EPA
United States
Environmental Protection
Agency

High-throughput Toxicokinetics

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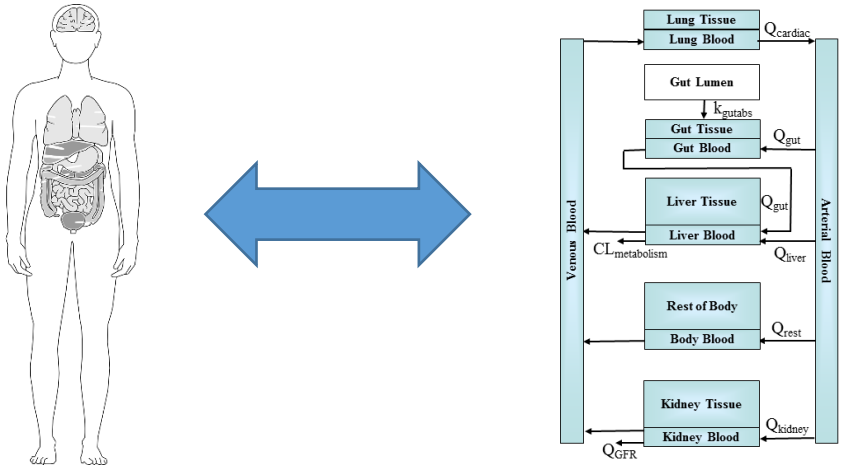
Date

Review from last time

- Toxicokinetics links external exposure to a chemical with internal body concentrations of that chemical by describing ADME
  - Absorption, Distribution, Metabolism, and Excretion of a chemical
- TK modeling can be used to answer questions like “If sperm concentration is reduced when someone has 68 ppt of TCDD in their blood, then what is the corresponding external exposure to TCDD, so EPA can set regulations accordingly?”
- TK models can be simple, empirical models (e.g. 1- or 2-compartment), or more detailed physiologically-based models (PBTK)
- PBTK models may be bespoke (chemical-specific model structure) or generic (same model structure for all chemicals, just different parameters)
- PBTK models are especially useful for extrapolation:
  - Inter-species extrapolation
  - Route-to-route extrapolation
  - Internal-external extrapolation (reverse TK)
  - Extrapolation to different chemicals
  - *In vitro-in vivo* extrapolation

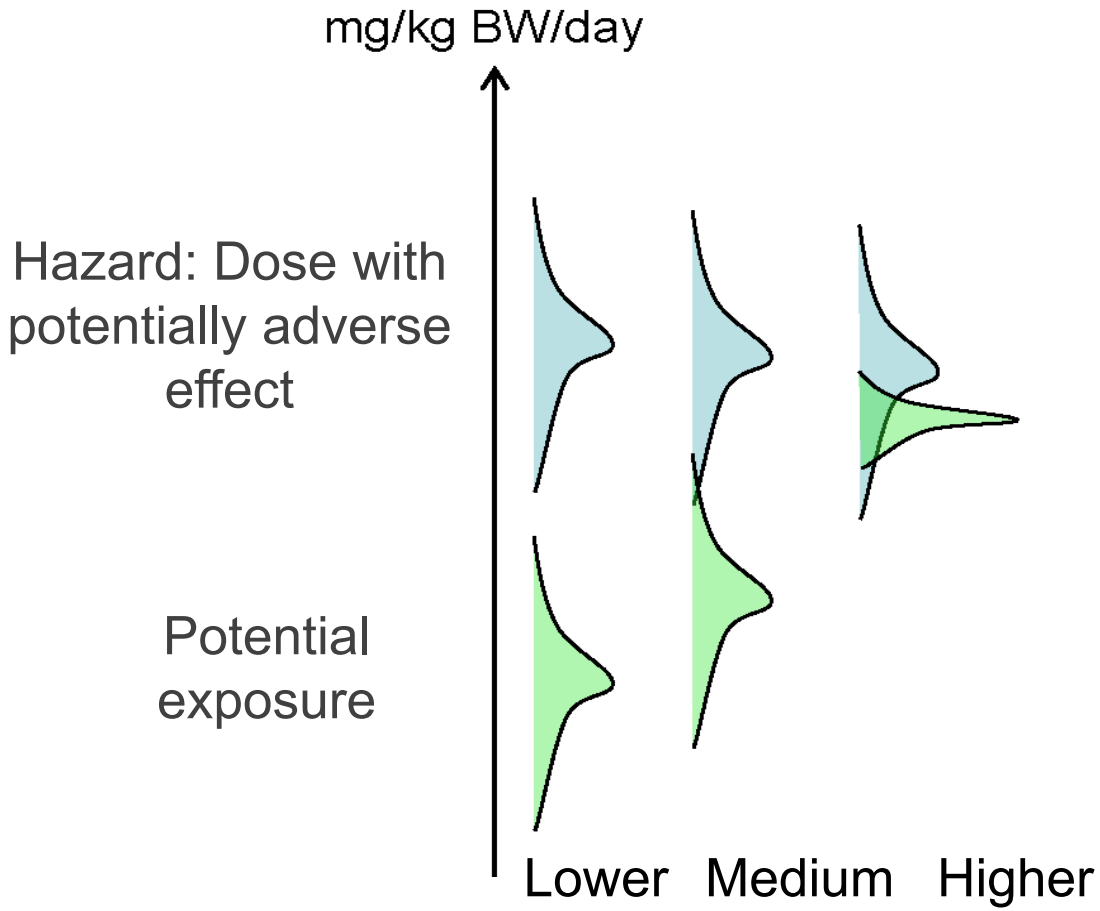


EPA United States Environmental Protection Agency

Overview for this lecture

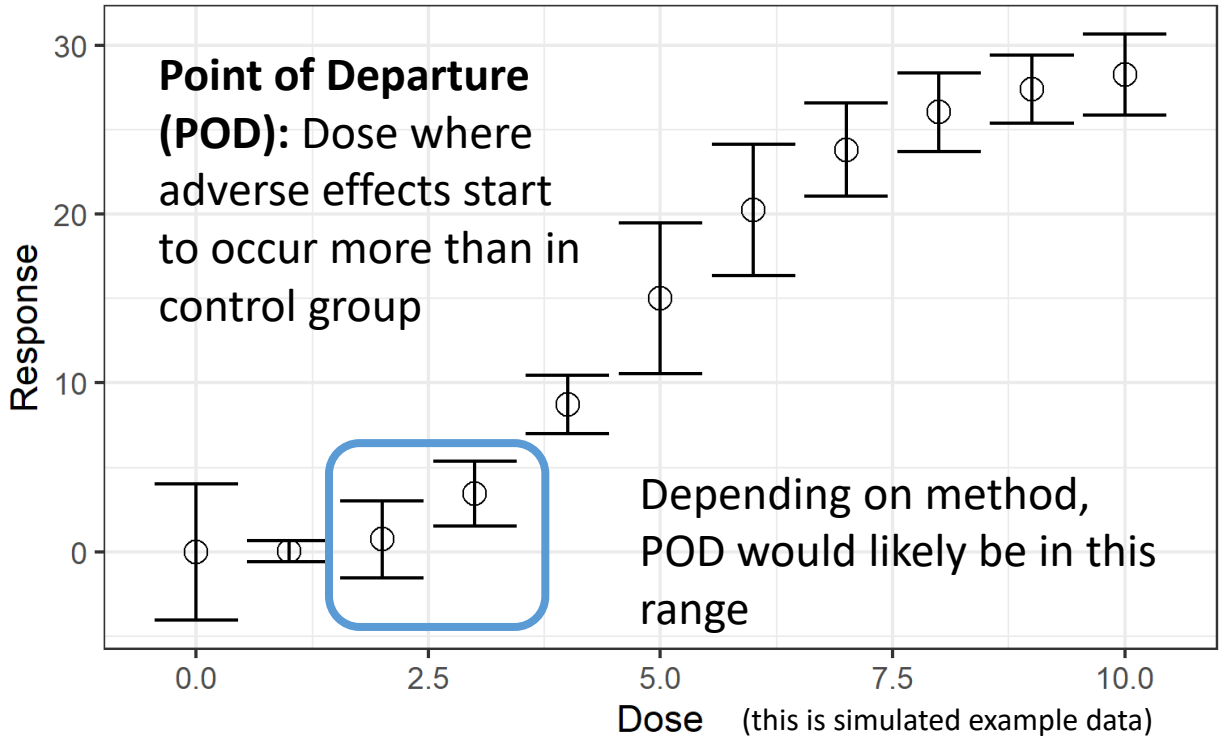
- Motivating scenario: Estimating potential chemical risk from hazard/toxicity and exposure… for 100s-1000s of chemicals?
- High-throughput hazard data
- High-throughput exposure modeling
- Connecting HT hazard and exposure through…. HT toxicokinetics!
- HT TK model structure & parameters
- How do we get chemical-specific TK parameters for 100s-1000s of chemicals?
- How do we incorporate human physiological variability?
- HTTK is available through a free, open-source R package

Risk is a function of both hazard and exposure

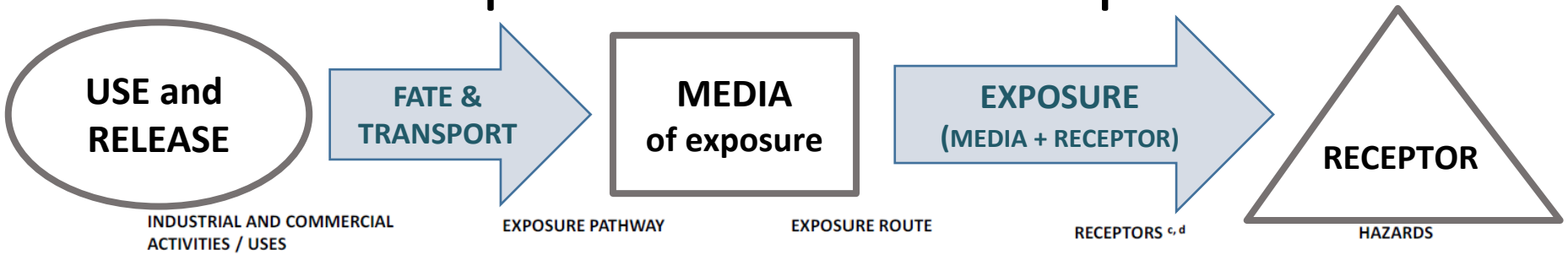


Traditionally hazard data comes from dose-response studies *in vivo*, one chemical at a time

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



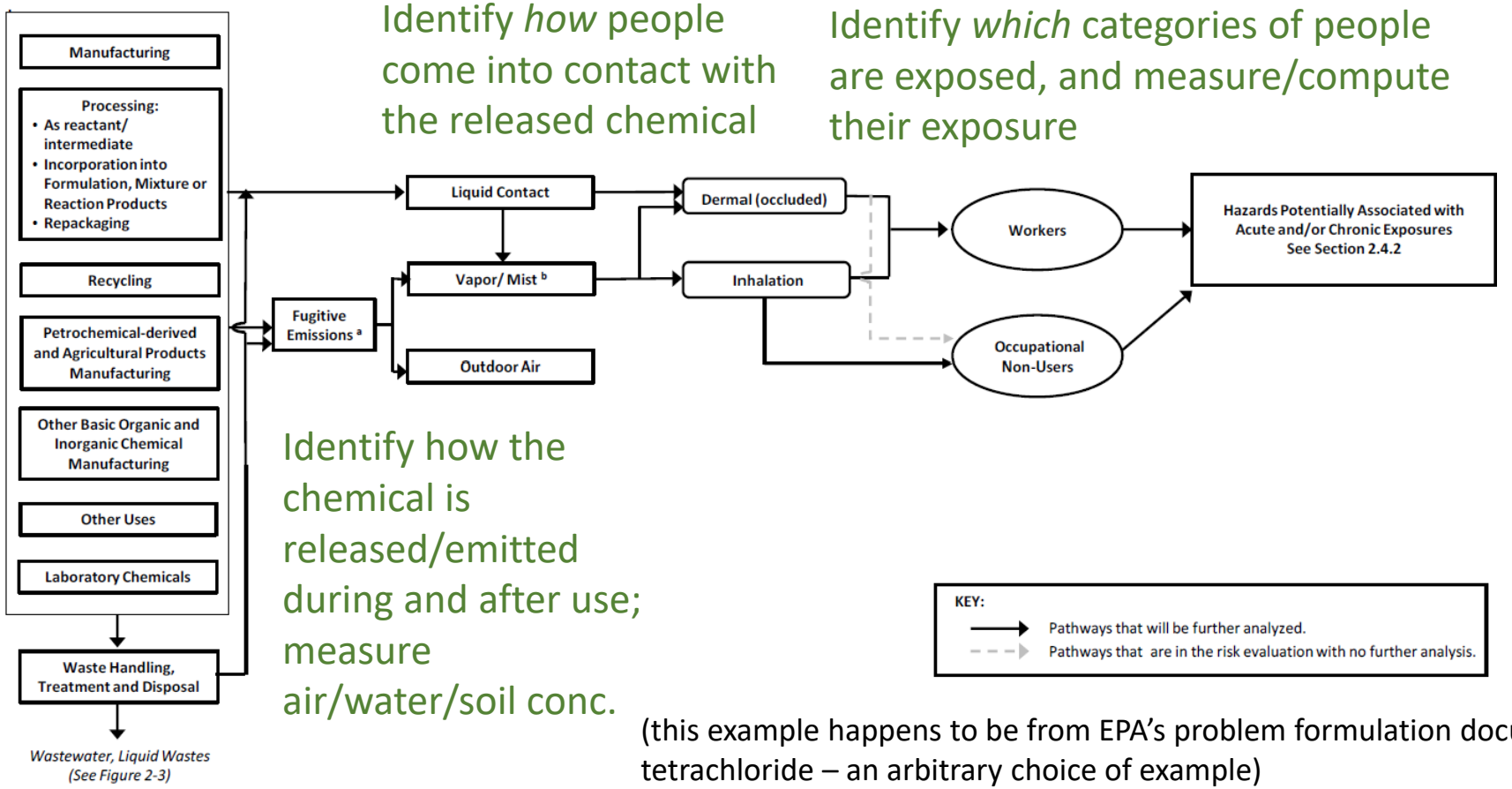
Traditional exposure assessment: Develop a conceptual model that is **chemical-specific** and **scenario-specific**



Identify specific uses of the chemical, in specific scenarios

Identify *how* people come into contact with the released chemical

Identify *which* categories of people are exposed, and measure/compute their exposure



Identify how the chemical is released/emitted during and after use; measure air/water/soil conc.

