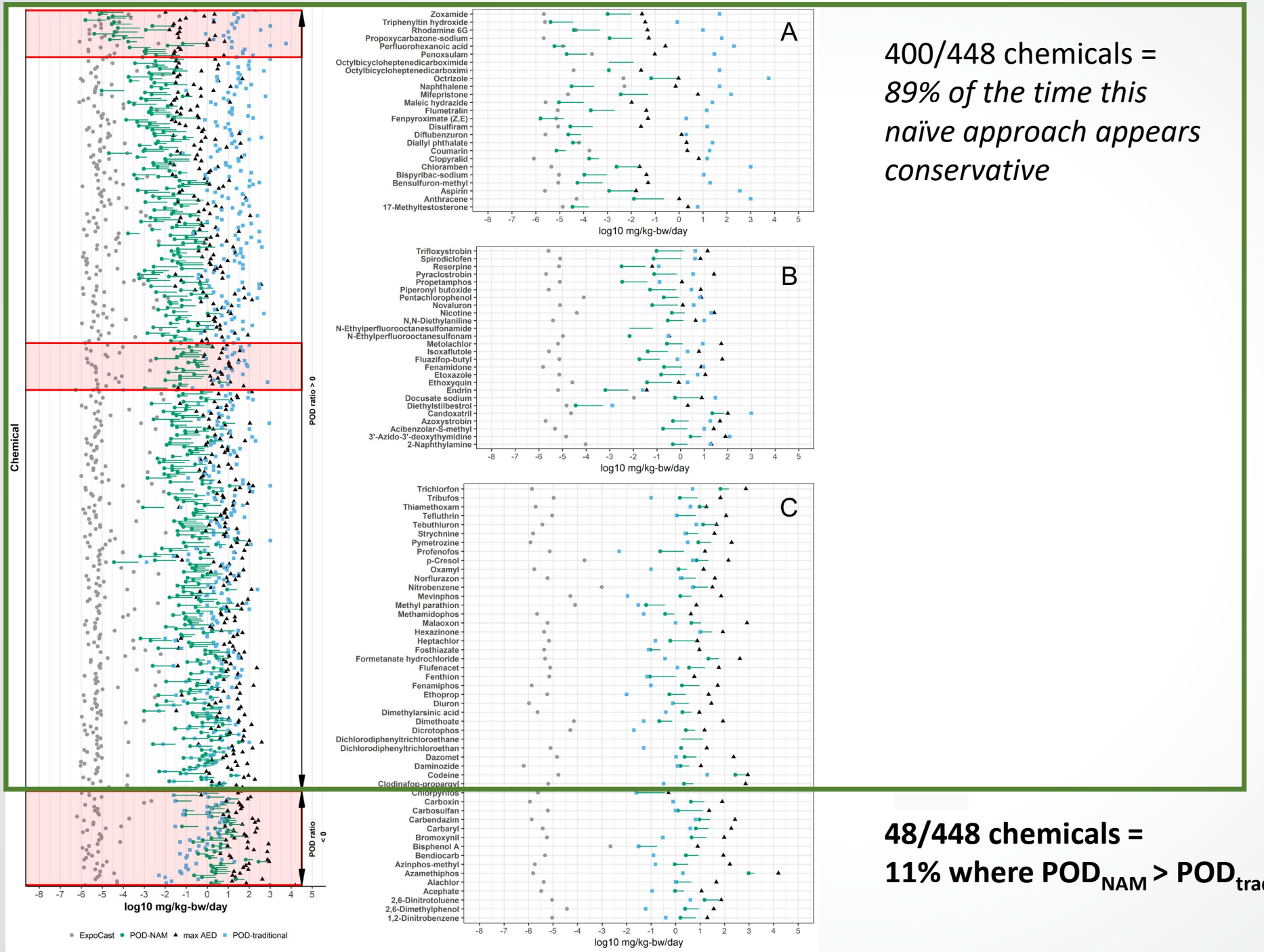


Figure 1, Paul Friedman et al. *2019*



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

