In Vitro to In Vivo Extrapolation Incorporating Toxicokinetics

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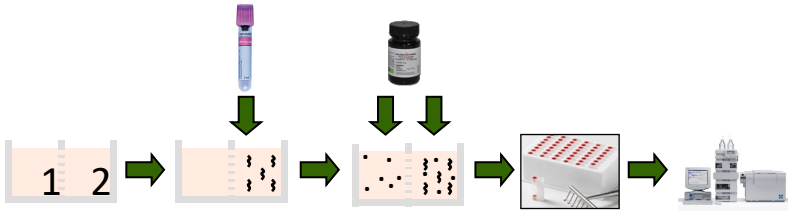
High-throughput toxicokinetic (HTTK) approaches enable in vitro to in vivo extrapolation (IVIVE) of dose for thousands of chemicals

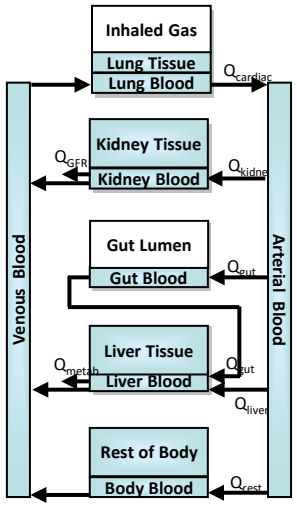
in vitro toxicokinetic data

Hepatic clearance from suspended hepatocytes



Plasma protein binding

Generic toxicokinetic models



= **httk**

Some high-level assumptions commonly employed to-date:

(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 PBTK).

Led by John Wambaugh, Barbara Wetmore, Caroline Ring, and colleagues

Many works have applied HTTK to prioritization and assessment case studies over the last decade

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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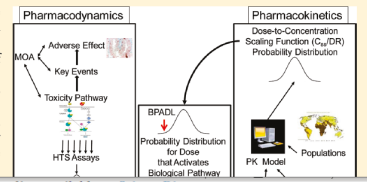
ABSTRACT: We describe a framework for estimating the human dose at which a chemical significantly alters a biological pathway *in vivo*, making use of *in vitro* assay data and an *in vitro*-derived pharmacokinetic model, coupled with estimates of population variability and uncertainty. The quantity 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

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*

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Research Article

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2018
Toxicology in Vitro
journal homepage: www.elsevier.com/locate/toxinvit

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
ELSEVIER
2019
Food and Chemical Toxicology
journal homepage: www.elsevier.com/locate/foodchemtox

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
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2020
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journal homepage: www.elsevier.com/locate/envint

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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2019

TOXICOLOGICAL SCIENCES, 2019, 1–24
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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
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2020
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journal homepage: www.elsevier.com/locate/taap

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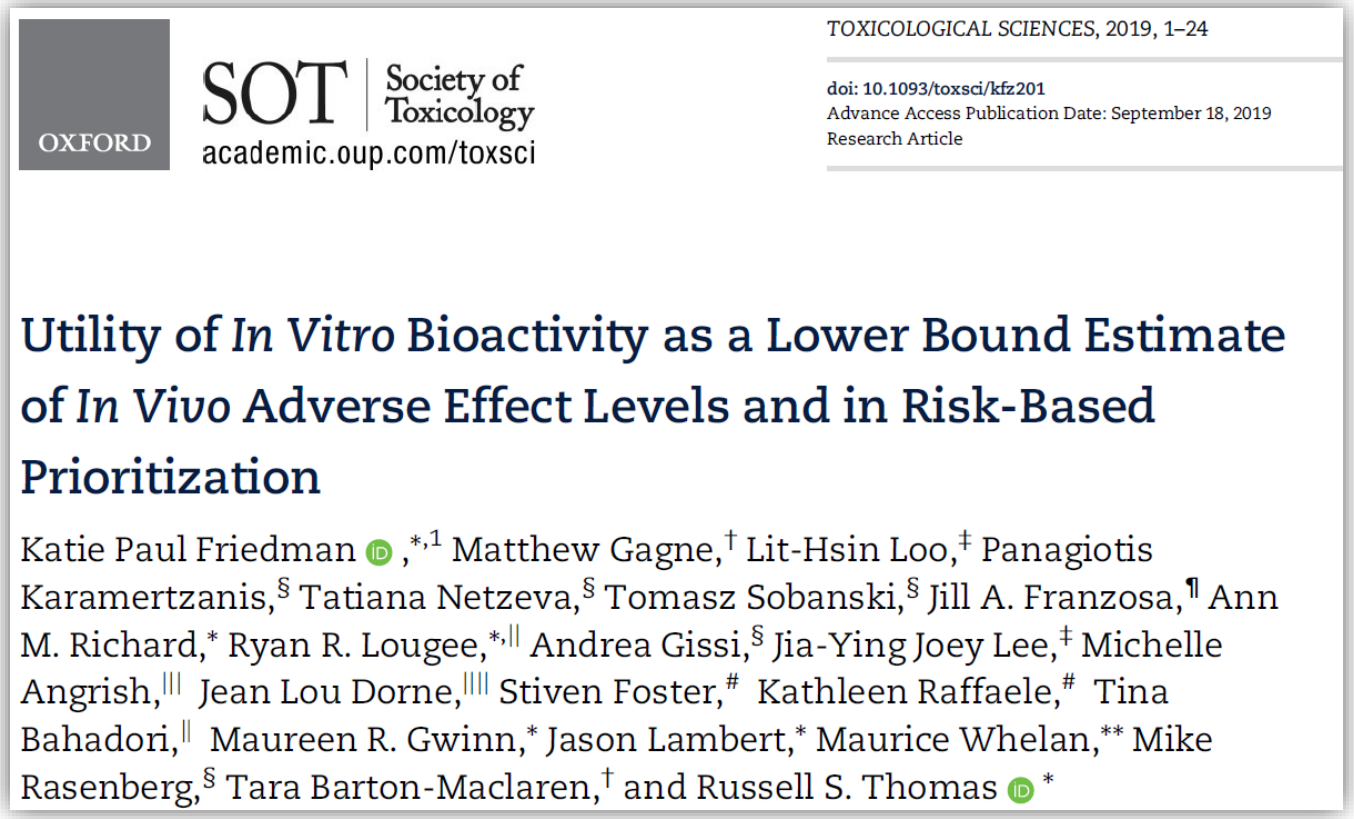
A subset of the papers describing the application of a high-throughput toxicokinetic approach – too many to fit

