EPA United States Environmental Protection Agency

EUROTOX 2023
LJUBLJANA – SLOVENIA
10–13 SEPTEMBER 2023

Modelling kinetics and dynamics of chemicals at the U.S. Environmental Protection Agency using the httk open-source platform

John Wambaugh, Sarah Davidson-Fritz, Annabel Meade, Miyuki Breen, Risa Sayre, Taylor Wall, Elaina Kenyon, Marina Evans, Barbara Wetmore, and Caroline Ring

Center for Computational Toxicology and Exposure

Symposium:
"Next generation risk assessment of food chemicals, environmental contaminants and pharmaceuticals using open-source modelling platforms: Perspectives from regulatory agencies, academia, and industry"

Send Questions to:
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http://orcid.org/0000-0002-4024-534X

US EPA Office of Research and Development

- The Office of Research and Development (ORD) is the scientific research arm of EPA
- 539 peer-reviewed journal articles in 2021
- Research is conducted by ORD's four national centers organized to address:
  - Public health and environmental assessment
  - Computational toxicology and exposure
  - Environmental measurement and modeling
  - Environmental 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

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Next Generation Risk Assessment Requires Toxicokinetics

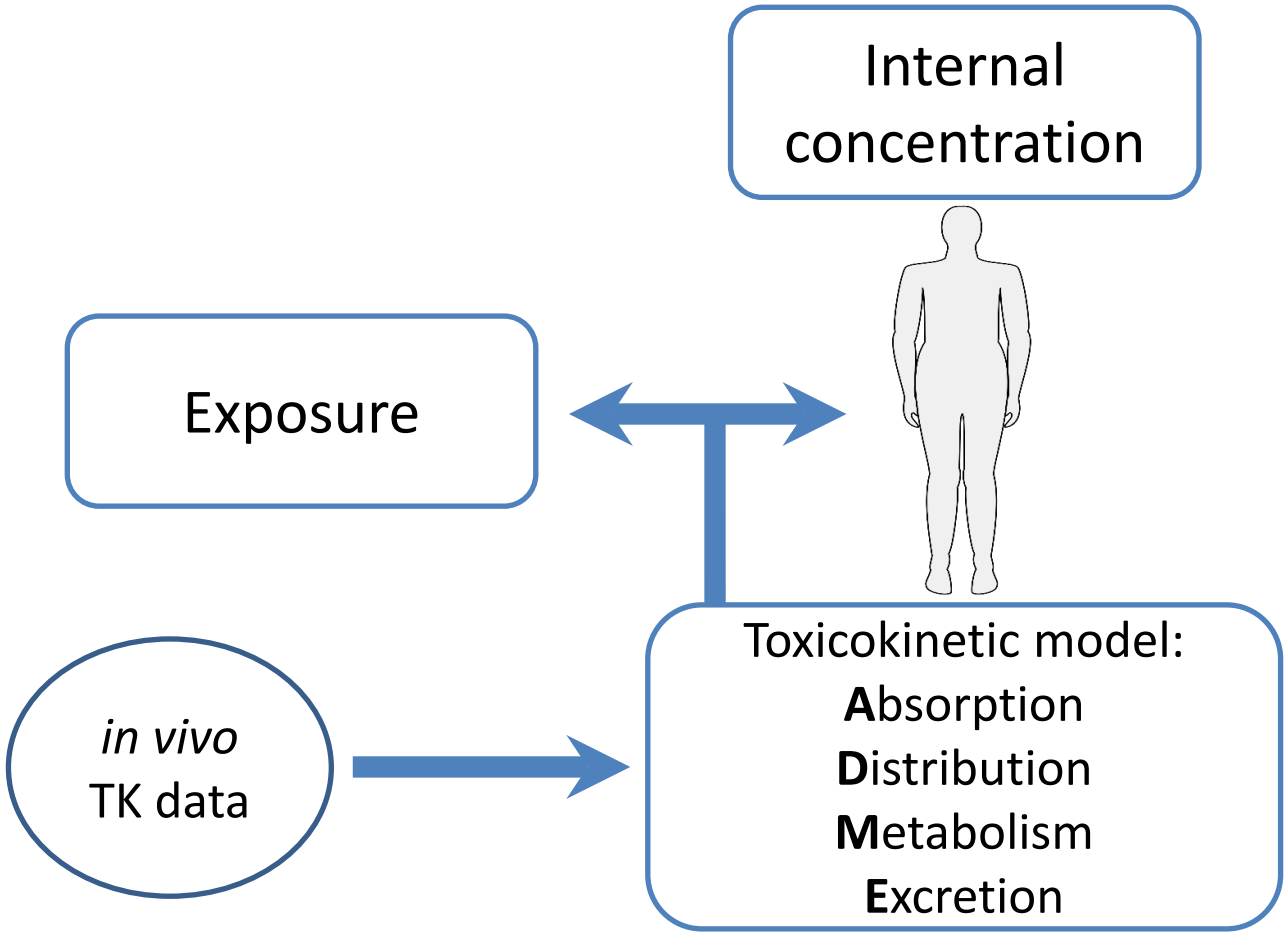
United States Environmental Protection Agency