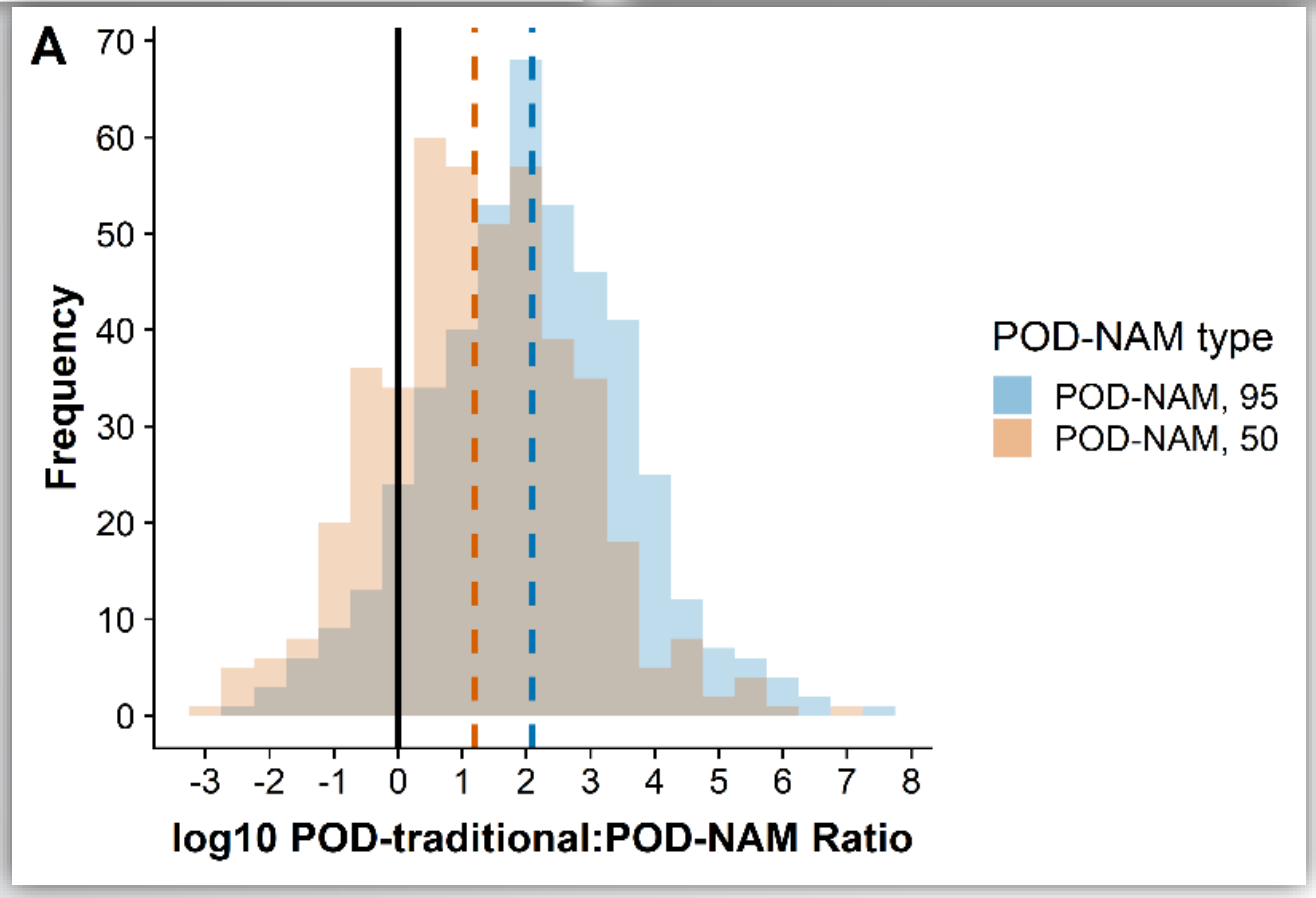
48/448 chemicals =
11% where $POD_{NAM} > POD_{traditional}$

$POD_{NAM} < POD_{traditional}$

(most of the time)

