EPA United States Environmental Protection Agency

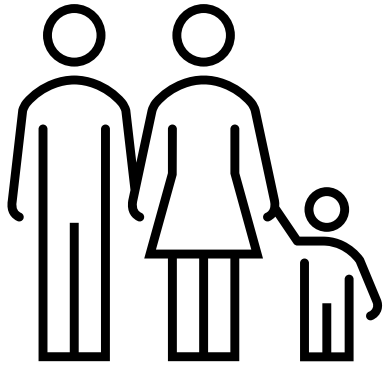
Utility of NAMs for risk-based prioritization

Caroline L. Ring[1]; Katie Paul Friedman[1]; Kristin K. Isaacs [1]; John F. Wambaugh[1]

[1]US Environmental Protection Agency, Center for Computational Toxicology and Exposure

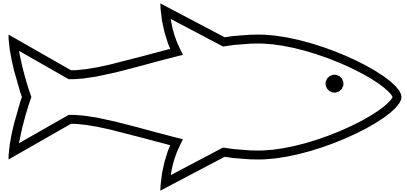
The views expressed in this presentation are those of the author(s) and do not necessarily reflect the views or policies of the U.S. EPA

Human & ecological populations are exposed to thousands of chemicals in the environment – which ones are highest priority?



CC-BY [franchiseopportunitiesphotos]

CC-BY [Joost Nelissen]

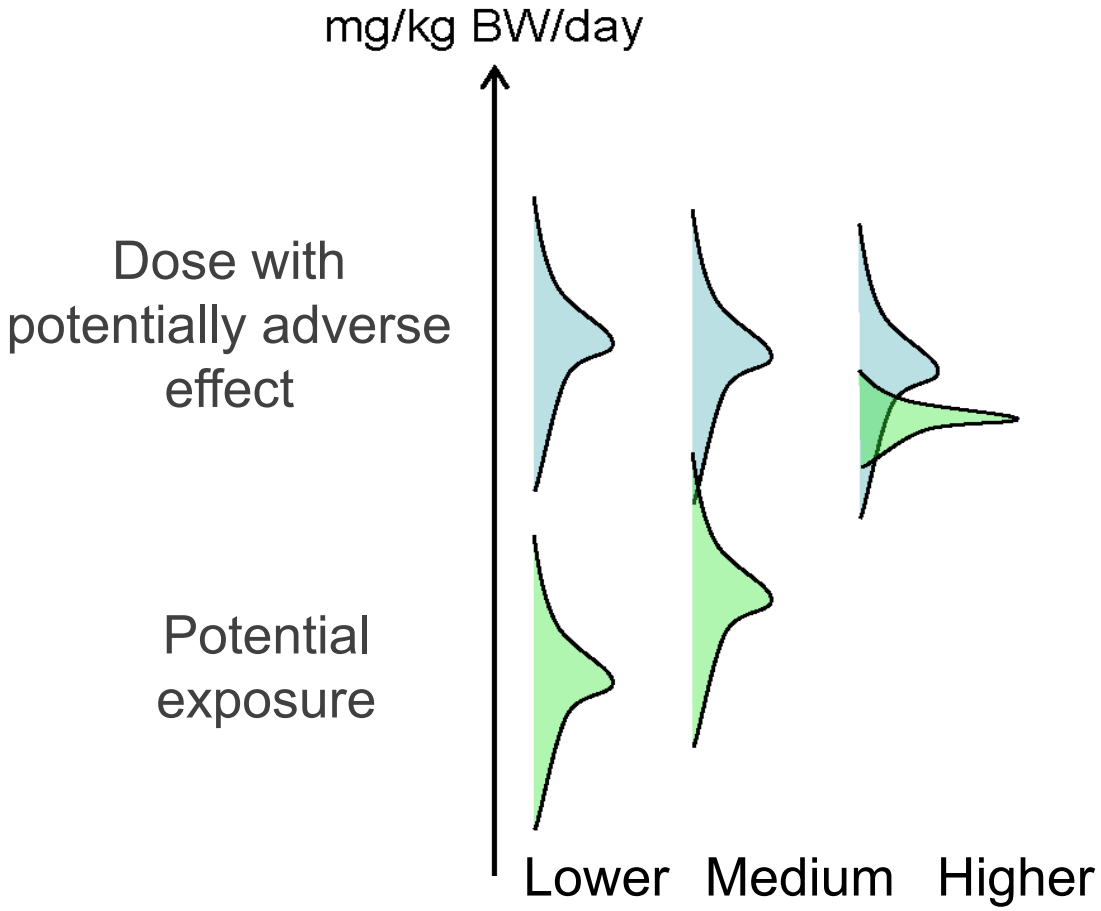


Schmidt, C. W. (2016)

CC-BY [Ali Karimian]

US EPA government photograph

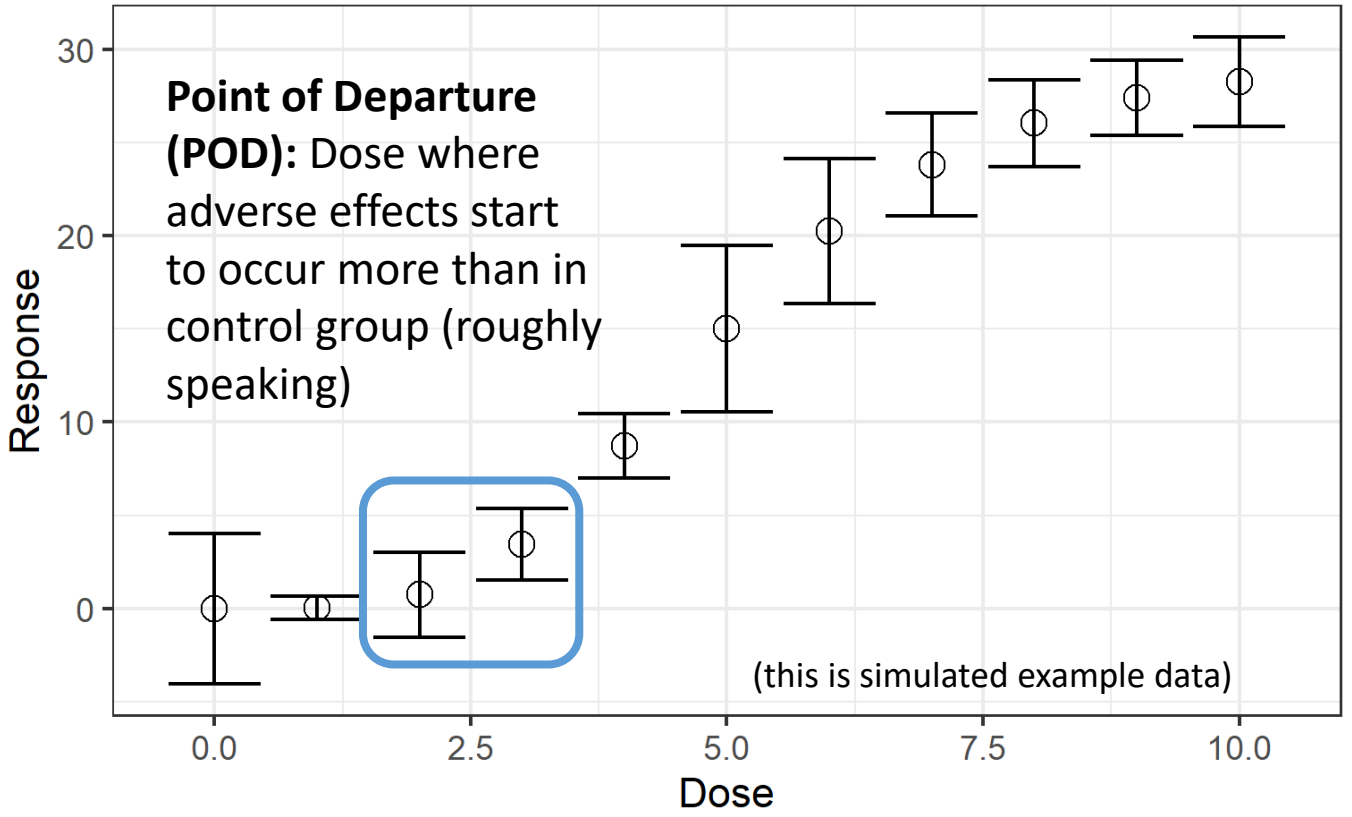
Risk-based prioritization is a function of both hazard and exposure



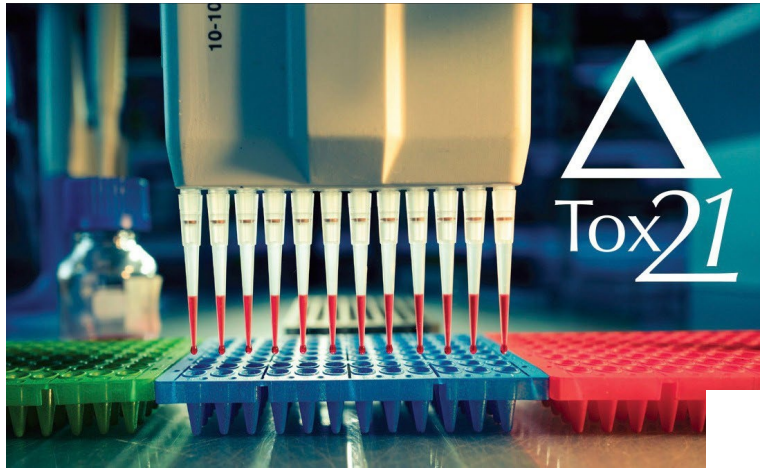
[NRC 2007; Bell et al. 2018; Bessems et al. 2014]

Traditional hazard data comes from studies *in vivo*, one chemical at a time

[Observe adverse effects in each dose group after days, weeks, months, or years of dosing]



New approach methodologies for hazard: *In vitro* high-throughput screening (HTS) assays, e.g. ToxCast/Tox21



Thousands of chemicals are screened in concentration-response across hundreds of *in vitro* assays for various kinds of biological activity (binding, signaling, viability…) – now with transcriptomics!