Next Generation Risk Assessment (NGRA)

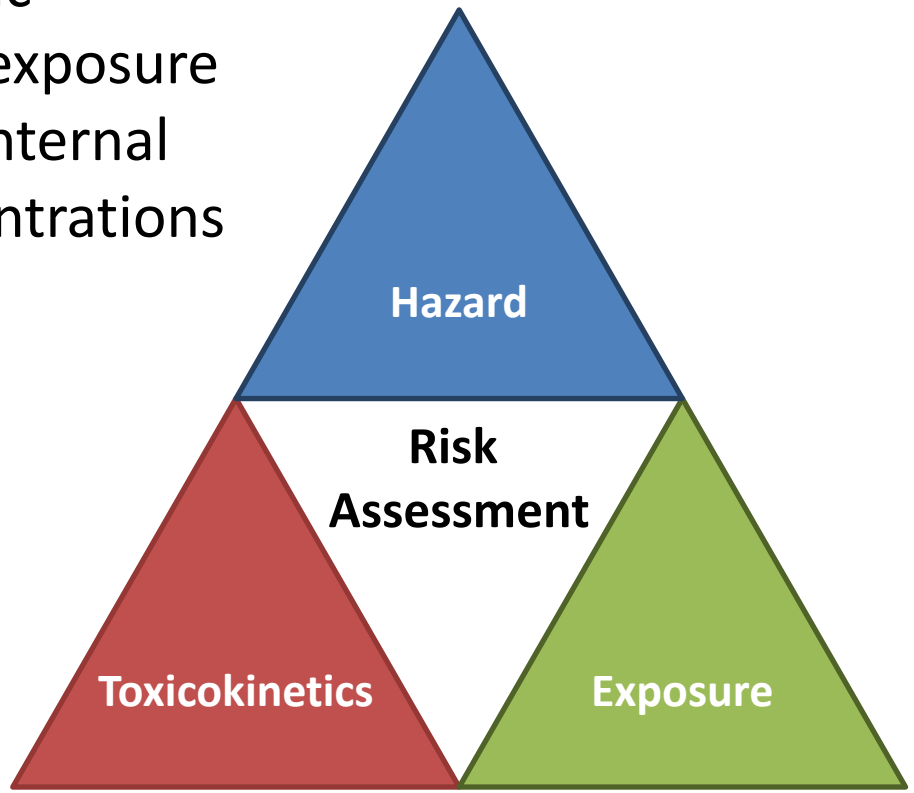
- Next generation risk assessment (NGRA) must incorporate endpoints from new approach methodologies (NAMs) that are often *in vitro*
- There is a need to translate *in vitro* measures into recognizable risk assessment concepts (*in vivo* point of departure, margin of exposure)
- This requires ***in vitro – in vivo*** **extrapolation (IVIVE)**
- Powerful tools (such as physiologically-base toxicokinetic modeling) exist for one-chemical-at-a-time IVIVE (such as SimCYP, Gastroplus, PK-Sim)

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)

Most Chemicals Do Not Have TK Data

- We need chemical-specific toxicokinetics (TK) for *in vitro-in vivo* extrapolation (IVIVE) (Coecke et al, 2013), **but:**
- 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