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



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doi: 10.1093/toxsci/kfz201
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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 *

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

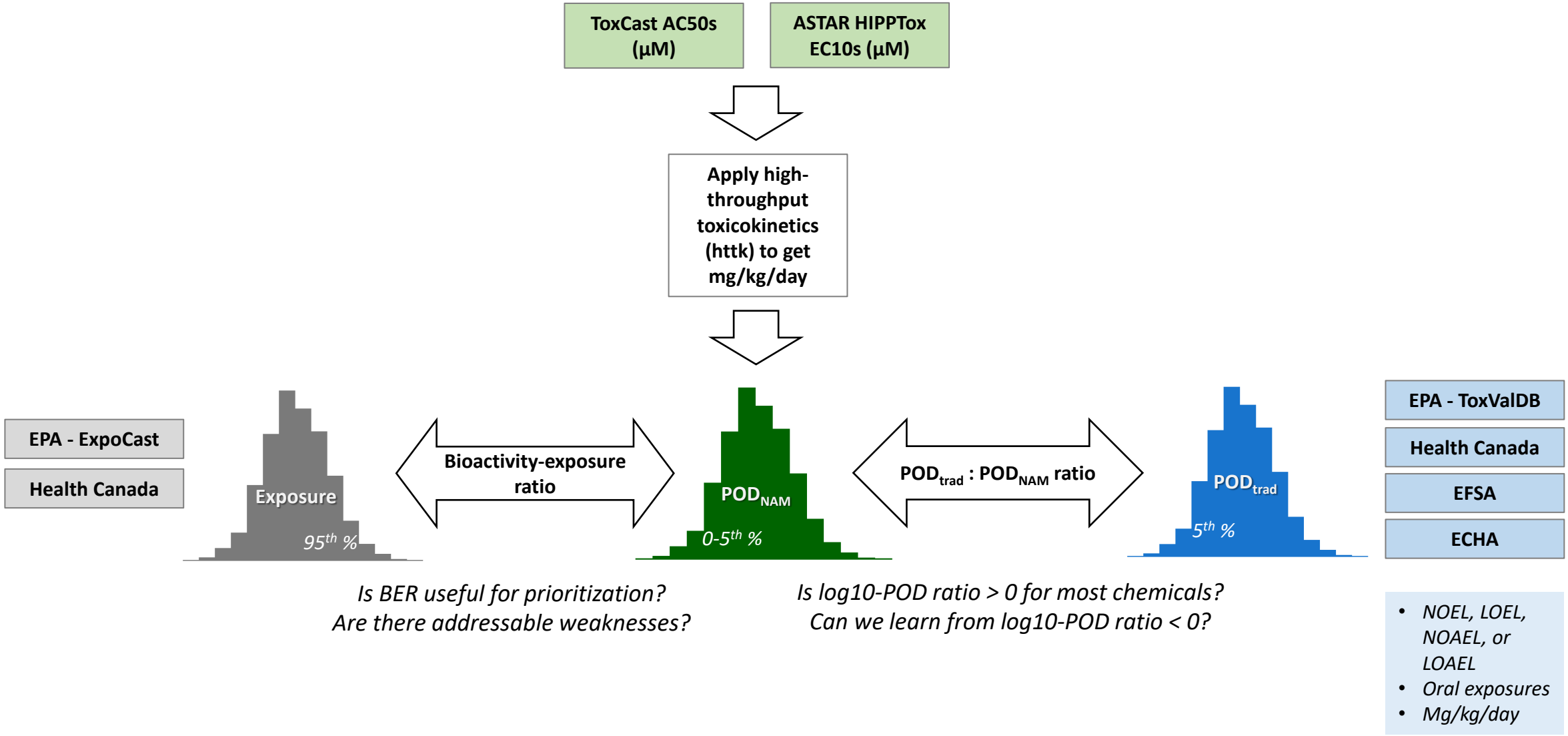
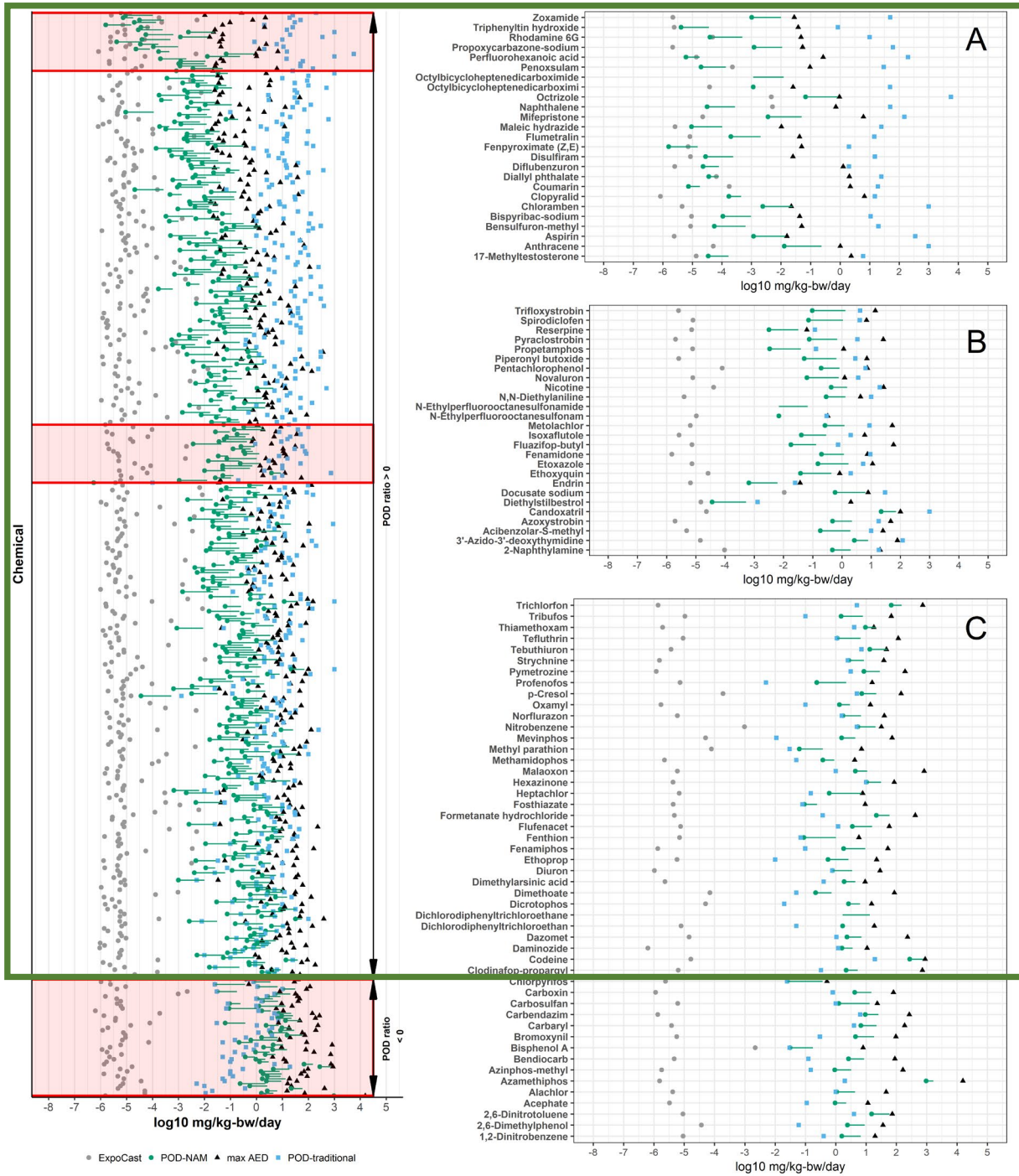