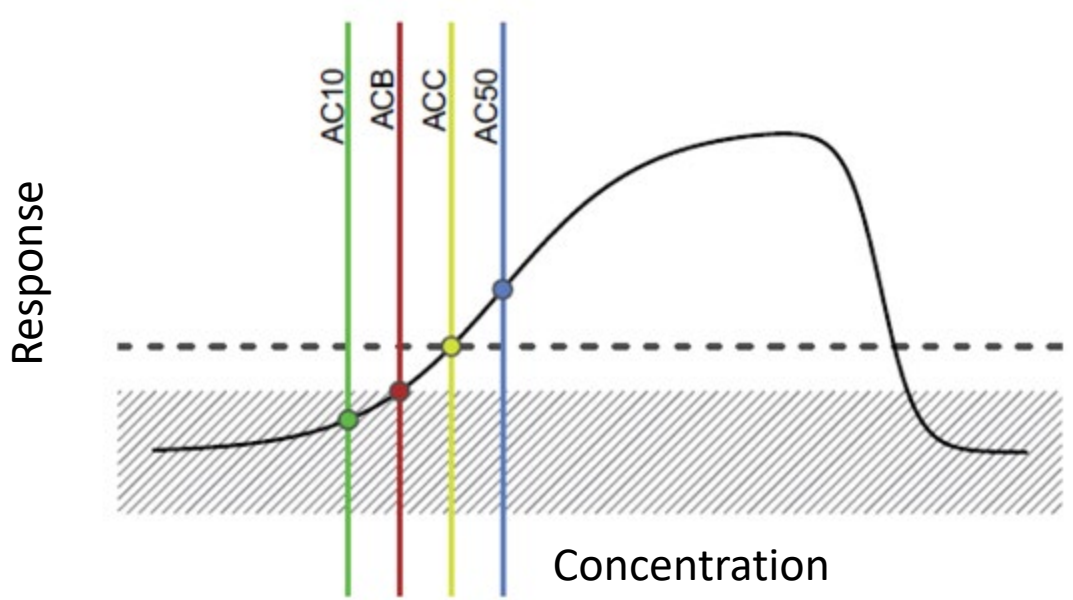
[Schmidt 2009; Dix et al. 2007; Kavlock et al. 2018; Filer *et al.*, 2016; Franzosa et al. 2021]

Data: For each chemical, ***in vitro* concentrations associated with bioactivity** in each assay, if any

All data are public:

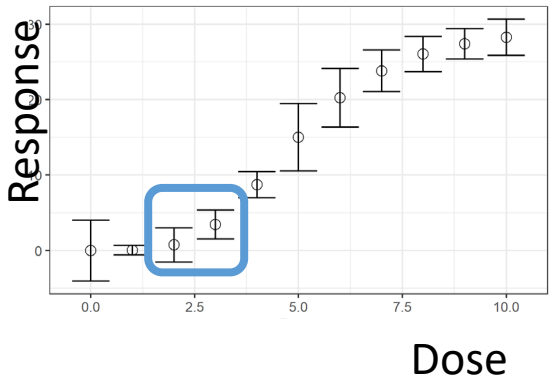
http://comptox.epa.gov/dashboard/

https://www.epa.gov/chemical-research/exploring-toxcast-data-downloadable-data

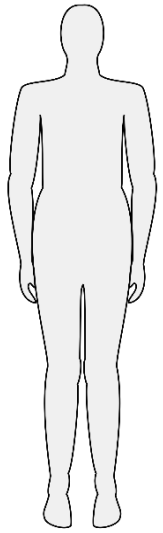


Convert *in vitro* concentration to equivalent "*in vivo*" POD using toxicokinetic modeling

Equivalent dose *in vivo*

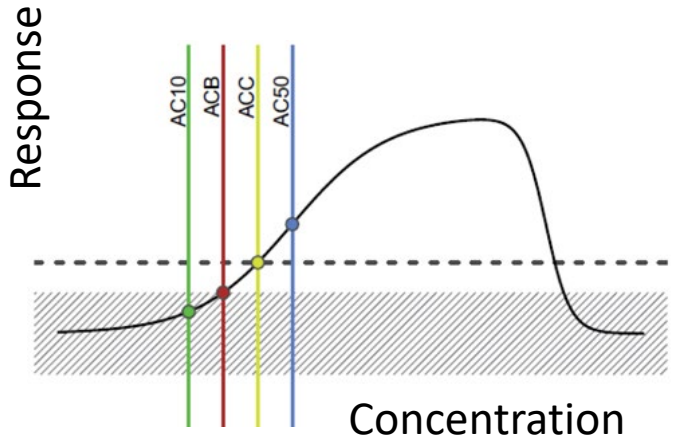


[Tan et al. 2007;
Rotroff et al. 2010;
Wetmore et al. 2015]



Toxicokinetic model:
Absorption
Distribution
Metabolism
Excretion

In vitro bioactive concentration – assumed as body concentration

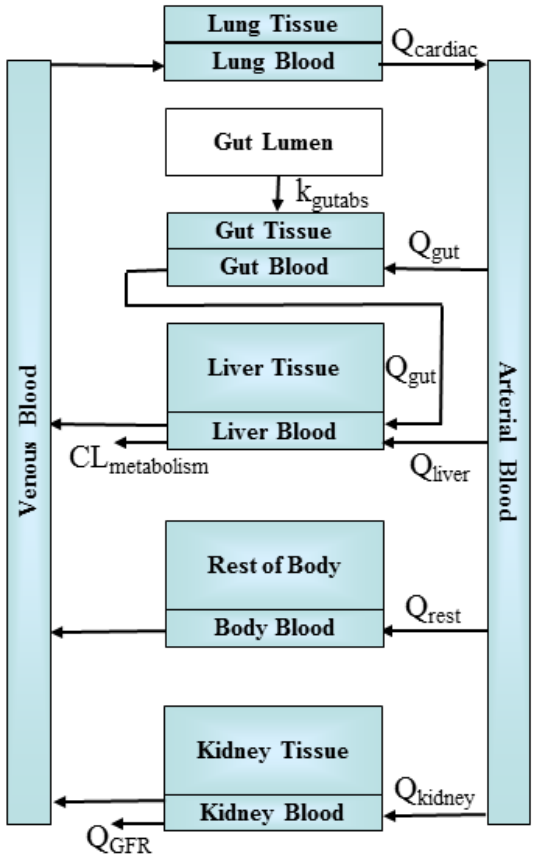


New approach methodologies for toxicokinetics: *High-throughput* toxicokinetics (HTTK)

Generic physiologically-based TK (PBTK) model: can be parameterized for many chemicals with minimal chemical-specific data requirements

+

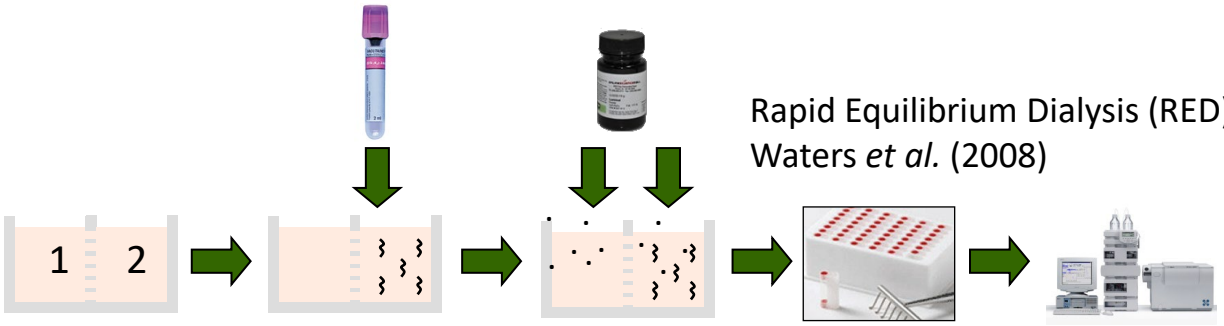
In vitro measurements of the minimal chemical-specific TK model parameters (hepatic clearance rate & plasma protein binding)



Wambaugh et al. (2015)
Pearce et al. (2017a)
Ring et al. (2017)
Linakis et al. (2020)
Breen et al. (2021)

Cryo-preserved hepatocyte suspension
Shibata *et al.* (2002)

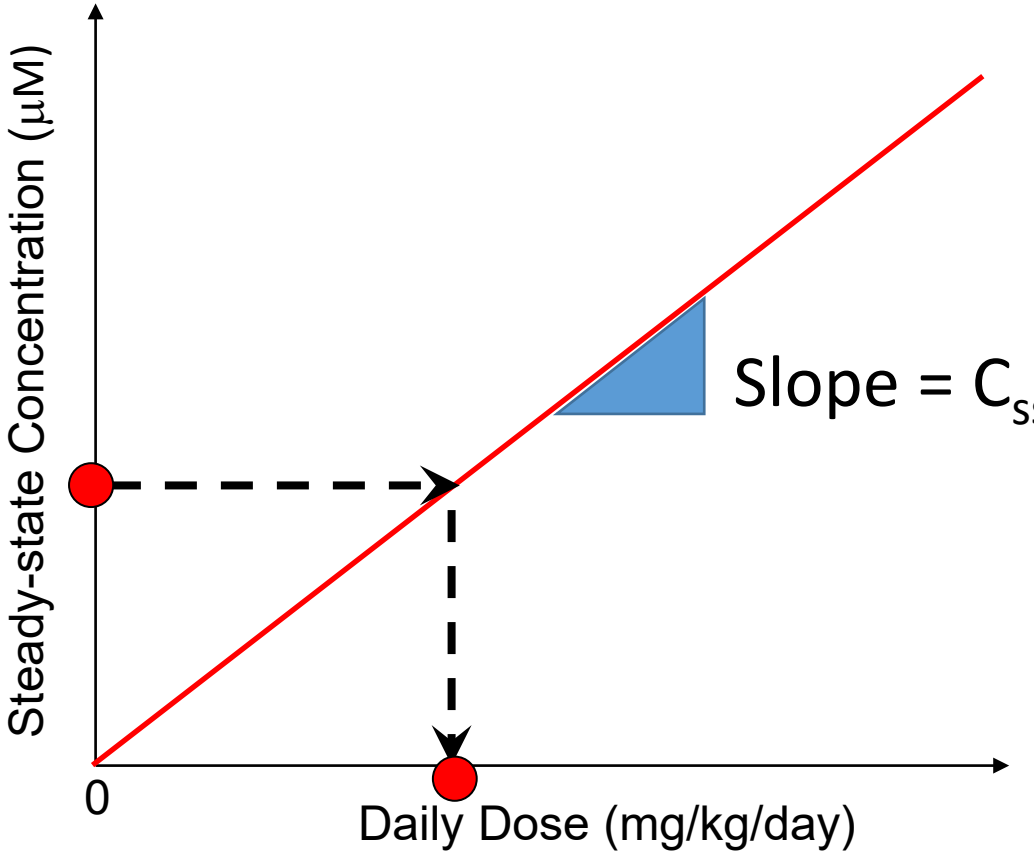
Rapid Equilibrium Dialysis (RED)
Waters *et al.* (2008)