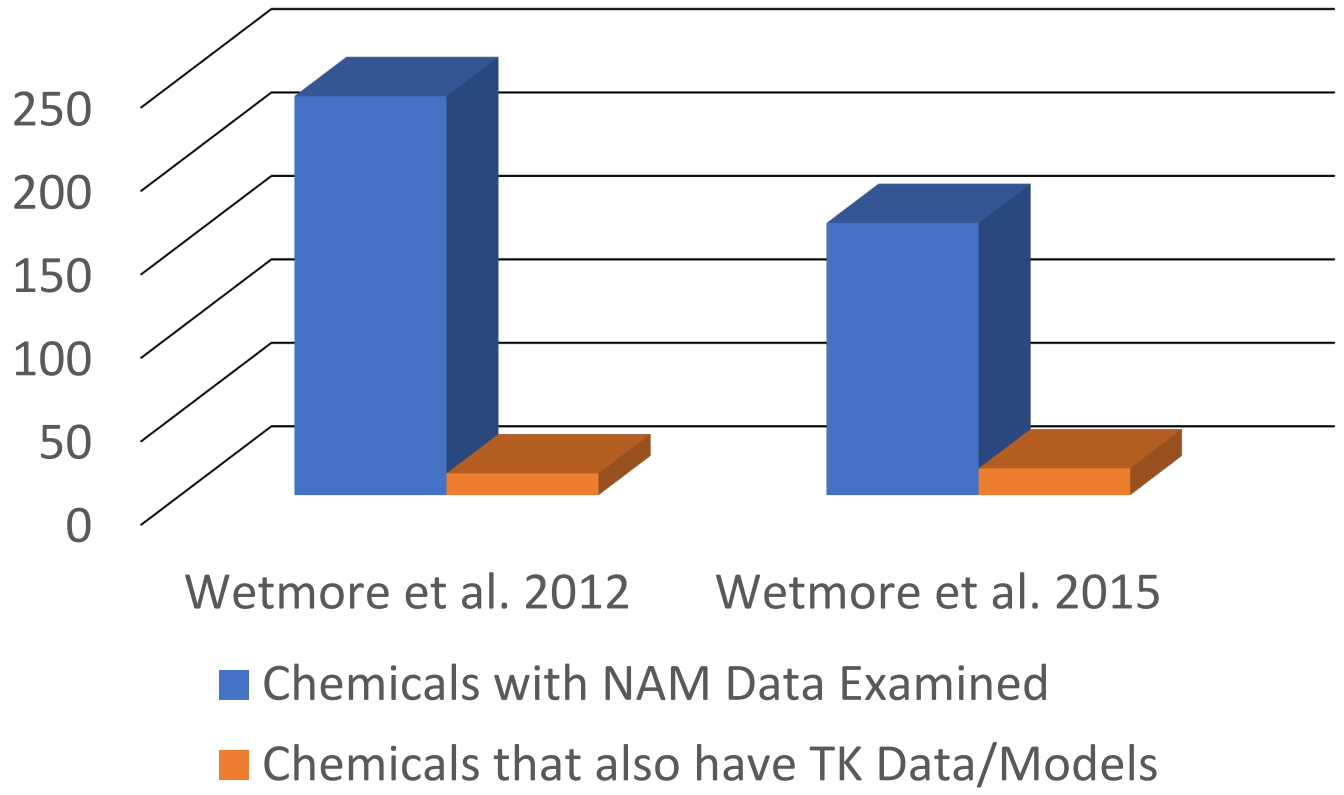


Figure modified from Bell et al. (2018)

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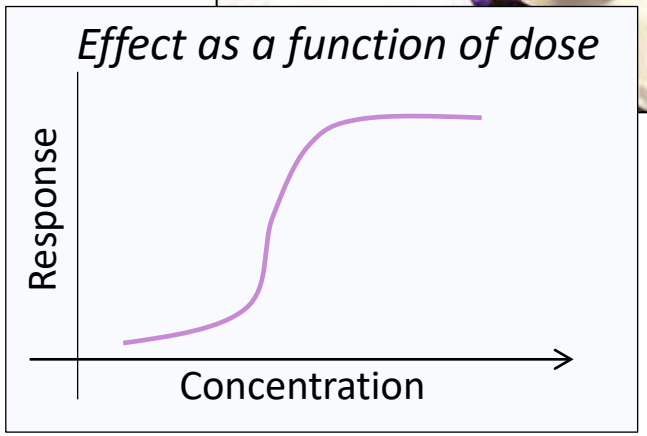
HTTK

High Throughput Toxicokinetics

- NAMs often include multiple assay formats as part of a screening battery
- NAMs are intended to allow screening of large libraries of chemicals – hundreds to thousands
- High throughput tools are needed for IVIVE
- High(er) throughput toxicokinetics (HTTK) provides the needed IVIVE for NGRA

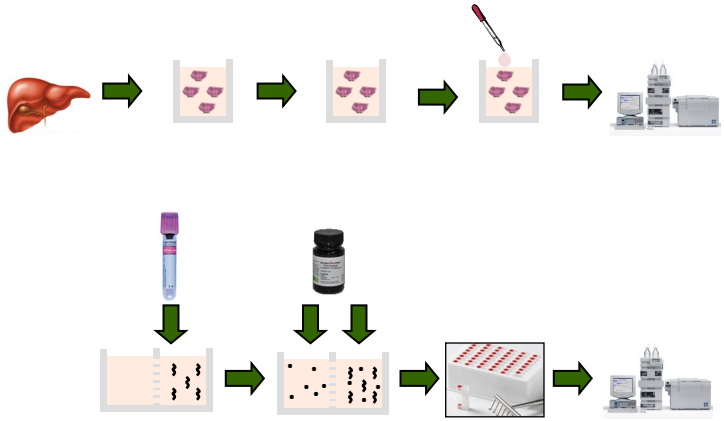


High Throughput Toxicokinetics (HTTK):
A New Approach Methodology (NAM) for Exposure

- 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)
  - In addition to using a standardized (generic) model, this approach also standardizes the parameters and *in vitro* measurements needed to describe a chemical
- HTTK can provide **open-source data and models** for evaluation and use by the broader scientific community (Pearce et al, 2017)
- While there is more data for pharmaceuticals, these data are often proprietary