Figure 3, Paul Friedman et al. *2019*

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

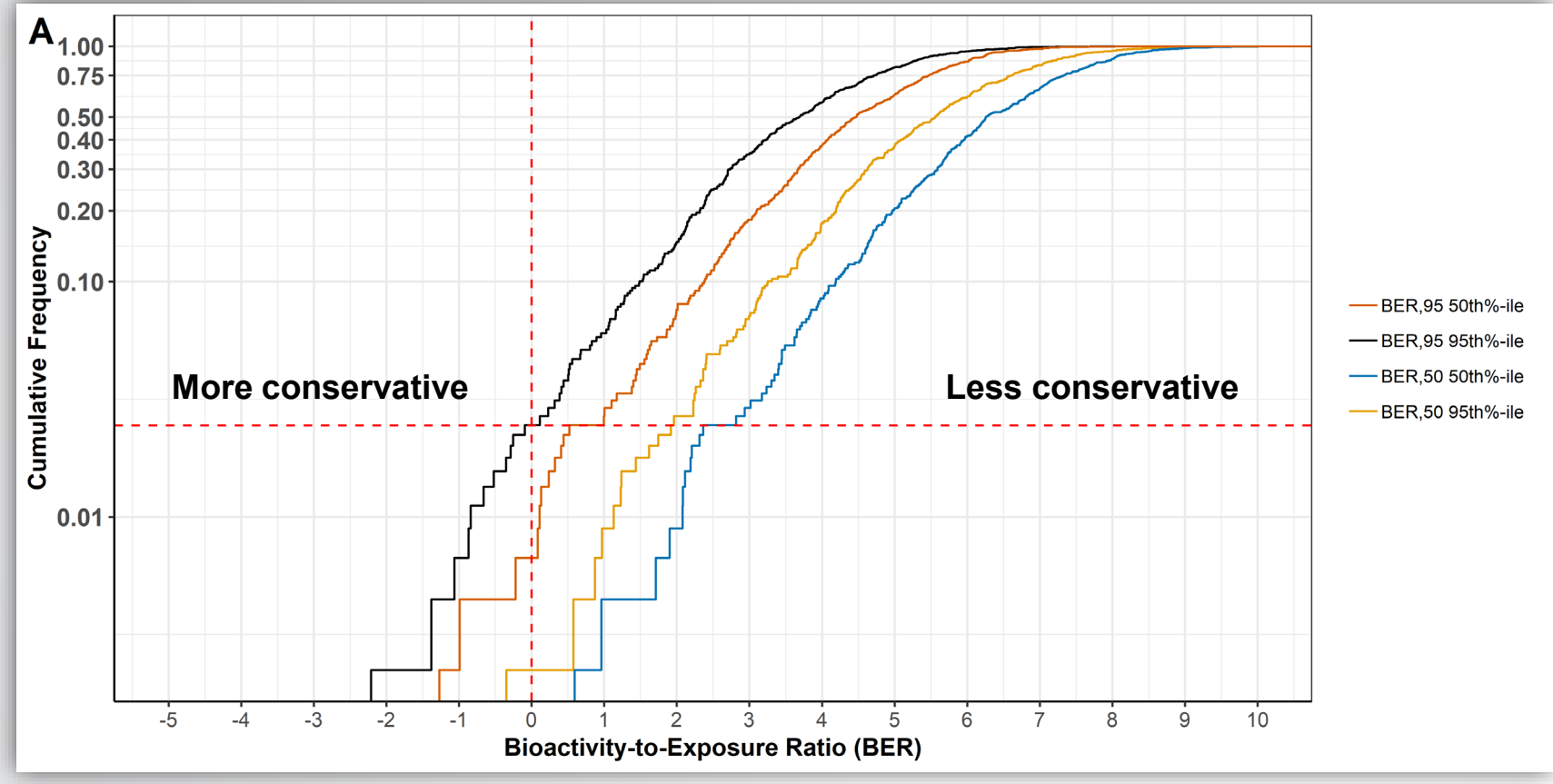


$POD_{NAM,95}$ includes interindividual variability in the in vitro to in vivo extrapolation process to a greater extent and is more often a conservative estimate of $POD_{traditional}$.

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.

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



- <u>Make choices based on tolerable uncertainty (i.e., based on use case).</u>
- 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.