Rotroff et al. (2010)
Wetmore et al. (2012)
Wetmore et al. (2015)
Wambaugh et al. (2019)

For high-throughput applications: focus on steady-state plasma concentration (C_{ss}) & simple TK models where C_{ss} is linear with dose

Concentration of interest: e.g. *in vitro* bioactive concentration



Slope = C_{ss} for 1 mg/kg/day

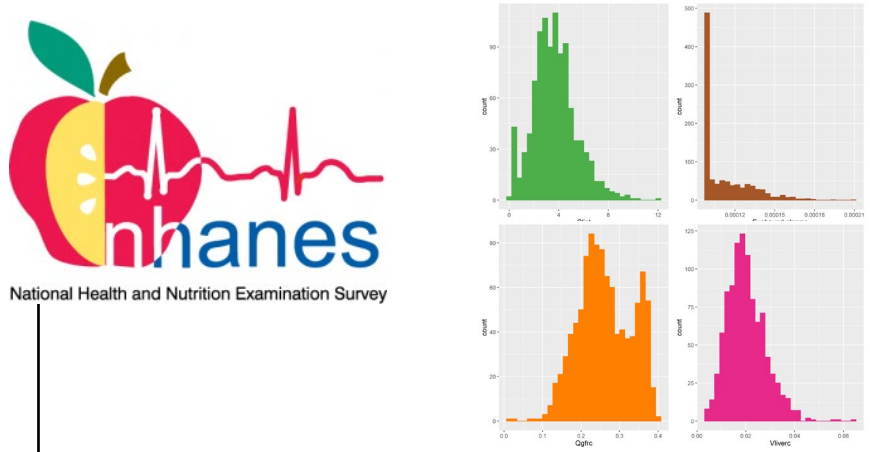
in `httk` R package:
`calc_analytic_css()`

Equivalent dose

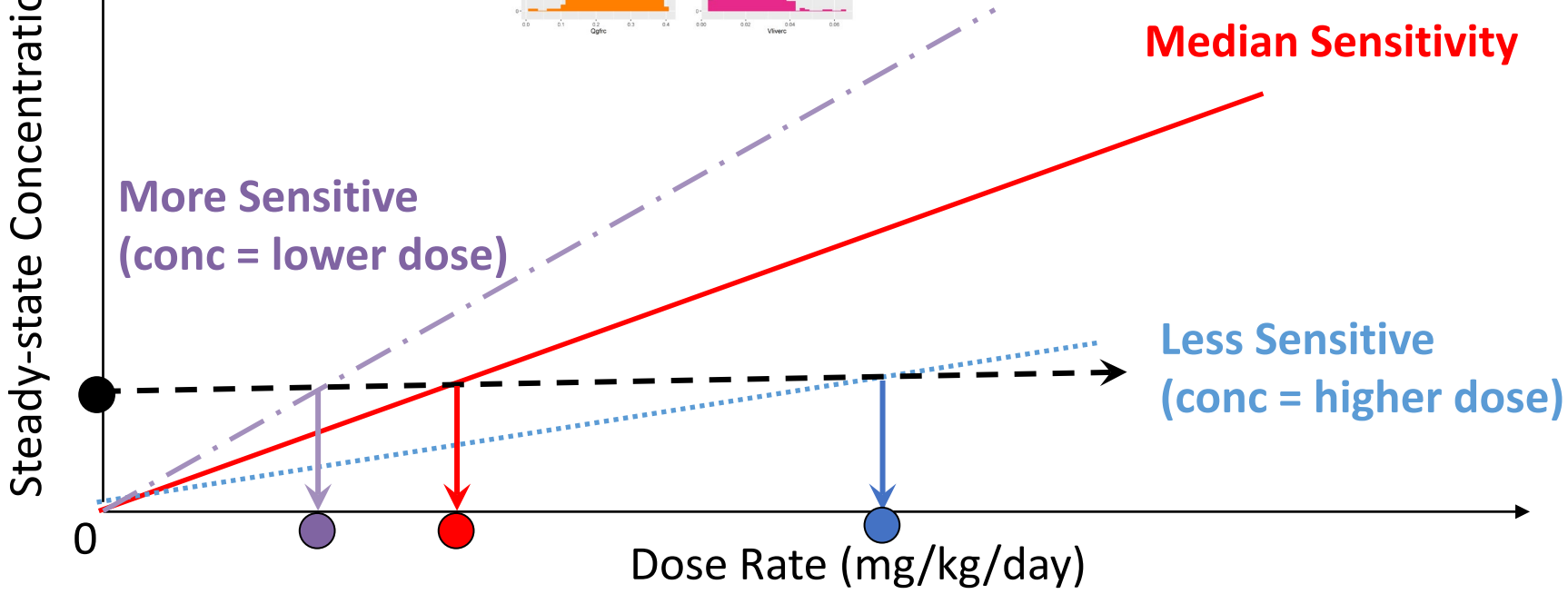
Wetmore *et al*. (2012)

Inter-individual variability in TK is modeled using a correlated Monte Carlo approach based on CDC NHANES

Ring et al. 2017

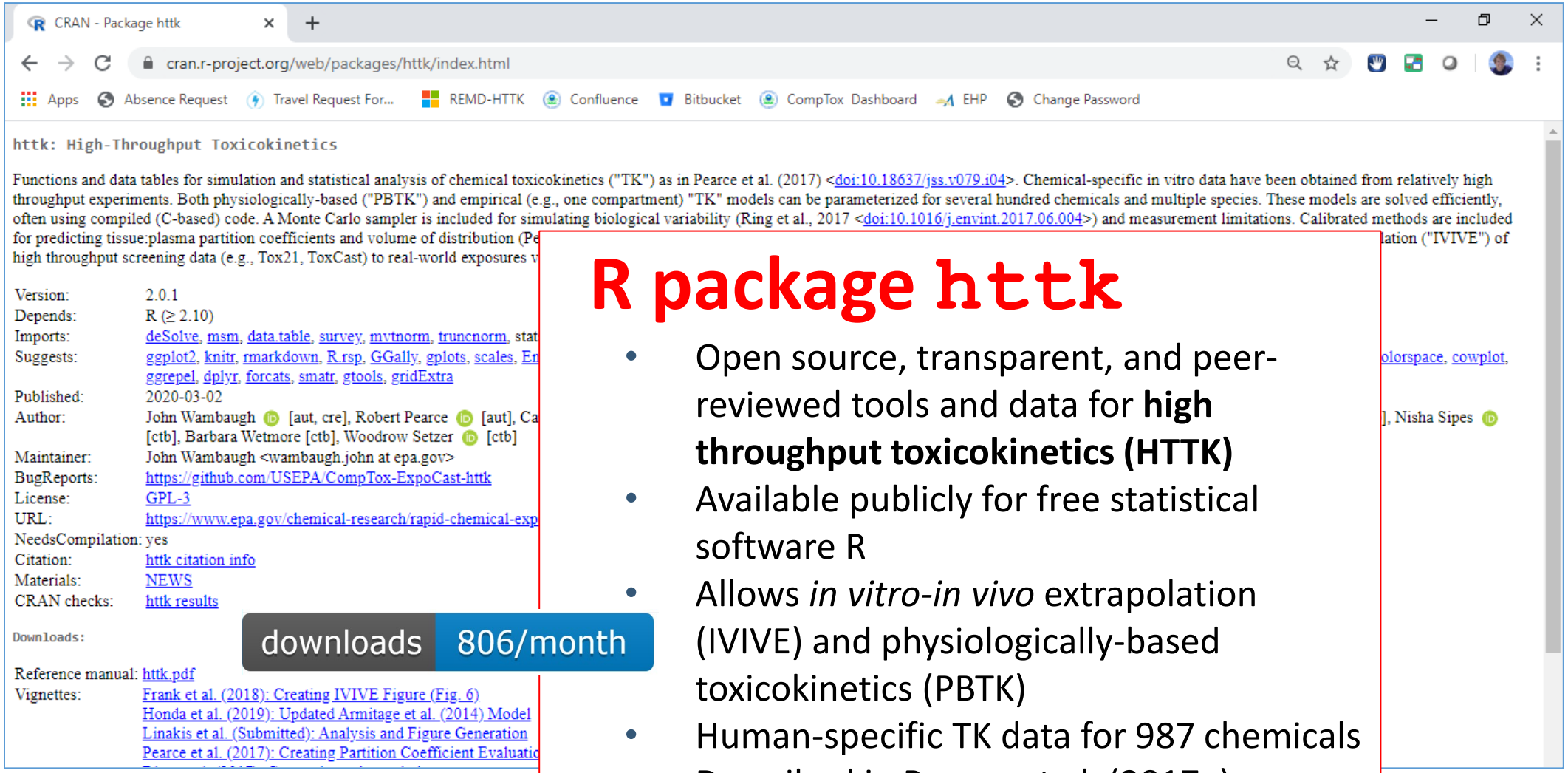


Sample population TK parameters & calculate resulting population Css-dose slope distribution



