EPA
United States
Environmental Protection
Agency

The Physicians Committee for Responsible Medicine
Summer School on Innovative Approaches in Science 2022

Toxicokinetic Human Variability Simulations Help Establish a Risk Context

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Office of Research and Development

U.S. Environmental Protection Agency

Data and Diversity:
Improving Coverage of Relevant Populations
(Toxicology Track)

Thursday, June 9, 2022

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US EPA Office of Research and Development

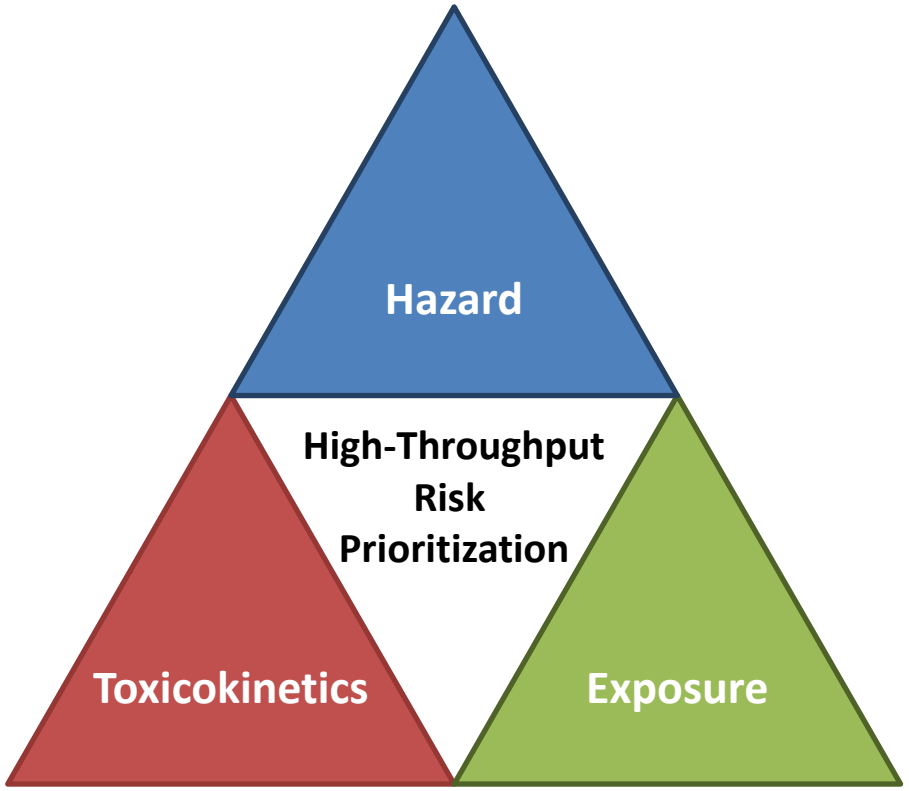
- The Office of Research and Development (ORD) is the scientific research arm of EPA
  - 543 peer-reviewed journal articles in 2019
- Research is conducted by ORD's four national centers, and three offices organized to address:
  - Public health and env. assessment; comp. tox. and exposure; env. measurement and modeling; and env. solutions and emergency response.
- 13 facilities across the United States
- Research conducted by a combination of Federal scientists (including uniformed members of the **Public Health Service**); contract researchers; and postdoctoral, graduate student, and post-baccalaureate trainees

ORD Facility in
Research Triangle Park, NC

Calculating Chemical Risk

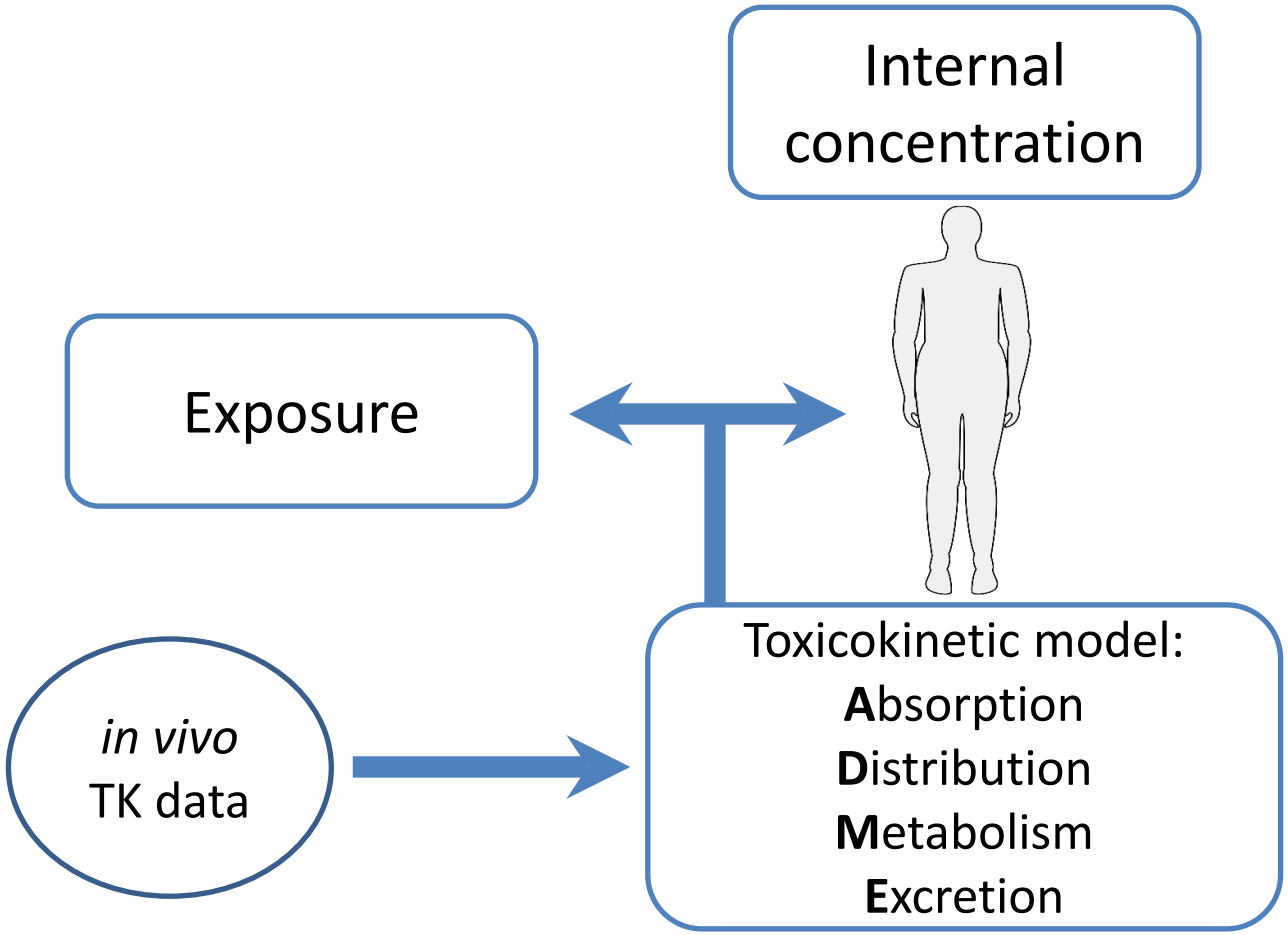
- High throughput risk prioritization based upon *in vitro* screening requires comparison to exposure (for example, NRC, 1983)
  - Data obtained *in vitro* must be placed in an *in vivo* context: *in vitro-in vivo* extrapolation (IVIVE)

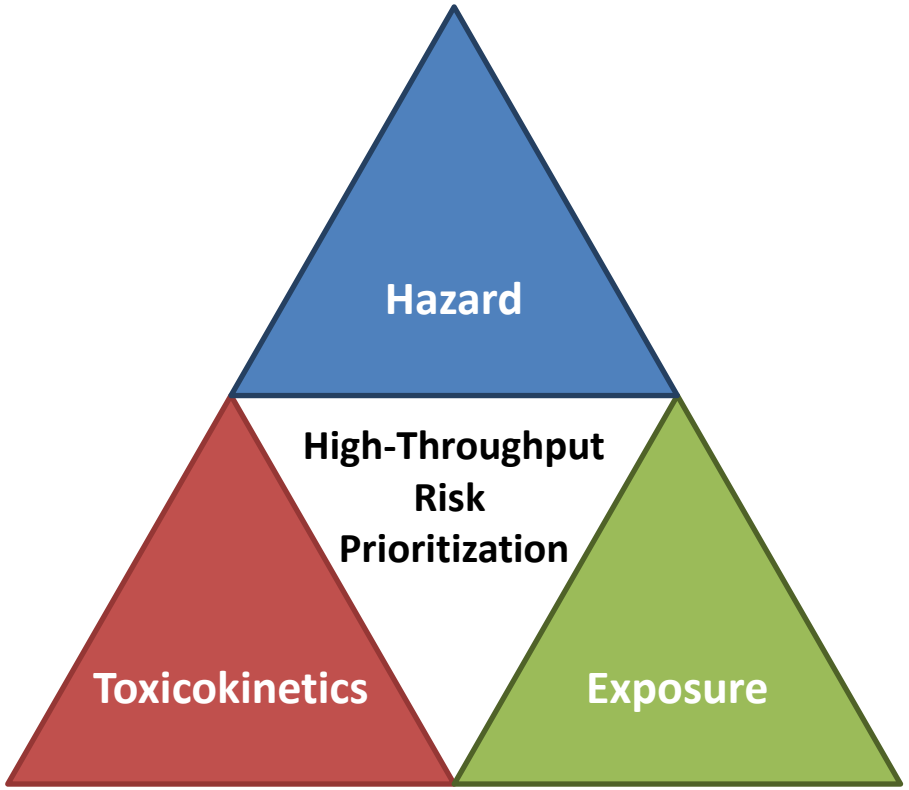
- Information must be relevant to the scenario, for example, consumer, ambient, or occupational exposure.