Figure 1, Paul Friedman et al. *2019*



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

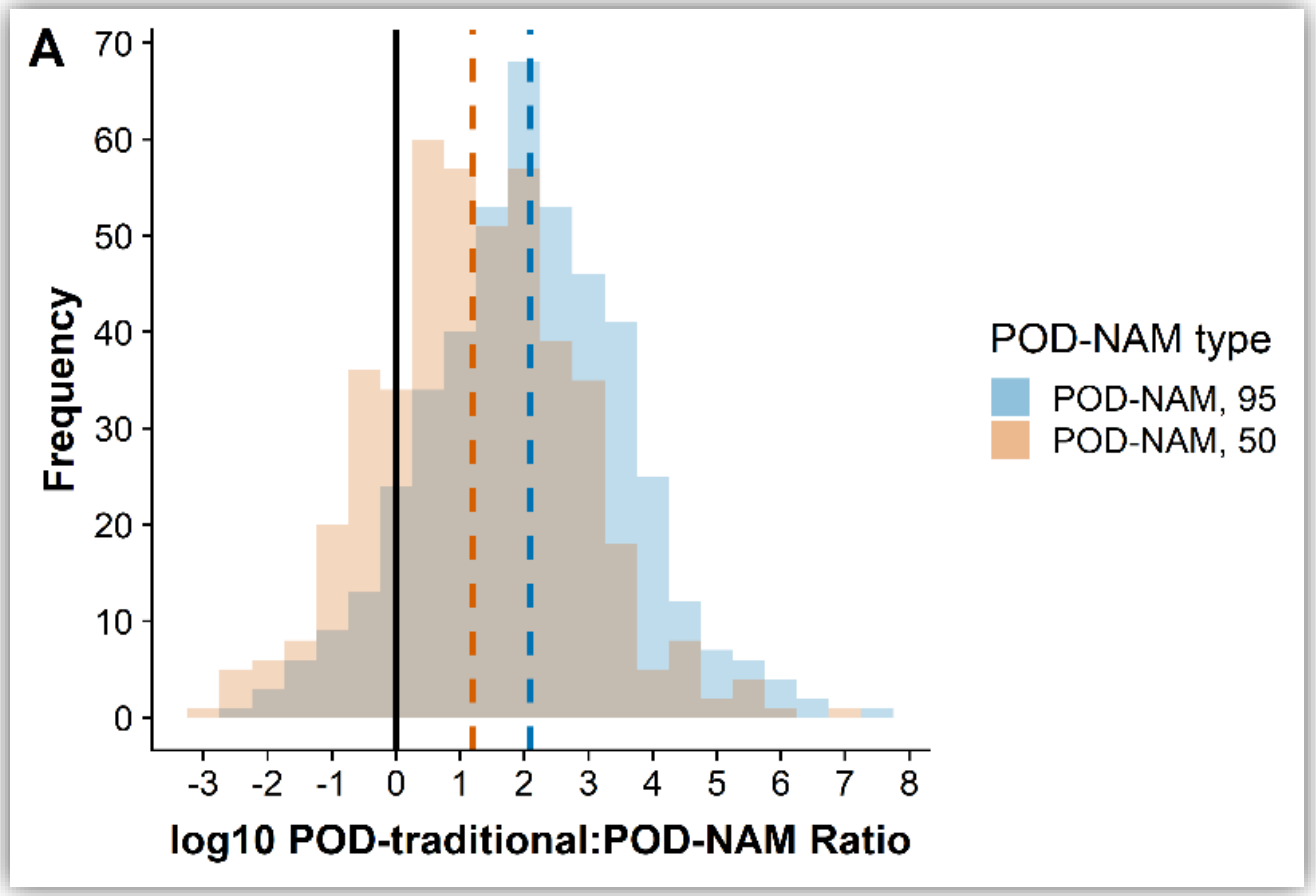
48/448 chemicals =
11% where $POD_{NAM} > POD_{traditional}$