HTTK models, data, & algorithms are freely available in R package `httk`

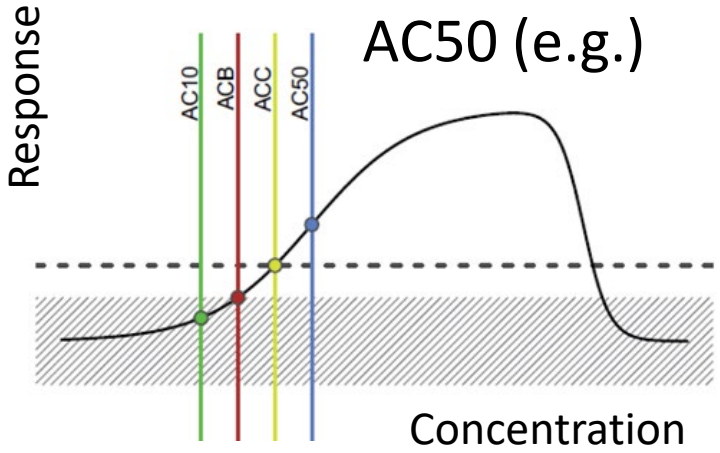
https://CRAN.R-project.org/package=httk

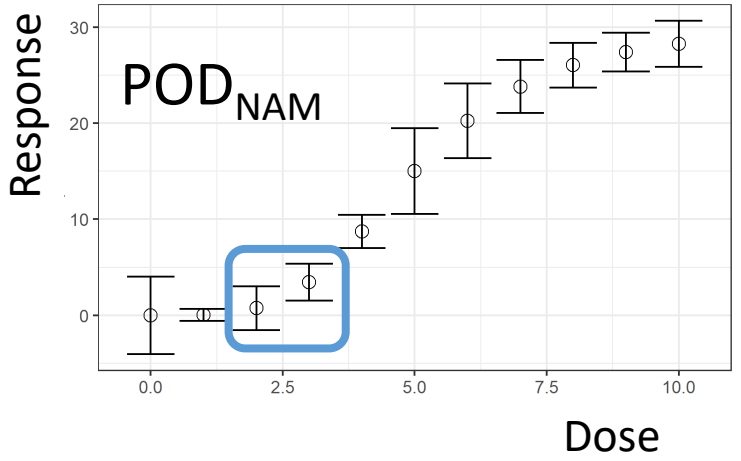


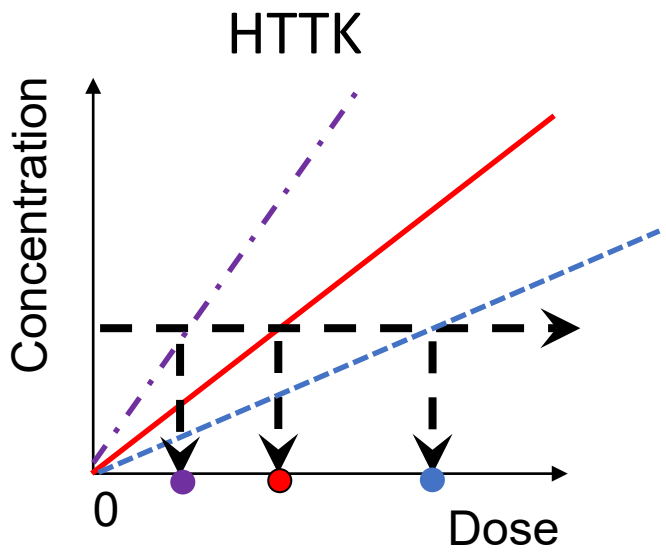
R package `httk`

- Open source, transparent, and peer-reviewed tools and data for **high throughput toxicokinetics (HTTK)**
- Available publicly for free statistical software R
- Allows *in vitro-in vivo* extrapolation (IVIVE) and physiologically-based toxicokinetics (PBTK)
- Human-specific TK data for 987 chemicals
- Described in Pearce et al. (2017a)

So: We can map *in vitro* bioactive *concentration* to equivalent "*in vivo*" POD_{NAM} (*dose*) using HTTK