Toxicokinetics

- Toxicokinetics describes the absorption, distribution, metabolism, and excretion of a chemical by the body:
  - Chemical-specific
  - Links exposure with internal concentrations

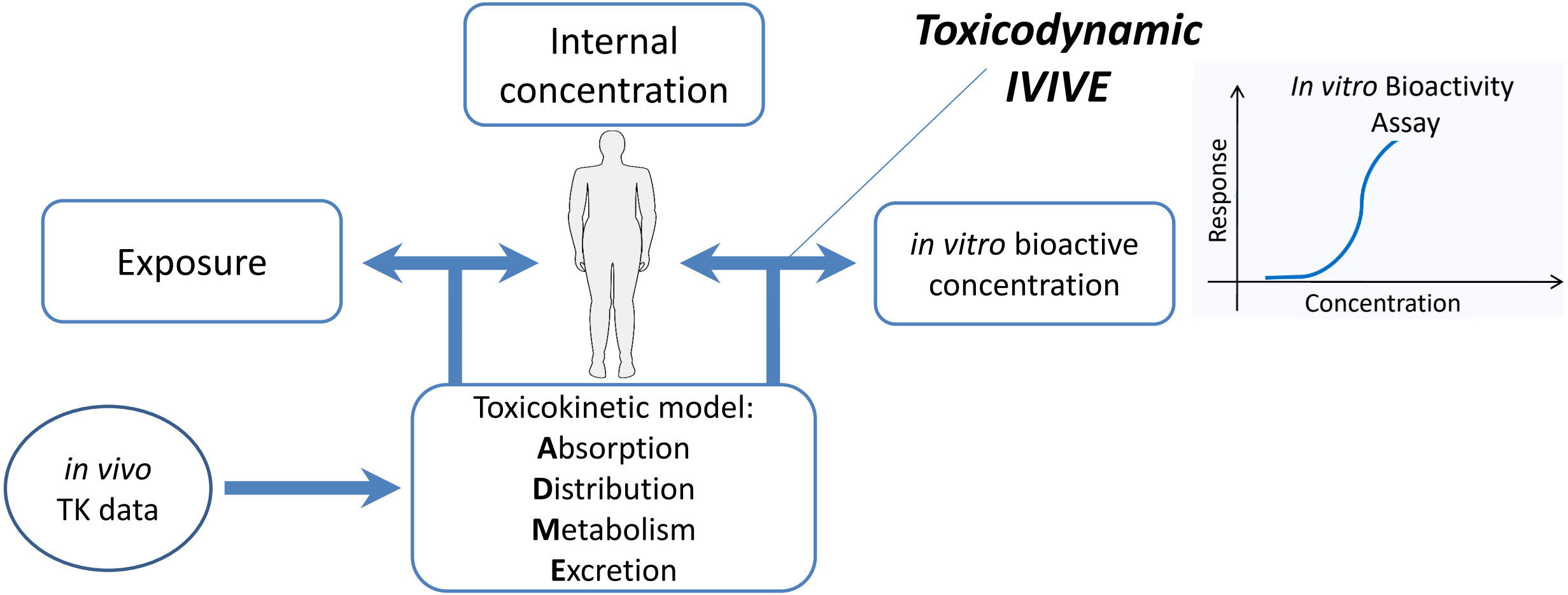




Breen *et al.* (2021)

In Vitro-In Vivo Extrapolation (IVIVE)

- Translation of *in vitro* high throughput screening requires chemical-specific toxicokinetic models
  - Needed for anywhere from dozens to thousands of chemicals



Breen *et al.* (2021)

Most Chemicals lack Toxicokinetic Data

- Most non-pharmaceutical chemicals – for example, flame retardants, plasticizers, pesticides, solvents – do not have human *in vivo* TK data.
- Non-pesticidal chemicals are unlikely to have any *in vivo* TK data, even from animals

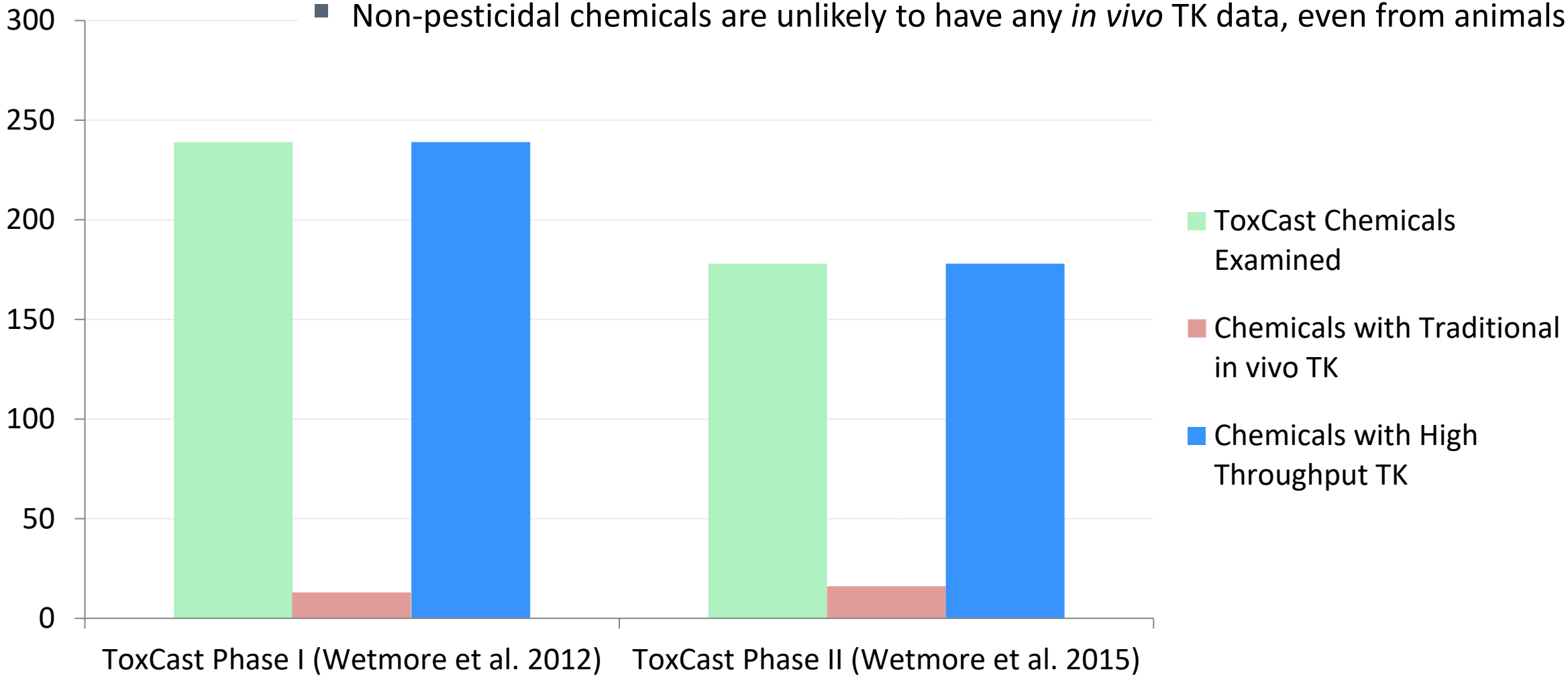


Figure from Bell *et al.* (2018)

High Throughput Toxicokinetics (HTTK)

- To provide toxicokinetic data for larger numbers of chemicals collect *in vitro*, high throughput toxicokinetic (HTTK) data
(for example, Rotroff *et al.*, 2010, Wetmore *et al.*, 2012, 2015)
- HTTK methods have been used by the pharmaceutical industry to determine range of efficacious doses and to prospectively evaluate success of planned clinical trials
(Jamei, *et al.*, 2009; Wang, 2010)
- The **primary goal** of HTTK is to provide a human dose context for bioactive *in vitro* concentrations from HTS (that is*, in vitro-in vivo* extrapolation, or **IVIVE**)
(for example, Wetmore *et al.*, 2015)
- A **secondary goal** is to provide **open-source data and models** for evaluation and use by the broader scientific community (Pearce *et al.*, 2017a)

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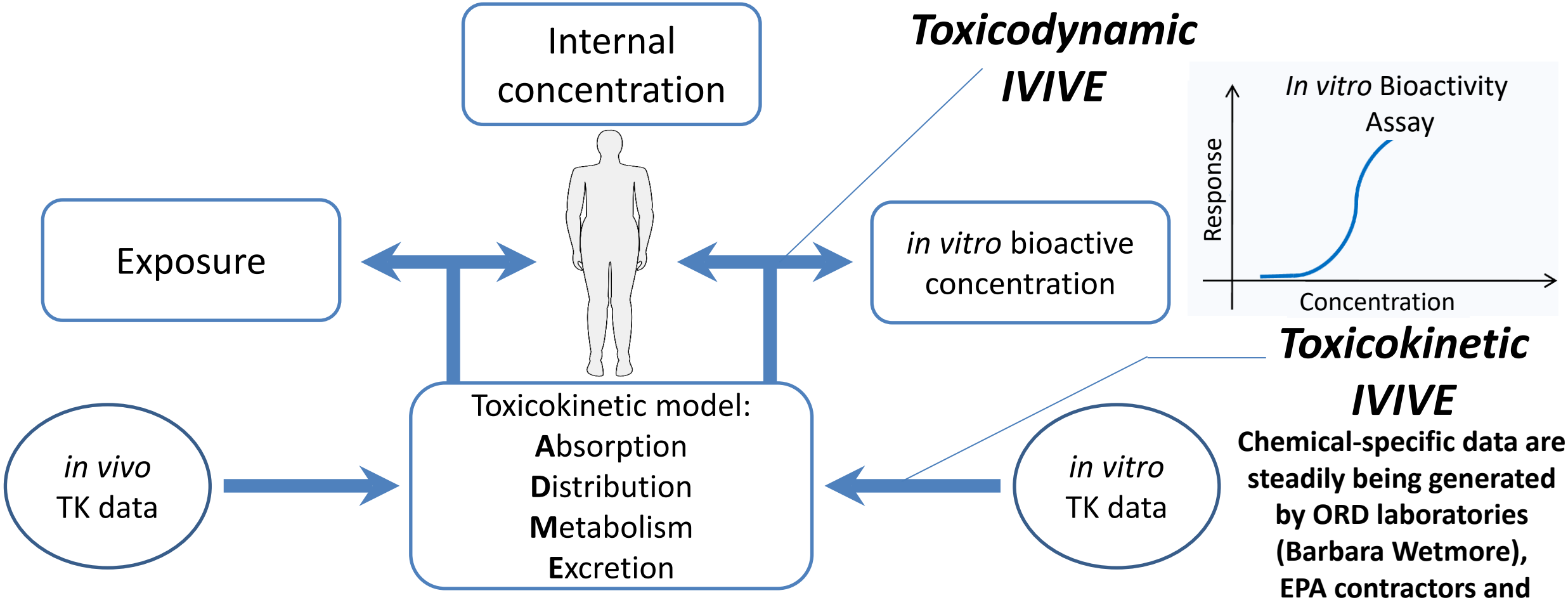
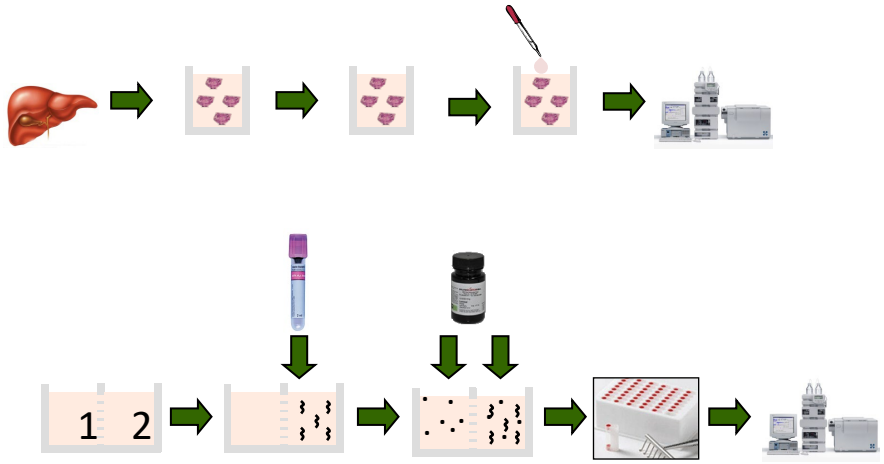