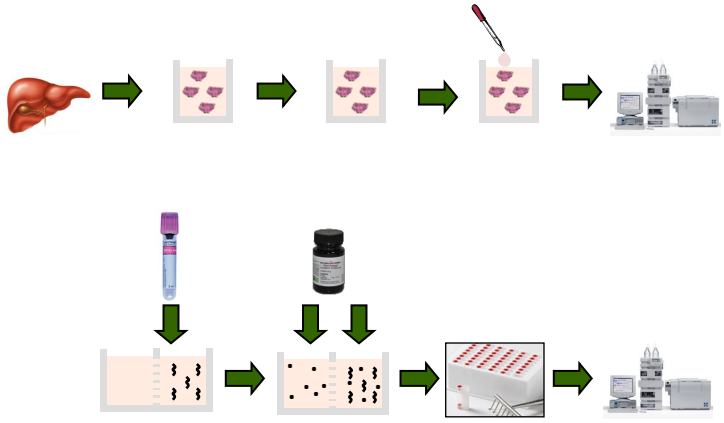
The Elements of High Throughput Toxicokinetics

In vitro toxicokinetic data



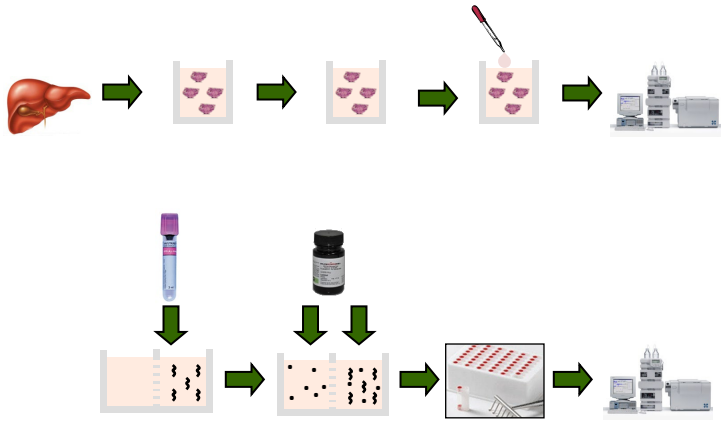
In vitro toxicokinetic data

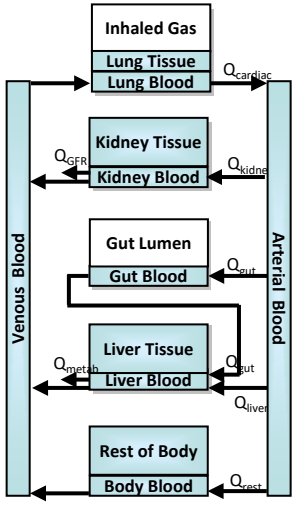
Typically, intrinsic hepatic clearance and fraction unbound in plasma



The Elements of High Throughput Toxicokinetics

In vitro toxicokinetic data + generic toxicokinetic model

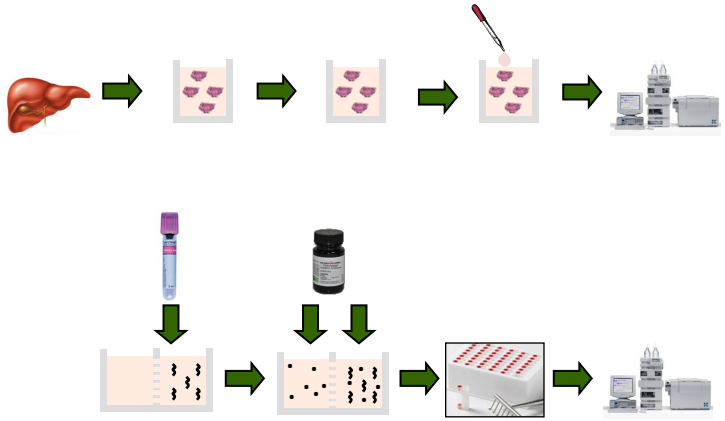


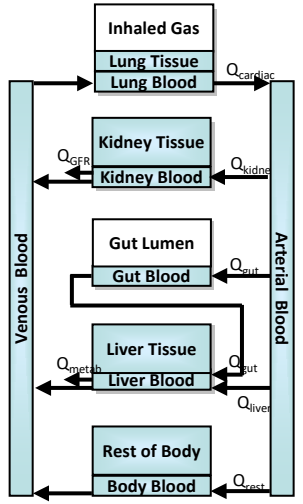


The Elements of High Throughput Toxicokinetics

In vitro **toxicokinetic data + generic toxicokinetic model**

= high(er) throughput toxicokinetics





= *httk*

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Open Source Data

Chemical-Specific HTTK Data

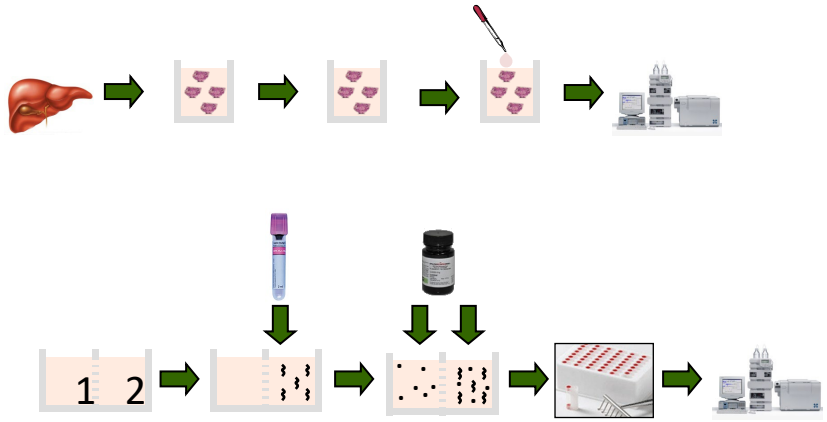
- A decade of publications have made available *in vitro* data characterizing > 1,000 chemicals