(this example happens to be from EPA's problem formulation document for carbon tetrachloride – an arbitrary choice of example)

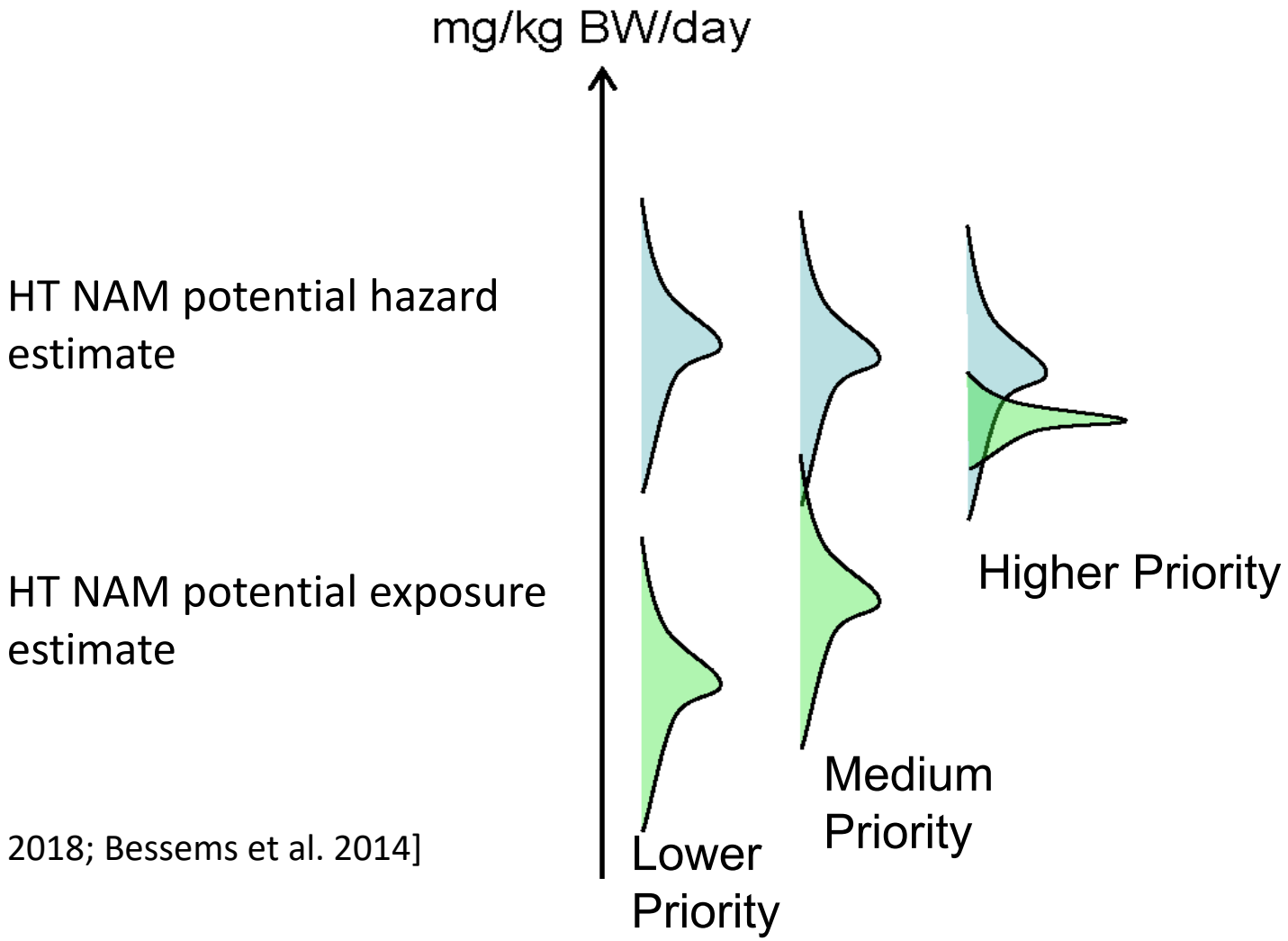
EPA has thousands of chemicals to consider, with hundreds of new ones added yearly: the traditional chemical-specific approach doesn't scale!

- Most non-food, non-drug, non-pesticide chemicals, ranging from industrial waste to dyes to packing materials, are covered by the Toxic Substances Control Act (TSCA) and come under EPA's purview
- Currently 41,953 "active" (currently-used) chemicals on TSCA inventory, and hundreds of new ones listed every year

Need some way to rapidly prioritize these chemicals according to potential risk, to decide where to invest resources for "deeper dives"

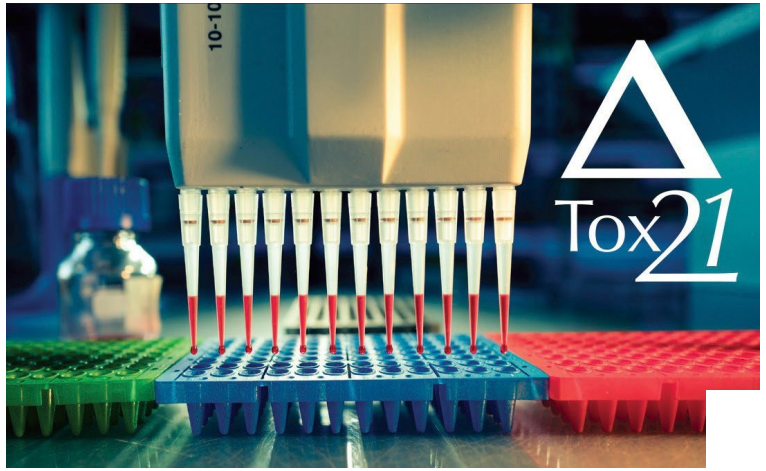
Schmidt, C. W. (2016)

Potential hazard and exposure, and potential risk, can be estimated rapidly for large numbers of chemicals using high-throughput (HT) New Approach Methodologies (NAMs) for hazard and exposure.



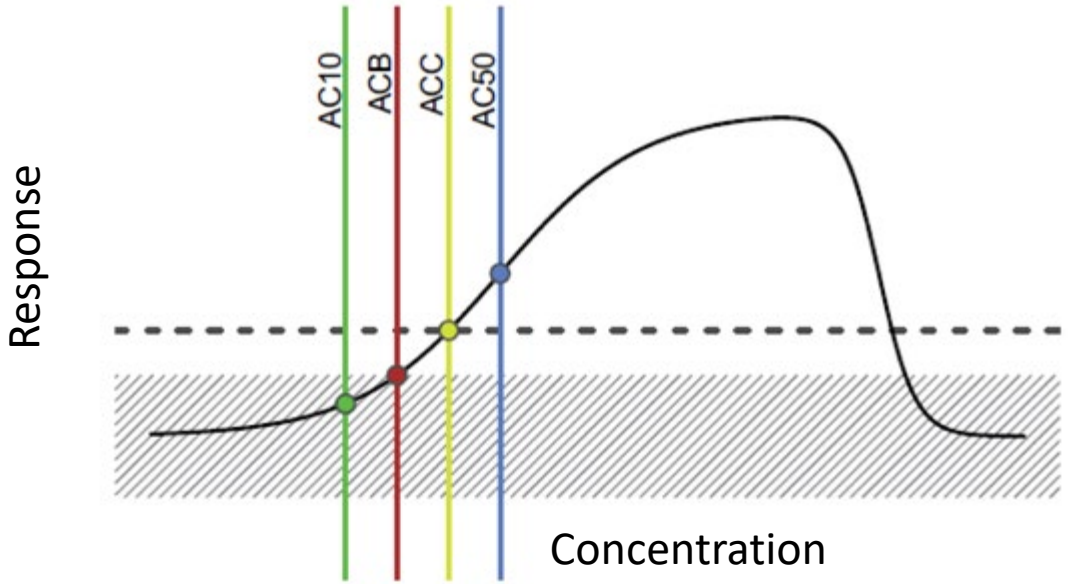
[NRC 2007; Bell et al. 2018; Bessems et al. 2014]

Potential chemical hazard can be rapidly screened using *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 bioactivity (binding, signaling, viability…)

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

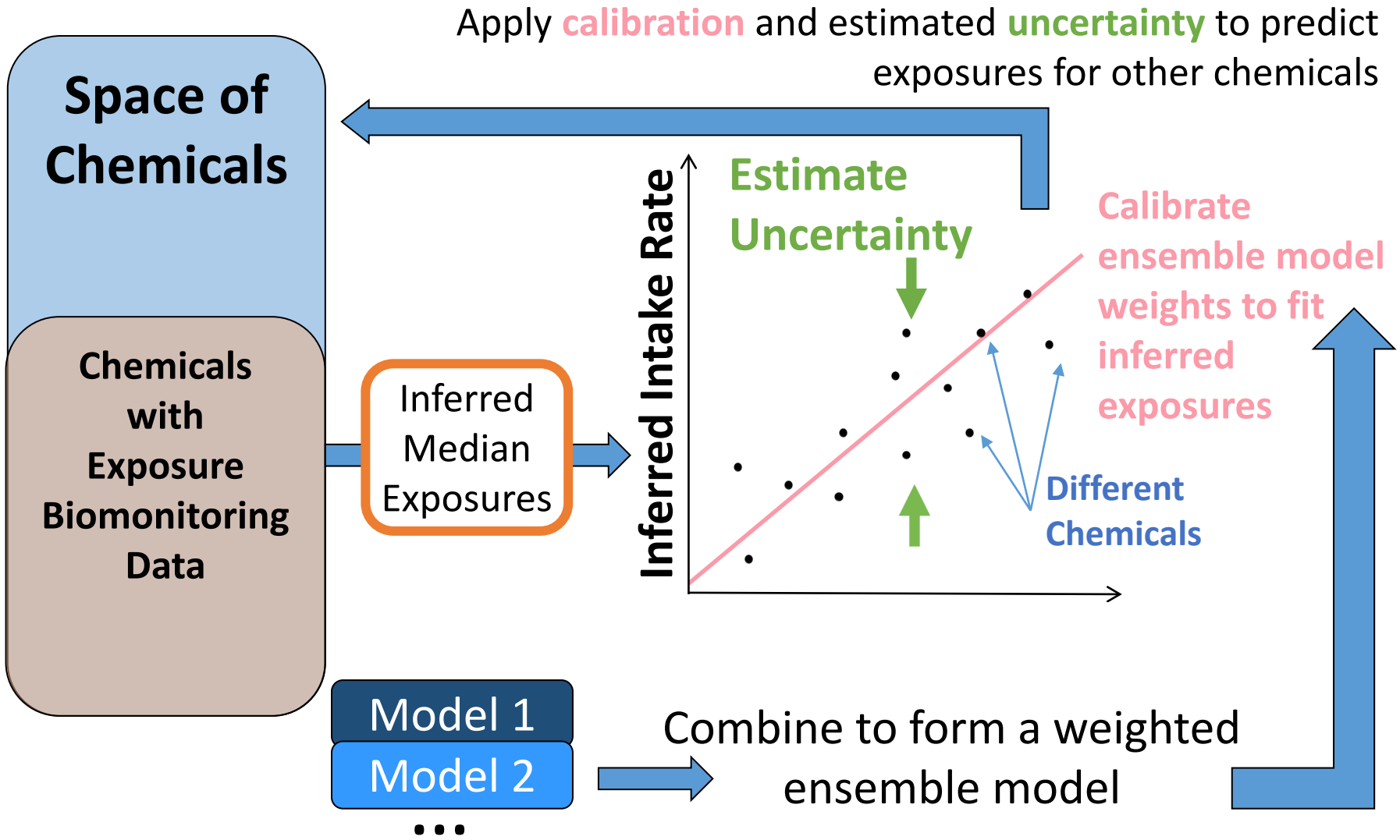


[Schmidt 2009; Dix et al. 2007; Kavlock et al. 2018]

Potential external exposures can be predicted using computational methods, e.g. the SEEM Framework (Systematic Empirical Evaluation of Models)

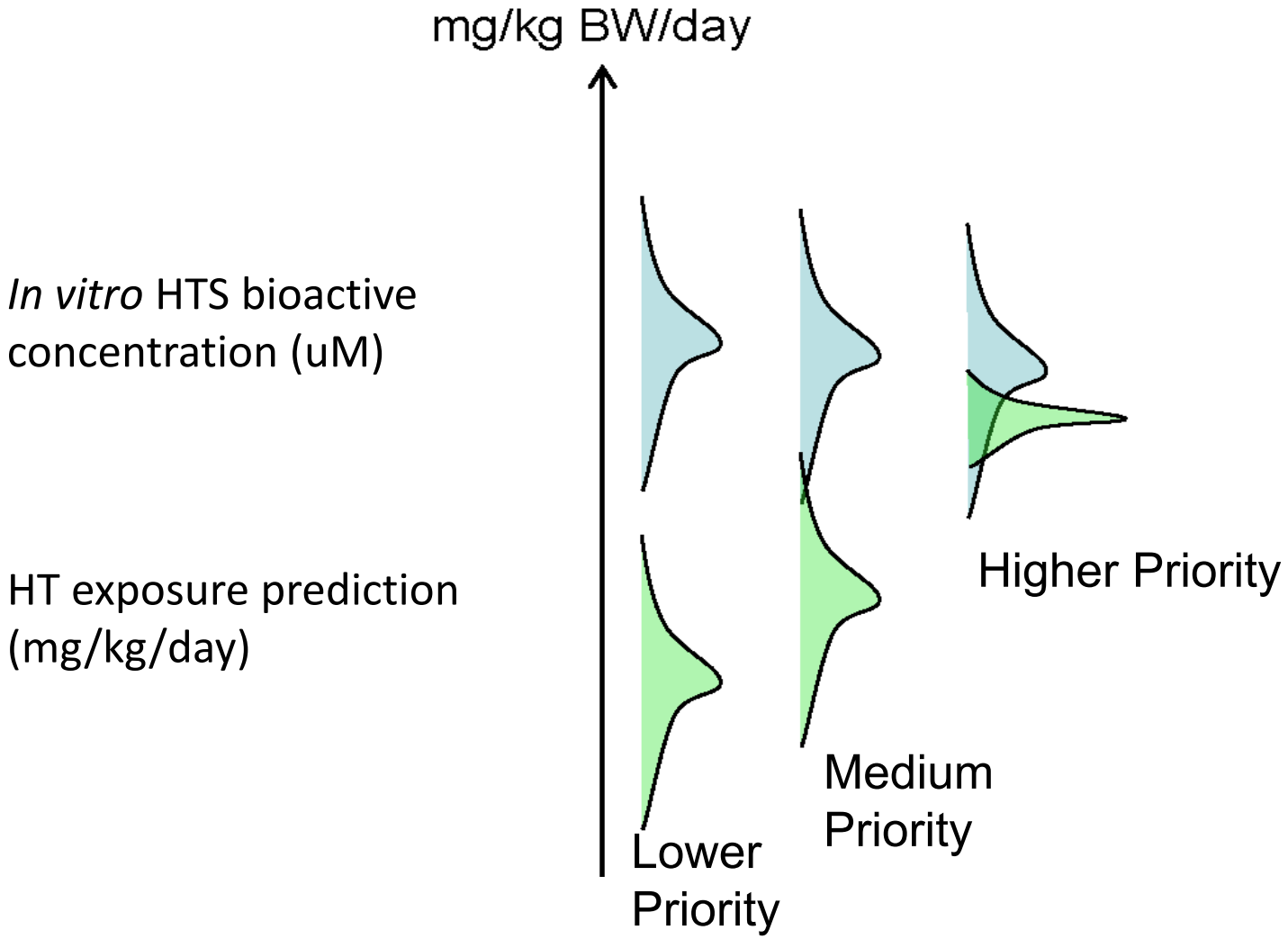
We use Bayesian methods to incorporate multiple HT exposure models with exposure biomonitoring data to make consensus exposure predictions for data-poor chemicals
(Wambaugh et al., 2013, 2014; Ring et al., 2018)

Available as R package: https://github.com/HumanExposure/SEEM3RPackage

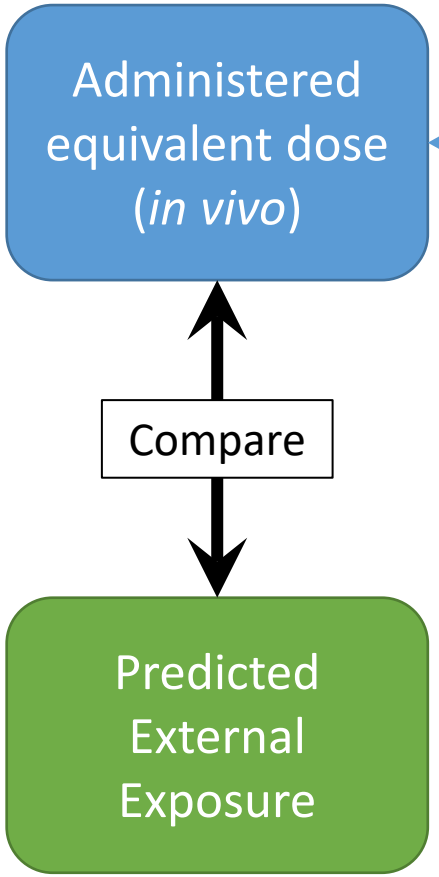


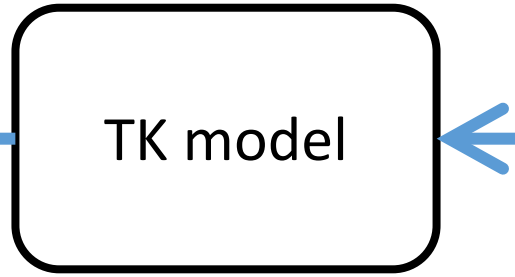
Slide adapted from John Wambaugh

HT NAMs let us rapidly predict hazard and exposure for many chemicals – but how can we compare a hazard in terms of *in vitro* concentration to an exposure in terms of external dose?