Breen *et al.* (2021)

High Throughput Toxicokinetics (HTTK)

In vitro toxicokinetic data



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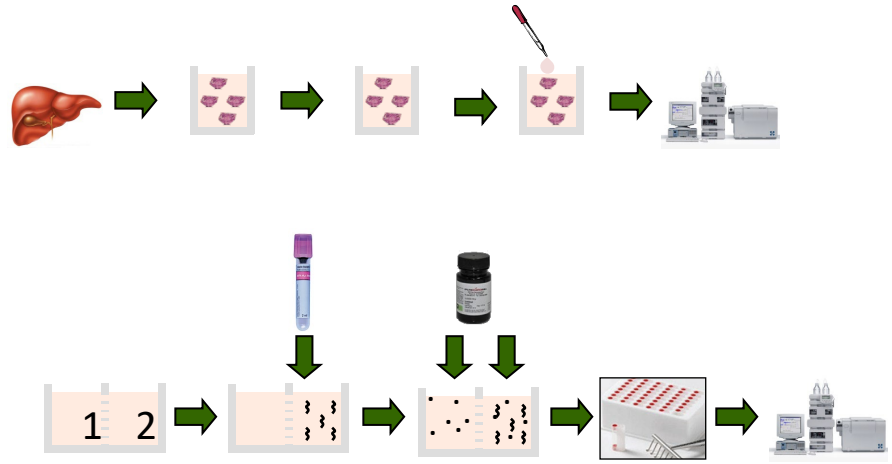
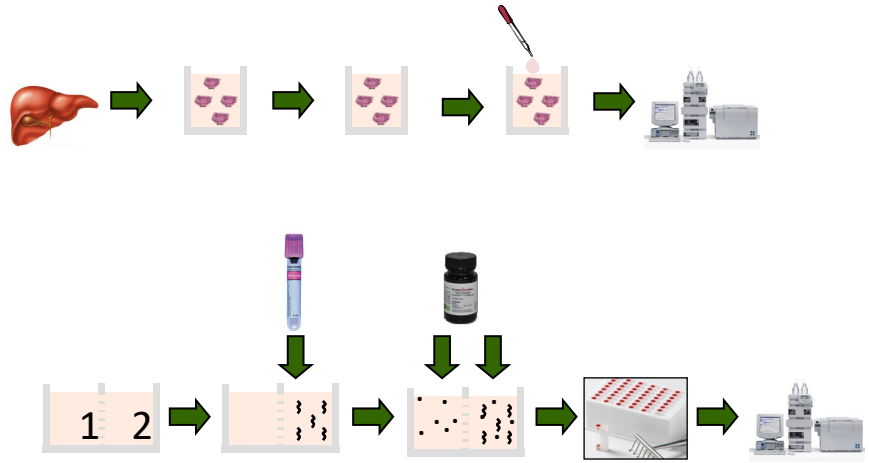
In vitro toxicokinetic data

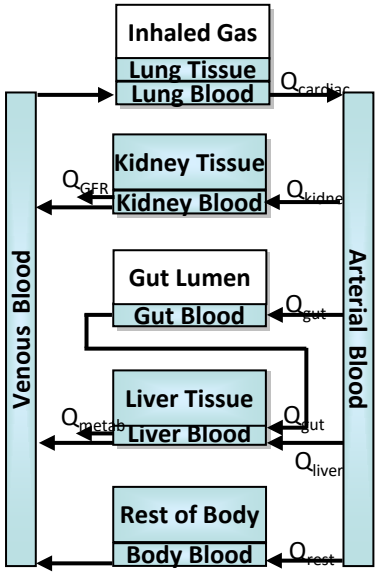


Rotroff *et al.* (2010)
Wetmore *et al.* (2012)
Wetmore *et al.* (2015)
Wambaugh *et al.* (2019)

High Throughput Toxicokinetics (HTTK)

In vitro toxicokinetic data + generic toxicokinetic model





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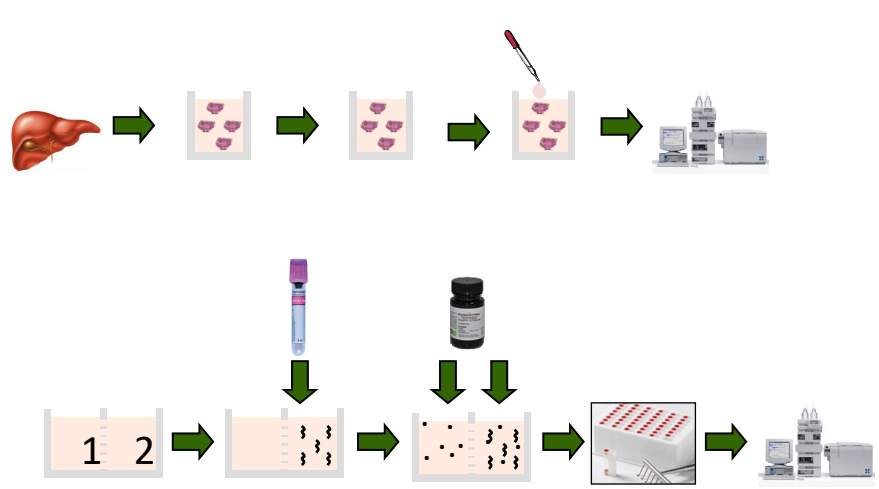

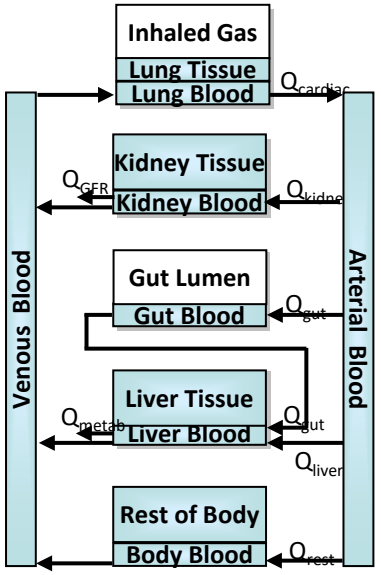

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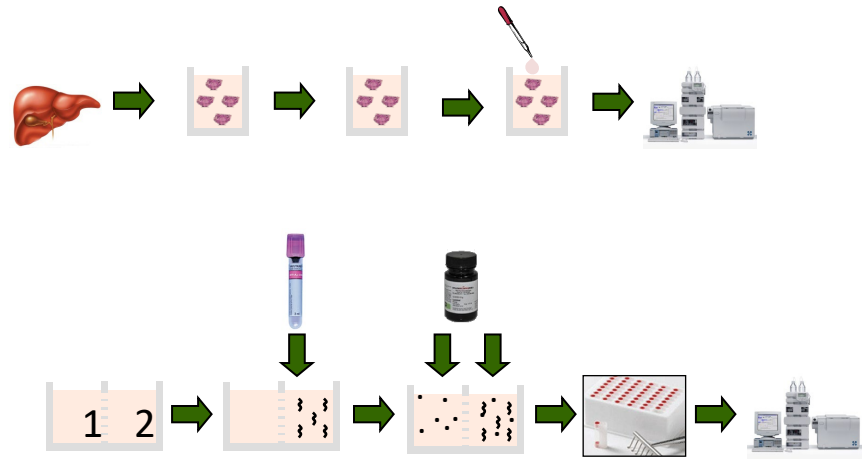
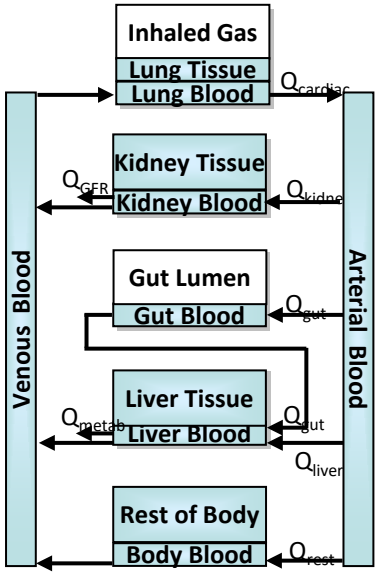
Pearce *et al.* (2017a)
Ring *et al.* (2017)
Linakis *et al.* (2020)

High Throughput Toxicokinetics (HTTK)

In vitro toxicokinetic data + generic toxicokinetic model = high(er) throughput toxicokinetics





= *httk*

Rotroff *et al.* (2010)
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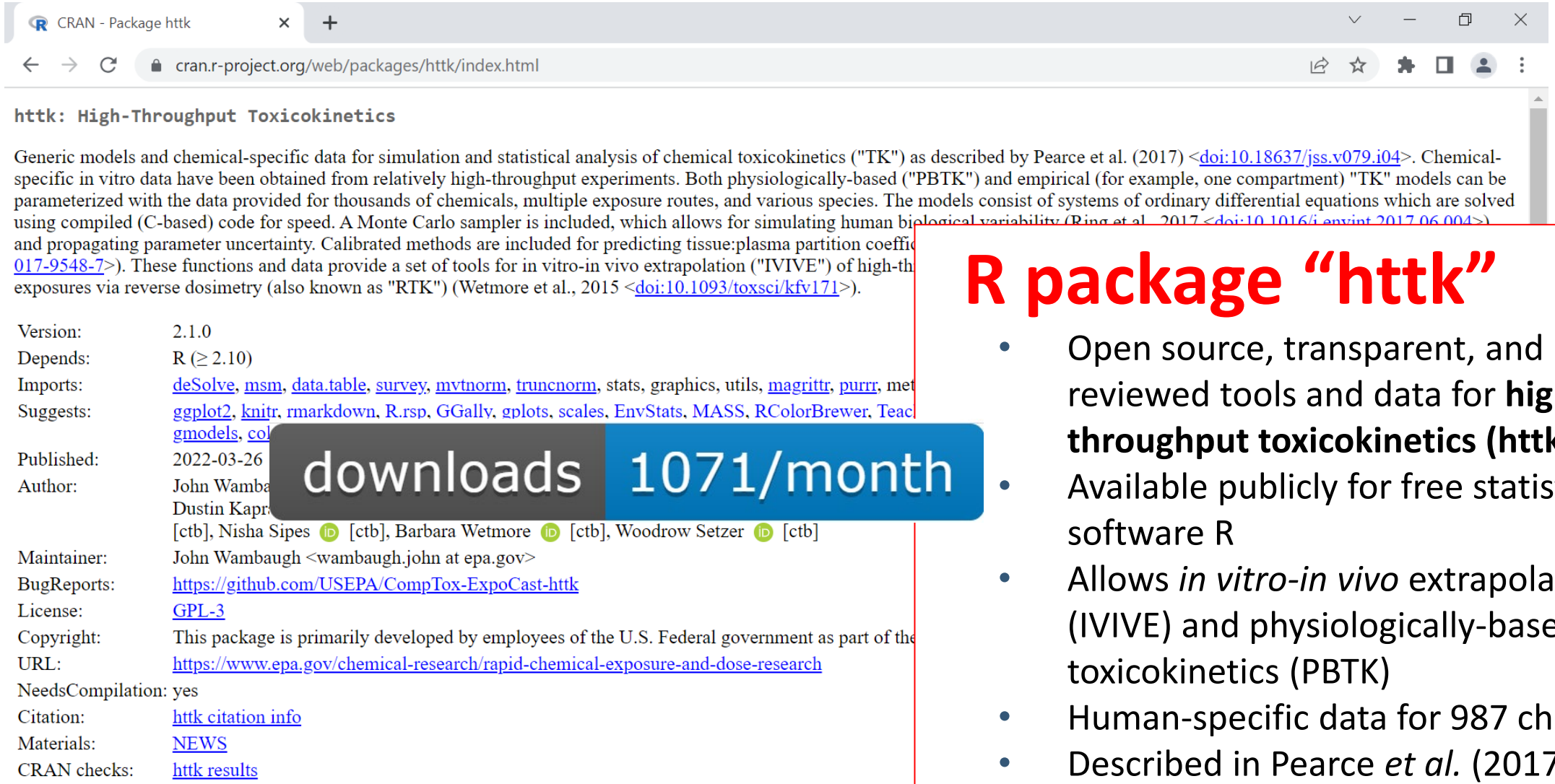
Open Source Tools and Data for HTTK