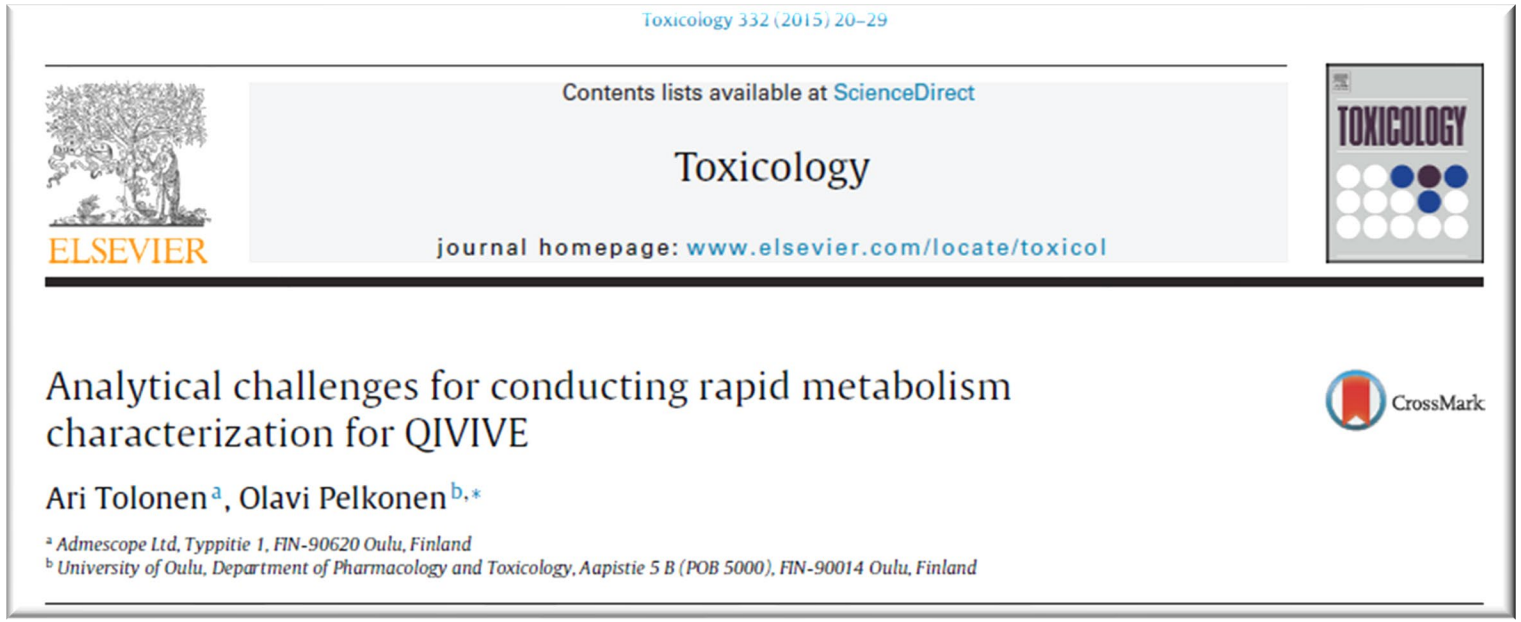
TK Process	In Vitro Assays	In Vivo TK Predicted	Assay Limitations	Chemical Limitations	Time and Labor	Impact	QSPR
Metabolism	Hepatocyte suspension,	Hepatic Metabolism	Relatively short timescales (< 4h)	Soluble, non-volatile chemicals	High Throughput	Clearance, steady-state concentration, half-life	Yes (pharma and commercial chemicals)
	microsome assays	Metabolism (including liver, kidney, gut, lung)	Many individual microsomes to be assayed	Soluble, non-volatile chemicals	High Throughput	Individuals microsomes can be expressed in multiple tissues and allow insight into human variability	Yes (pharma)
	Hepatocyte spheroids	Hepatic Metabolism	Expense, throughput	Soluble, non-volatile chemicals	In vitro but low throughput	Two week or greater running times for slowly metabolized chemicals	No
	individual bacterial species, intestinal content culture	Gut metabolism	anaerobic conditions, probably pH dependent, microbe-dependent	Soluble, non-volatile chemicals	low throughput	absorption of chemicals, alternative metabolism	No
	Vmax and KM	intrinsic clearance	Low Throughput, ideally would have method for parent and metabolites	soluble, non-volatile chemicals	low throughput	Saturation (non-linear) metabolism	No
Distribution	Plasma protein binding		Tradeoffs between speed and sensitivity	Soluble, non-volatile chemicals	High Throughput	Peak conc., partition coefficients	Yes (pharma and commercial)
	Blood:plasma ratio	Blood:plasma ratio, first pass hepatic metabolism		Soluble, non-volatile chemicals	High Throughput	More accurate prediction of systemic bioavailability	Yes (pharma and commercial)
	Transporters						
Absorption	Caco2, PAMPA		Mostly qualitative, skewed toward predicting “well absorbed”	Soluble, non-volatile chemicals	In vitro but low throughput	Identifies key routes of exposure	Yes (pharma only)
Elimination	Plasma protein binding		Tradeoffs between speed and sensitivity	Soluble, non-volatile chemicals	High Throughput	Peak conc., partition coefficients	Yes (pharma and commercial)