$POD_{NAM} < POD_{traditional}$

(most of the time)

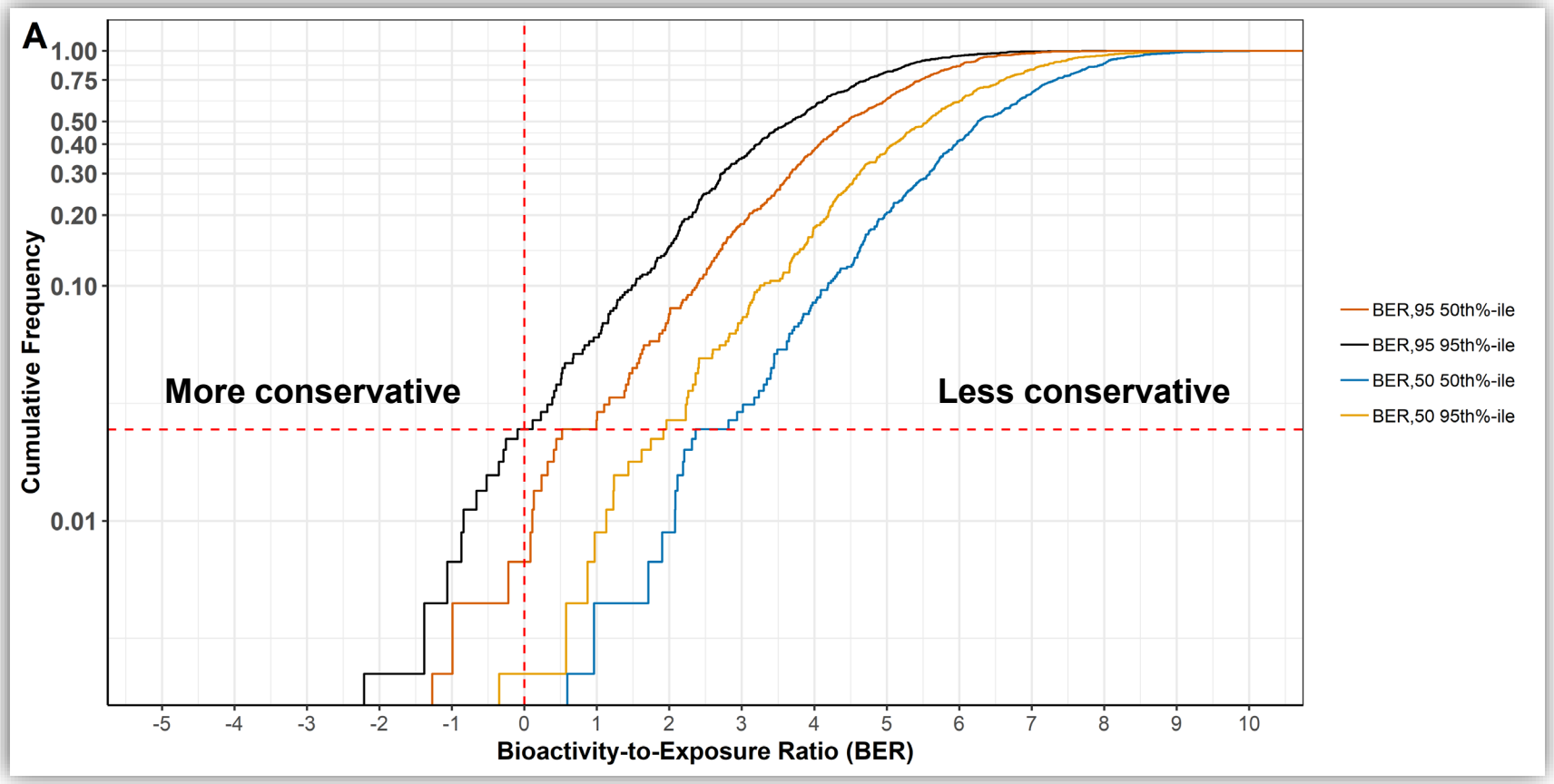
Figure 3, Paul Friedman et al. *2019*

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 and is more often a conservative estimate of $POD_{traditional}$.

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



BER_{95} , 95^{th} percentile did not prioritize an unreasonable number of substances.