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 data for 987 chemicals
- Described in Pearce *et al.* (2017a)

Modules within R Package "httk"

Feature	Description	Reference
Chemical Specific *In Vitro* Measurements	Metabolism and protein binding for ~1000 chemicals in human and ~200 in rat	Wetmore *et al.* (2012, 2013, 2015), plus others
Chemical-Specific *In Silico* Predictions	Metabolism and protein binding for ~8000 Tox21 chemicals	Sipes *et al.* (2017)
Generic toxicokinetic models	One compartment, three compartment, physiologically-based oral, intravenous, and inhalation (PBTK)	Pearce *et al.* (2017a), Linakis *et al.* (2020)
Tissue partition coefficient predictors	Modified Schmitt (2008) method	Pearce *et al.* (2017b)
Variability Simulator	Based on NHANES biometrics	Ring *et al.* (2017)
In Vitro Disposition	Armitage et al. (2014) model	Honda *et al.* (2019)
Uncertainty Propagation	Model parameters can be described by distributions reflecting uncertainty	Wambaugh *et al.* (2019)

In Vitro - In Vivo Extrapolation (IVIVE)

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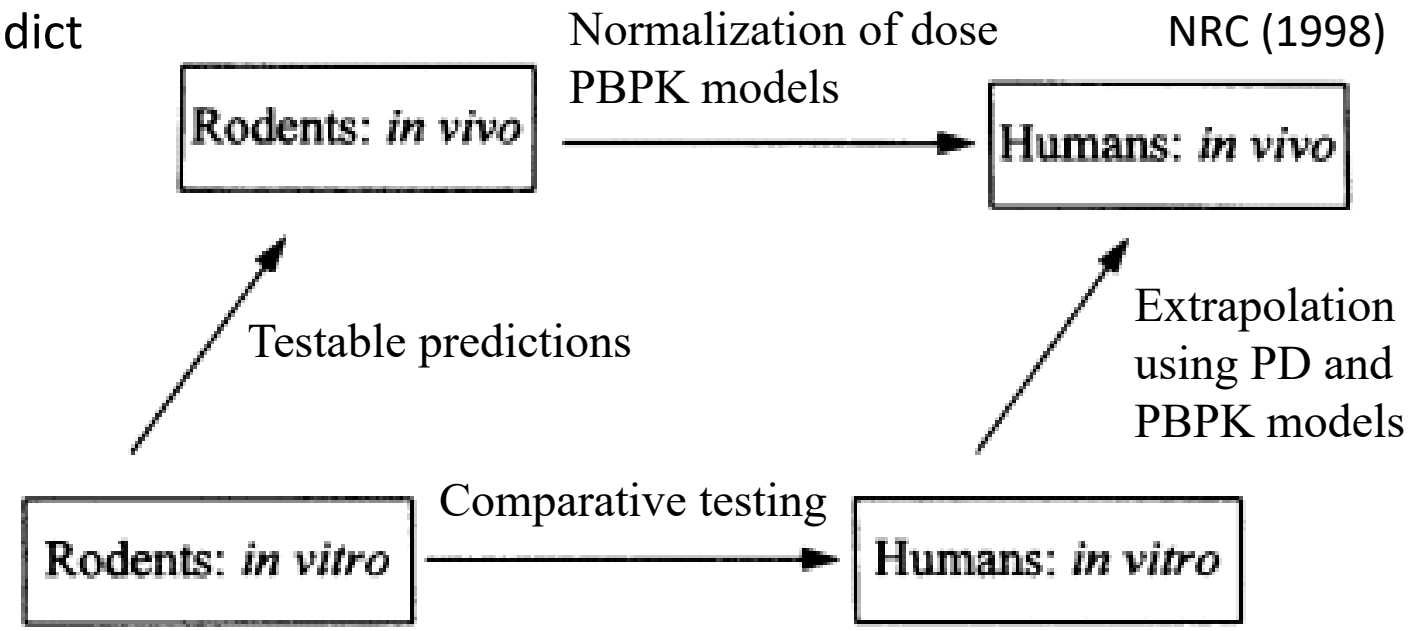

"The Parallelogram Approach" (Sobels, 1982)

slide modified from one by Barbara Wetmore

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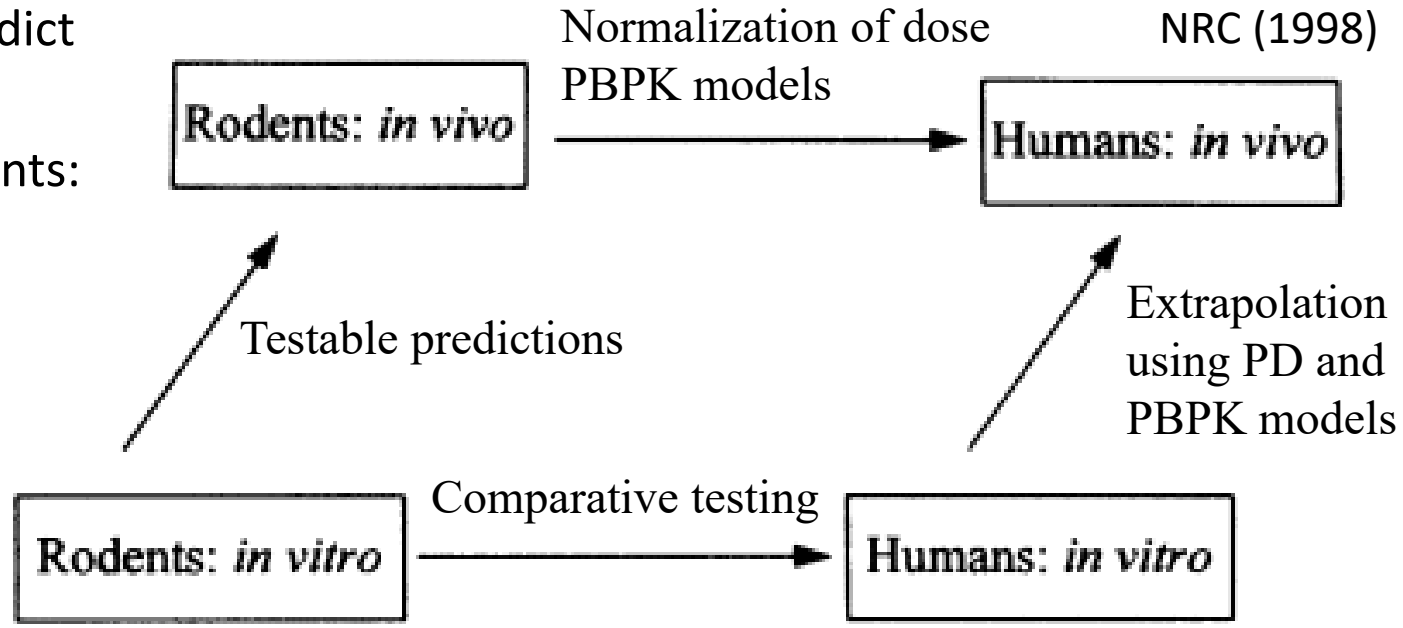
- IVIVE can be broken down into two components:
  - IVIVE-PK/TK (**Pharmacokinetics/Toxicokinetics**):
    - Fate of molecules/chemicals in body
    - Considers absorption, distribution, metabolism, excretion (ADME)
    - Can use empirical PK or physiologically-based (PBPK)



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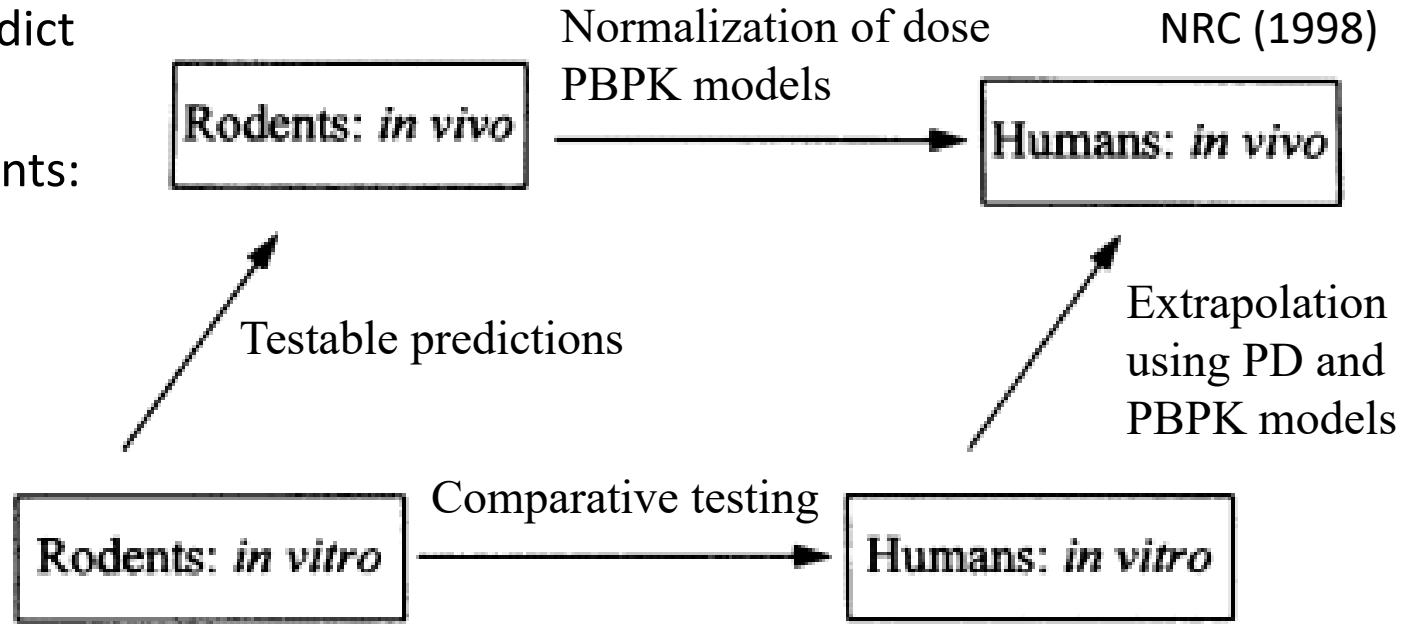
  - IVIVE-PD/TD (**Pharmacodynamics/Toxicodynamics**):
    - Effect of molecules/chemicals at biological target *in vivo*
    - Perturbation as adverse/therapeutic effect, reversible/ irreversible effects