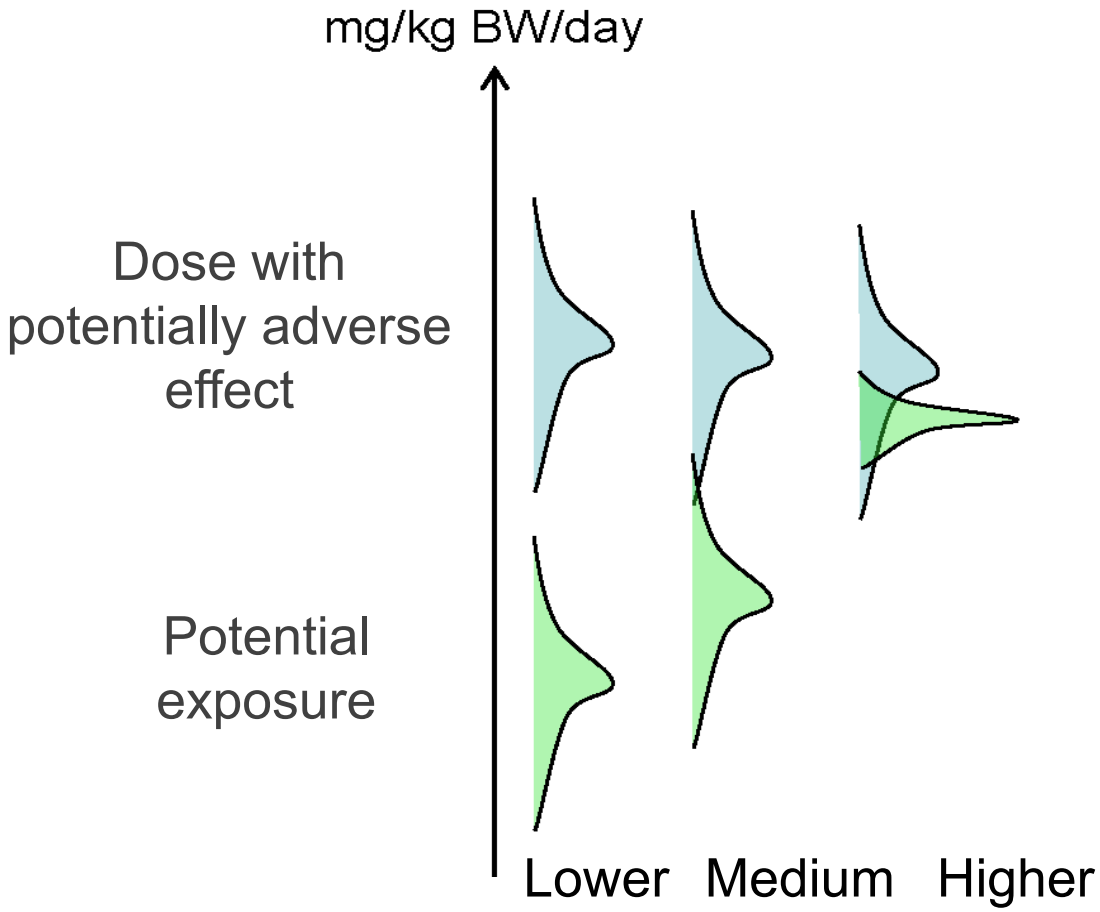




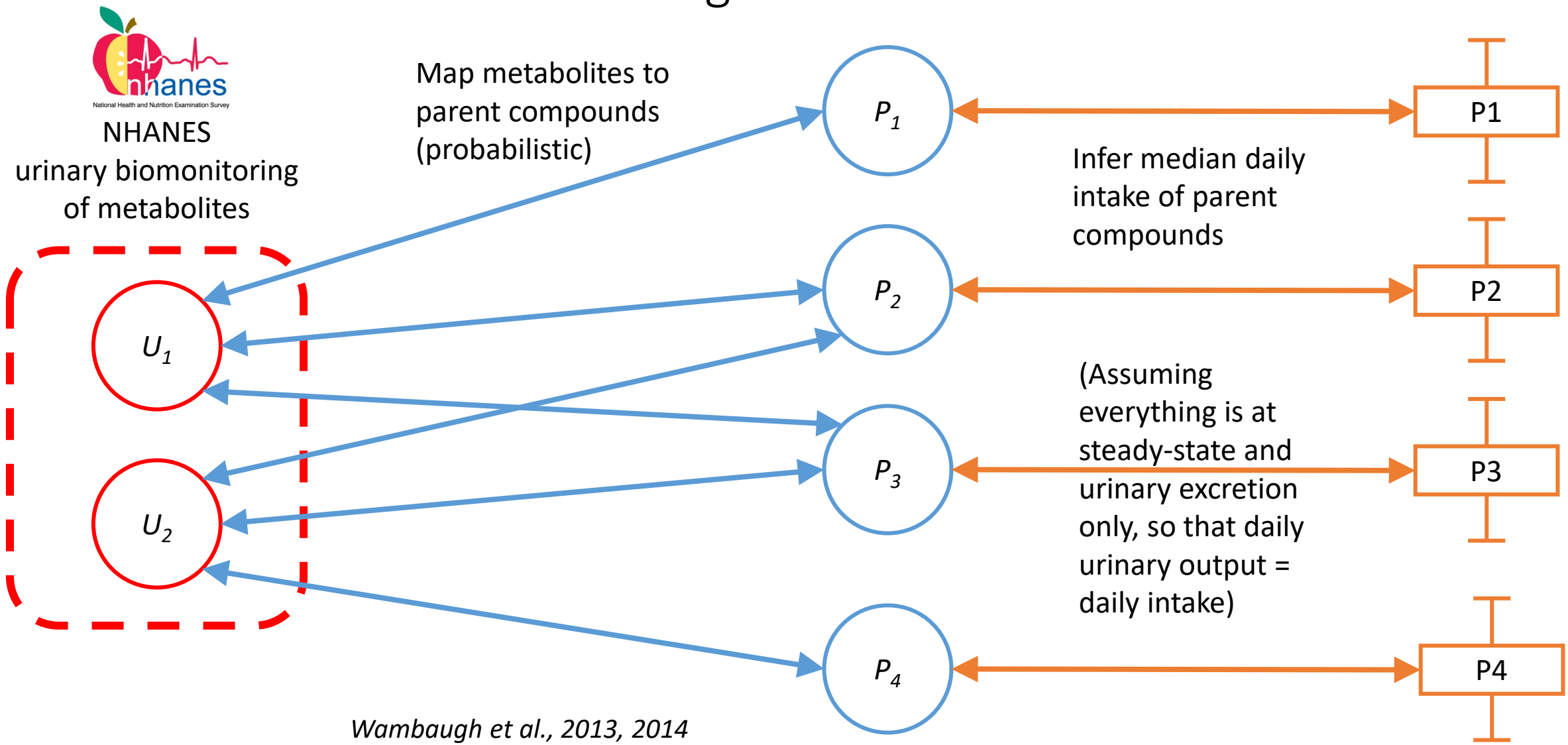


Note that this is like a data-driven UF_H (human TK variability)

Risk is a function of both hazard and *exposure* – new approach methodologies for exposure



ExpoCast: Bayesian inference of external exposures from internal biomonitoring data

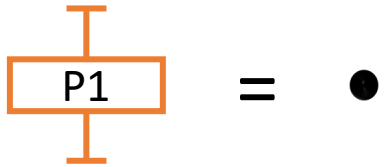


Wambaugh et al., 2013, 2014
Stanfield et al., 2021

SEEM3: A consensus model for aggregate exposure

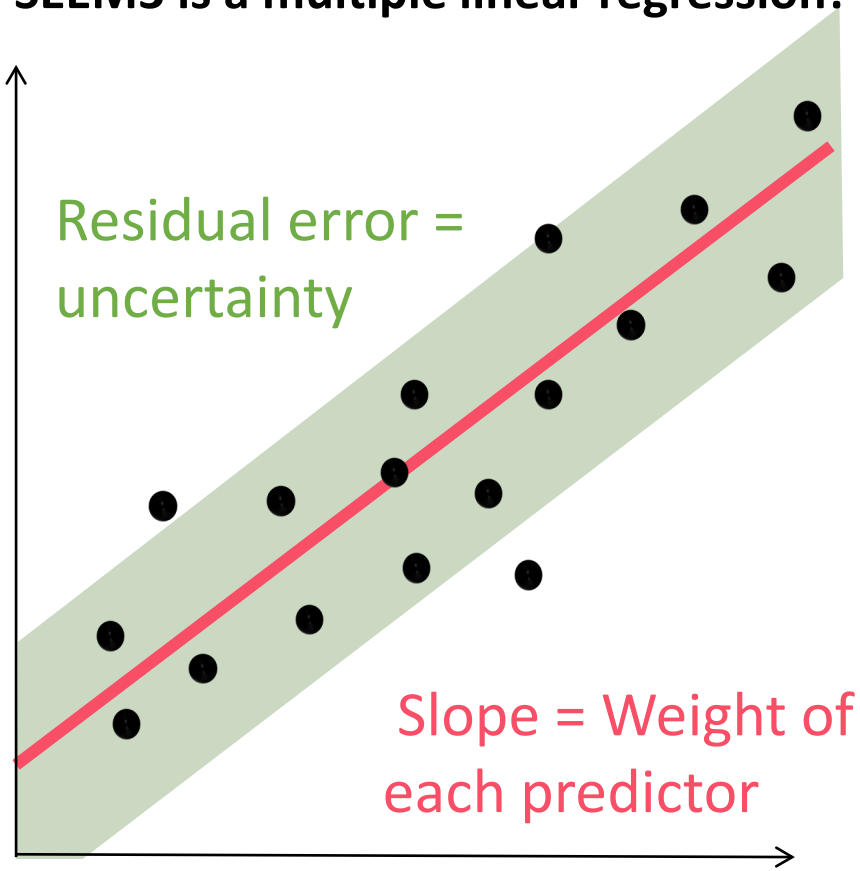
SEEM3 = Systematic Empirical Evaluation of Models, version 3
Ring et al. (2019)

Train model on inferred exposures from NHANES biomonitoring data



Bayesian inference = *Probabilistic* estimates of intercept, slopes, and uncertainty

SEEM3 is a multiple linear regression!



Intercept = Exposure when all predictors at mean value

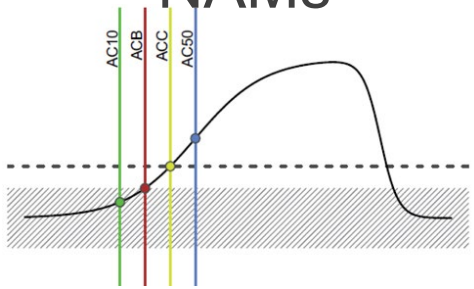
Exposure Predictors:

- Predictions of HT exposure models (USETox, RAIDAR, FINE, SHEDS-HT…)
- Chemical production volume (U.S.)
- Existing EPA pesticide exposure assessments
- Presence on Stockholm Convention list of banned persistent organic pollutants

Missing predictor data: Impute mean

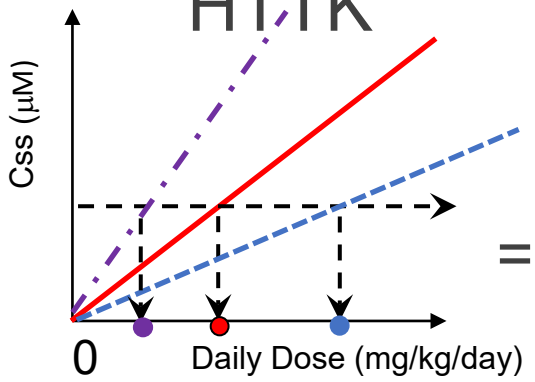
Bioactivity-exposure ratio (BER):
POD_{NAM} ÷ NAM-predicted exposure