See Coecke et al. (2013), Breen et al. (2021)

Analytical Chemistry is a Key Bottleneck

- Most HTTK *in vitro* assays require chemical-specific method to quantify changes in chemical concentration
- Assays requires ~2 mg neat compound – sometimes hard to procure
- For some chemicals "typical" methods like liquid or gas chromatography mass spectrometry do not work
- Other chemicals are obscured by matrix effects – for example, similar biological components of the assay





Toxicology 332 (2015) 20–29

Contents lists available at ScienceDirect

Toxicology

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

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Analytical challenges for conducting rapid metabolism characterization for QIVIVE

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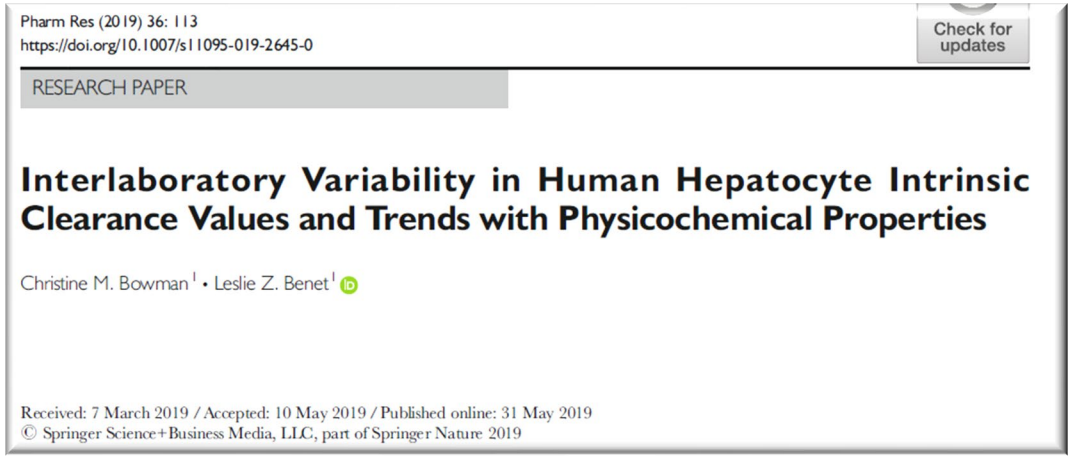
Lots of help from Kathy Coutros and Tony Williams

Best Practices for HTTK Data

- Multiple governments and organizations continuing to collect *in vitro* data for HTTK

- Various approaches, including R package "httk" try to summarize these data

- EPA is interested in standardizing data analysis
  - Project led by Sarah Davidson-Fritz
  - Working on new R package "invitroTKstats"
  - Ensure all necessary measurements and metadata are recorded
  - Structure data to support potential future databases



Pharm Res (2019) 36: 113
https://doi.org/10.1007/s11095-019-2645-0

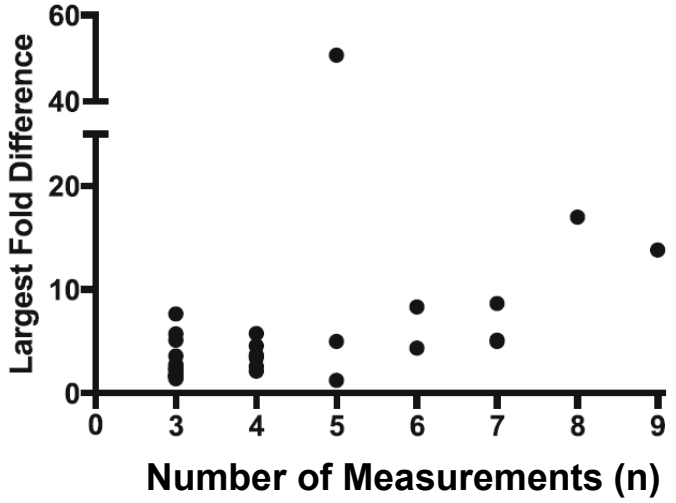
Check for updates

RESEARCH PAPER

Interlaboratory Variability in Human Hepatocyte Intrinsic Clearance Values and Trends with Physicochemical Properties

Christine M. Bowman[1] • Leslie Z. Benet[1]

Received: 7 March 2019 / Accepted: 10 May 2019 / Published online: 31 May 2019



n	3	4	5	6	7	8	9
# values	17	6	3	2	3	1	1
Mean Largest Dif.	2.8	3.7	19	6.3	6.3	17	14
SD	1.8	1.3	28	2.8	2.1	-	-