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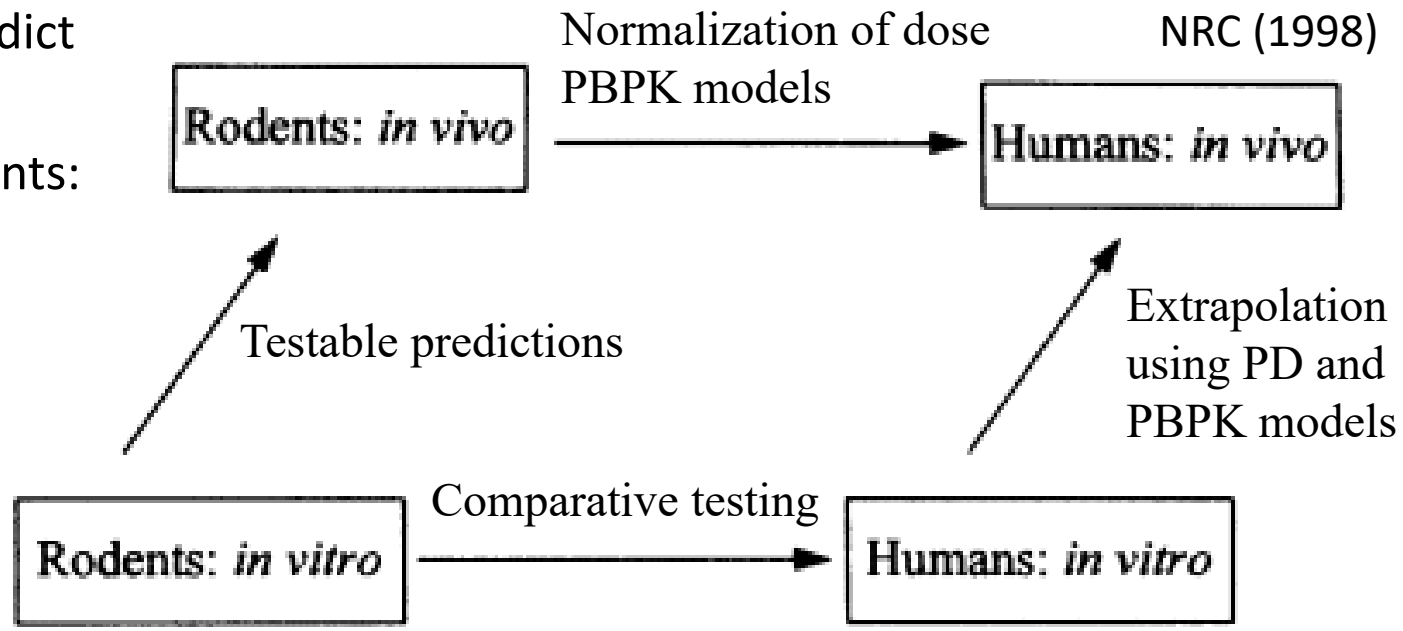

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HTTK only covers toxicokinetic extrapolation

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IVIVE by Scaling Factor

- There are many approaches to IVIVE, but we choose a relatively simple one:
- We make various assumptions that allow conversion of an *in vitro* concentration $[\boldsymbol{X}]$ (µM) into an **administered equivalent dose** (AED) with units of mg/kg body weight/day:

$$\mathbf{AED} = \boldsymbol{F_{IVIVE}} \times [\boldsymbol{X}]$$

- **AED** is the **external dose rate** that would be needed to **cause a given steady-state plasma concentration**
- F_{IVIVE} is a scaling factor that varies by chemical

IVIVE by Scaling Factor

- For a given chemical, $F_{IVIVE} = 1 / C_{ss,95}$
- $C_{ss,95}$ is the steady-state plasma concentration as the result of a 1 mg/kg/day exposure

$$\mathbf{AED_{95}} = \frac{[\boldsymbol{X}]}{\boldsymbol{C_{ss,95}}}$$

- The "95" refers to the upper 95th percentile – due to human variability and measurement uncertainty there are a range of possible C_{ss} values
- All of this assumes that the individuals have enough time to come to "steady-state" with respect to their daily exposures

Don't forget:

$$\mu\boldsymbol{M} = \mathbf{1000}\frac{\mathbf{1}}{\boldsymbol{MW}}\frac{\boldsymbol{mg}}{\boldsymbol{L}}$$

Population simulator for HTTK

Correlated Monte Carlo sampling of physiological model parameters built into R “httk” package (Pearce et al., 2017):

Sample CDC National Health and Nutrition Examination Survey (NHANES) biometrics for actual individuals:

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

Slide from Caroline Ring

Ring *et al*. (2017)

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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.)

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(+ residual marginal variability)

Predict physiological quantities

Tissue masses
Tissue blood flows
GFR (kidney function)
Hepatocellularity

(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.)

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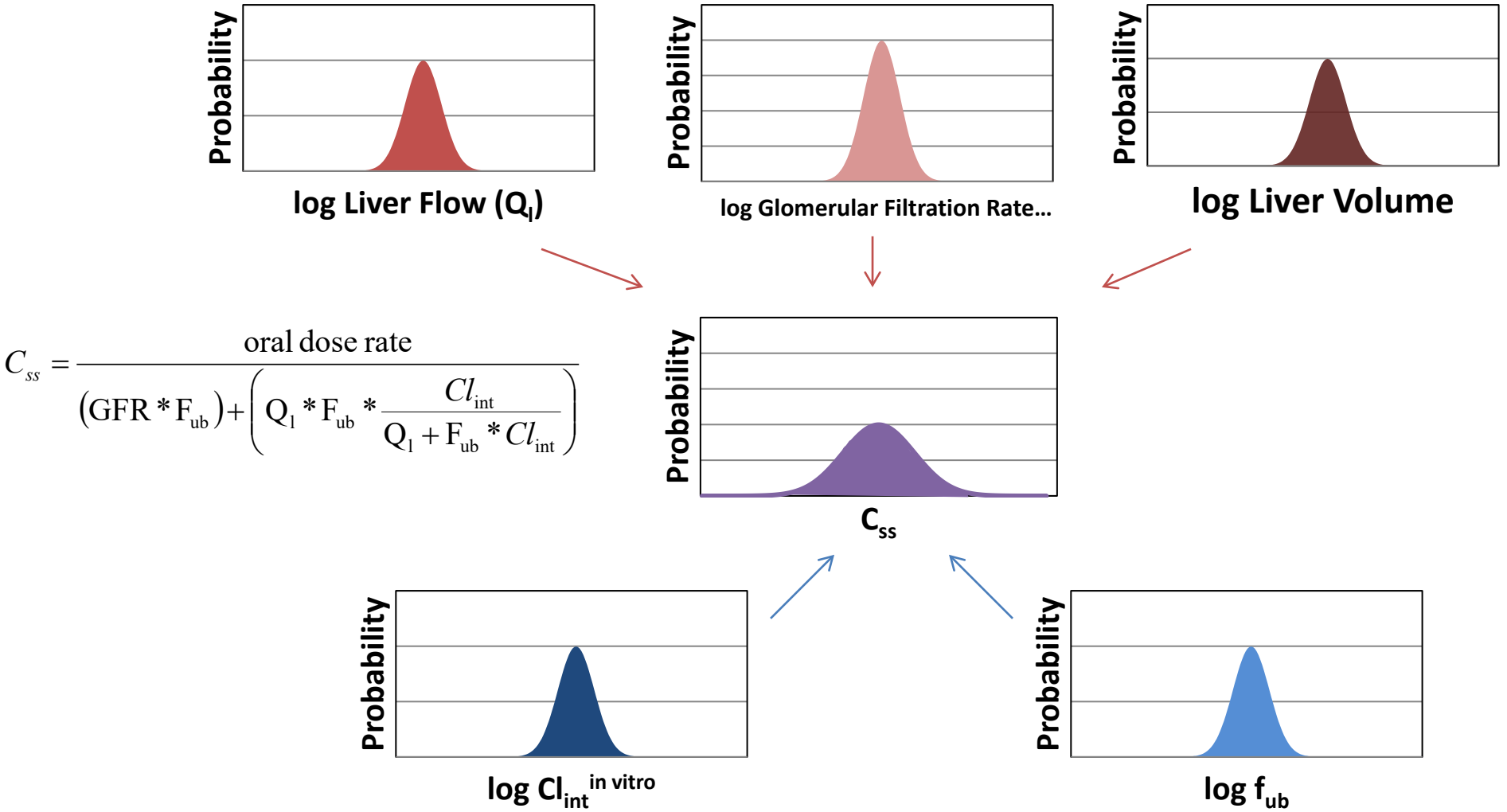
Monte Carlo Sampling