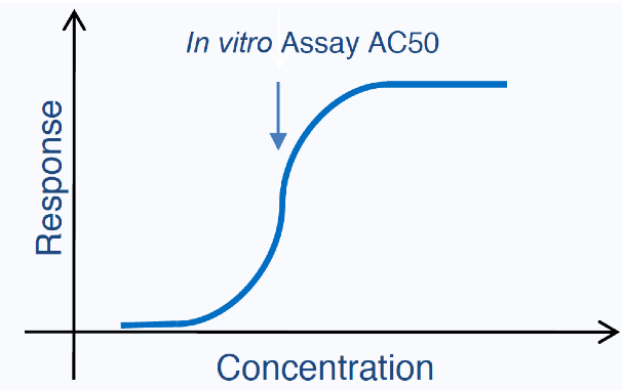
In vitro HTS bioactive concentration can be compared to predicted external exposures with *in vitro-in vivo* extrapolation (IVIVE) – using reverse toxicokinetics!





Assume equivalent to bioactive internal dose *in vivo*

[Tan et al. 2007;
Rotroff et al. 2010;
Wetmore et al. 2012, 2013, 2015]



High-throughput chemical prioritization requires *high-throughput* TK (HTTK)

Goal: A TK model that allows reverse TK to be performed rapidly, for large numbers of chemicals.

Characteristics of HTTK modeling needed to achieve that goal:

- A **generic** PBTK model
  - assumes the same ADME processes can apply to all chemicals
- A PBTK model with **minimal chemical-specific TK parameter**s
  - Minimize the number of parameters that take different values for different chemicals
- A PBTK model whose **chemical-specific TK parameters can be measured *in vitro*,** rather than having to be measured *in vivo*
  - Look for existing *in vitro* experimental methods to measure TK parameters – pharmaceutical industry has been working on this for years
- A PBTK model that is **not too computationally intensive**
  - feasible to solve for hundreds or thousands of chemicals, even when doing reverse TK
- A PBTK model that allows **quantification of uncertainty & variability** in its predictions

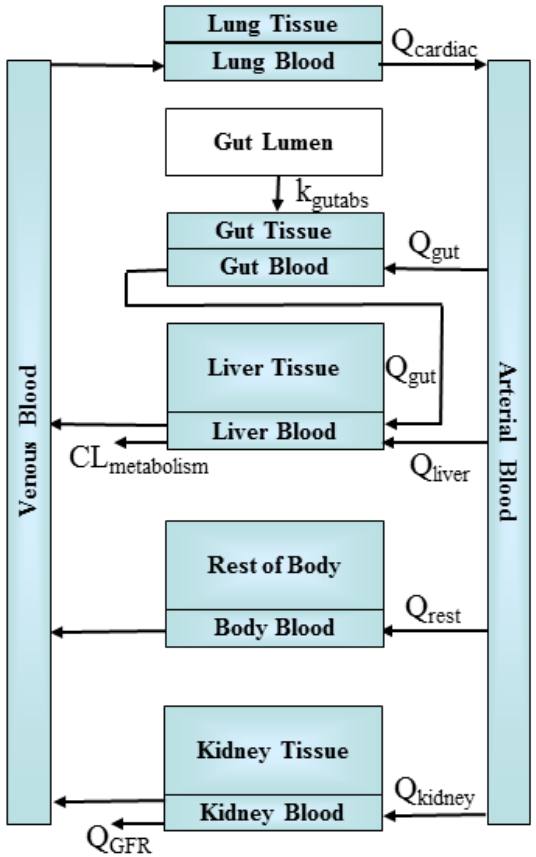
High-throughput TK (HTTK)

Generic physiologically-based TK (PBTK) model

+

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

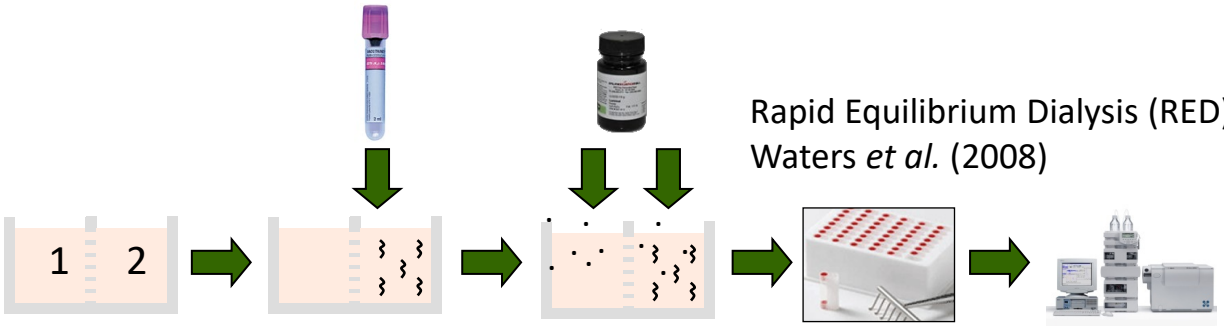
Assume clearance via hepatic metabolism (of chemical not bound to plasma proteins) & passive renal filtration



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

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

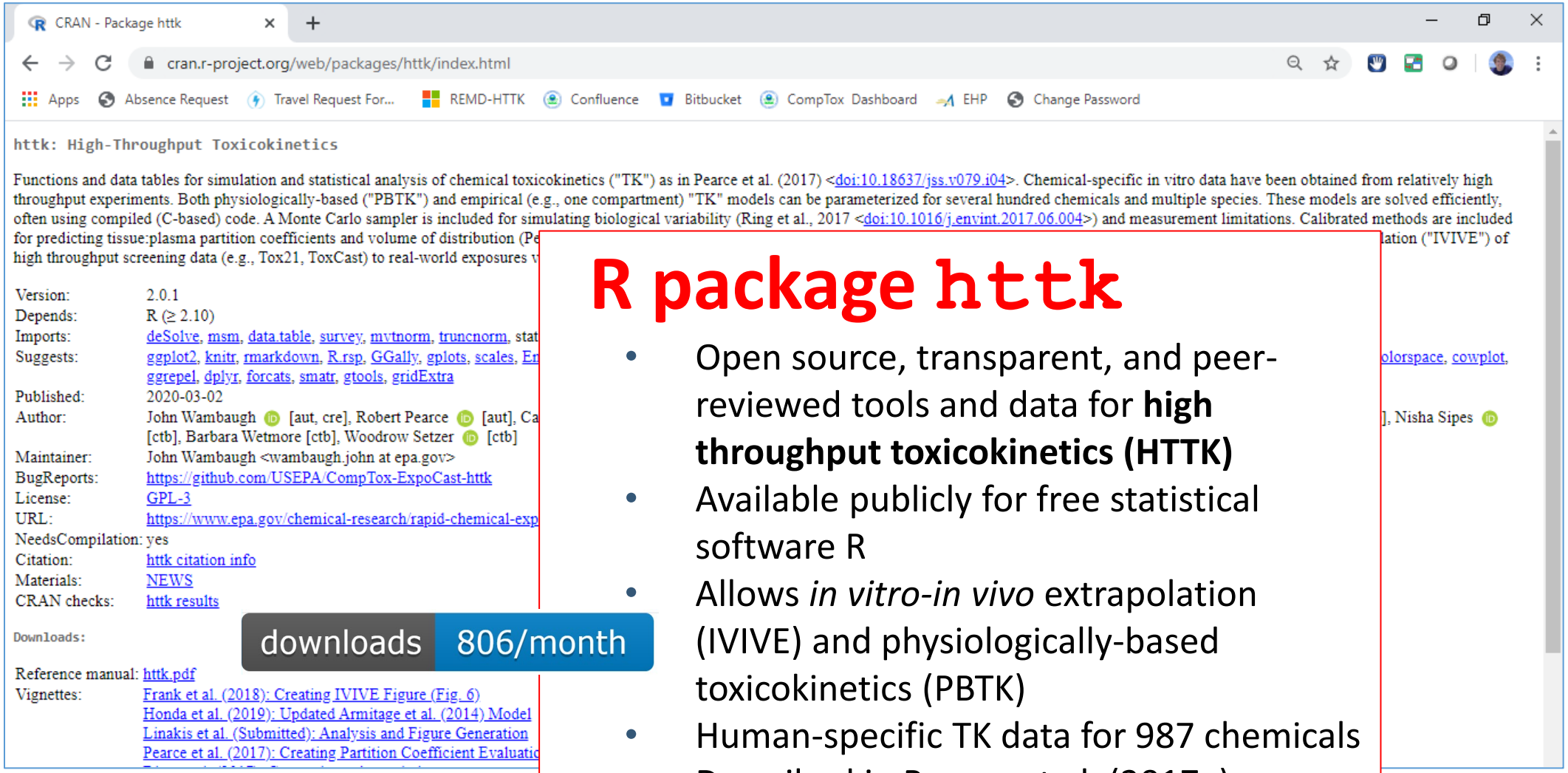
Rapid Equilibrium Dialysis (RED)
Waters *et al.* (2008)



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

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

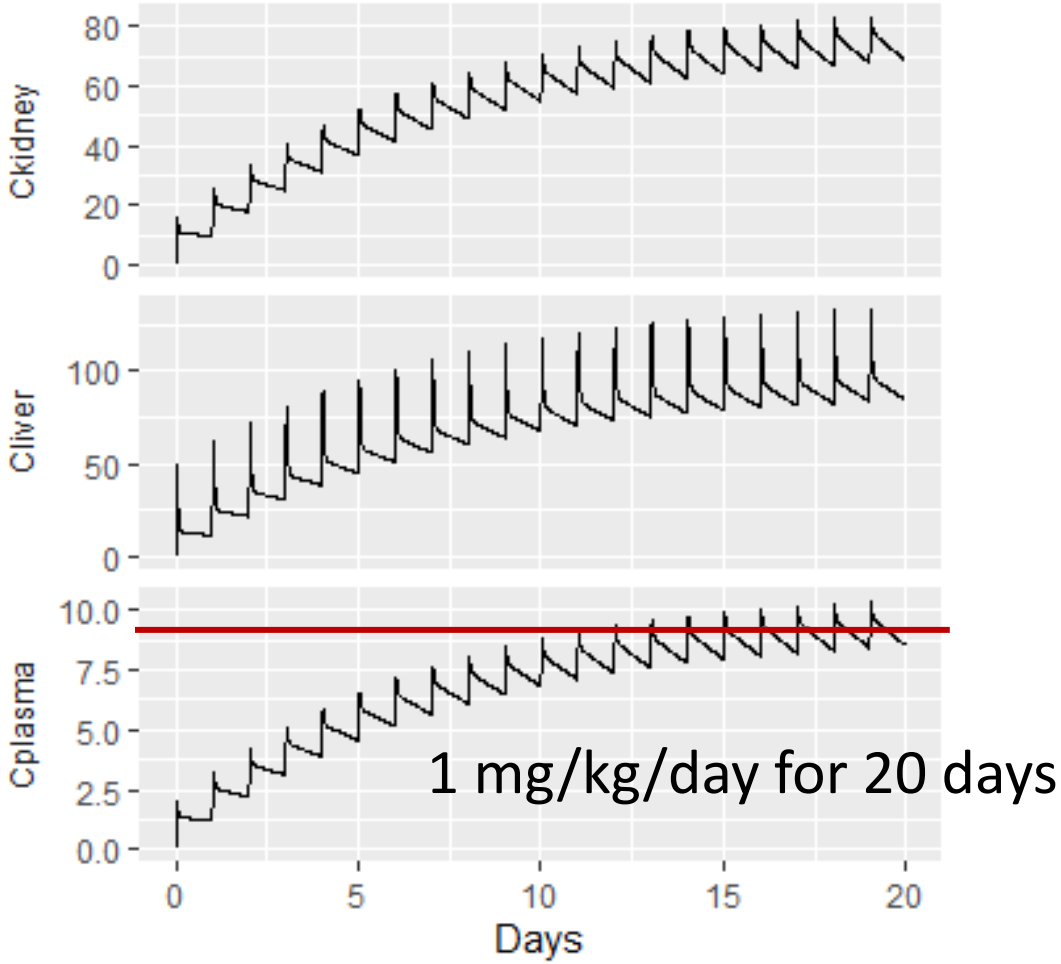
https://CRAN.R-project.org/package=httk



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)

For screening purposes, we are usually interested in long-term, low-level exposures, so we focus on the steady-state plasma concentration (Css) after long-term repeated dosing