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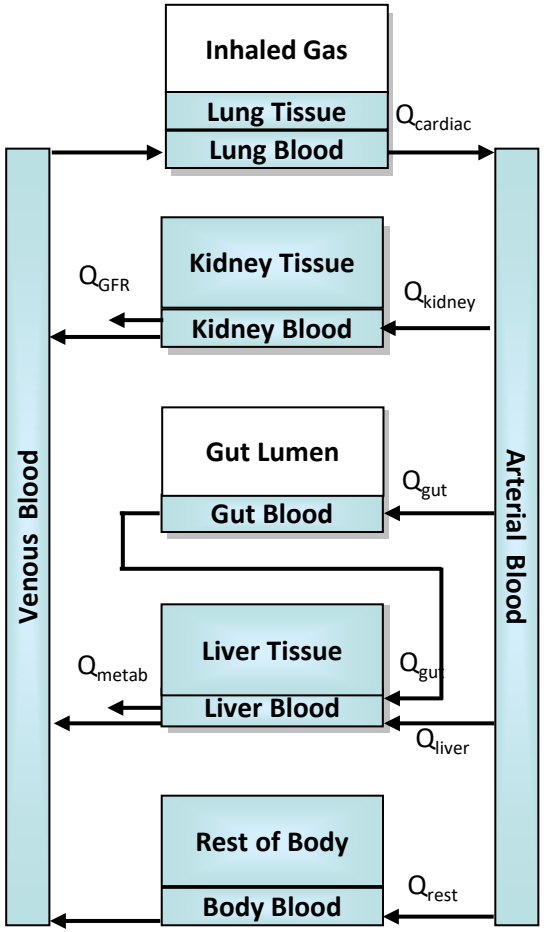
Open Source Models

Generic PBK/PBPK/PBTK Models

- A standardized physiology is assumed, regardless of chemical:
  - The same parameters such as volumes, flows, and rates are used
  - The same processes are included (hepatic metabolism, glomerular filtration) or omitted
- A fixed set of descriptors (such as rate of metabolism and protein binding) are varied from chemical to chemical and potentially measured in vitro
- The generic model is implemented once, reducing the likelihood of coding errors and enhancing documentation
- We can estimate the accuracy of a generic model for a new chemical using performance across multiple chemicals where data happen to exist



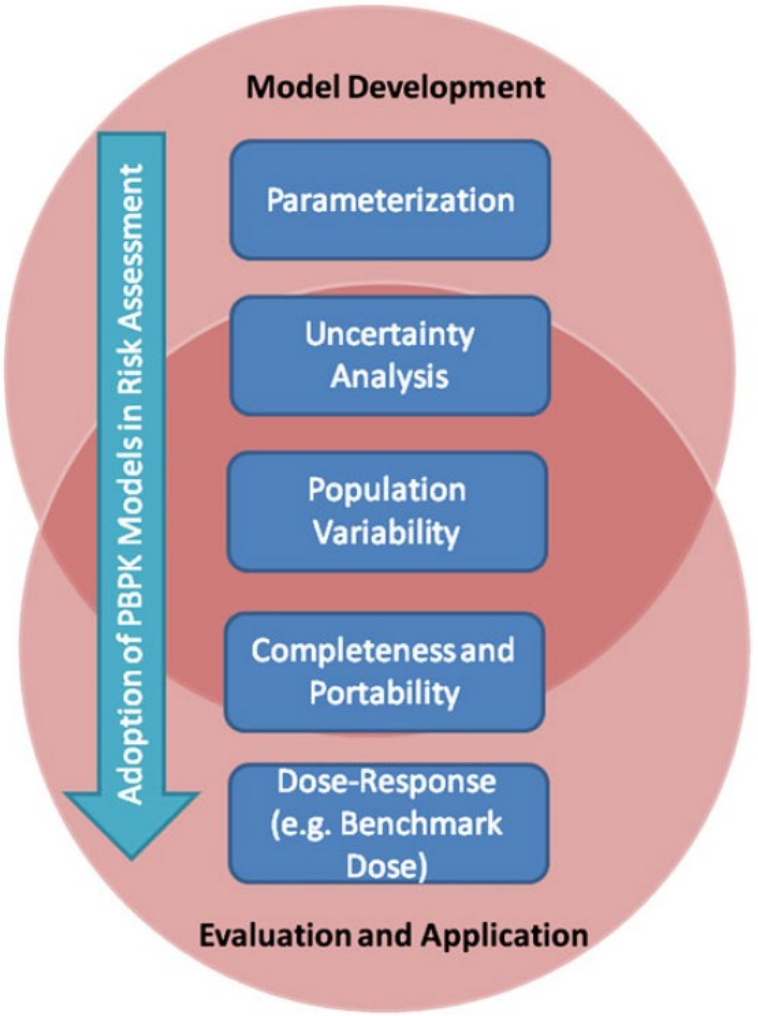
Open Source, Verifiable, Reproducible

TOXICOLOGICAL SCIENCES 126(1), 5–15 (2012)
doi:10.1093/toxsci/kfr295
Advance Access publication November 1, 2011