- Can be used for variability and uncertainty



Variability

Different crayons have different colors…

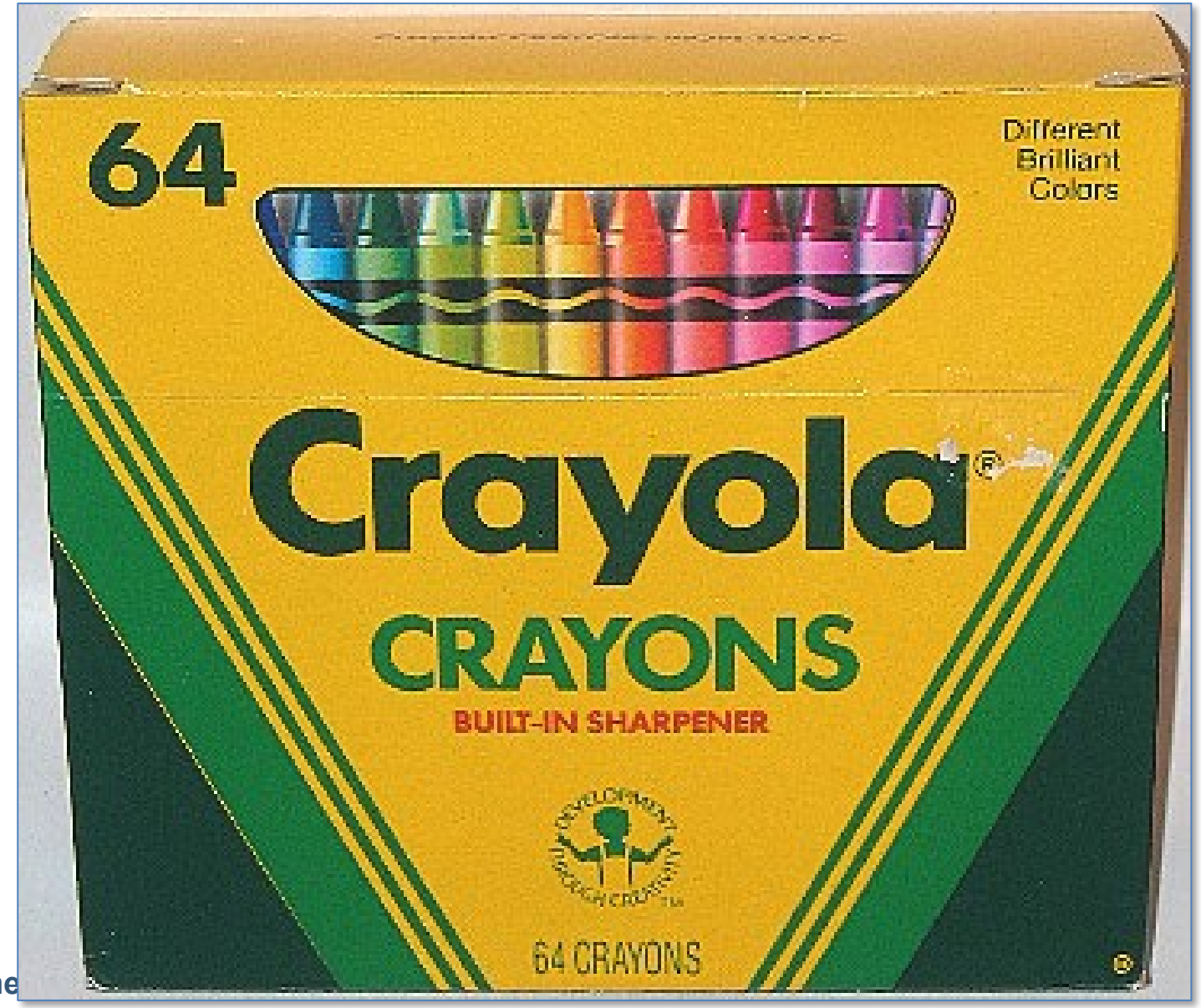
Variability

Different crayons have different colors, and none of them are the “average” color

Uncertainty

Until I open the box, I don't know what colors I have...

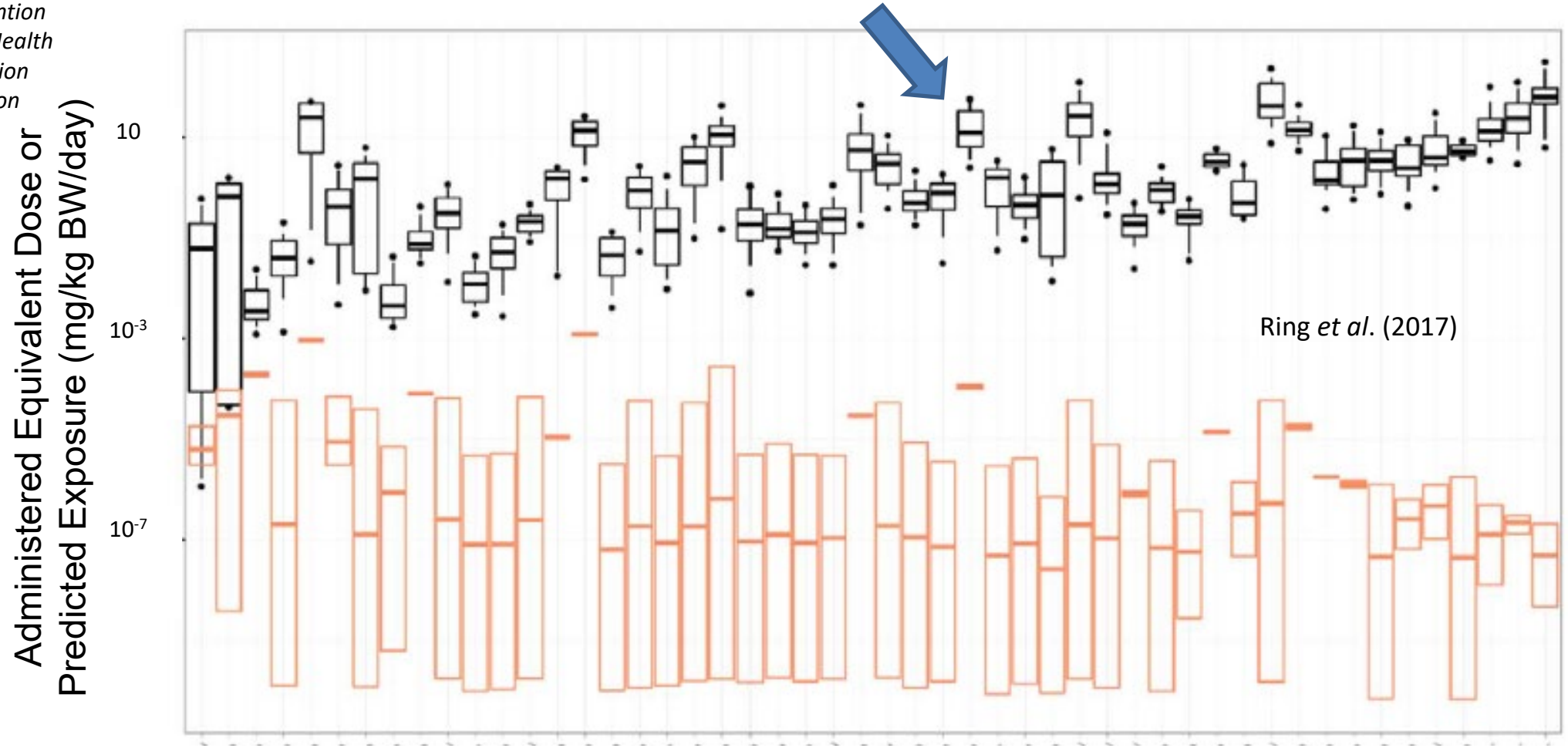
...especially if my nine-year-old has been around.



IVIVE Allows Chemical Prioritization

CDC NHANES: U.S. Centers for Disease Control and Prevention National Health and Nutrition Examination Survey

In Vitro Screening + IVIVE can estimate doses needed to cause bioactivity (Wetmore *et al.*, 2015)



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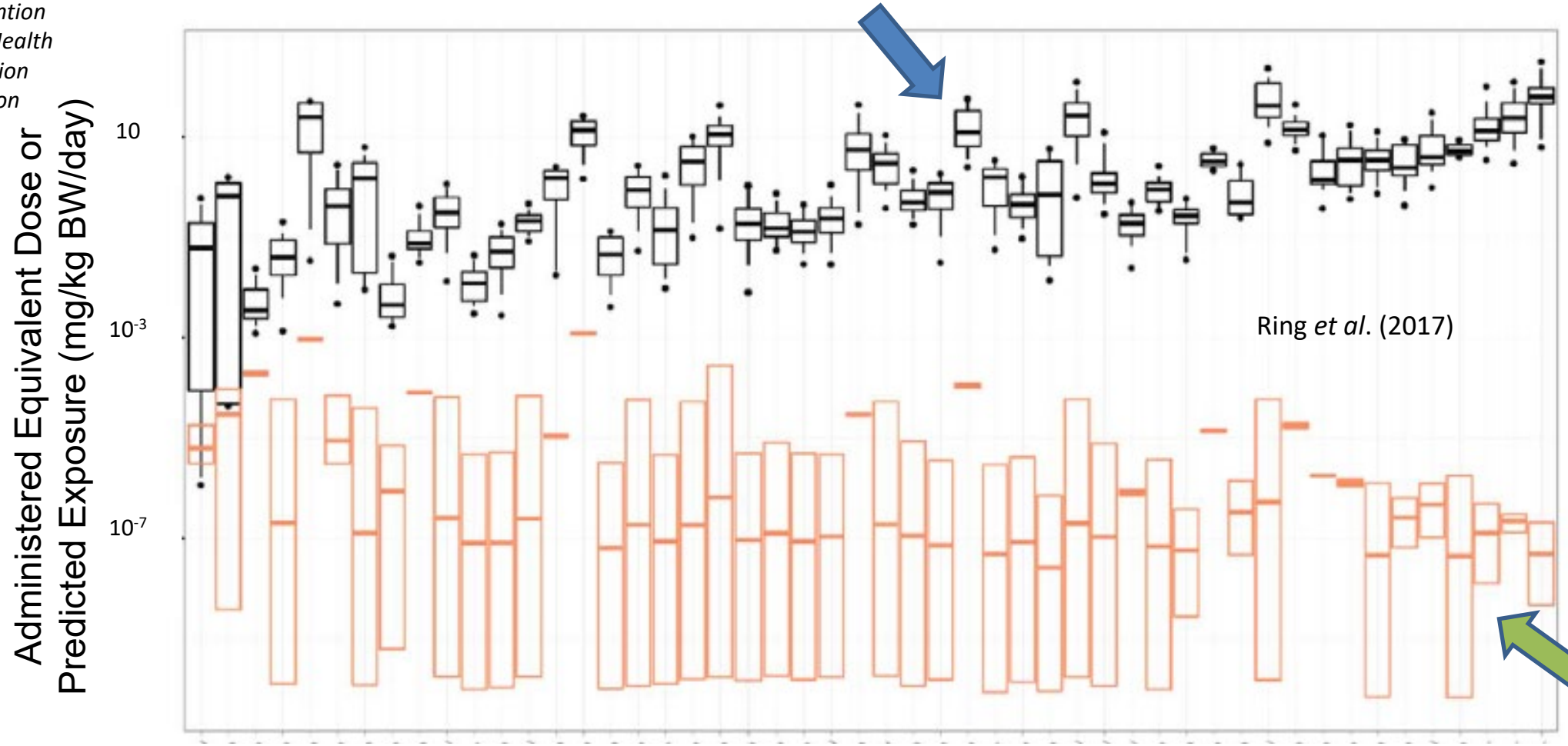

Exposure intake rates can be inferred from biomarkers (Wambaugh *et al.*, 2014)