The BER selected reflects the level of conservatism and uncertainty considered within a screening assessment.

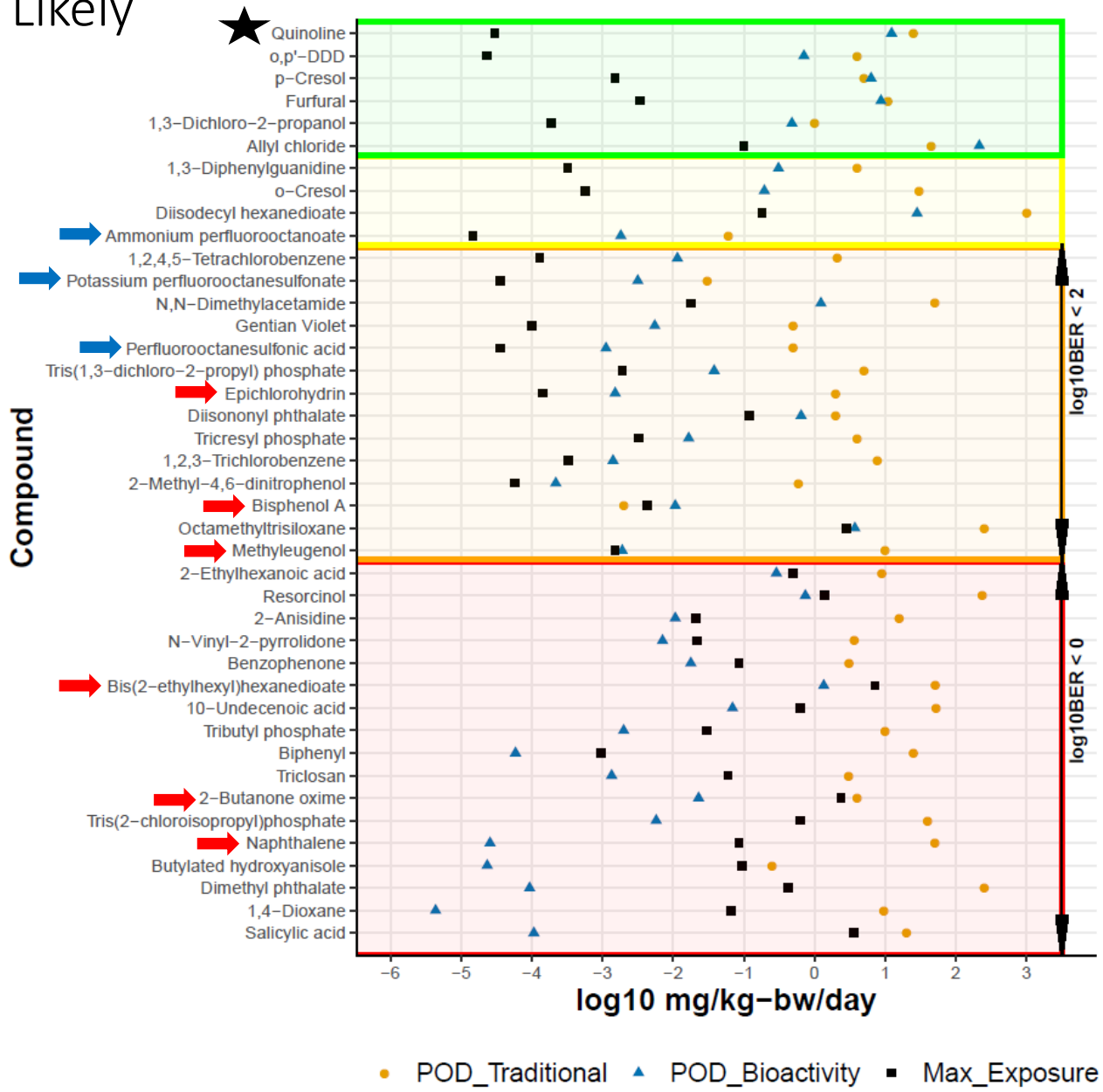
Case study learnings and limitations

- 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 thresholds of toxicological concern.
- When combined with high-throughput exposure estimates, this approach provides a reasonable basis for risk-based prioritization and screening level risk assessments.

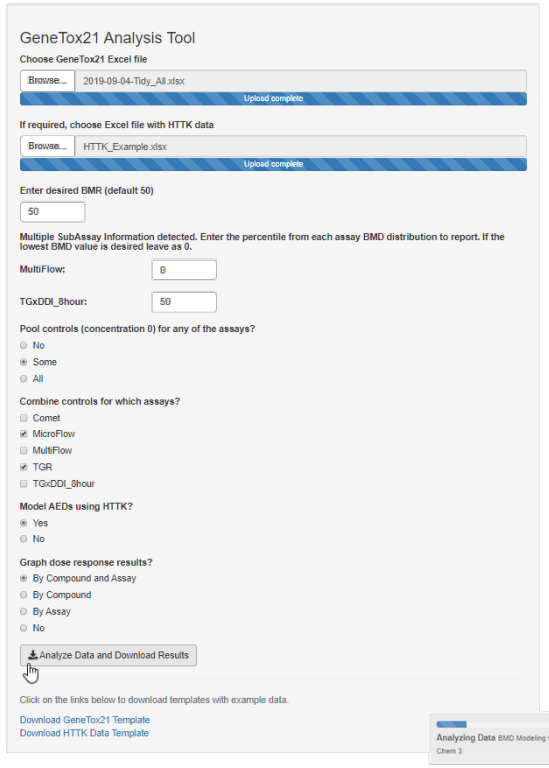
- Specific types of chemicals may be currently outside the domain of applicability due to assay limitations, e.g., organophosphate insecticides: how do we identify these in the future?
- This is the largest retrospective look at this to-date; but what if new chemicals perform differently?
- Additional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