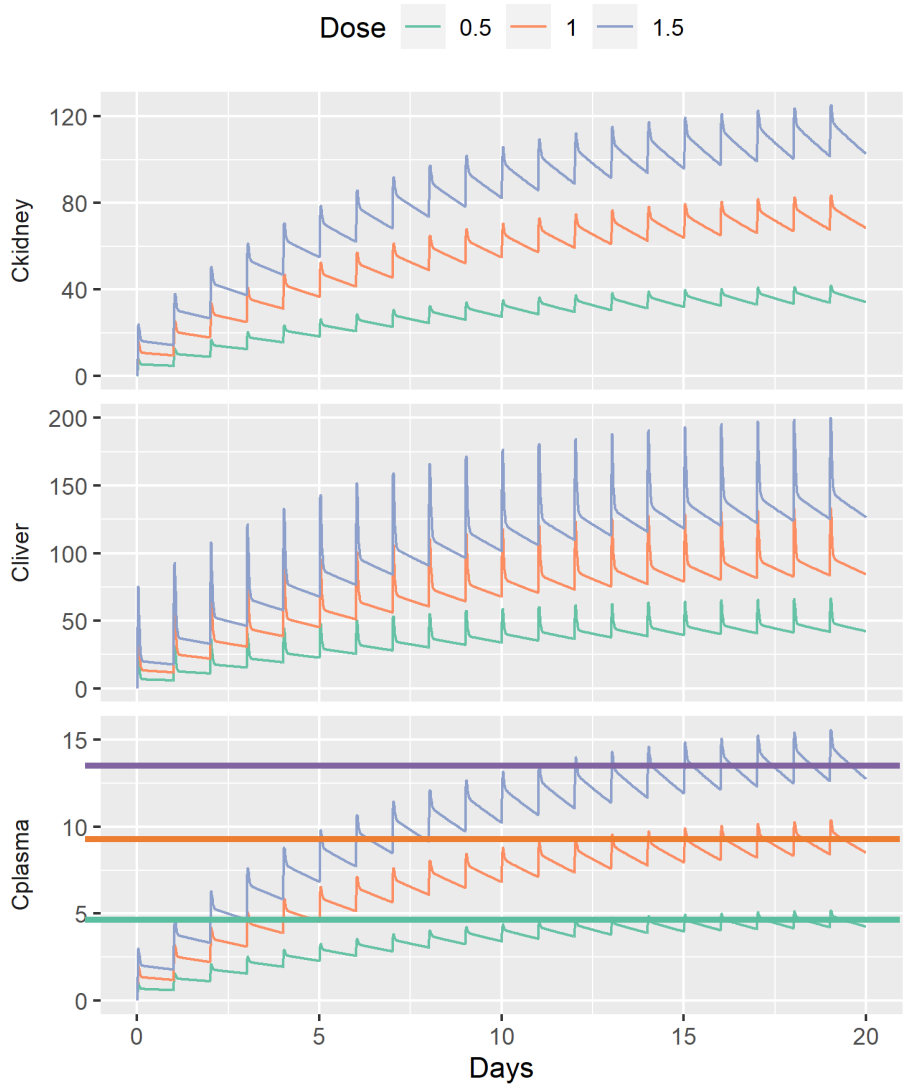


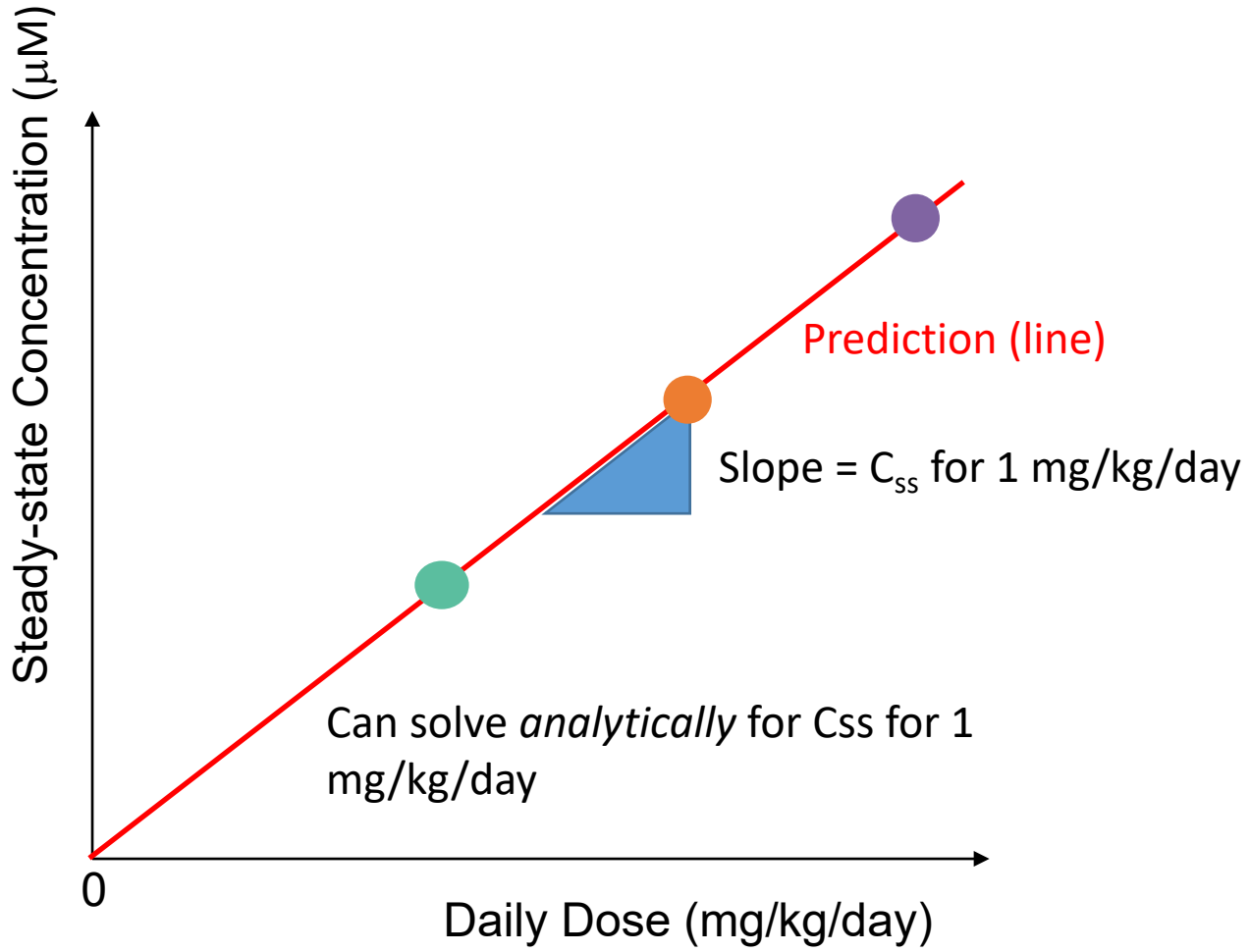
Using a summary metric of internal dose simplifies the computational load.

We no longer need to store and analyze the full concentration vs. time trace for each chemical.

Instead we need to store only *one* number for each chemical: TK model-predicted Css.

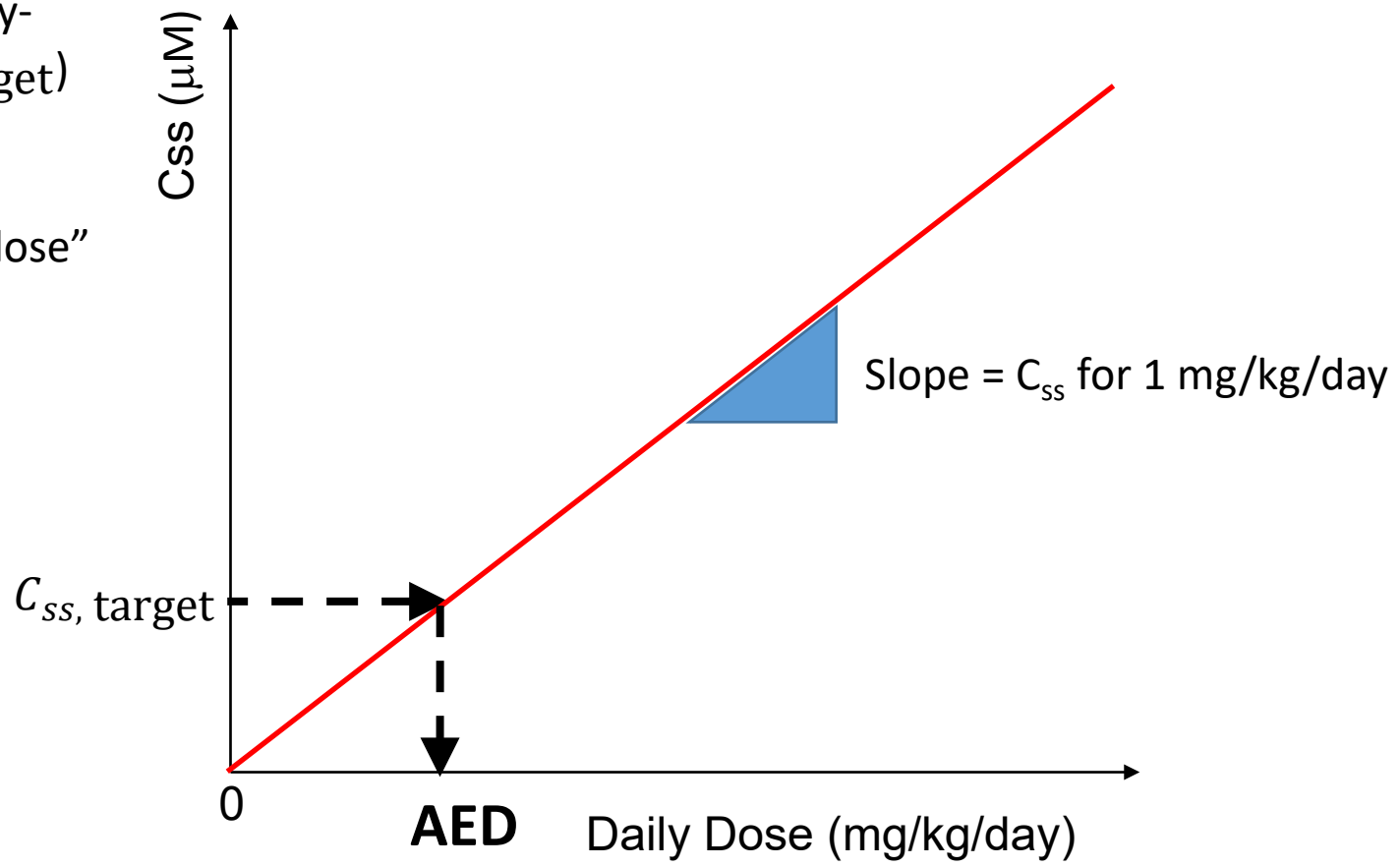
We use relatively simple TK models where Css has a linear relationship with dose





Linear relationship makes reverse TK quick & easy

- Calculate slope (Css for dose = 1 mg/kg/day)
- Graphically:
  - start with the "target" concentration on the y-axis *(in vitro* bioactive concentration $C_{ss,\,\mathrm{target}}$)
  - go over to the Css-dose line
  - drop down to the x-axis
  - then read off the "administered equivalent dose" (AED) on the x-axis.
- Mathematically: $\mathrm{AED} = \frac{\mathrm{C_{ss},\,target}}{\mathrm{slope}}$



Wetmore *et al*. (2012)

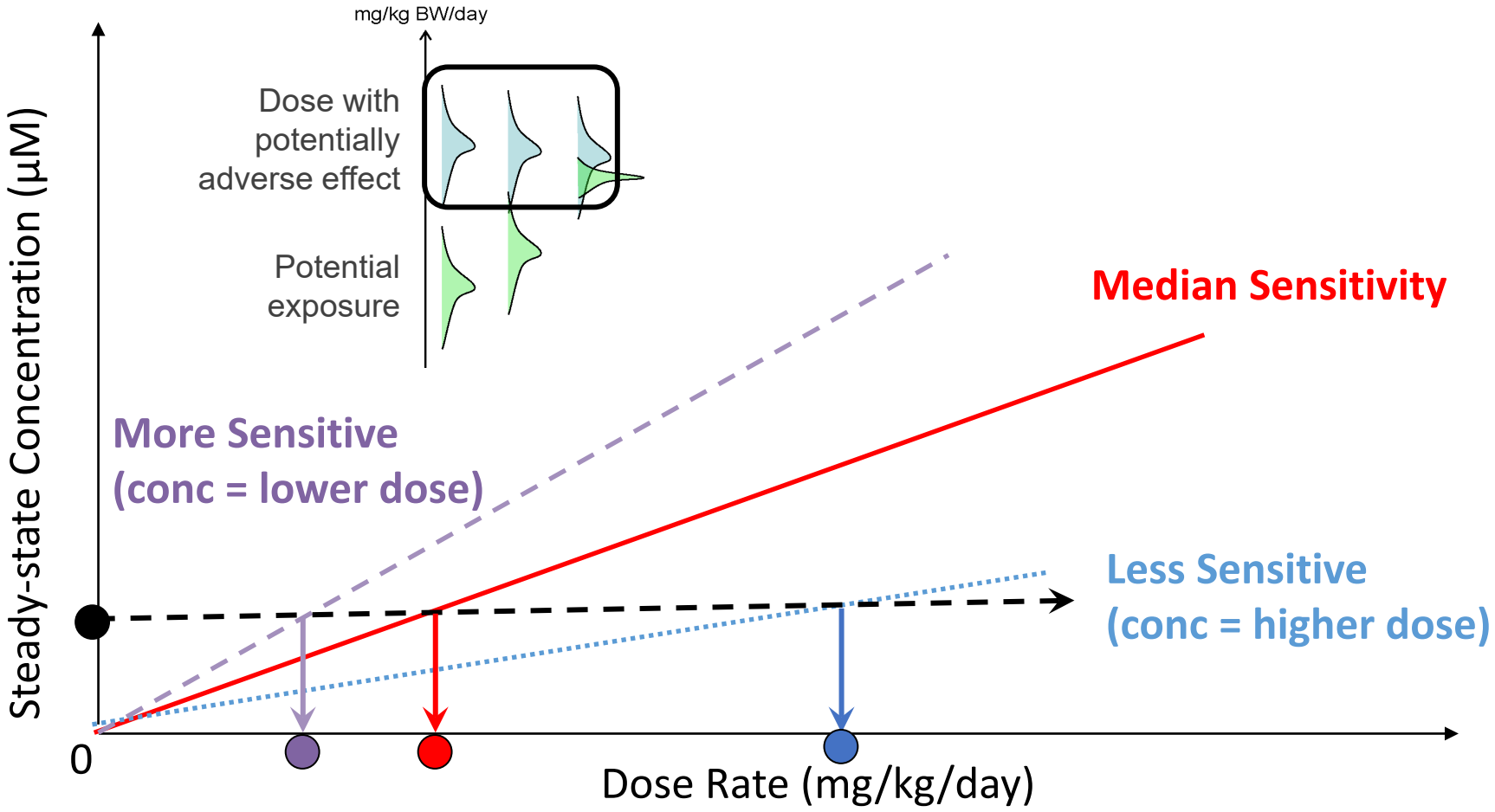
Q: What determines the slope of the line?
A: The TK model parameters.

Chemical-specific parameters	
Intrinsic hepatic clearance rate	Measured in HT *in vitro* assays (Rotroff *et al.* 2010; Wetmore *et al.* 2012, 2014, 2015; Wambaugh *et al.* 2019)
Fraction unbound to plasma protein	
Tissue:blood partition coefficients	Predict from phys-chem properties and tissue properties (Pearce et al., 2017b)
Physiological parameters	
Tissue masses (including body weight)	Gathered from data available in the published literature [Wambaugh et al. 2015; Pearce et al. 2017a]
Tissue blood flows	
Glomerular filtration rate (passive renal clearance)	
Hepatocellularity	

TK model parameters represent biology — so they have population variability

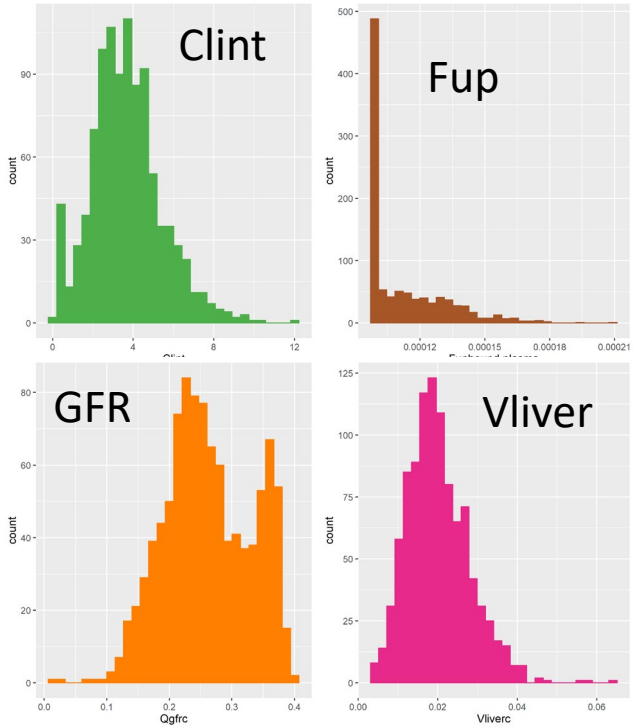
Chemical-specific parameters	
Intrinsic hepatic clearance rate	Represent chemical-body interactions — vary with individual genetics, environmental factors, age, etc.
Fraction unbound to plasma protein	
Tissue:blood partition coefficients (for compartmental models)	
Physiological parameters	
Tissue masses (including body weight)	Represent physiology — vary with individual genetics, environmental factors, age, etc.
Tissue blood flows	
Glomerular filtration rate (passive renal clearance)	
Hepatocellularity	

That means the slope of the line varies across the population — so a single *in vitro* concentration corresponds to a *distribution* of external doses.