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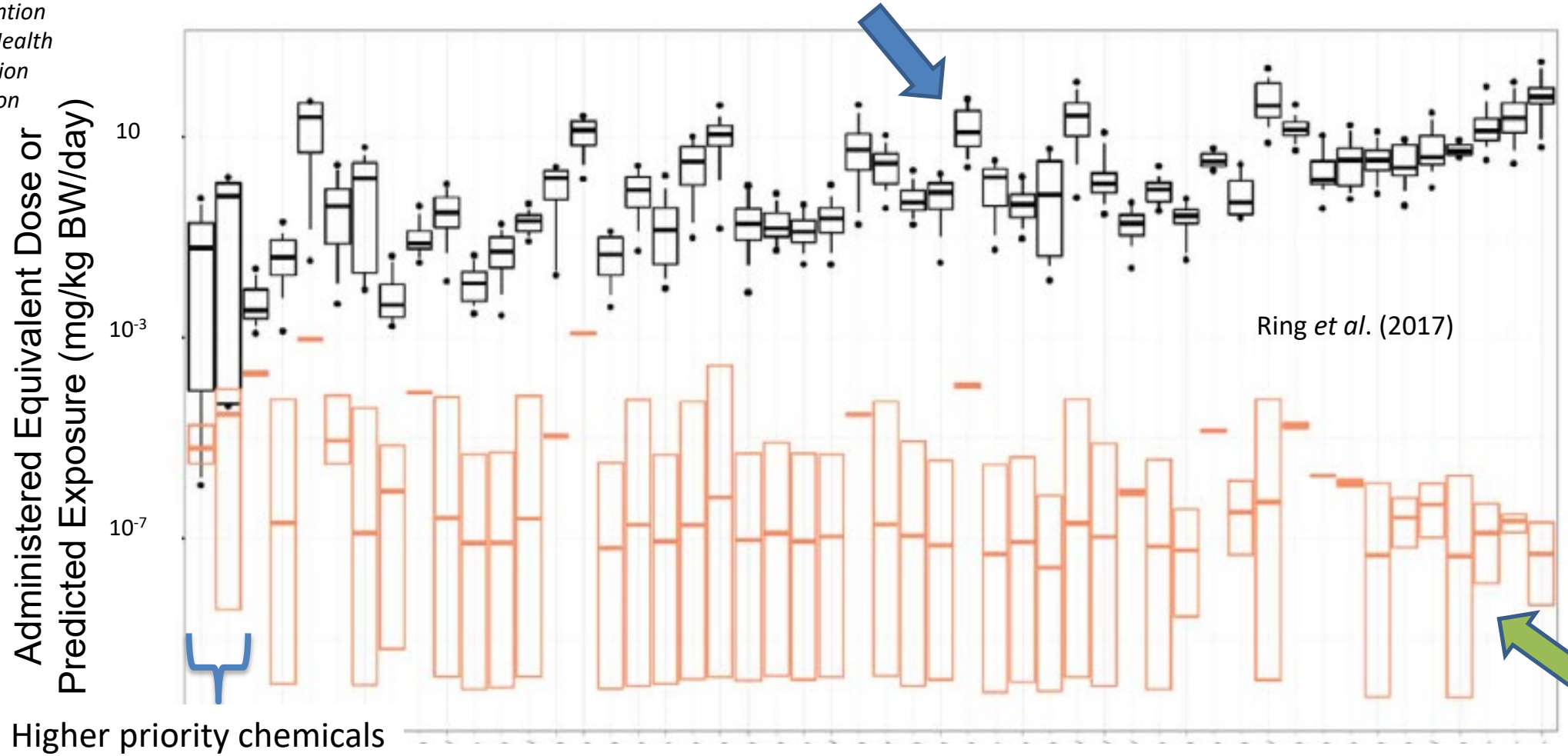

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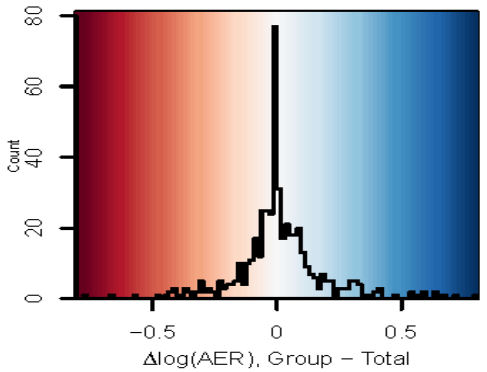
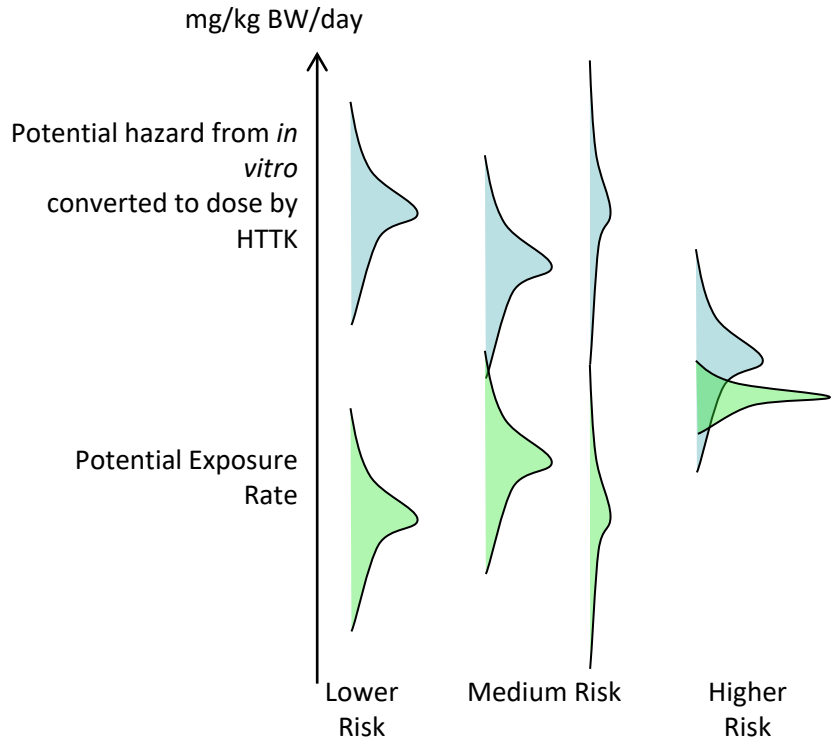
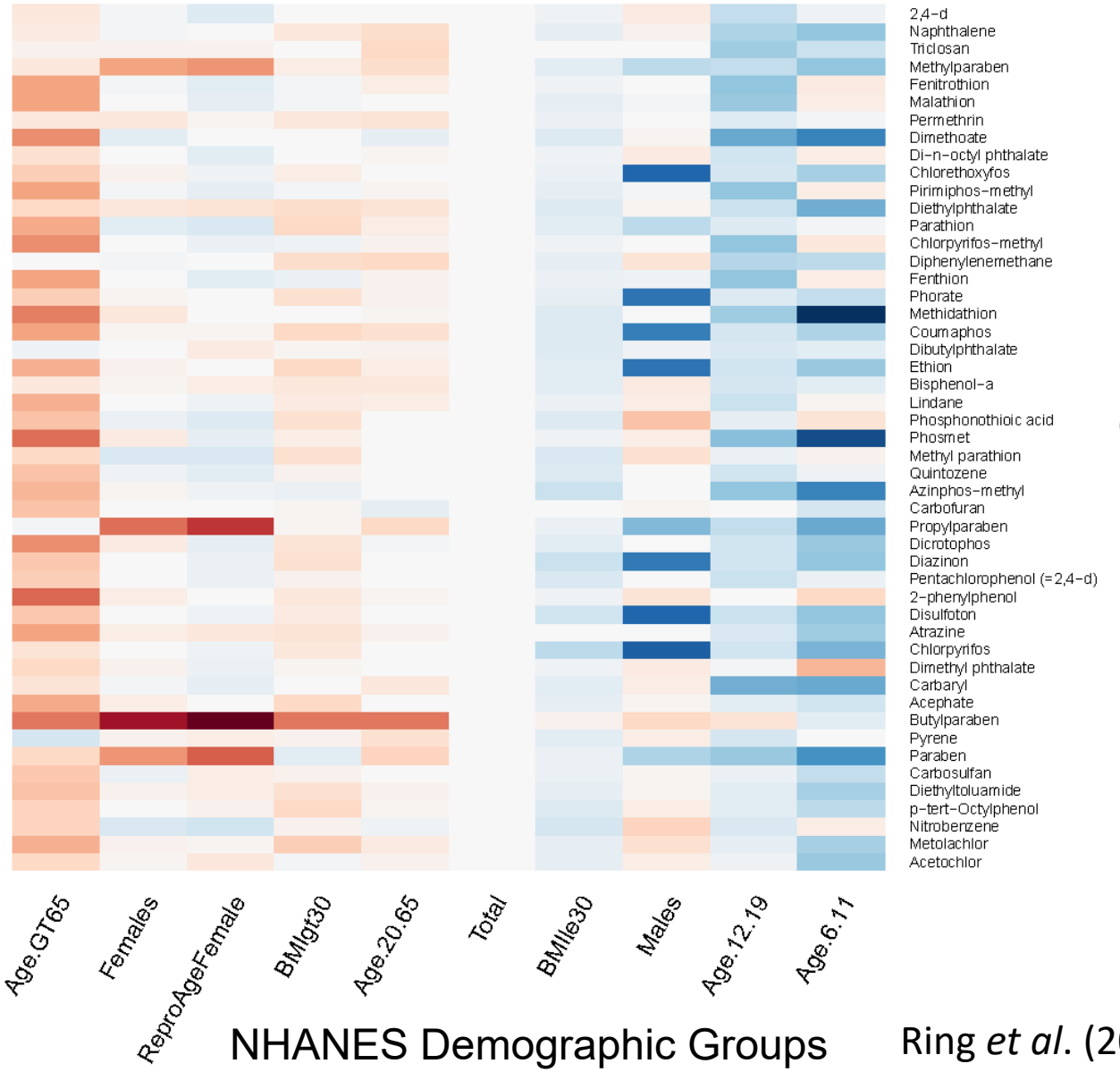
Life-stage and Demographic Specific Predictions

- We use HTTK to calculate margin between bioactivity and exposure for specific populations







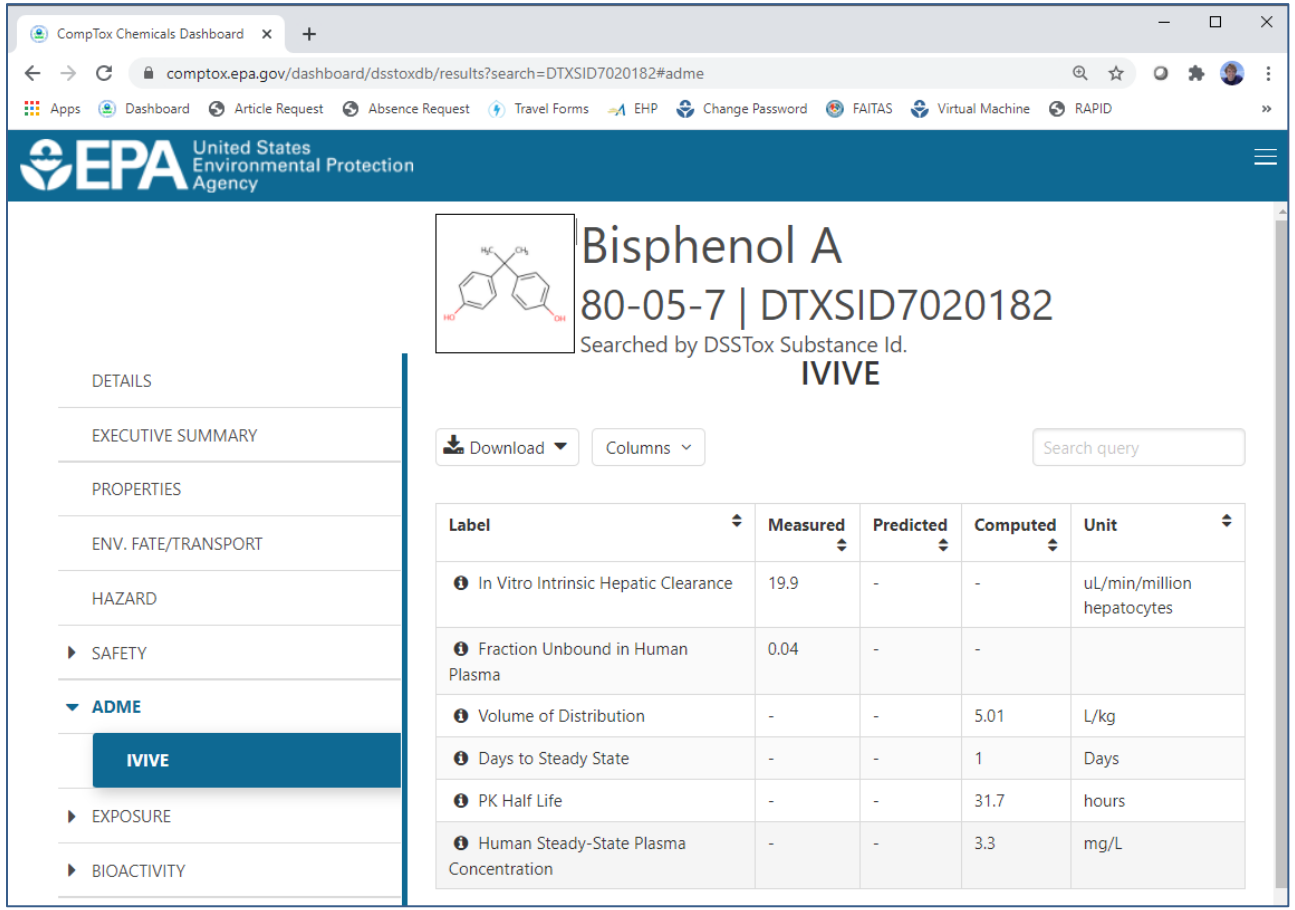
Ring *et al.* (2017)