Chemicals Concluded Toxic Under CEPA More Likely to have Low BERs

- Health Canada conducted follow-up study to support development of guidance Science Approach Document
- Results show that $POD_{Bioactivity}$ lower than $POD_{Traditional}$ for 38 out of 41 chemicals
- All **non-genotoxic** compounds considered toxic to human health (red arrows) or ecotoxic (blue arrows) had a BER < ~100
- One toxic chemical (**Quinoline**), considered as a potential genotoxin, was identified as low priority using this approach (star)
- There are only five assays in ToxCast that measure DNA damage or stalled replication and these have low sensitivity
- Thus, a parallel approach that builds on these experiences but uses genotoxicity assays is needed



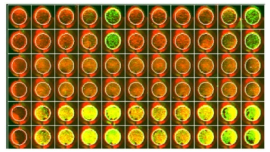
Complementary Approach that Includes Genetic Toxicology Data is Needed



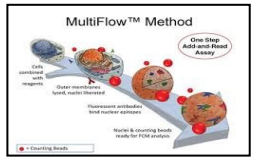
https://mbeal.shinyapps.io/genetox21_app/

25 Reference Chemicals

PrediScreen

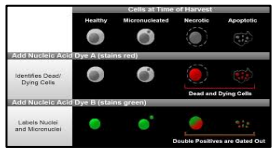


MultiFlow

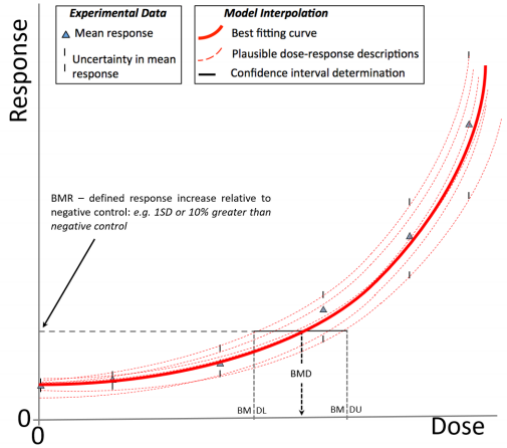


ToxTracker

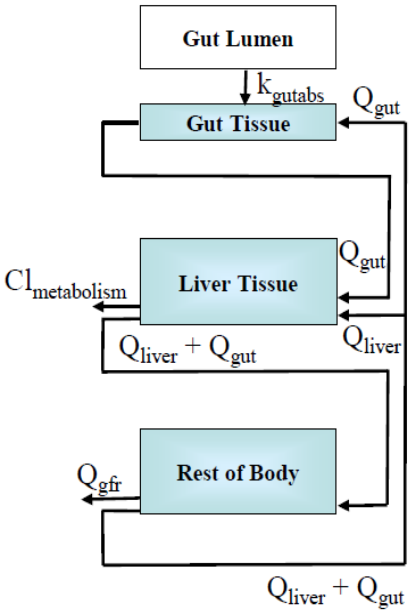
MicroFlow



TGR



Benchmark Concentration Modeling



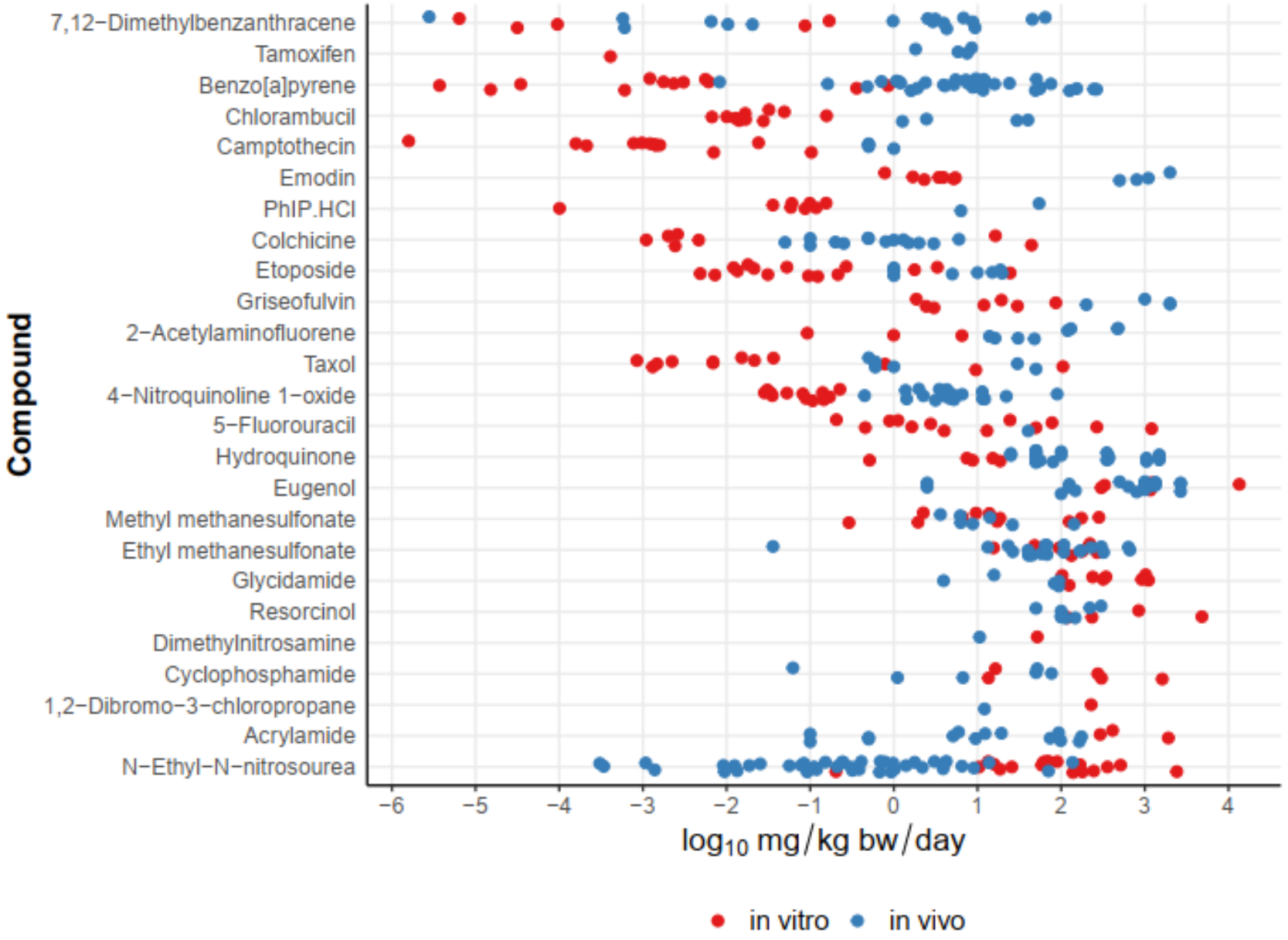
***in vitro* to *in vivo* Extrapolation (IVIVE)**