In vitro hazard
NAMs



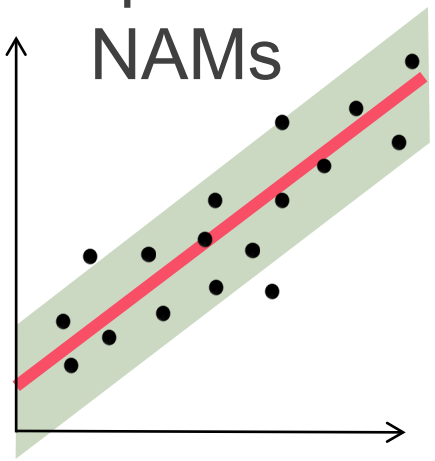
X

HTTK

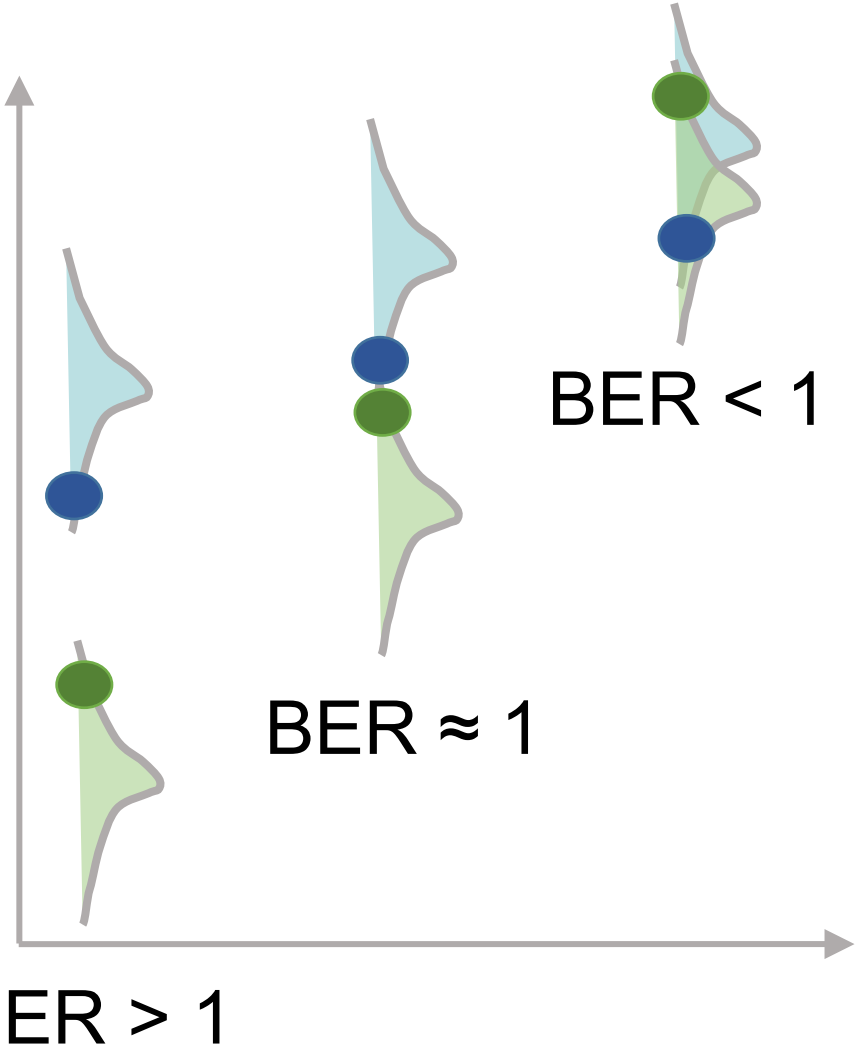


= POD_{NAM}

Exposure
NAMs

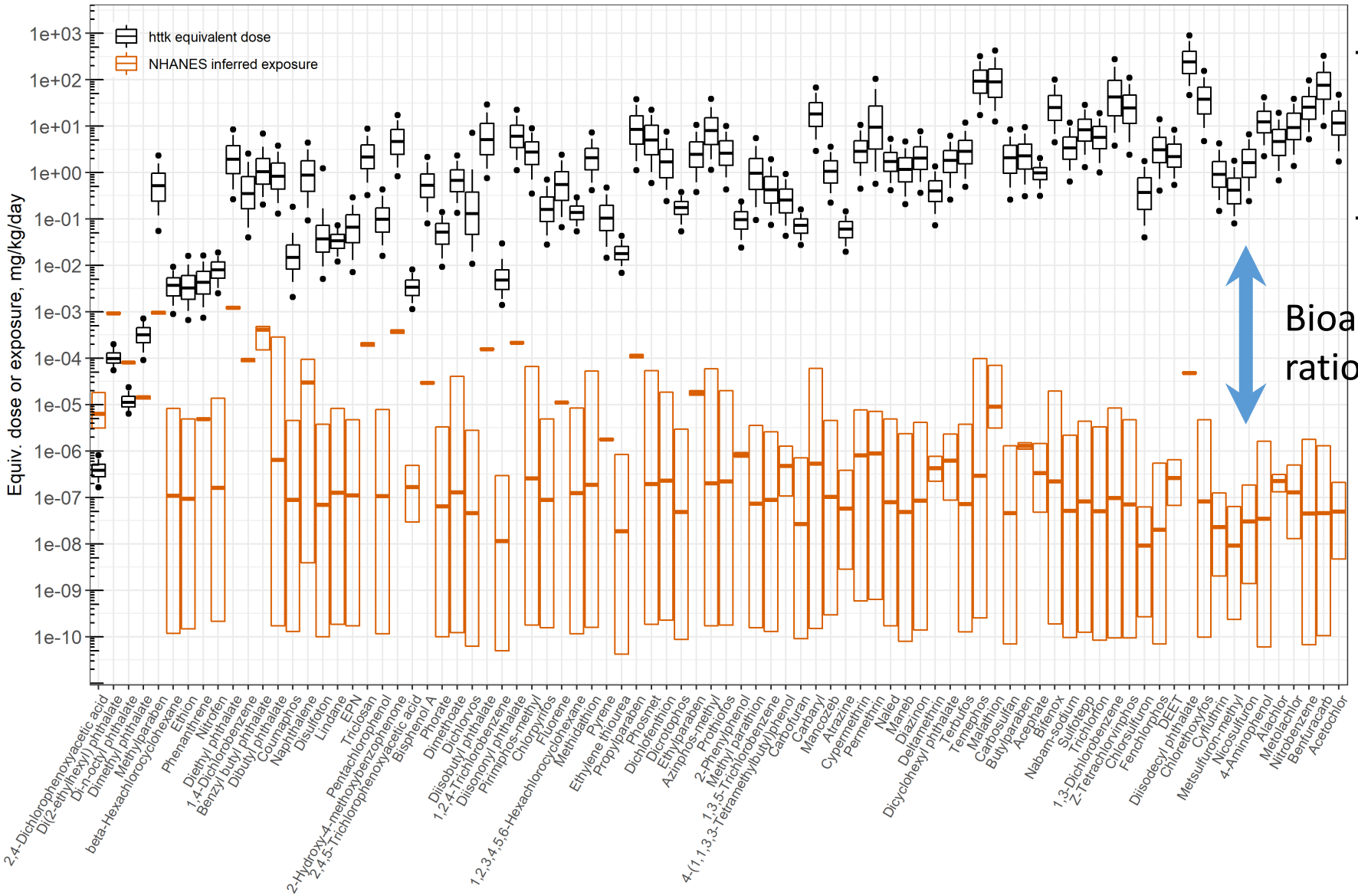


= Daily
exposure rate
(mg/kg/day)



Wetmore et al. (2015)
Paul Friedman et al. (2017)

Example: BER-based prioritization of 84 chemicals



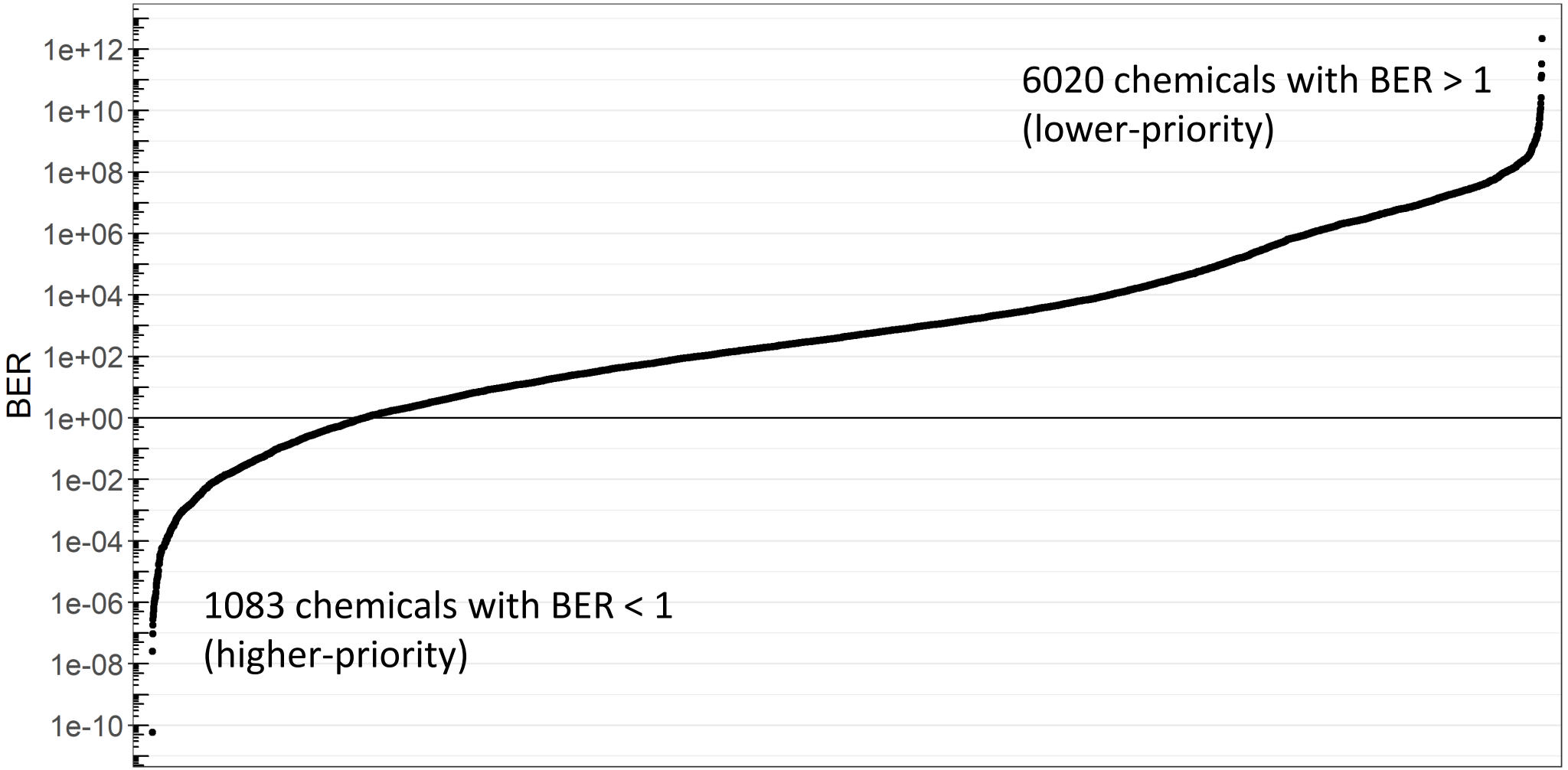
Population distributions of equivalent dose for 10th percentile ToxCast AC50 (bottom point = most-sensitive 5%)

Bioactivity-exposure ratio (BER)

Population median aggregate exposures with 95% credible interval, inferred from NHANES urinary biomonitoring data

Updated version of analysis from Ring et al. (2017)

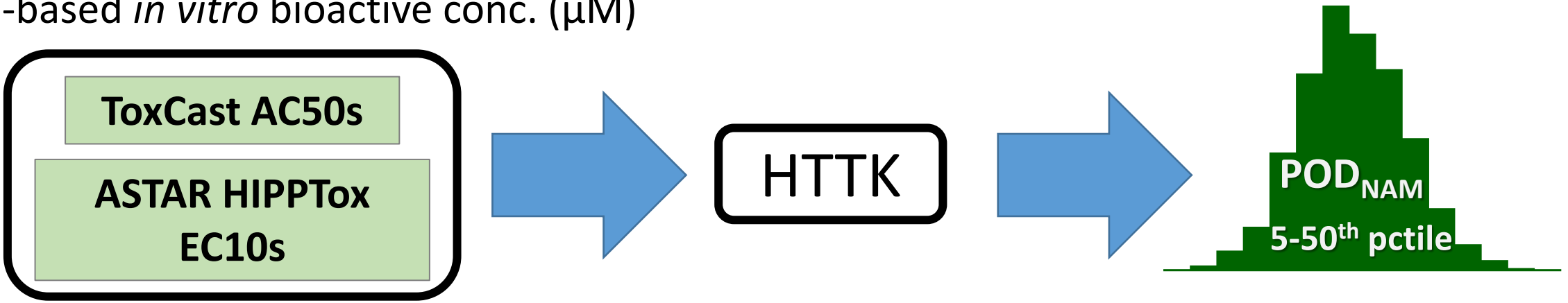
An even-more high-throughput application: BER prioritization of 7104 chemicals based on HTTK IVIVE of ToxCast AC50s and HT exposure predictions from SEEM3 model