HTTK on the CompTox Chemicals Dashboard

- The CompTox Chemicals Dashboard provides $C_{ss,95}$ values for >1000 chemicals

https://comptox.epa.gov/dashboard/

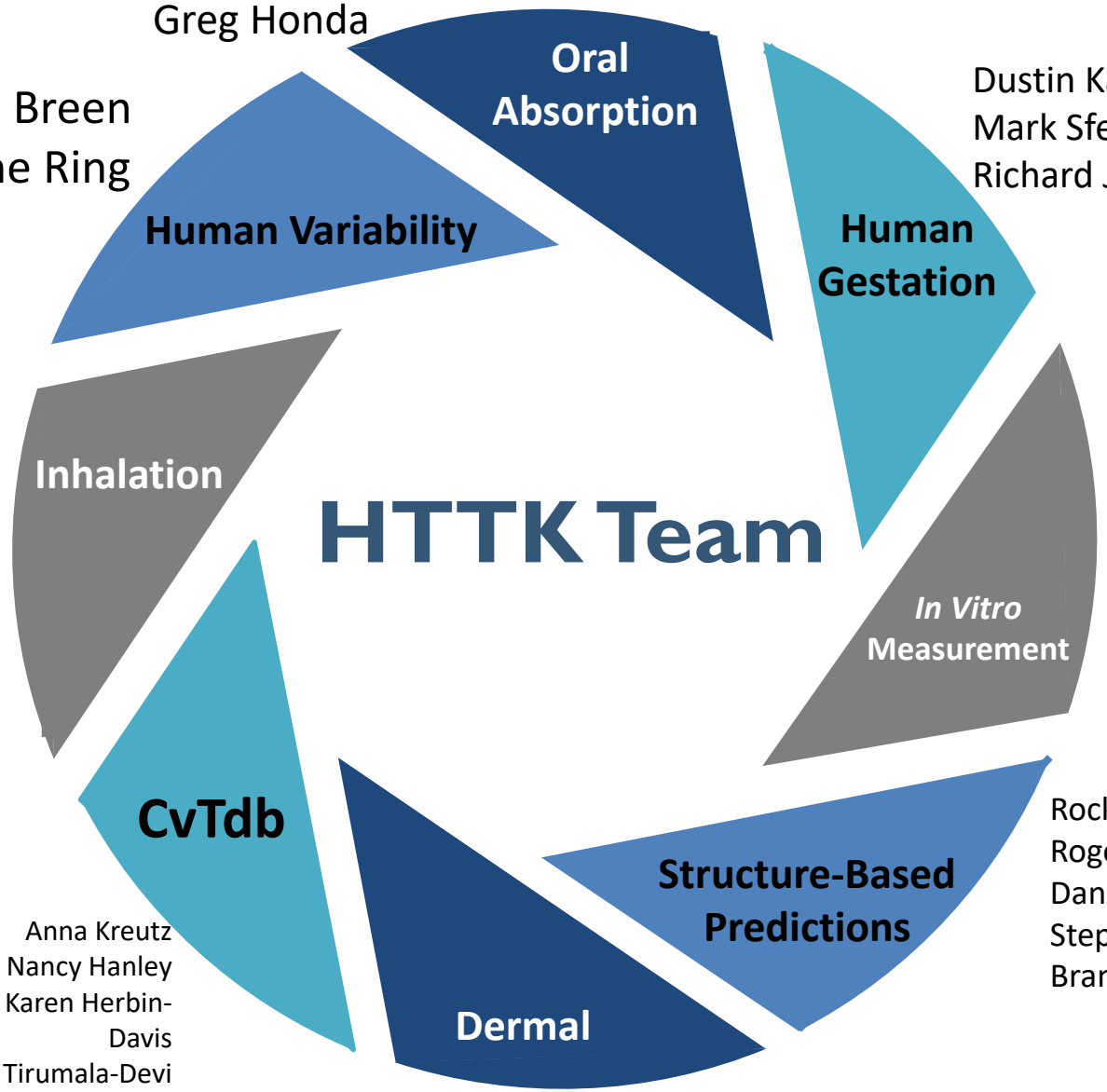
- We use EPA's R package "httk" to provide IVIVE predictions
- The value reported is calculated assuming a 1 mg/kg/day dose rate
- We give the upper 95th percentile of the calculated values based on a Monte Carlo simulation of human variability and uncertainty



Conclusions

- HTTK allows dosimetric adjustment of high-throughput screening (HTS) data
  - Thousands of chemicals
  - Open source, free, and evaluated software
- HTTK accounts for human population diversity using biometrics from the CDC NHANES to predict toxicokinetic model parameters
  - Variability is simulated using a Monte Carlo approach
- Breen et al. (in preparation) updates R package “httk” to the most recent three NHANES cohorts and adds children under the age of 6
- Toxicodynamic variability is not included
- HTTK in vitro parameters are generated from pooled adult tissues

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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References

- Armitage, J. M., Wania, F., & Arnot, J. A. (2014). Application of mass balance models and the chemical activity concept to facilitate the use of in vitro toxicity data for risk assessment. Environmental science & technology, 48(16), 9770-9779.
- Bell, Shannon M., *et al.* (2018) "In vitro to in vivo extrapolation for high throughput prioritization and decision making." Toxicology In Vitro 47 213-227.
- Bosgra, Sieto, *et al.* "An improved model to predict physiologically based model parameters and their inter-individual variability from anthropometry." Critical reviews in toxicology 42.9 (2012): 751-767.
- Breen, Miyuki, *et al.* "High-throughput PBTK models for in vitro to in vivo extrapolation." *Expert Opinion on Drug Metabolism & Toxicology* 17.8 (2021): 903-921.
- Breen, Miyuki, *et al.* "Simulating Toxicokinetic Variability to Identify Susceptible and Highly Exposed Populations". In preparation.
- Honda, Gregory S., *et al.* "Using the concordance of in vitro and in vivo data to evaluate extrapolation assumptions." PloS one 14.5 (2019): e0217564.
- Jamei, Masoud, *et al.* "The Simcyp® population-based ADME simulator." Expert opinion on drug metabolism & toxicology 5.2 (2009): 211-223.
- Linakis, Matthew *et al.* "Development of a Generalized Inhalation Model for use with the High-Throughput Toxicokinetics (httk) Package in R", Journal of Exposure Science & Environmental Epidemiology (2020): 1-12.
- McNally, Kevin, *et al.* "PopGen: a virtual human population generator." Toxicology 315 (2014): 70-85.
- National Research Council. (1983). Risk Assessment in the Federal Government: Managing the Process Working Papers. National Academies Press.
- Pearce, Robert G., *et al.* "httk: R Package for High-Throughput Toxicokinetics." Journal of Statistical Software, (2017a)
- Pearce, Robert G., *et al.* "Evaluation and calibration of high-throughput predictions of chemical distribution to tissues." Journal of pharmacokinetics and pharmacodynamics 44.6 (2017b): 549-565.
- Price, Paul S., *et al.* "Modeling interindividual variation in physiological factors used in PBPK models of humans." Critical reviews in toxicology 33.5 (2003): 469-503.
- Ring, Caroline L., *et al.* "Identifying populations sensitive to environmental chemicals by simulating toxicokinetic variability." *Environment International* 106 (2017): 105-118.
- Rotroff, Daniel M., *et al.* "Incorporating human dosimetry and exposure into high-throughput in vitro toxicity screening." Toxicological Sciences 117.2 (2010): 348-358.
- Schmitt, Walter. "General approach for the calculation of tissue to plasma partition coefficients." Toxicology in Vitro 22.2 (2008): 457-467.
- Sipes, Nisha S., *et al.* "An intuitive approach for predicting potential human health risk with the Tox21 10k library." Environmental science & technology 51.18 (2017): 10786-10796.
- Sobels, F. H. (1982) "The parallelogram; an indirect approach for the assessment of genetic risks from chemical mutagens." In: Progress in Mutation Research, Vol. 3 (K. C. Bora, G. R. Douglas, and E. R. Nestman, Eds.), Elsevier, Amsterdam, pp. 233-327.
- Wambaugh, J. F., *et al.* (2014). High throughput heuristics for prioritizing human exposure to environmental chemicals. Environmental science & technology, 48(21), 12760-12767.
- Wambaugh, John F., *et al.* "Toxicokinetic triage for environmental chemicals." Toxicological Sciences 147.1 (2015): 55-67.
- Wambaugh, John F., *et al.* (2019): "Assessing Toxicokinetic Uncertainty and Variability in Risk Prioritization" Toxicological Sciences, 172(2), 235-251.
- Wang, Ying-Hong. "Confidence assessment of the Simcyp time-based approach and a static mathematical model in predicting clinical drug-drug interactions for mechanism-based CYP3A inhibitors." Drug Metabolism and Disposition 38.7 (2010): 1094-1104.
- Wetmore, Barbara A., *et al.* "Integration of dosimetry, exposure and high-throughput screening data in chemical toxicity assessment." *Tox. Sciences* (2012)
- Wetmore, Barbara A., *et al.* "Relative impact of incorporating pharmacokinetics on predicting in vivo hazard and mode of action from high-throughput in vitro toxicity assays." toxicological sciences 132.2 (2013): 327-346.
- Wetmore, Barbara A., *et al.* "Incorporating high-throughput exposure predictions with dosimetry-adjusted in vitro bioactivity to inform chemical toxicity testing." Toxicological Sciences 148.1 (2015): 121-136.

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