Population variability in IVIVE can be quantified using a Monte Carlo approach

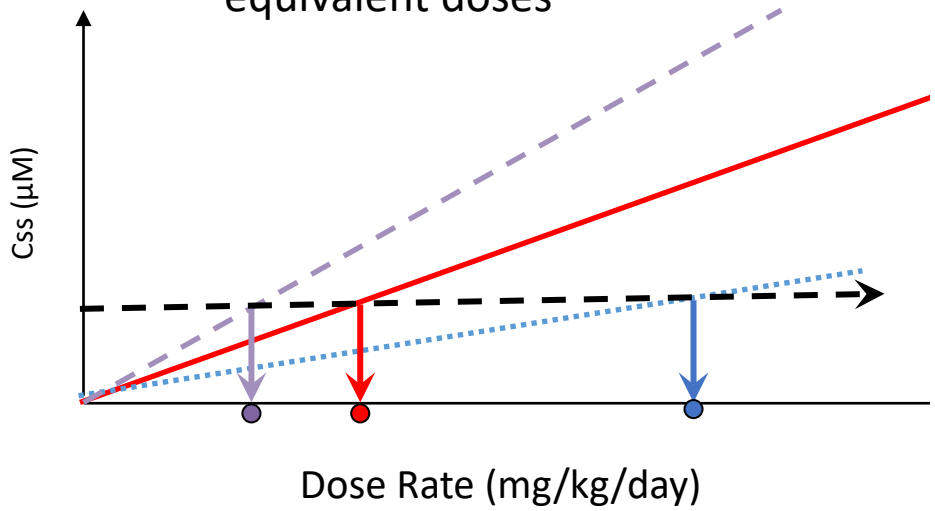
Draw samples from population distribution of TK model parameters



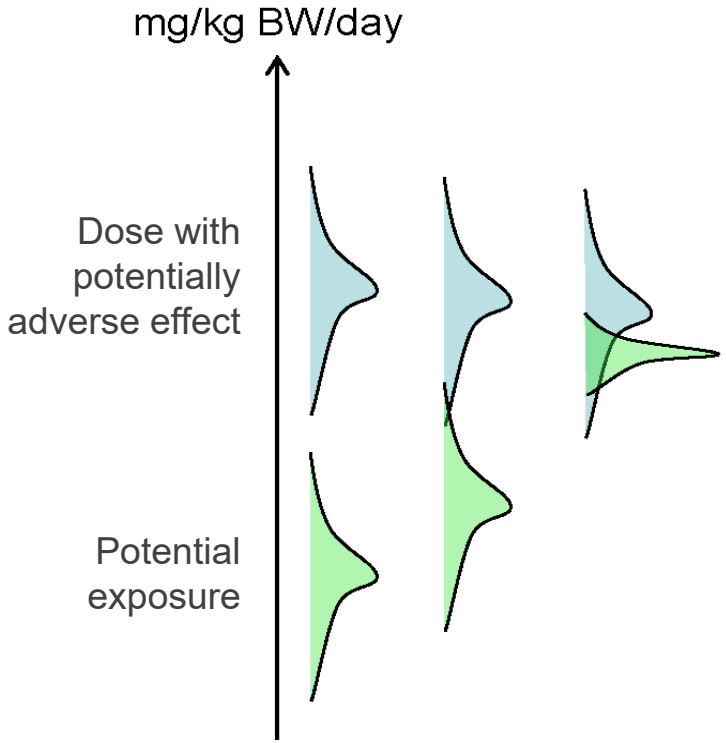
(+ other params)

Calculate Css-dose slope (TK model-predicted Css for dose = 1 mg/kg/day) for each sampled set of TK model parameters

Get resulting distribution of equivalent doses



Compare equivalent dose distribution to potential exposure distribution to calculate potential risk

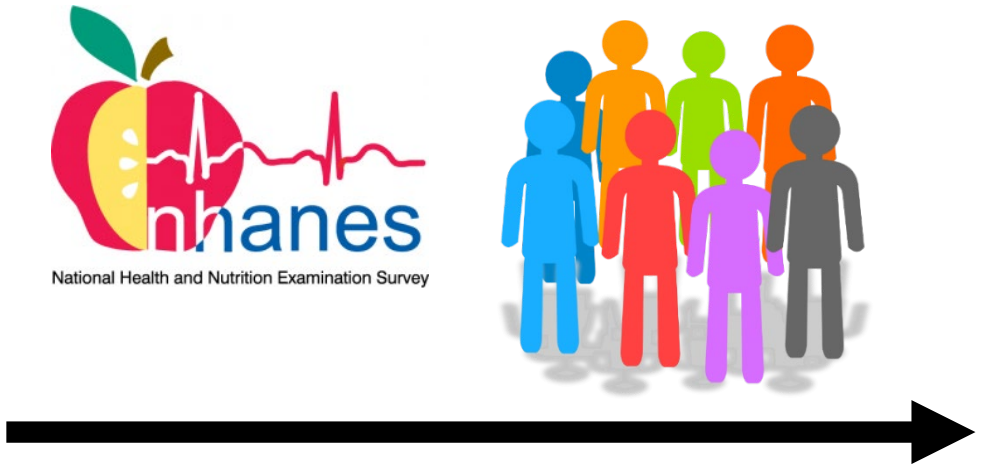


Sample from estimated population distribution of physiological TK parameters using a *correlated* Monte Carlo approach (**HTTK-Pop**)

Based on physiology data measured as part of the US CDC National Health and Nutrition Examination Survey (NHANES) — publicly available on the web at https://www.cdc.gov/nchs/nhanes/index.htm

Sample NHANES-measured quantities for actual individuals:

- Sex
- Race/ethnicity
- Age
- Height
- Weight
- Serum creatinine
- Hematocrit



Regression equations from literature (McNally *et al.*, 2014) (+ residual marginal variability)

(Similar approach used in SimCYP [Jamei et al. 2009], GastroPlus, PopGen [McNally et al. 2014], P3M [Price et al. 2003], physB [Bosgra et al. 2012], etc.)

Predict physiological TK quantities (as used by generic TK model) for each individual:

- Tissue masses
- Tissue blood flows
- GFR (kidney function)
- Hepatocellularity

Ring *et al*. (2017)

HTTK-Pop can generate simulated populations with user-specified demographics if desired

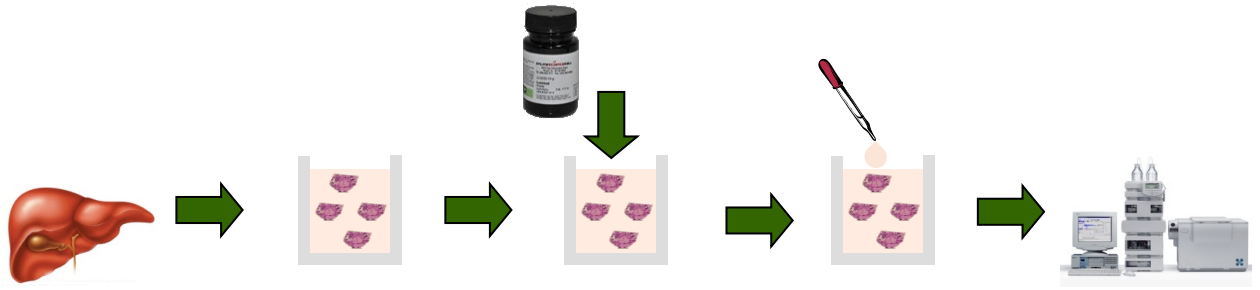
User can specify…	Example	Default if not specified
Age limits in years	Ages 6-11 years	All NHANES (0-79 years)
Age limits in months	Ages 0-36 months	All NHANES (0-79 years)
# of males and females	1000 males, 0 females	Randomly selected from NHANES respondents
BMI category	BMI > 25 (overweight & obese)	Randomly selected from NHANES respondents

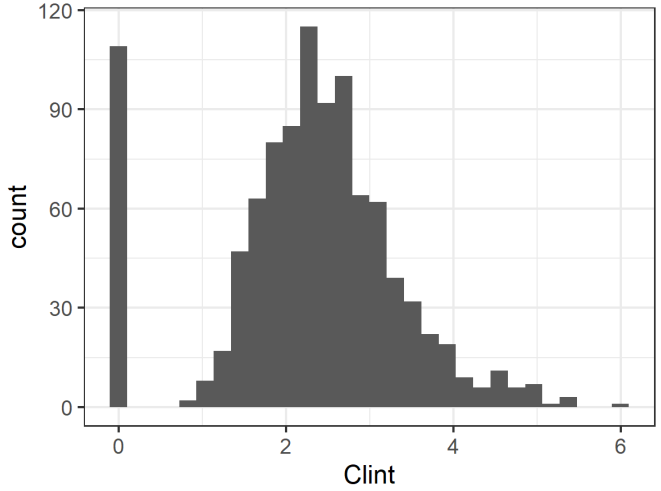
HTTK-Pop produces samples of physiological TK model parameters based on NHANES respondents in the specified demographic groups

Also: chemical-specific parameters measured *in vitro* carry measurement uncertainty

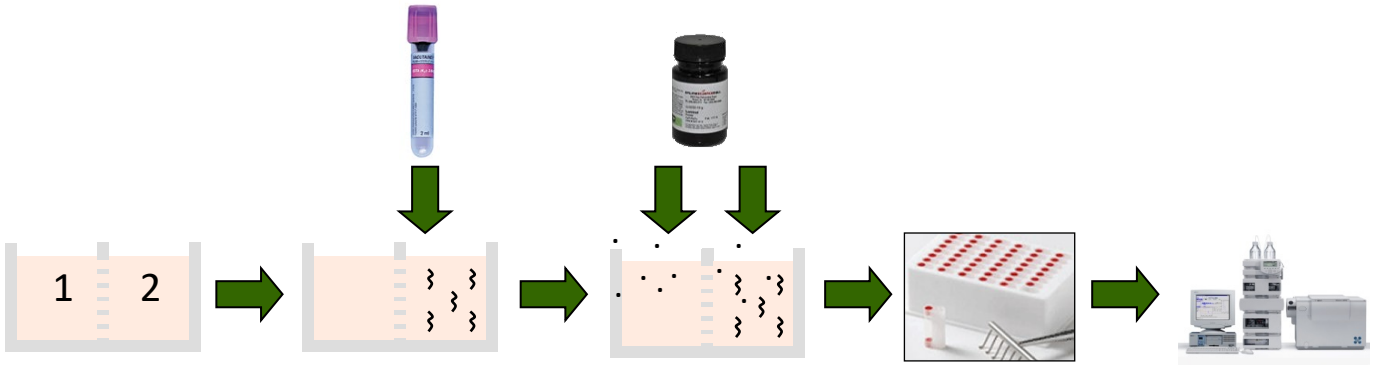
Result: A *distribution* of possible values for the chemical-specific parameter

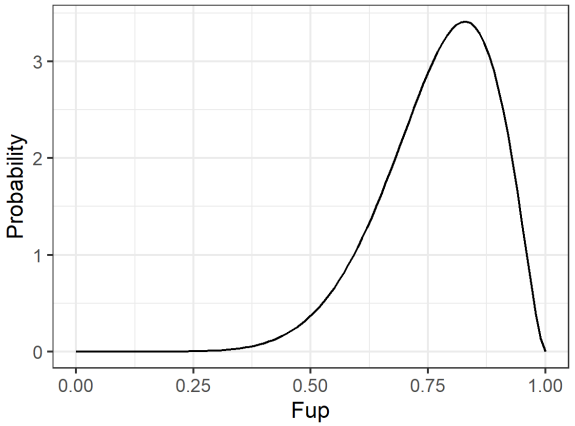
CLint: Cryo-preserved hepatocyte suspension
Shibata *et al.* (2002)





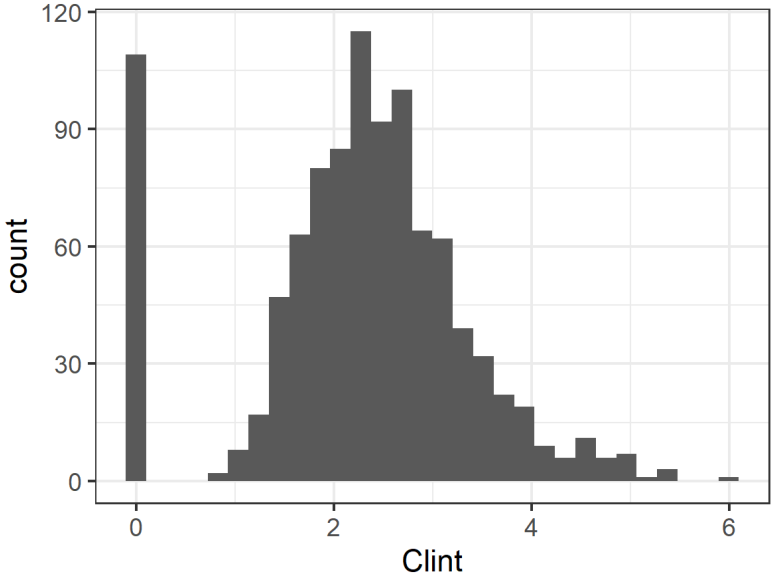
Fup: Rapid Equilibrium Dialysis (RED)
Waters *et al.* (2008)



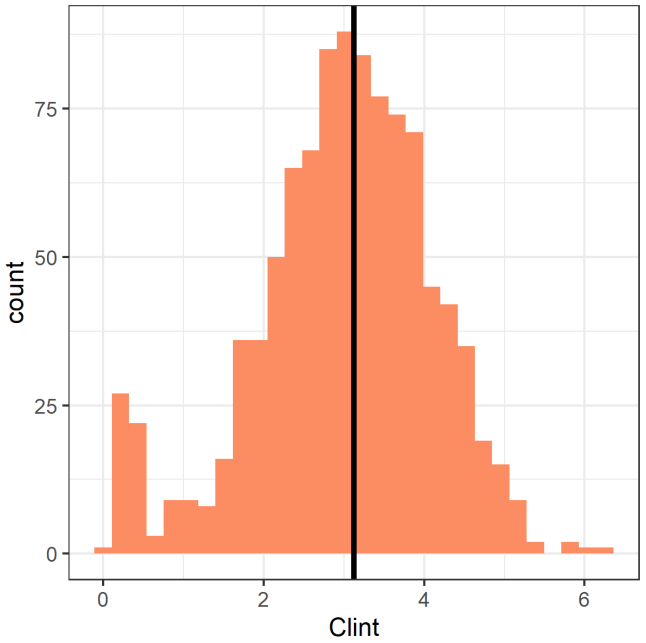


Chemical-specific TK parameters: Two-stage Monte Carlo approach to modeling both *measurement uncertainty* and *population variability*

Step 1: Draw 1 sample from uncertainty distribution and treat as "population average" value

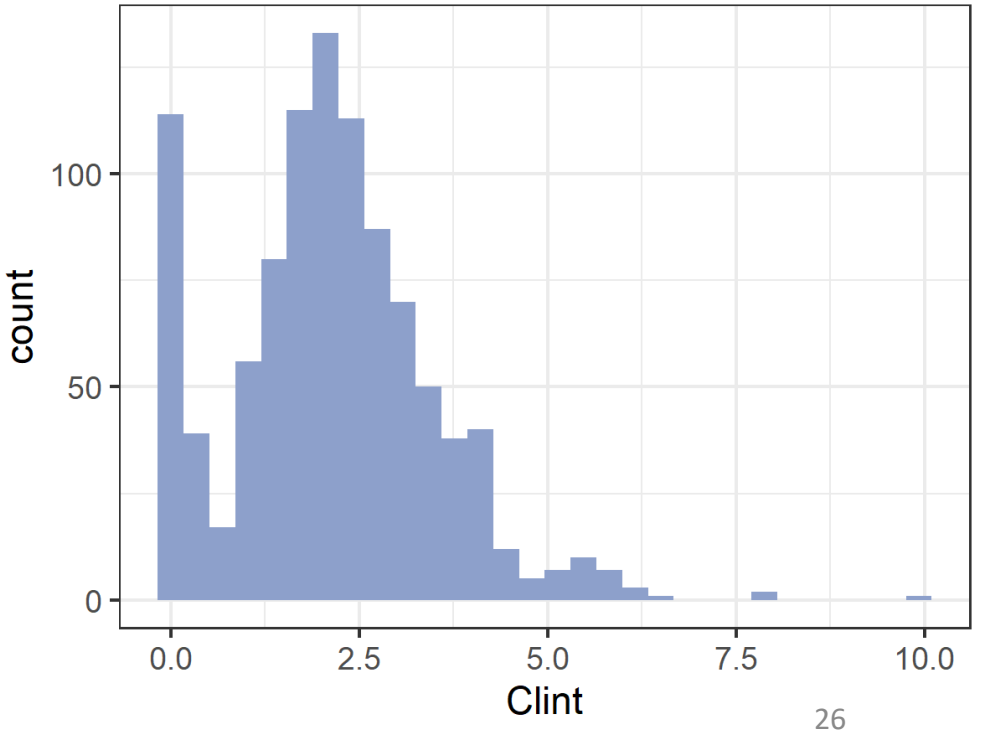


Step 2: Assume population variability (30% CV) around the sampled "population average" value from Step 1, and draw 1 sample



For CLint: Add 5% "poor metabolizers" (10% of original pop. average)

Repeat Steps 1 and 2 for each simulated individual to get sampled values that include both uncertainty & variability



Wambaugh et al. (2019)