Genotoxic Administered Equivalent Dose (G-AED; mg/kg bw/day)

Health and Environmental Sciences Institute

Genetic Toxicology Technical Committee

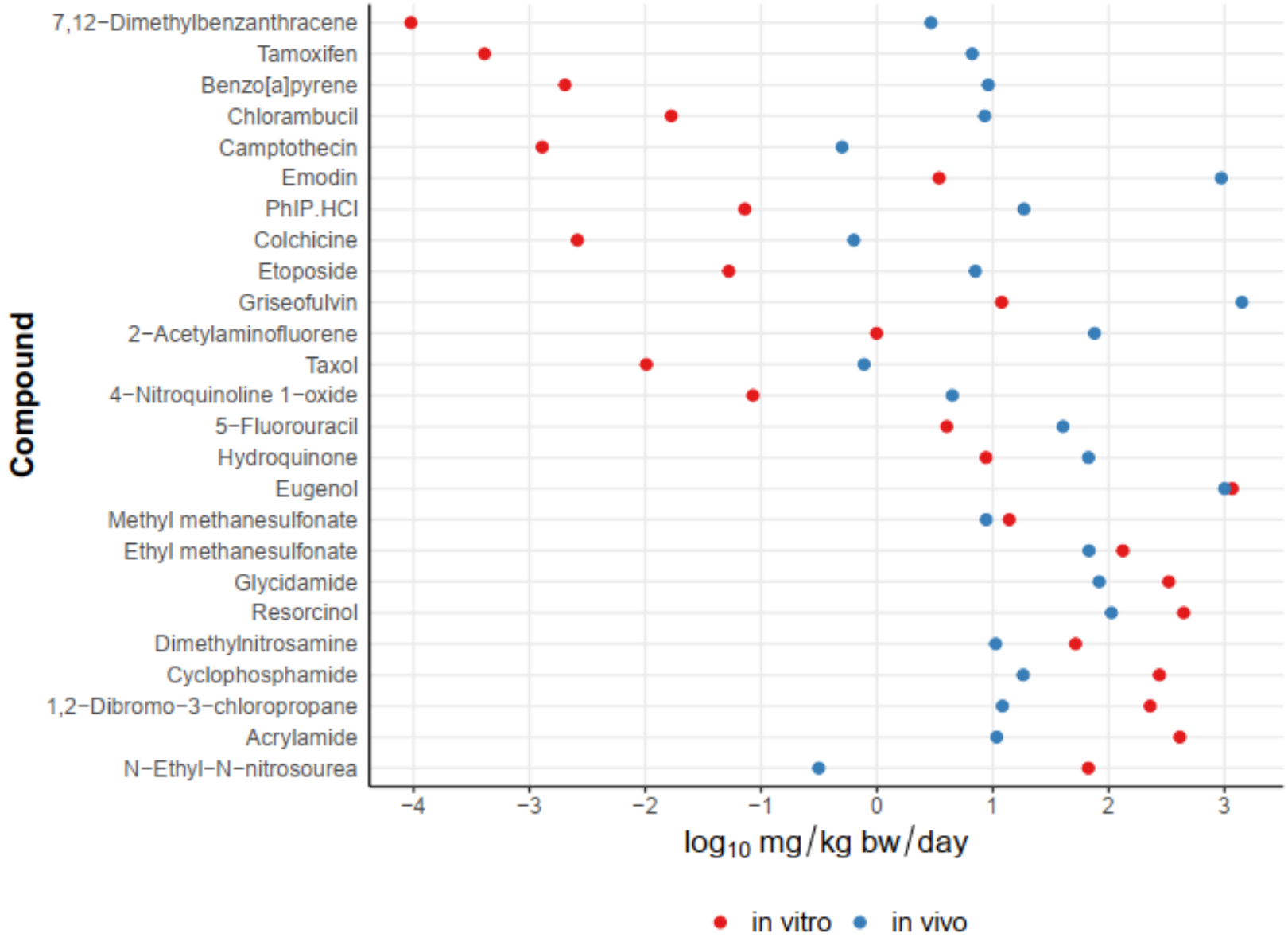
IVIVE Application to Genetox Data Provides Protective PODs