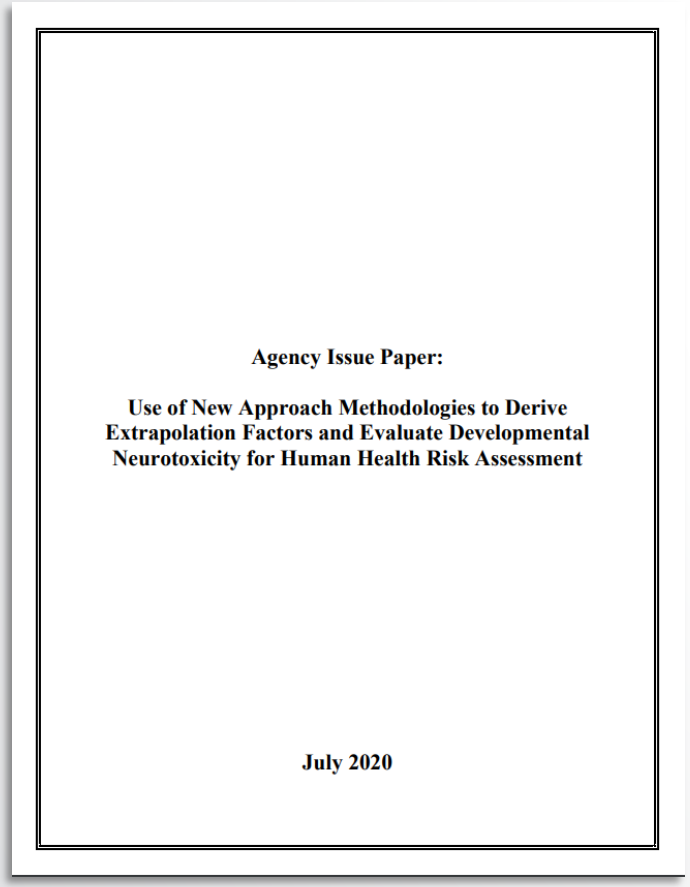
BER_{95} , 95th percentile did not prioritize an unreasonable number of substances; the BER selected reflects the level of conservatism and uncertainty considered within a screening assessment.

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.

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

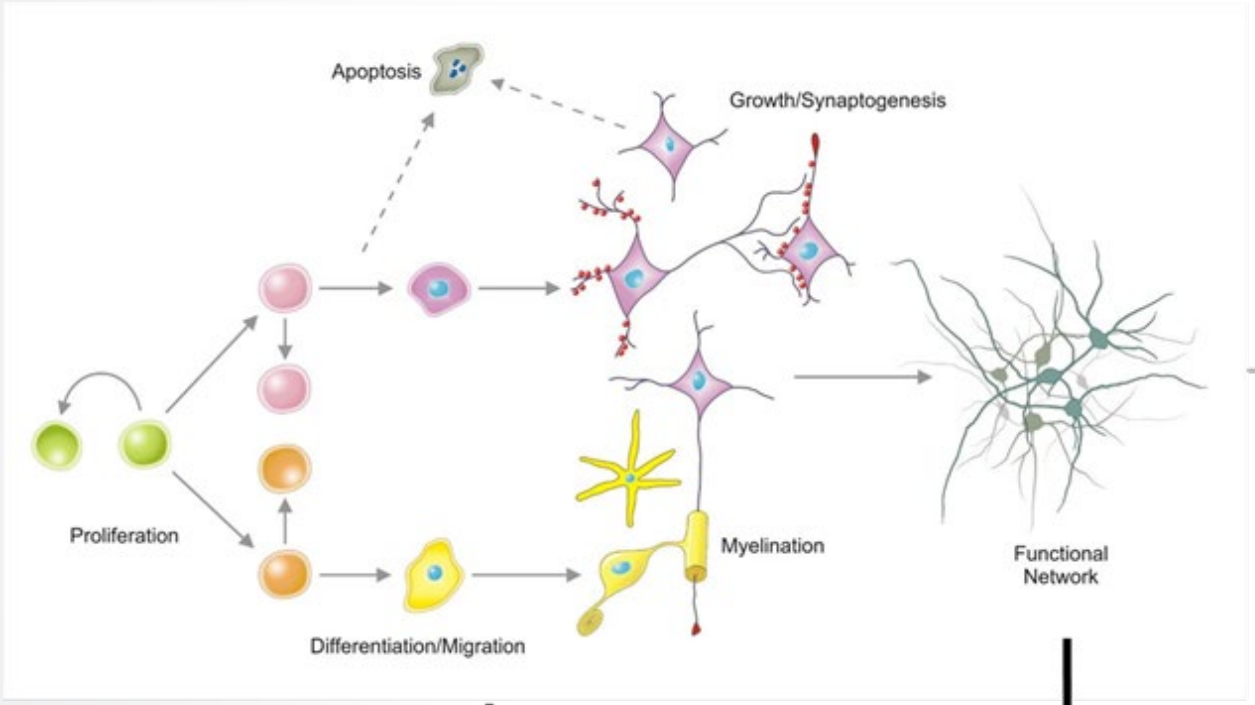
ORD DNT NAMs Team: Josh Harrill, Tim Shafer, Katie Paul Friedman