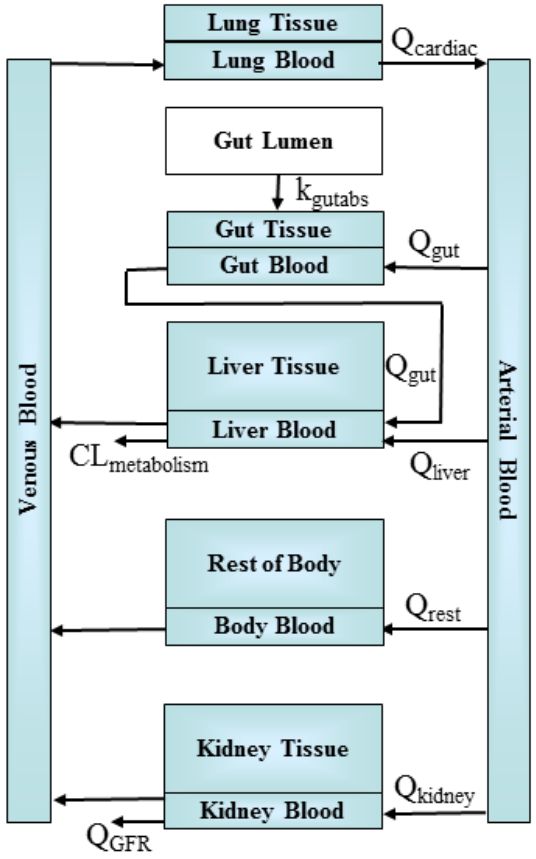
Putting it all together: A table of HTTK model parameters for each "simulated individual" in a "simulated population", for a given chemical

SEQN	Demographics		Body measures		Tissue volumes	Blood flows	GFR	Hepatocellularity	Fup	Clint
	Sex	Age	Ht	Wt						
67184	M	42	171	55	[…]	[…]	[…]	[…]	[…]	[…]
52034	M	0.5	73	9	[…]	[…]	[…]	[…]	[…]	[…]
64847	F	11	154	47	[…]	[…]	[…]	[…]	[…]	[…]
51787	F	22	166	87	[…]	[…]	[…]	[…]	[…]	[…]
49889	M	9	147	50	[…]	[…]	[…]	[…]	[…]	[…]
64606	F	59	169	115	[…]	[…]	[…]	[…]	[…]	[…]
45549	F	50	165	80	[…]	[…]	[…]	[…]	[…]	[…]
[…]	[…]	[…]	[…]	[…]	[…]	[…]	[…]	[…]	[…]	[…]

NB: This is fake data for illustration purposes

Putting it all together: Evaluate Css at 1 mg/kg/day (Css-dose slope) for each "simulated individual" for a given chemical

SEQN	[Physiological TK parameters]	Fup	Clint
67184	[…]	[…]	[…]
52034	[…]	[…]	[…]
64847	[…]	[…]	[…]
51787	[…]	[…]	[…]
49889	[…]	[…]	[…]
64606	[…]	[…]	[…]
45549	[…]	[…]	[…]
[…]	[…]	[…]	[…]

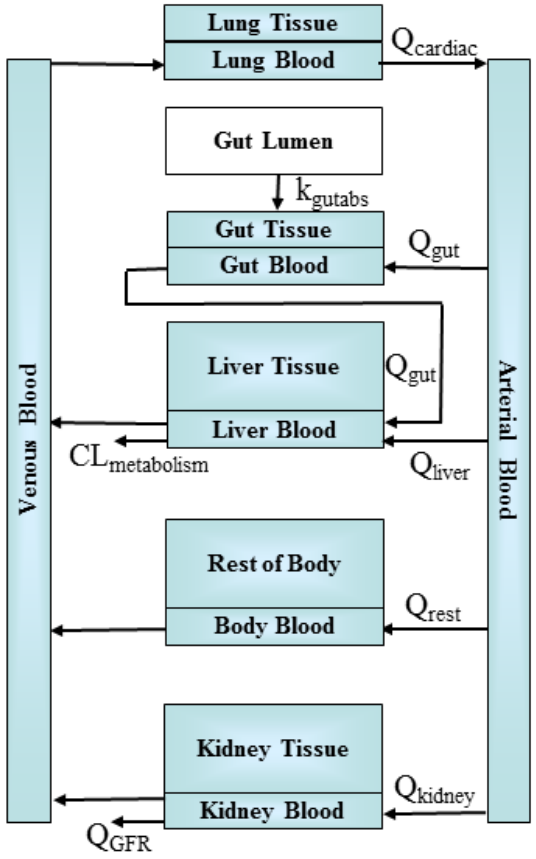


NB: This is fake data for illustration purposes – these slopes may not really correspond to these individuals

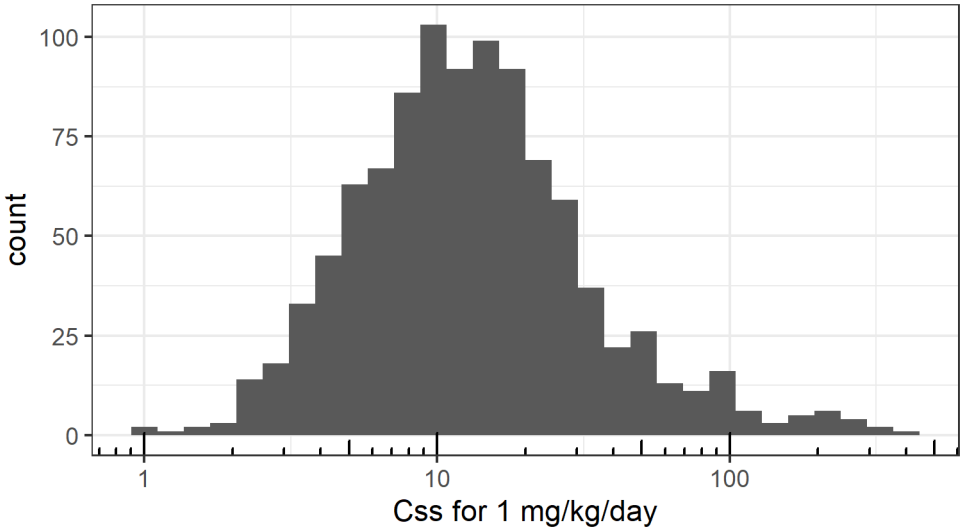
SEQN	Css at 1 mg/kg/day
67184	10.110
52034	25.710
64847	18.040
51787	14.460
49889	18.650
64606	8.481
45549	6.886
[…]	[…]

Putting it all together: Evaluate Css at 1 mg/kg/day (Css-dose slope) for each "simulated individual" for a given chemical

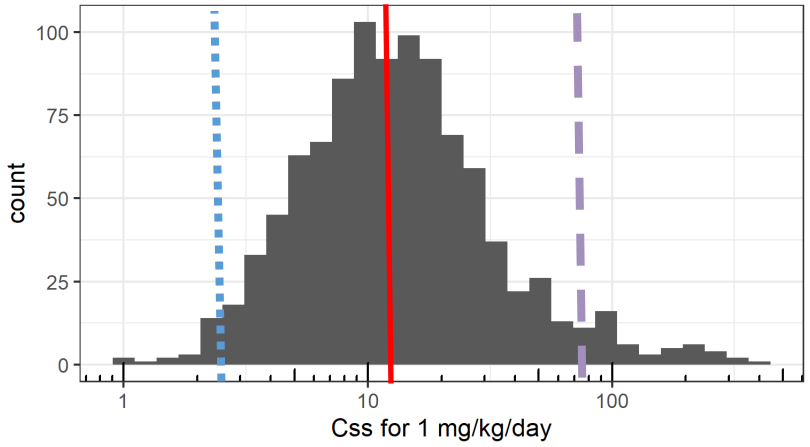
SEQN	[Physiological TK parameters]	Fup	Clint
67184	[…]	[…]	[…]
52034	[…]	[…]	[…]
64847	[…]	[…]	[…]
51787	[…]	[…]	[…]
49889	[…]	[…]	[…]
64606	[…]	[…]	[…]
45549	[…]	[…]	[…]
[…]	[…]	[…]	[…]

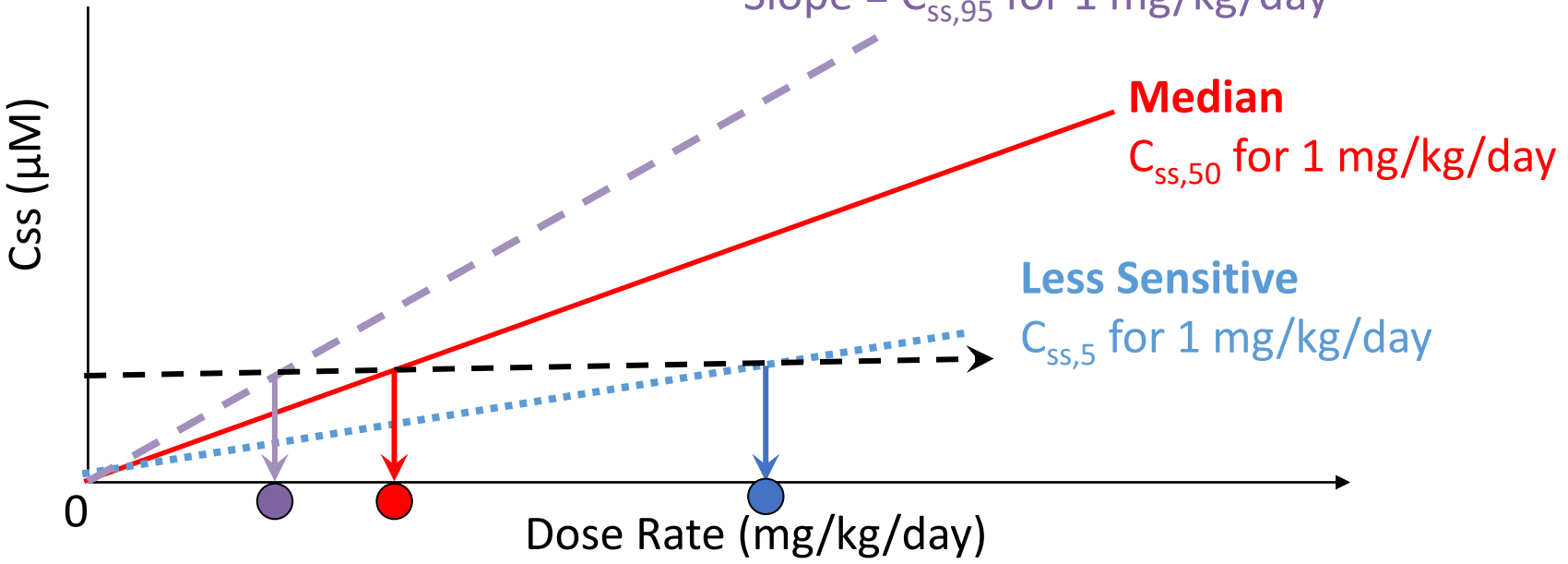


Result: Samples characterize a *distribution* of Css-dose slope values

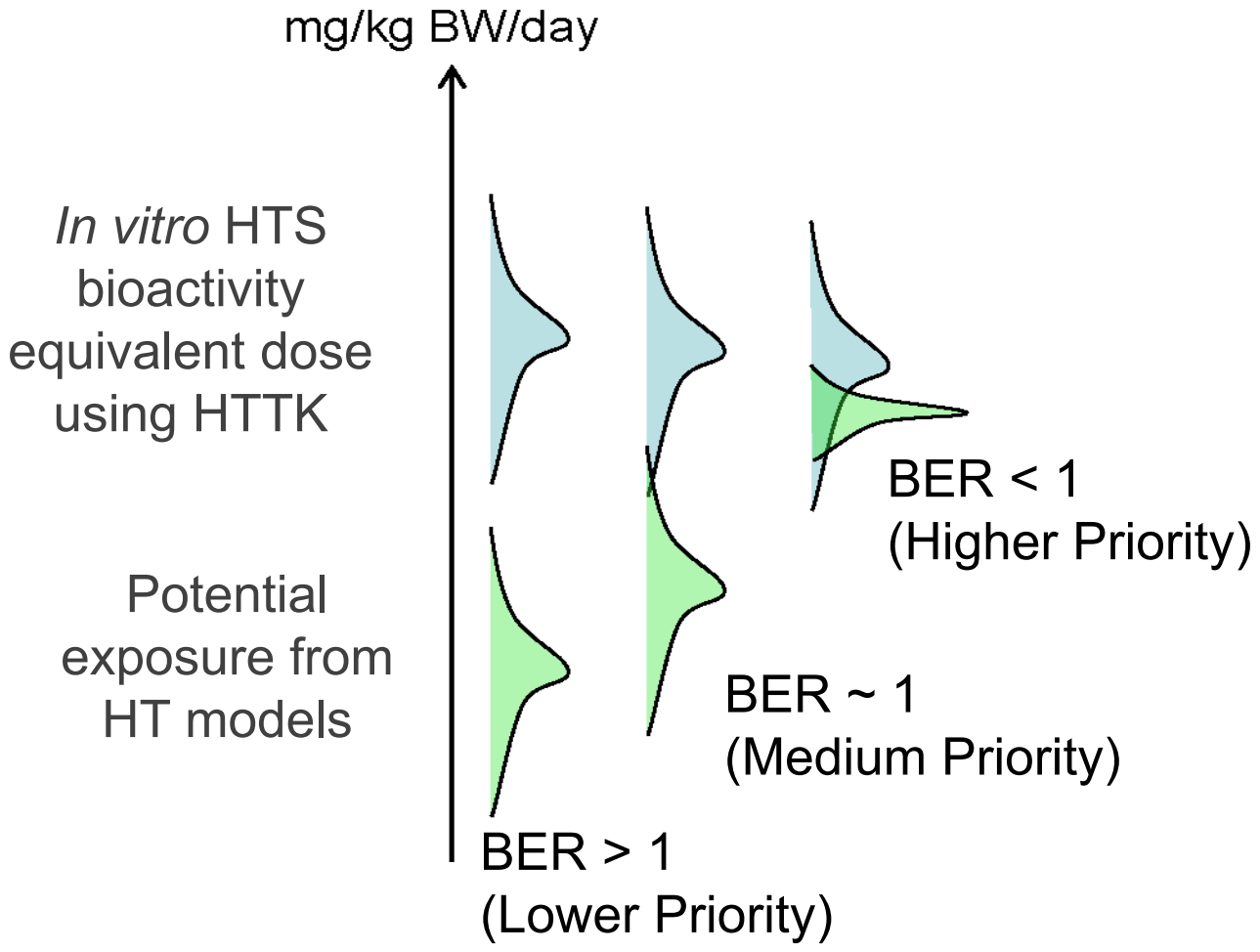


Steeper slopes have lower equivalent doses – 95th percentile slope = “most-sensitive” 5% of the population

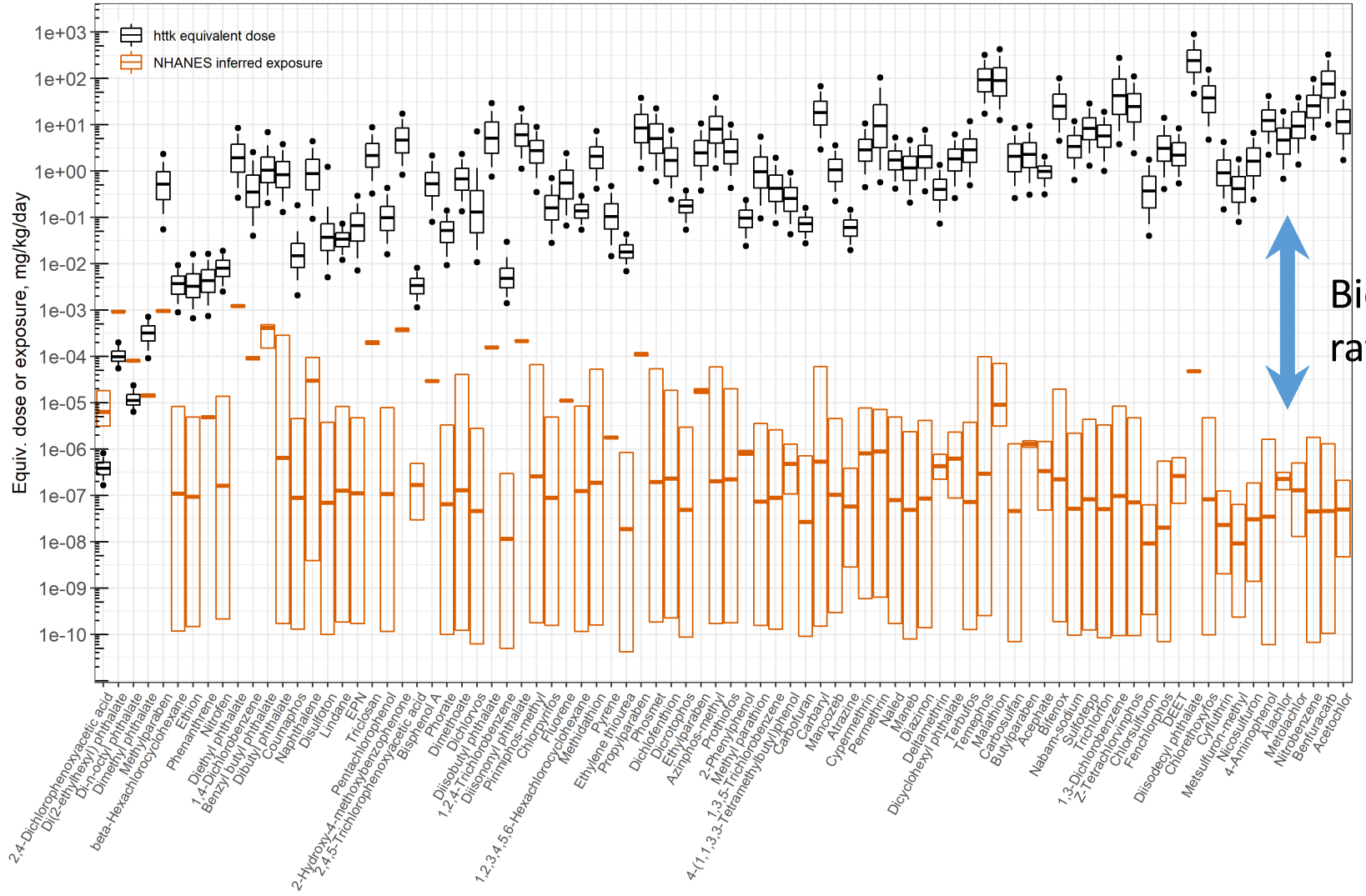




Then, we compare the low-end equivalent dose to the high-end potential exposure to calculate "Bioactivity-Exposure Ratio" (BER).