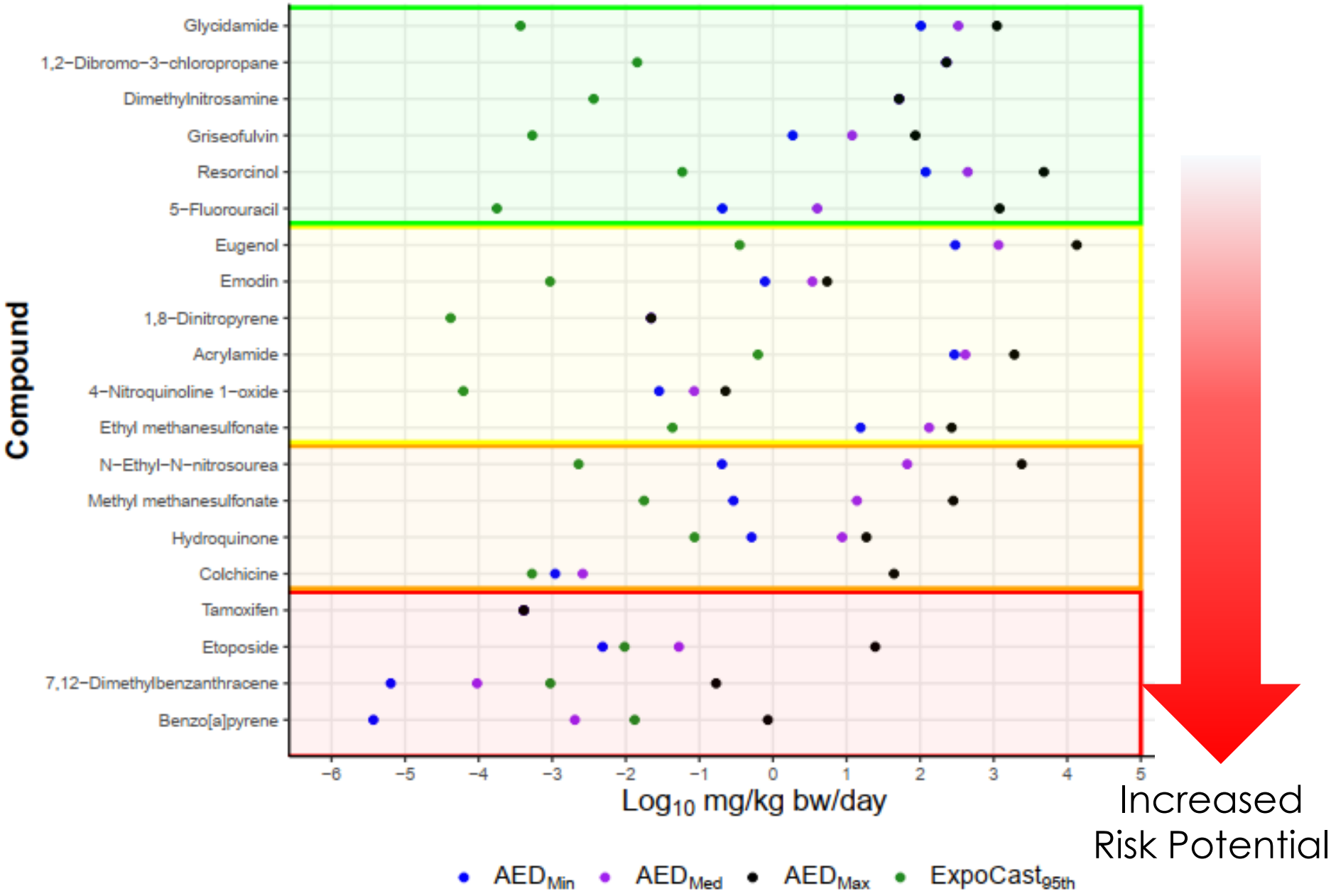
(1) Median AED Lower than Median *in vivo* POD for Most Chemicals

(2) AEDs that are not protective tend to be within one order of magnitude of *in vivo* POD

(3) ENU positive control had an AED that was much higher than POD



Bioactivity Exposure Ratios Help to Identify Chemicals with the Highest Potential for Concern



Conclusions and Future Directions

- **Reverse dosimetry is a powerful tool for deriving NAM-based PODs for different chemical screening and assessment applications**
- **IVIVE supports *in vitro* testing strategy for deriving conservative PODs**
  - Protective trend first demonstrated with bioactivity data from ToxCast
  - Trend consistent with genotoxicity NAM endpoints
  - Opportunity to explore other models to enhance the approach for chemicals where the PODs were not conservative
    - Decision trees that include thresholds of toxicological concern or other *in silico* alerts
    - Higher tier PBTK models
    - Mass balance modeling to account for *in vitro* disposition
    - Refinement of assumptions on a chemical basis in IVIVE, e.g. bioavailability, renal transport, restrictive clearance
- **IVIVE/Genetox approach could support chemical safety evaluation without the use of animals**
  - Rapid screening and priority setting
  - Guidance documents
- **Need to build confidence using a broad chemical space**
  - Genetic toxicology case study limited to well-established genotoxicants
  - Prospective case studies needed to evaluate emerging chemicals of concern
  - 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.

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ACCELERATING THE PACE OF CHEMICAL RISK ASSESSMENT

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