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

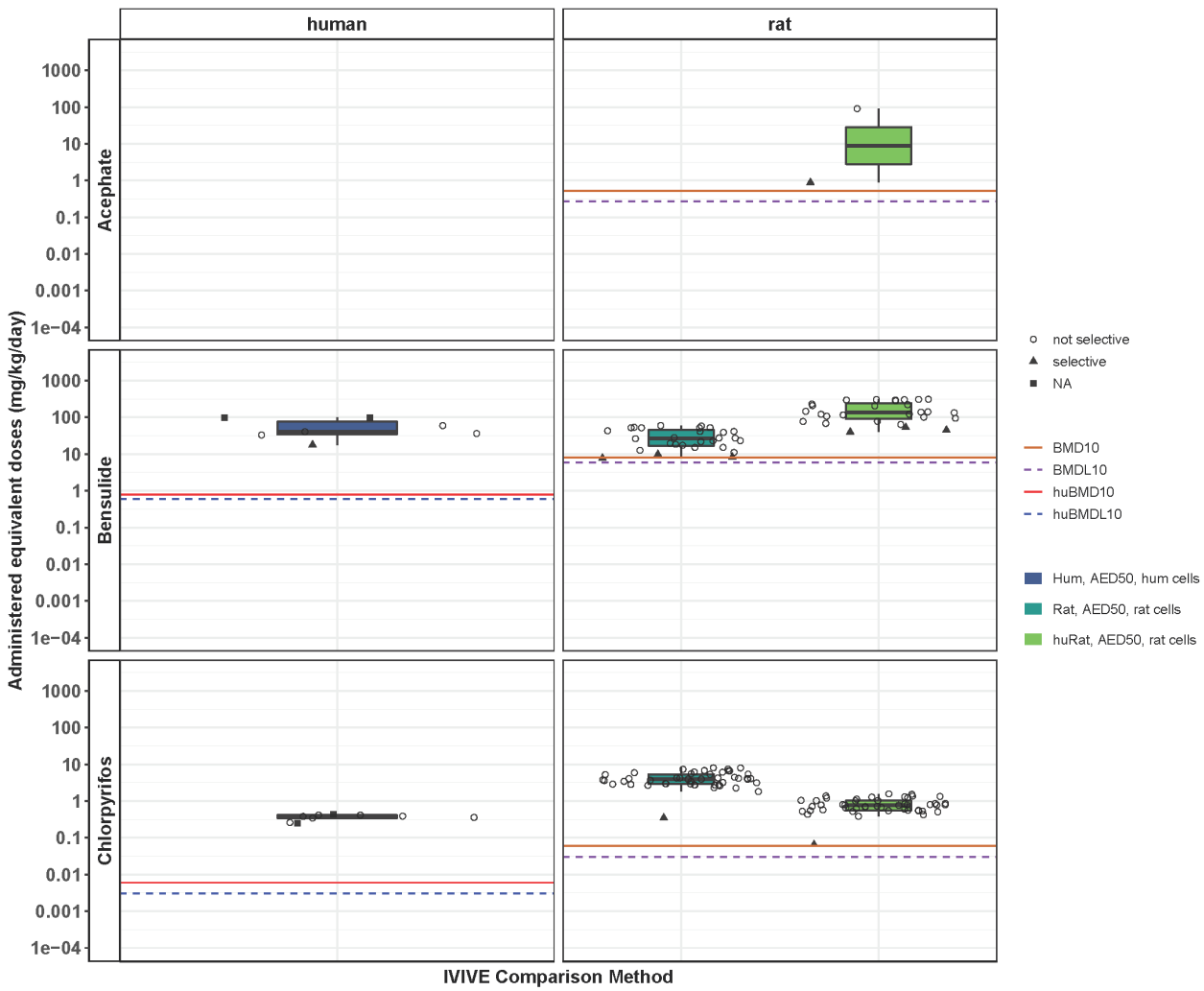
https://beta.regulations.gov/document/EPA-HQ-OPP-2020-0263-0006
Code here: https://www.epa.gov/sap/use-new-approach-methodologies-nams-derive-extrapolation-factors-and-evaluate-developmental