Example: BER-based prioritization of 84 chemicals, using IVIVE of ToxCast AC50s.



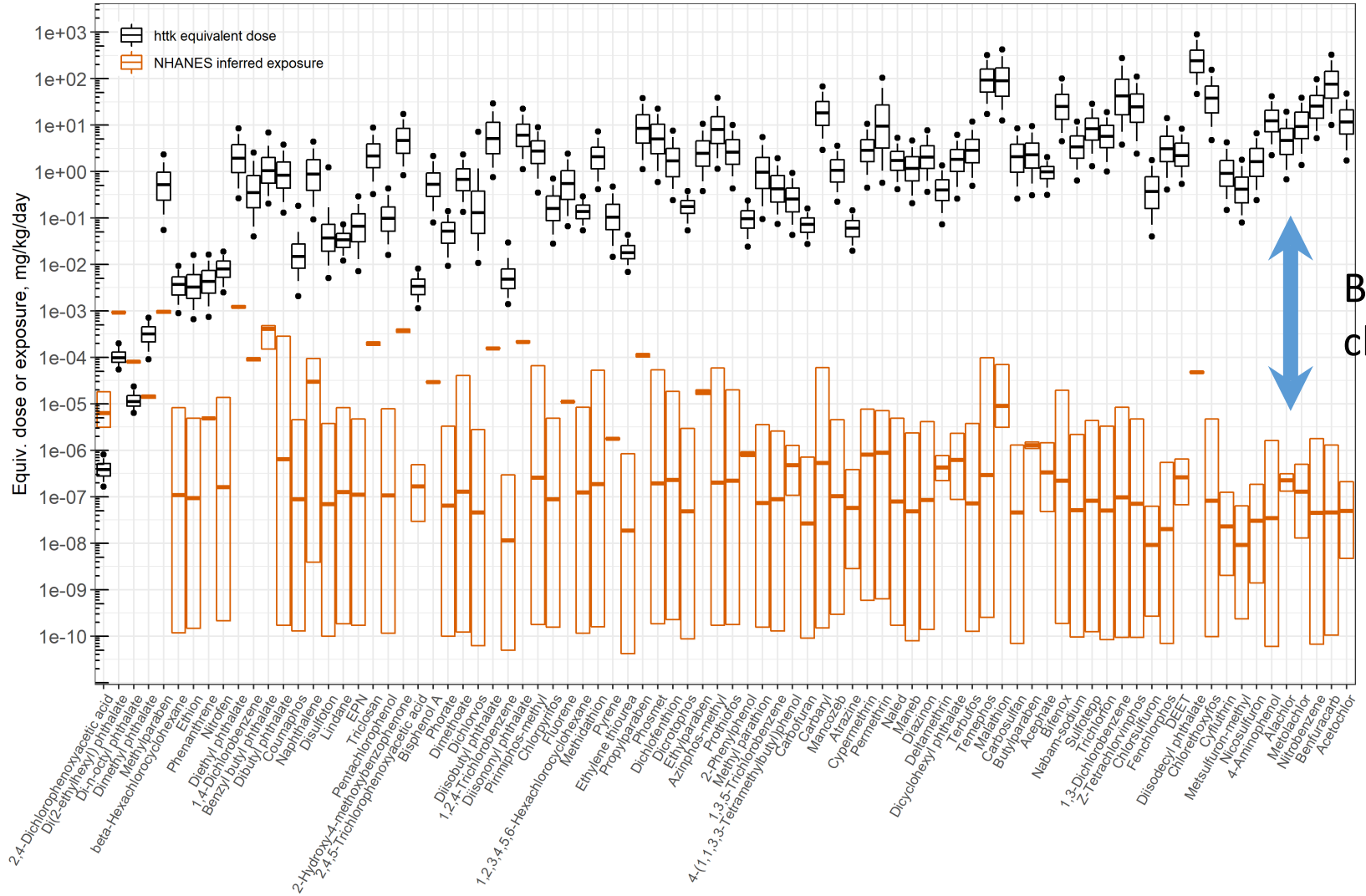
Population distributions of equivalent dose for 10th percentile ToxCast AC50 (bottom point = most-sensitive 5%)

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)

How might this prioritization change for potentially-sensitive subpopulations?



Equivalent dose might shift if subpopulation TK distribution is different from the overall US population

BER might therefore shift — changing prioritization?

Exposures might shift if subpopulation-specific NHANES-inferred exposures were different from overall US population

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

Evaluating potentially-sensitive subpopulations

- Potential population median exposures were inferred from NHANES urine biomonitoring data for 10 subpopulations of interest (Wambaugh et al. 2014; Ring et al. 2017):
  - ages 6-11
  - ages 12-19
  - ages 66+
  - men
  - women
  - reproductive-aged women (age 18-45)
  - BMI < 30
  - BMI > 30
- Used HTTK-Pop to simulate population TK variability for the same 10 subpopulations & calculate equivalent doses for ToxCast AC50s.
- Computed BERs for each chemical and each subpopulation.

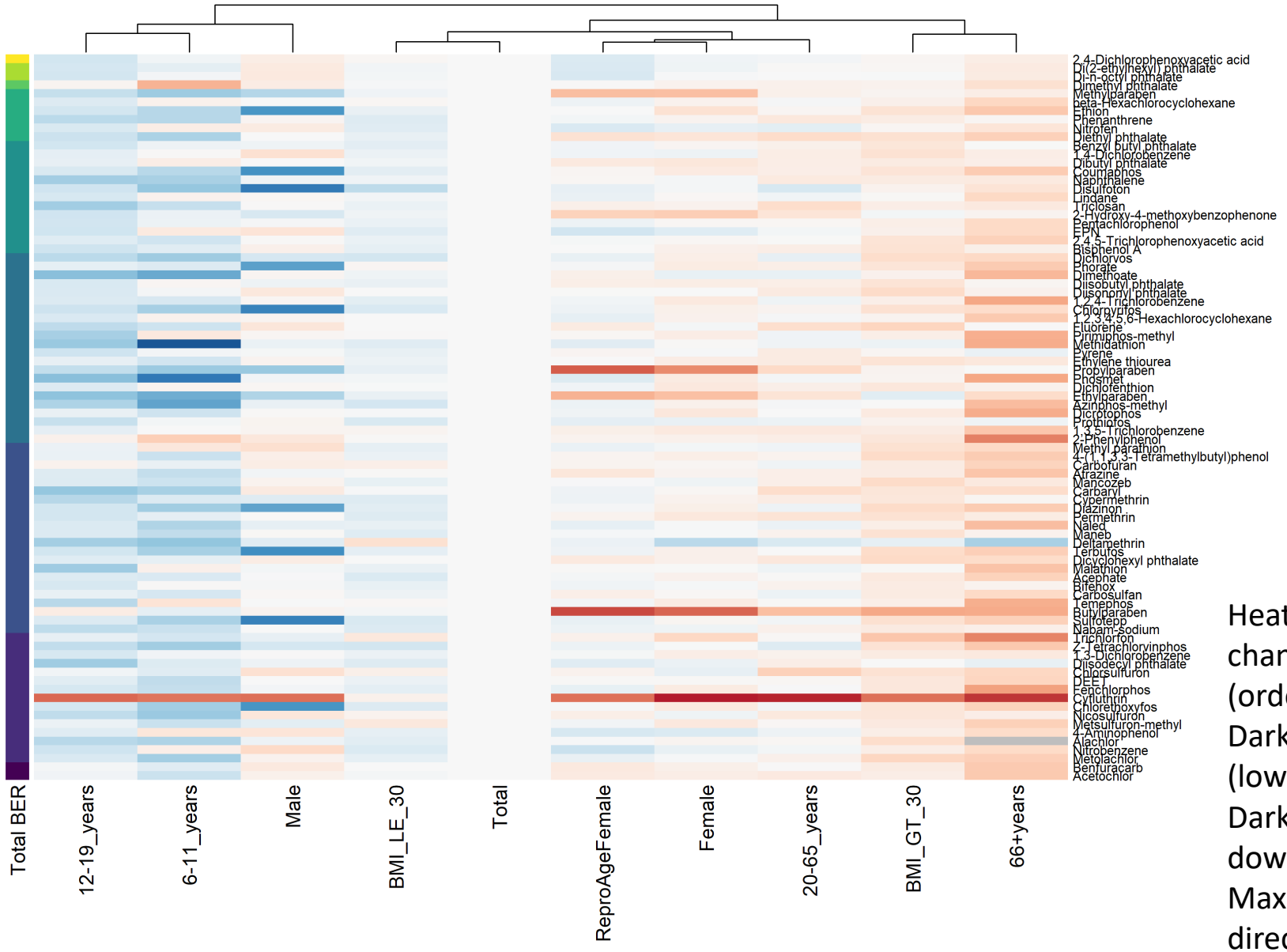
How much did BERs change, relative to the BER for the same chemical in the Total US population?

How different are subpopulation BERs vs. Total population?

Rows: Chemicals (listed in same order as for Total population BER rankings)

Sidebar colors indicate BER order of magnitude in Total population

Columns: Potentially-sensitive subpopulations



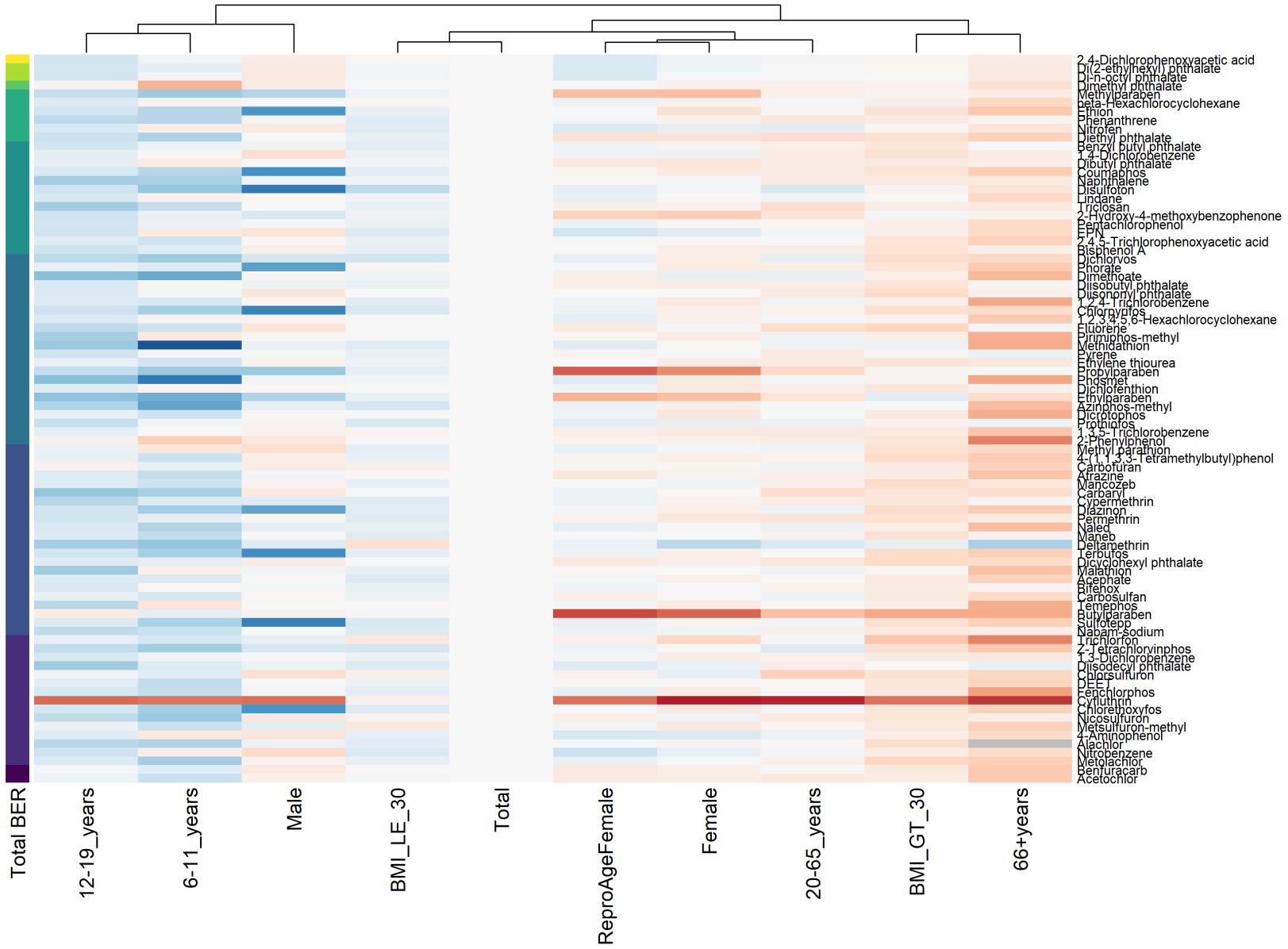
Updated version of analysis from Ring et al. (2017)

Heatmap colors: BER change vs. Total population (order of magnitude)
Darker blue = BER shifts up (lower priority)
Darker red = BER shifts down (higher priority)
Max shift = 10x either direction

How different are subpopulation BERs vs. Total population?

For these chemicals & subpopulations, BER shifts aren't big enough to substantially change chemical prioritization.

However, we do see some chemical-specific shifts — and some broader subpopulation-wide shifts across chemicals — illustrating the potential of subpopulation-specific prioritization.