Case study: Is POD_{NAM} health-protective vs. traditional *in vivo* POD? (Paul Friedman et al. 2019)

In vivo PODs (mg/kg/day)

NAM-based *in vitro* bioactive conc. (µM)



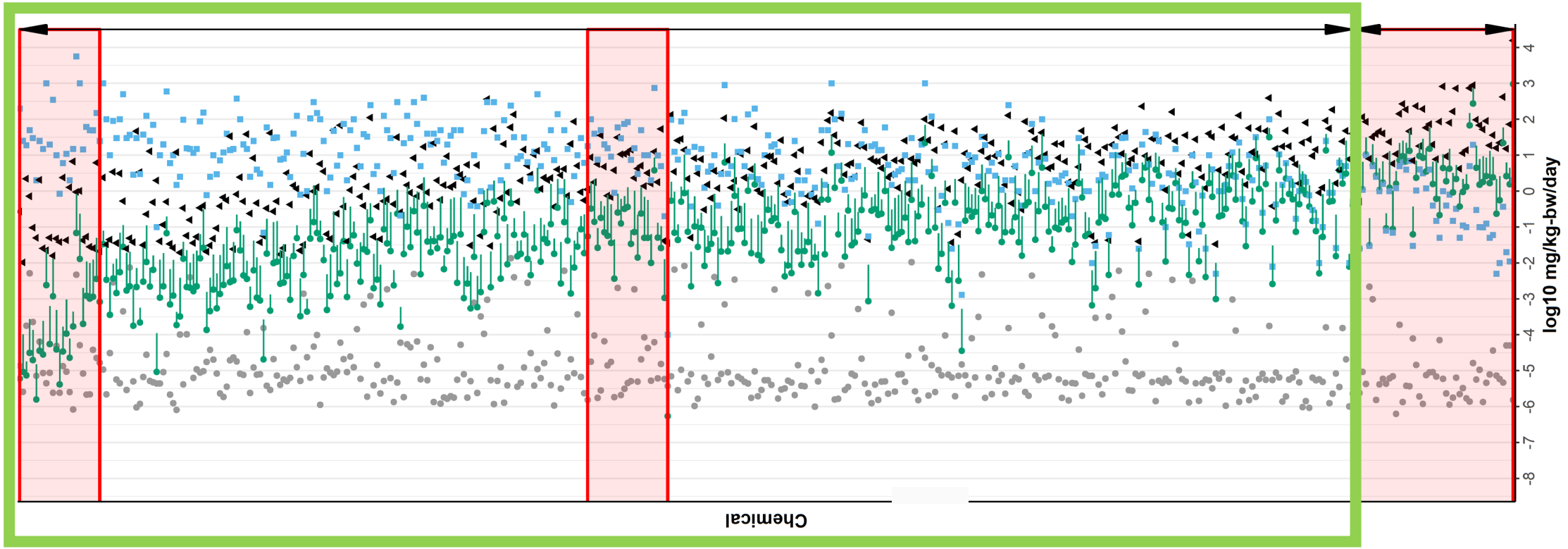
$POD_{NAM} < POD_{traditional}$ (most of the time)

Adapted from Figure 3, Paul Friedman et al. *2019*

$POD_{traditional}$

POD_{NAM} (median – most-sensitive 5%)

NAM-pred exposure



400/448 chemicals = *89% of the time,*
$POD_{NAM} < POD_{trad}$

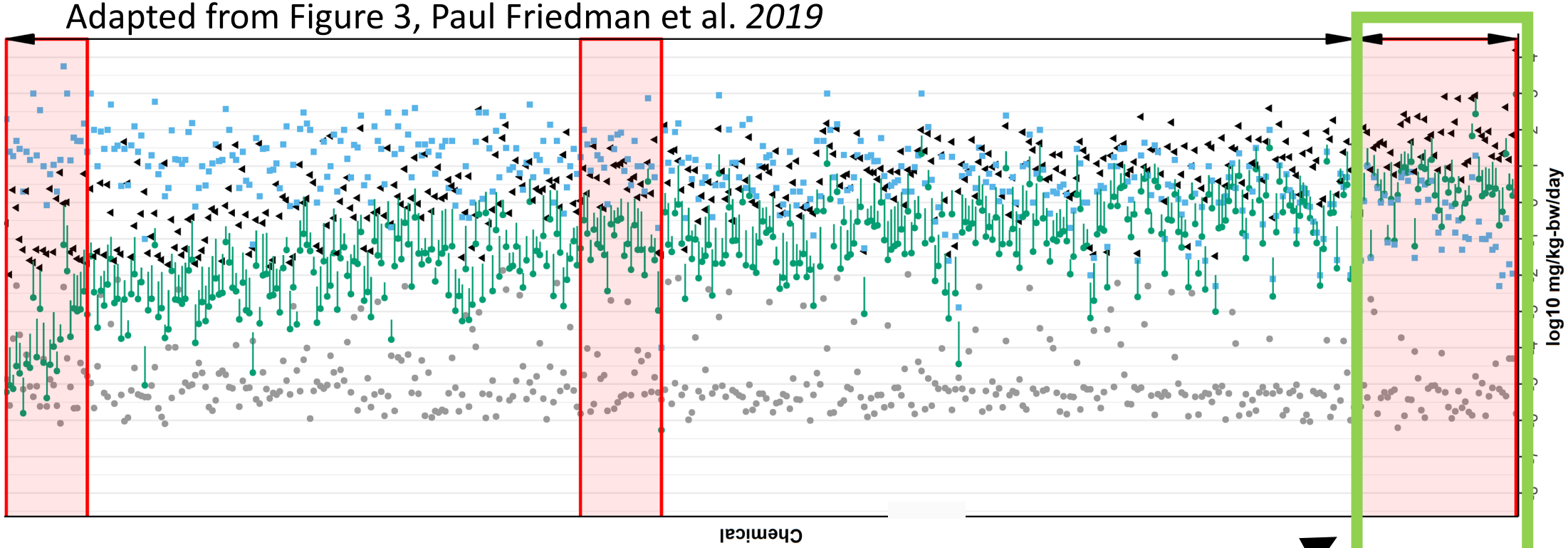
When POD_{NAM} > $POD_{traditional}$, specific chemical features are more likely

$POD_{traditional}$

POD_{NAM} (median – most-sensitive 5%)