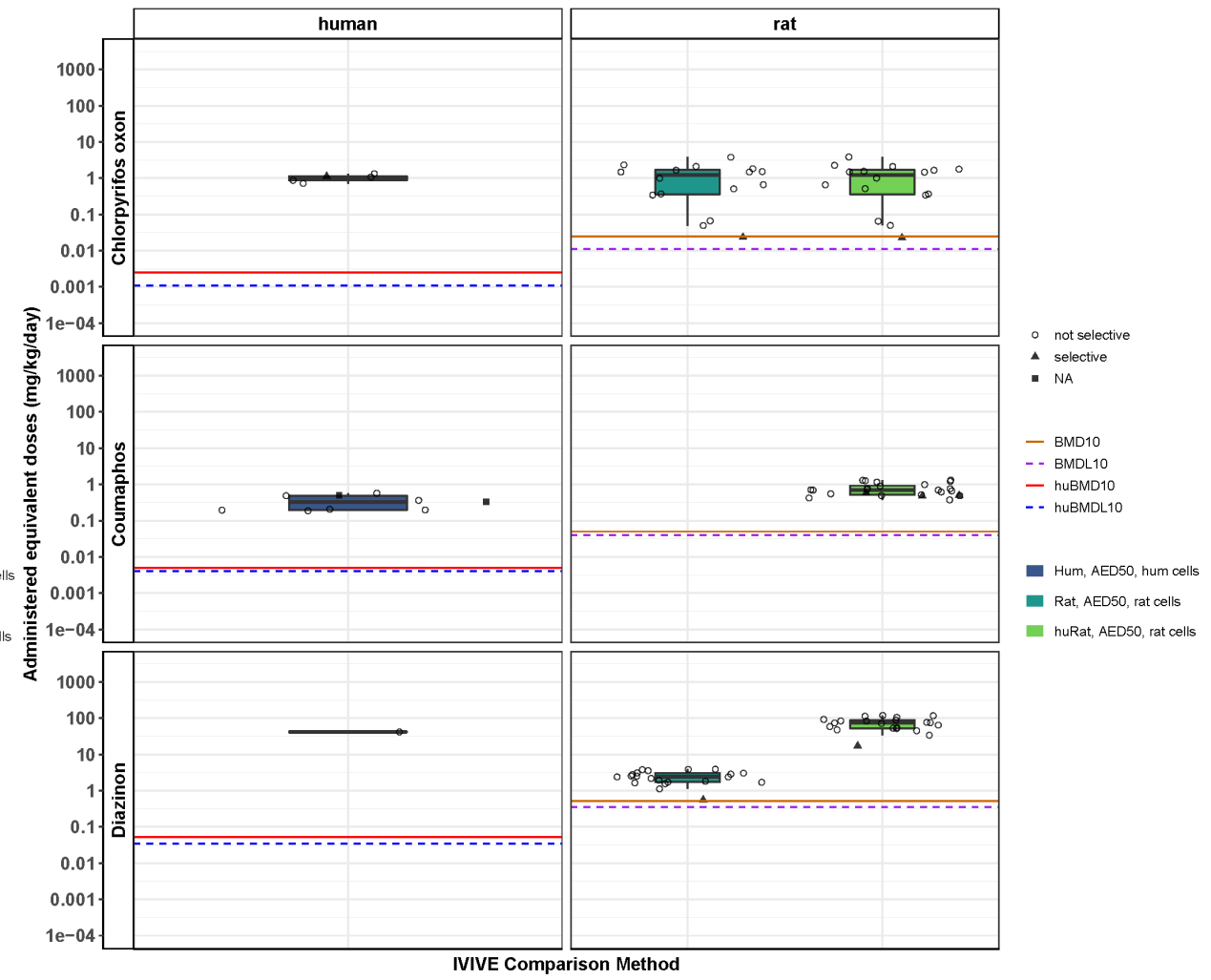
Phenotypic Screening for DNT Hazard

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



Example: AED50 to BMD/BMDL10 comparisons





Employing toxicokinetic and toxicodynamic NAMs

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

- 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?