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

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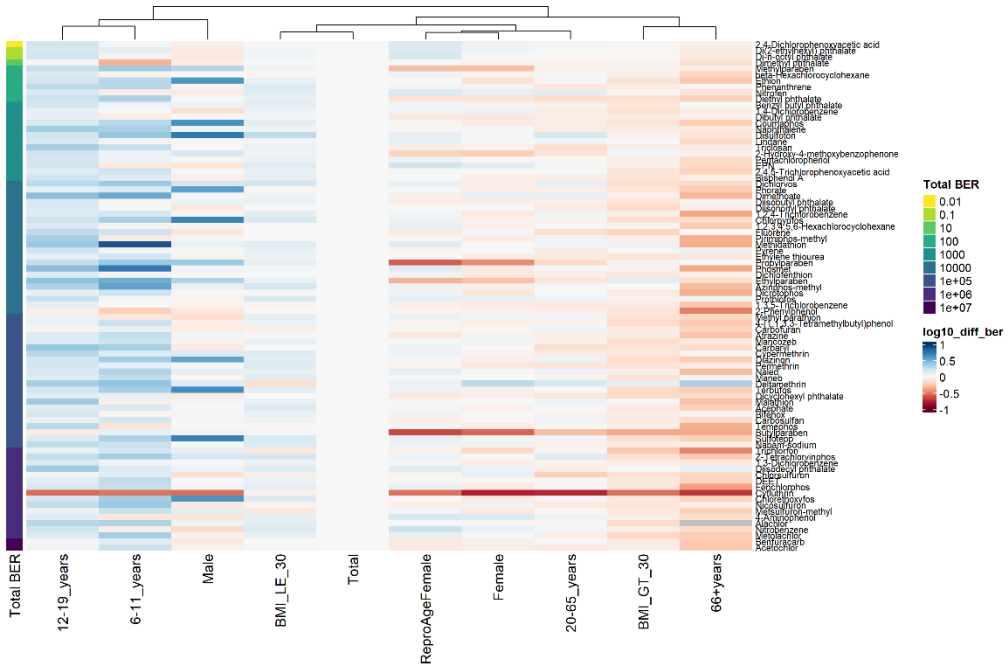
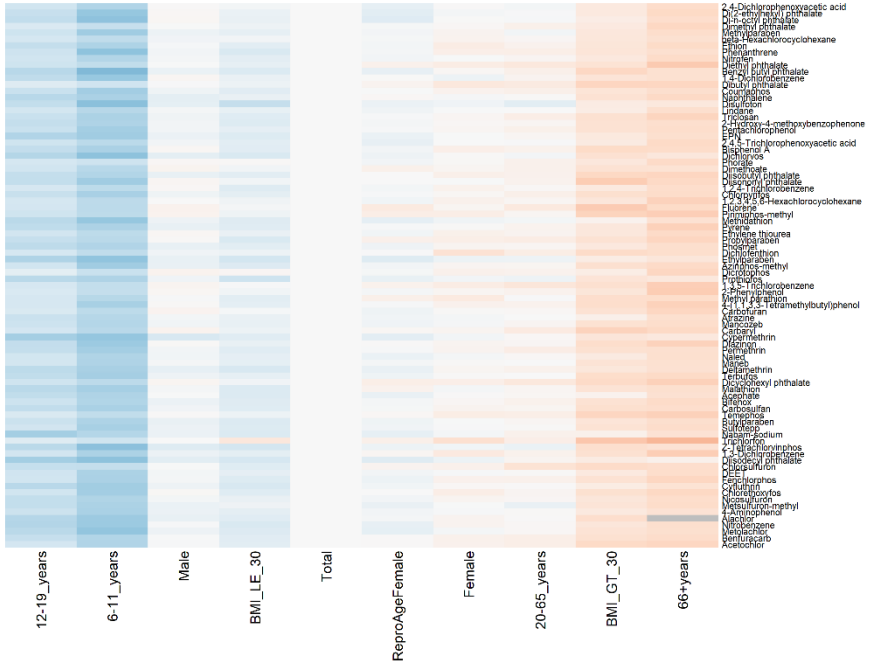
Are BER shifts driven by shifts in equivalent dose, or shifts in exposure, or both?

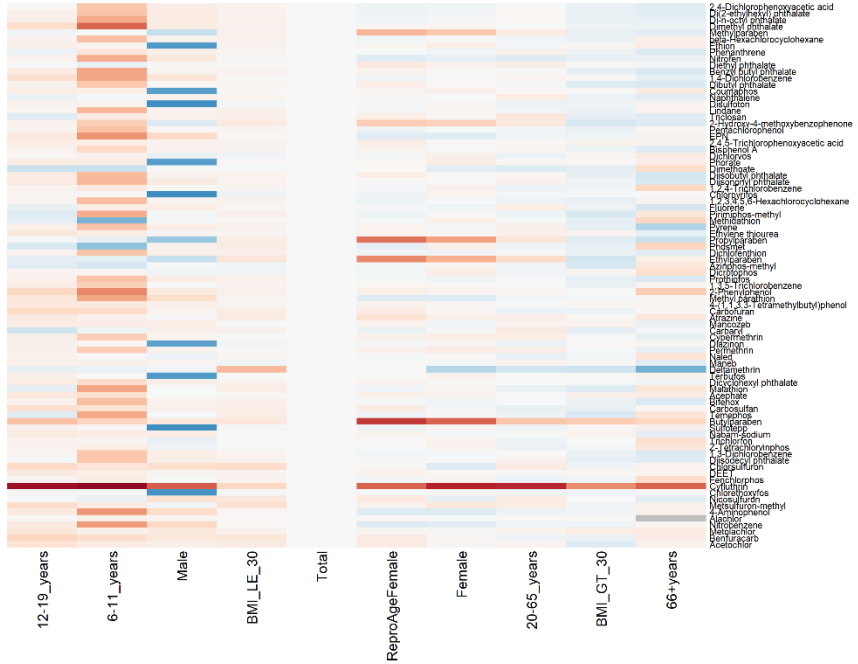
BER



Equiv. dose

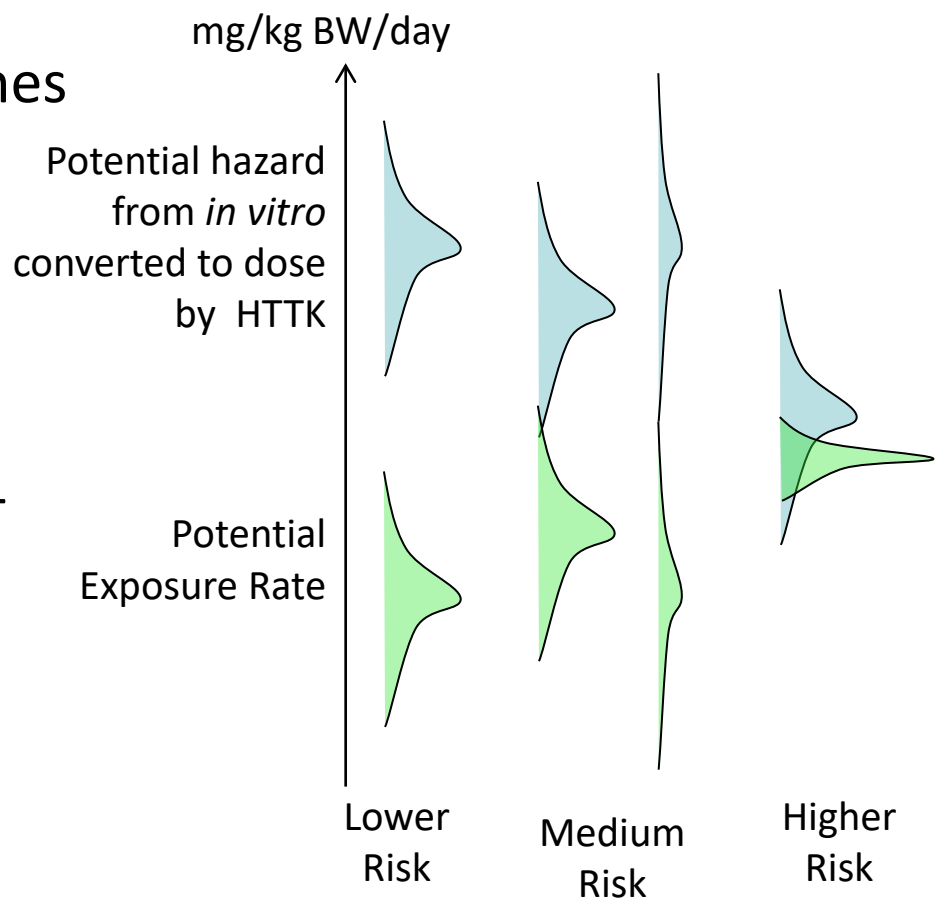


Exposure



Summary

- We would like to know more about the risk posed by thousands of chemicals in the environment – which ones should we start with?
- We can use *in vitro* high-throughput screening (HTS) assays when *in vivo* toxicology data are not available
- To extrapolate *in vitro* HTS data to equivalent *in vivo* doses, we use high-throughput toxicokinetics (HTTK) -- generic model that can be parameterized with *in vitro* data
- HTTK methods are available through the free, open source R package "httk"
- Simulating population variability and measurement uncertainty for TK parameters allows us to examine potential risk for potentially sensitive sub-populations



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

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Active work is ongoing to update and expand HTTK, HTTK-Pop, and exposure models!

- HTTK-Pop is being updated to include the most recent NHANES physiology data (2013-2018) (Breen et al., in prep)
- New HT-PBTK models are being developed
  - an inhalation TK model (Linakis et al., 2020; Breen et al., in prep) – currently available in httk package (though not yet for IVIVE/reverse TK)
  - a dermal TK model (Evans et al., in prep) — not yet available in httk package, but watch this space
  - a gestational/fetal TK model (Kapraun et al., 2018; Kapraun et al., in prep) — not yet available in httk package, but watch this space
- HT exposure models are being updated (Stanfield et al., in prep)

More things you can do with `httk`

- Time-dependent TK modeling (concentration vs. time predictions for a given dose)
  - One-, two-, and three-compartment models, along with PBTK models
- Get summary internal dose metrics other than steady-state concentration
  - Mean concentration
  - Peak concentration
  - AUC
- Inter-species extrapolation
- Route-to-route extrapolation
- Extrapolation across life stages
- Do you have measured chemical-specific TK parameters for chemicals that aren't already in `httk`? Add them as new rows to `httk`'s built-in tables of TK model parameters, so you can run all `httk` functions for your new chemicals.
- Use *in silico* predictions for chemical-specific TK parameters (Sipes *et al.* 2017; Pradeep et al. 2020; Mansouri et al. 2021; Dawson et al. 2021)
- Use the HTTK-Pop module separately to generate a sample of population physiology, body measures, demographics for use in other modeling applications (e.g. population exposure models [East et al., 2020])

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Thank you!

Questions?

Contact me at ring.caroline@epa.gov

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References

1. National Research Council 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. Washington, DC: The National Academies Press. https://doi.org/10.17226/11970
2. Bell SM, Chang X, Wambaugh JF, et al. In vitro to in vivo extrapolation for high throughput prioritization and decision making. Toxicology in Vitro. 2018 2018/03/01/;47:213-227.
3. Bessems JG, Loizou G, Krishnan K, et al. PBTK modelling platforms and parameter estimation tools to enable animal-free risk assessment: recommendations from a joint EPAA–EURL ECVAM ADME workshop. Regulatory Toxicology and Pharmacology. 2014;68(1):119-139.
4. Schmidt CW. TOX 21: new dimensions of toxicity testing. National Institute of Environmental Health Sciences; 2009.
5. Dix DJ, Houck KA, Martin MT, et al. The ToxCast program for prioritizing toxicity testing of environmental chemicals. Toxicological Sciences. 2007;95(1):5-12.
6. Kavlock RJ, Bahadori T, Barton-Maclaren TS, et al. Accelerating the Pace of Chemical Risk Assessment. Chemical Research in Toxicology. 2018 2018/05/21;31(5):287-290.
7. Wambaugh JF, Setzer RW, Reif DM, Gangwal S, Mitchell-Blackwood J, Arnot JA, et al. High-throughput models for exposure-based chemical prioritization in the ExpoCast project. Environ Sci Technol. 2013;47(15):8479-88.
8. Wambaugh JF, Wang A, Dionisio KL, Frame A, Egeghy P, Judson R, et al. High throughput heuristics for prioritizing human exposure to environmental chemicals. Environ Sci Technol. 2014;48(21):12760-7.
9. Ring CL, Arnot JA, Bennett DH, Egeghy PP, Fantke P, Huang L, et al. Consensus Modeling of Median Chemical Intake for the U.S. Population Based on Predictions of Exposure Pathways. Environ Sci Technol. 2019;53(2):719-32.
10. Tan Y-M, Liao KH, Clewell HJ. Reverse dosimetry: interpreting trihalomethanes biomonitoring data using physiologically based pharmacokinetic modeling. Journal of Exposure Science and Environmental Epidemiology. 2007;17(7):591-603.

11. Rotroff DM, Wetmore BA, Dix DJ, et al. Incorporating human dosimetry and exposure into high-throughput in vitro toxicity screening. Toxicological Sciences. 2010;117(2):348-358
12. Wetmore BA, Wambaugh JF, Allen B, et al. Incorporating High-Throughput Exposure Predictions With Dosimetry-Adjusted In Vitro Bioactivity to Inform Chemical Toxicity Testing. Toxicological Sciences. 2015 Nov;148(1):121-36
13. Wambaugh JF, Wetmore BA, Pearce R, Strope C, Goldsmith R, Sluka JP, et al. Toxicokinetic Triage for Environmental Chemicals. Toxicol Sci. 2015;147(1):55-67.
14. Pearce RG, Setzer RW, Strope CL, et al. Httk: R package for high-throughput toxicokinetics. Journal of Statistical Software. 2017a;79(1):1-26.
15. Ring CL, Pearce RG, Setzer RW, et al. Identifying populations sensitive to environmental chemicals by simulating toxicokinetic variability. Environment International. 2017 2017/09/01/;106:105-118.
16. Linakis, M. W., et al. (2020). "Development and Evaluation of a High Throughput Inhalation Model for Organic Chemicals " Journal of Exposure Science & Environmental Epidemiology.
17. Shibata Y, Takahashi H, Chiba M, Ishii Y. Prediction of hepatic clearance and availability by cryopreserved human hepatocytes: an application of serum incubation method. Drug Metab Dispos. 2002;30(8):892-6.
18. Waters NJ, Jones R, Williams G, Sohal B. Validation of a rapid equilibrium dialysis approach for the measurement of plasma protein binding. J Pharm Sci. 2008;97(10):4586-95.
19. Wetmore BA, Wambaugh JF, Ferguson SS, et al. Integration of dosimetry, exposure, and high-throughput screening data in chemical toxicity assessment. Toxicological Sciences. 2012 Jan;125(1):157-74.
20. Wetmore BA. Quantitative in vitro-to-in vivo extrapolation in a high-throughput environment. Toxicology. 2015;332:94-101.

21. Wambaugh JF, Wetmore BA, Ring CL, Nicolas CI, Pearce RG, Honda GS, et al. Assessing Toxicokinetic Uncertainty and Variability in Risk Prioritization. Toxicol Sci. 2019;172(2):235-51.
22. Sipes NS, Wambaugh JF, Pearce R, et al. An Intuitive Approach for Predicting Potential Human Health Risk with the Tox21 10k Library. Environmental Science & Technology. 2017 2017/09/19;51(18):10786-10796.
23. Pearce RG, Setzer RW, Davis JL, Wambaugh JF. Evaluation and calibration of high-throughput predictions of chemical distribution to tissues. J Pharmacokinet Pharmacodyn. 2017b;44(6):549-65.
24. Jamei M, Marciniak S, Feng K, et al. The Simcyp® population-based ADME simulator. Expert Opinion on Drug Metabolism & Toxicology. 2009;5(2):211-223.
25. McNally K, Cotton R, Hogg A, Loizou G. PopGen: A virtual human population generator. Toxicology. 2014;315:70-85.
26. Price PS, Conolly RB, Chaisson CF, Gross EA, Young JS, Mathis ET, et al. Modeling Interindividual Variation in Physiological Factors Used in PBPK Models of Humans. Critical Reviews in Toxicology. 2003;33(5):469-503.
27. Bosgra S, van Eijkeren J, Bos P, Zeilmaker M, Slob W. An improved model to predict physiologically based model parameters and their inter-individual variability from anthropometry. Crit Rev Toxicol. 2012;42(9):751-67.
28. Wetmore BA, Allen B, Clewell HJ, 3rd, et al. Incorporating population variability and susceptible subpopulations into dosimetry for high-throughput toxicity testing. Toxicological Sciences. 2014 Nov;142(1):210-24.
29. Kapraun DF, Wambaugh JF, Setzer RW, Judson RS. Empirical models for anatomical and physiological changes in a human mother and fetus during pregnancy and gestation. PLoS One. 2019;14(5):e0215906.