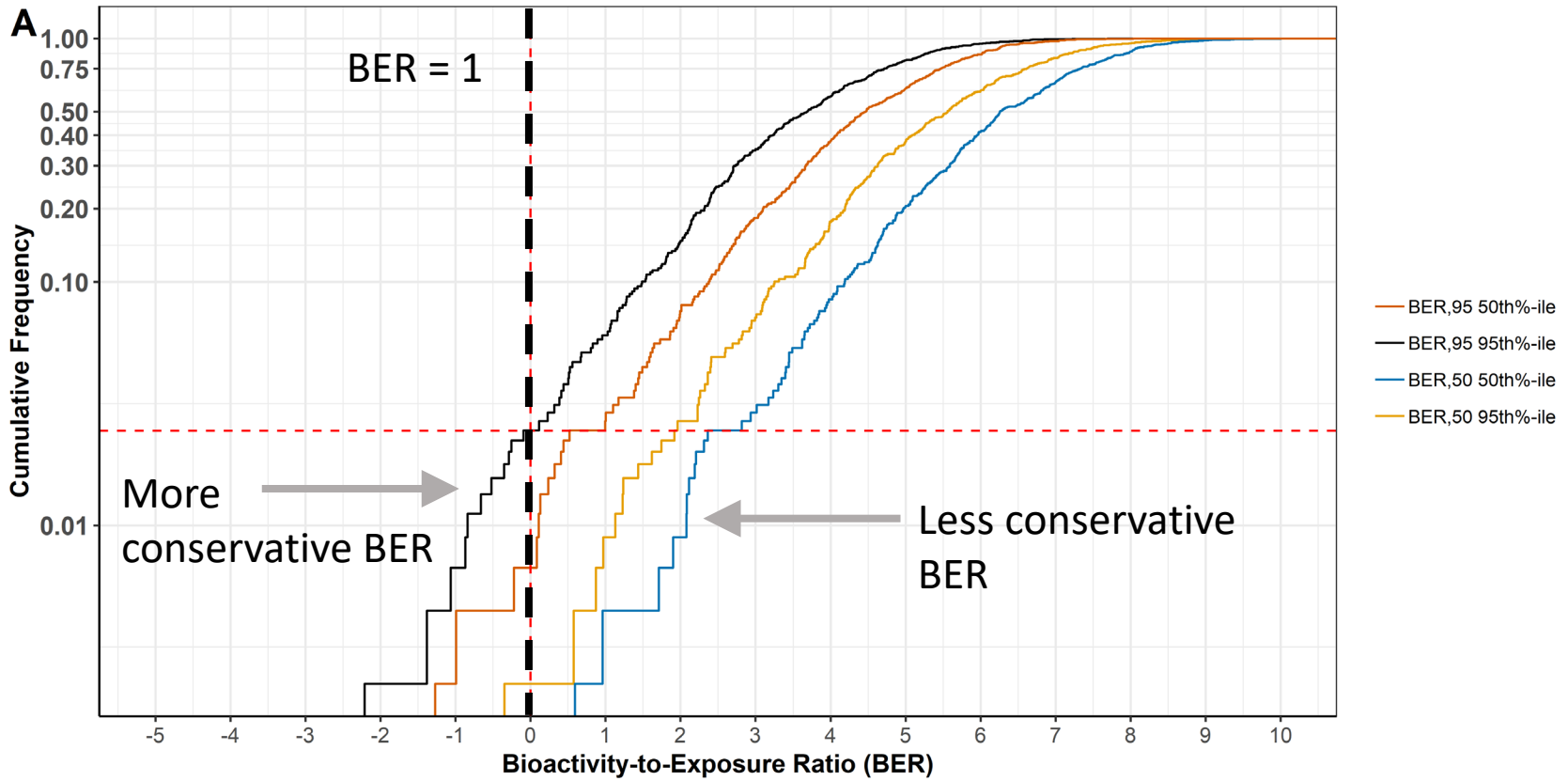
NAM-pred exposure

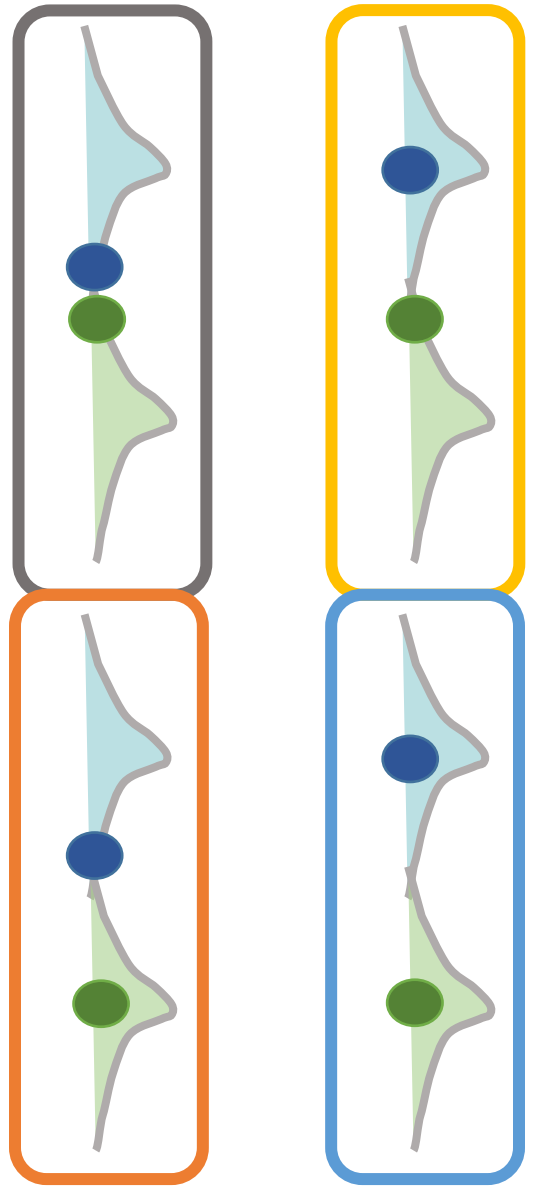
Adapted from Figure 3, Paul Friedman et al. *2019*



chemical features associated with organophosphate pesticides and carbamates more likely

BERs can be calculated to reflect different levels of uncertainty/conservatism based on use case





Adapted from Figure 4, Paul Friedman et al. *2019*

EPA United States Environmental Protection Agency

Conclusions

- Risk-based prioritization involves both hazard and exposure
- NAMs can help fill data gaps in both the hazard and the exposure components of risk
- *In vitro* bioactive concentrations can be converted to equivalent "in vivo" POD_{NAM} doses, using high-throughput toxicokinetics (HTTK)
- POD_{NAM} incorporates data-driven inter-individual TK variability via HTTK
- Exposure NAMs can rapidly predict median population aggregate exposures
- POD_{NAM} vs. exposure quantified using *bioactivity-exposure ratio* (BER)
- BER-based prioritization gives a useful starting point
- POD_{NAM} is typically more conservative than traditional *in vivo* PODs
- BER-based approach allows flexible consideration of uncertainty

NAMs for hazard, exposure, and toxicokinetics provide a useful way to rapidly prioritize chemicals

United States Environmental Protection Agency

References

1. Schmidt, C. W. TSCA 2.0: A New Era in Chemical Risk Management. *Environmental Health Perspectives* **124**, A182-A186, doi:doi:10.1289/ehp.124-A182 (2016).

2. National Research Council. *Toxicity Testing in the 21st Century*. (2007).

3. Bell, S. M. *et al.* In vitro to in vivo extrapolation for high throughput prioritization and decision making. *Toxicol In Vitro* **47**, 213-227, doi:10.1016/j.tiv.2017.11.016 (2018).

4. Bessems, J. G. *et al.* PBTK modelling platforms and parameter estimation tools to enable animal-free risk assessment: recommendations from a joint EPAA--EURL ECVAM ADME workshop. *Regul Toxicol Pharmacol* **68**, 119-139, doi:10.1016/j.yrtph.2013.11.008 (2014).

5. Schmidt, C. W. TOX 21: New Dimensions of Toxicity Testing. *Environmental Health Perspectives* **117**, A348-A353, doi:doi:10.1289/ehp.117-a348 (2009).

6. Dix, D. J. *et al.* The ToxCast program for prioritizing toxicity testing of environmental chemicals. *Toxicol Sci* **95**, 5-12, doi:10.1093/toxsci/kfl103 (2007).

7. Kavlock, R. J. *et al.* Accelerating the Pace of Chemical Risk Assessment. *Chemical Research in Toxicology* **31**, 287-290, doi:10.1021/acs.chemrestox.7b00339 (2018).

8. Filer, D. L., Kothiya, P., Setzer, R. W., Judson, R. S. & Martin, M. T. tcpl: the ToxCast pipeline for high-throughput screening data. *Bioinformatics* **33**, 618-620, doi:10.1093/bioinformatics/btw680 (2017).

9. Franzosa, J. A. *et al.* High-throughput toxicogenomic screening of chemicals in the environment using metabolically competent hepatic cell cultures. *npj Systems Biology and Applications* **7**, 7, doi:10.1038/s41540-020-00166-2 (2021).

10. Tan, Y. M., Liao, K. H. & Clewell, H. J., 3rd. Reverse dosimetry: interpreting trihalomethanes biomonitoring data using physiologically based pharmacokinetic modeling. *J Expo Sci Environ Epidemiol* **17**, 591-603, doi:10.1038/sj.jes.7500540 (2007).

11. Rotroff, D. M. *et al.* Incorporating human dosimetry and exposure into high-throughput in vitro toxicity screening. *Toxicol Sci* **117**, 348-358, doi:10.1093/toxsci/kfq220 (2010).

12. Wetmore, B. A. Quantitative in vitro-to-in vivo extrapolation in a high-throughput environment. *Toxicology* **332**, 94-101, doi:10.1016/j.tox.2014.05.012 (2015).

13. Wambaugh, J. F. *et al.* Toxicokinetic Triage for Environmental Chemicals. *Toxicol Sci* **147**, 55-67, doi:10.1093/toxsci/kfv118 (2015).

14. Pearce, R. G., Setzer, R. W., Strope, C. L., Wambaugh, J. F. & Sipes, N. S. httk: R Package for High-Throughput Toxicokinetics. *J Stat Softw* **79**, 1-26, doi:10.18637/jss.v079.i04 (2017).

15. Ring, C. L., Pearce, R. G., Setzer, R. W., Wetmore, B. A. & Wambaugh, J. F. Identifying populations sensitive to environmental chemicals by simulating toxicokinetic variability. *Environ Int* **106**, 105-118, doi:10.1016/j.envint.2017.06.004 (2017).

16. Linakis, M. W. *et al.* Development and evaluation of a high throughput inhalation model for organic chemicals. *J Expo Sci Environ Epidemiol* **30**, 866-877, doi:10.1038/s41370-020-0238-y (2020).

17. Breen, M., Ring, C. L., Kreutz, A., Goldsmith, M. R. & Wambaugh, J. F. High-throughput PBTK models for in vitro to in vivo extrapolation. *Expert Opin Drug Metab Toxicol* **17**, 903-921, doi:10.1080/17425255.2021.1935867 (2021).

18. Wetmore, B. A. *et al.* Integration of dosimetry, exposure, and high-throughput screening data in chemical toxicity assessment. *Toxicol Sci* **125**, 157-174, doi:10.1093/toxsci/kfr254 (2012).

19. Wetmore, B. A. *et al.* Incorporating High-Throughput Exposure Predictions With Dosimetry-Adjusted In Vitro Bioactivity to Inform Chemical Toxicity Testing. *Toxicol Sci* **148**, 121-136, doi:10.1093/toxsci/kfv171 (2015).

20. Wambaugh, J. F. *et al.* Assessing Toxicokinetic Uncertainty and Variability in Risk Prioritization. *Toxicol Sci* **172**, 235-251, doi:10.1093/toxsci/kfz205 (2019).

21. Wetmore, B. A. *et al.* Incorporating population variability and susceptible subpopulations into dosimetry for high-throughput toxicity testing. *Toxicol Sci* **142**, 210-224, doi:10.1093/toxsci/kfu169 (2014).

22. Wambaugh, J. F. *et al.* High-throughput models for exposure-based chemical prioritization in the ExpoCast project. *Environ Sci Technol* **47**, 8479-8488, doi:10.1021/es400482g (2013).

23. Wambaugh, J. F. *et al.* High throughput heuristics for prioritizing human exposure to environmental chemicals. *Environ Sci Technol* **48**, 12760-12767, doi:10.1021/es503583j (2014).

24. Stanfield, Z. *et al.* in *International Society of Exposure Science (ISES) Virtual Conference.*

25. Ring, C. L. *et al.* Consensus Modeling of Median Chemical Intake for the U.S. Population Based on Predictions of Exposure Pathways. *Environ Sci Technol* **53**, 719-732, doi:10.1021/acs.est.8b04056 (2019).

26. Paul Friedman, K. *et al.* Utility of In Vitro Bioactivity as a Lower Bound Estimate of In Vivo Adverse Effect Levels and in Risk-Based Prioritization. *Toxicological sciences : an official journal of the Society of Toxicology* **173**, 202-225, doi:10.1093/toxsci/kfz201 (2020).