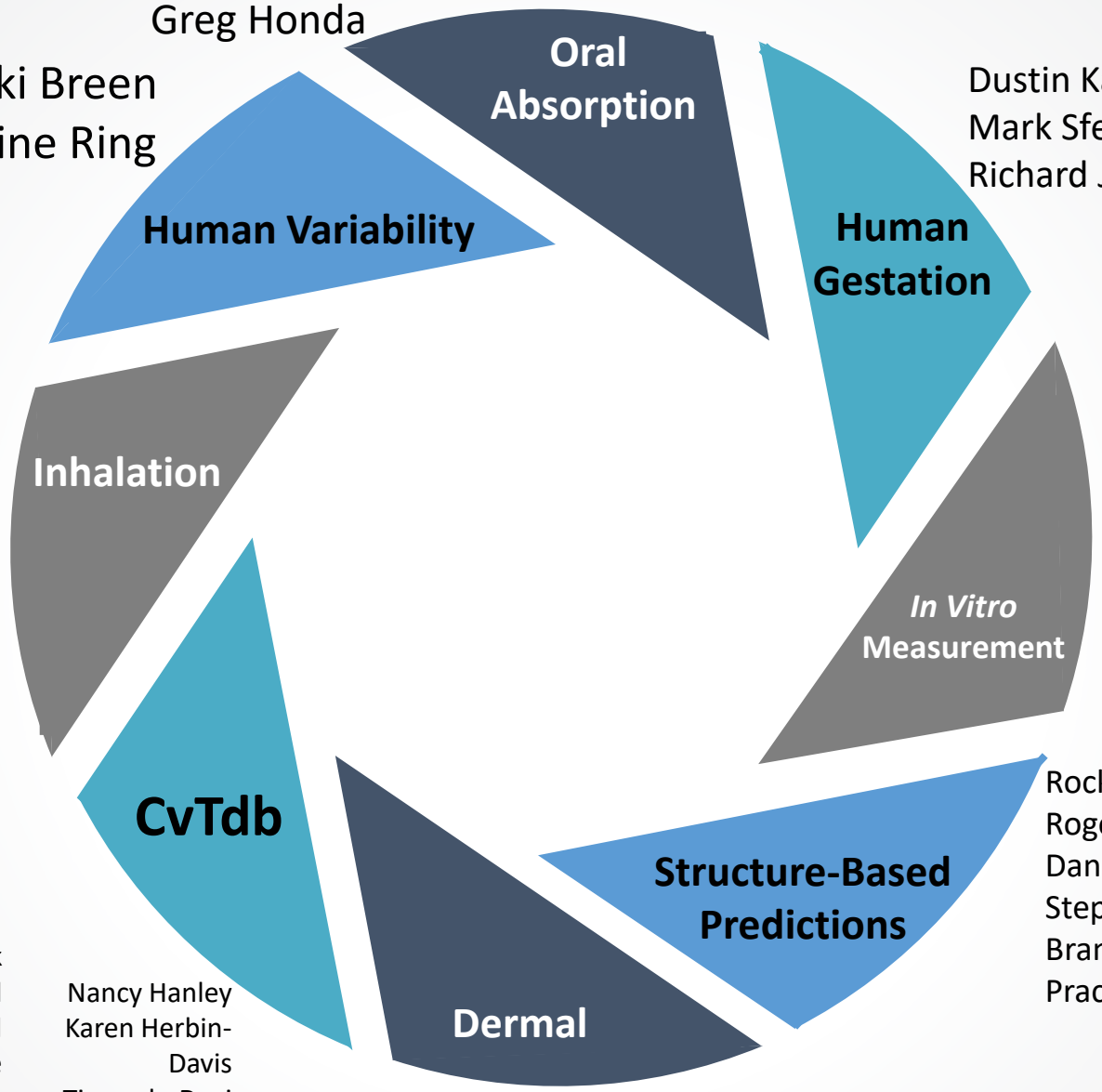
- **Chemical safety assessment with fewer resources is a motivator for rapid data acquisition and model development.**

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

Conclusions

- Reverse dosimetry is a powerful tool for deriving NAM-based points-of-departure for different chemical screening and assessment applications.
- The details of the choices made in the IVIVE approach have impacts on the POD_{NAM} derived, and uncertainties and assumptions should be explained.
  - R library(httk) provides a simple way for users to operationalize generic HTTK models and *in vitro* toxicokinetic data to derive POD_{NAM} from *in vitro* bioactivity data such as ToxCast data.
  - For some applications, conservative assumptions can be more tolerated.
  - Ongoing research will further inform sets of decisions for specific chemicals chemical assessment contexts (e.g., improvements and application of *in vitro* chemical disposition modeling).
- Ongoing work to compare POD_{NAM} to existing PODs as well as to values obtained through other PBTK approaches will provide important benchmarks on HTTK approaches to increase the acceptance of POD_{NAM} and BERs.

HTTK Team



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Funded by EPA's Office of Research and Development and Office of Science Coordination and Policy

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