Physiologically Based Pharmacokinetic Model Use in Risk Assessment—Why Being Published Is Not Enough

Eva D. McLanahan,*,[1] Hisham A. El-Masri,† Lisa M. Sweeney,‡ Leonid Y. Kopylev,|| Harvey J. Clewell,§ John F. Wambaugh,¶ and P. M. Schlosser||

"Although publication of a PBPK model in a peer-reviewed journal is a mark of good science, subsequent evaluation of published models and the supporting computer code is necessary for their consideration for use in [Human Health Risk Assessments]"



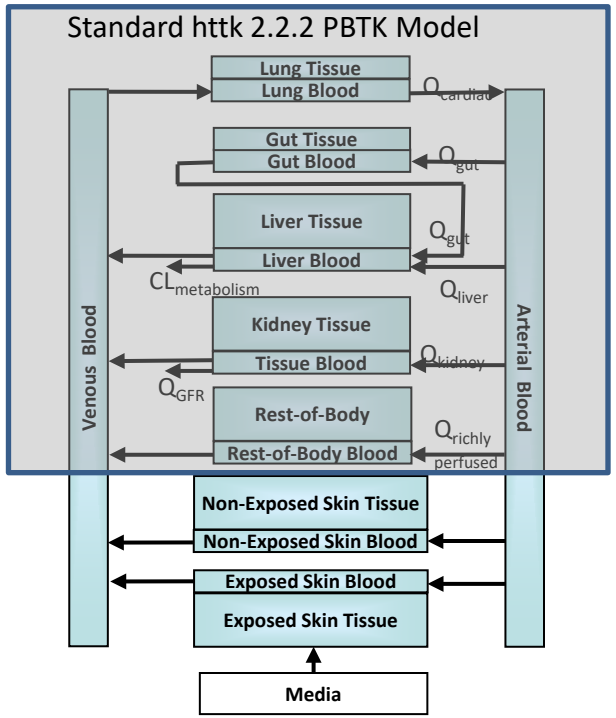
Key considerations during PBTK model development, evaluation, and applications for Human Health Risk Assessment

HT-PBTK Models from EPA

United States Environmental Protection Agency

Gas Inhalation Exposure Route

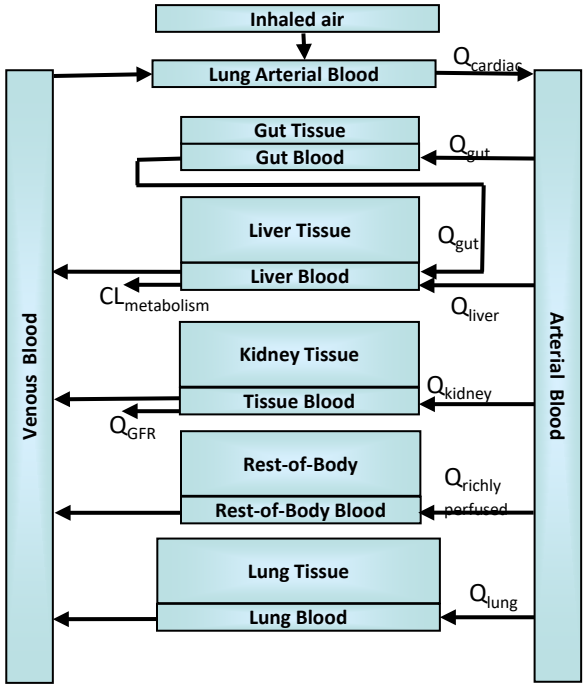
Linakis et al. (2020)



Dermal Exposure Route

EPA, Unilever

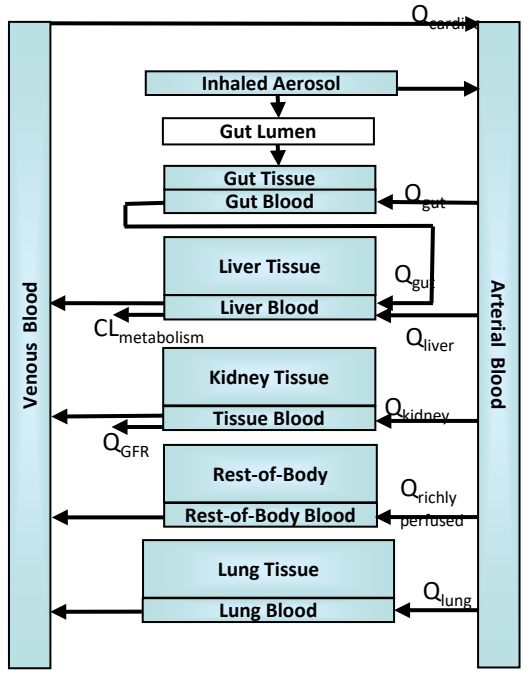
(Meade, in prep.)



Aerosol Inhalation Exposure Route (including APEX model)

EPA, USAF

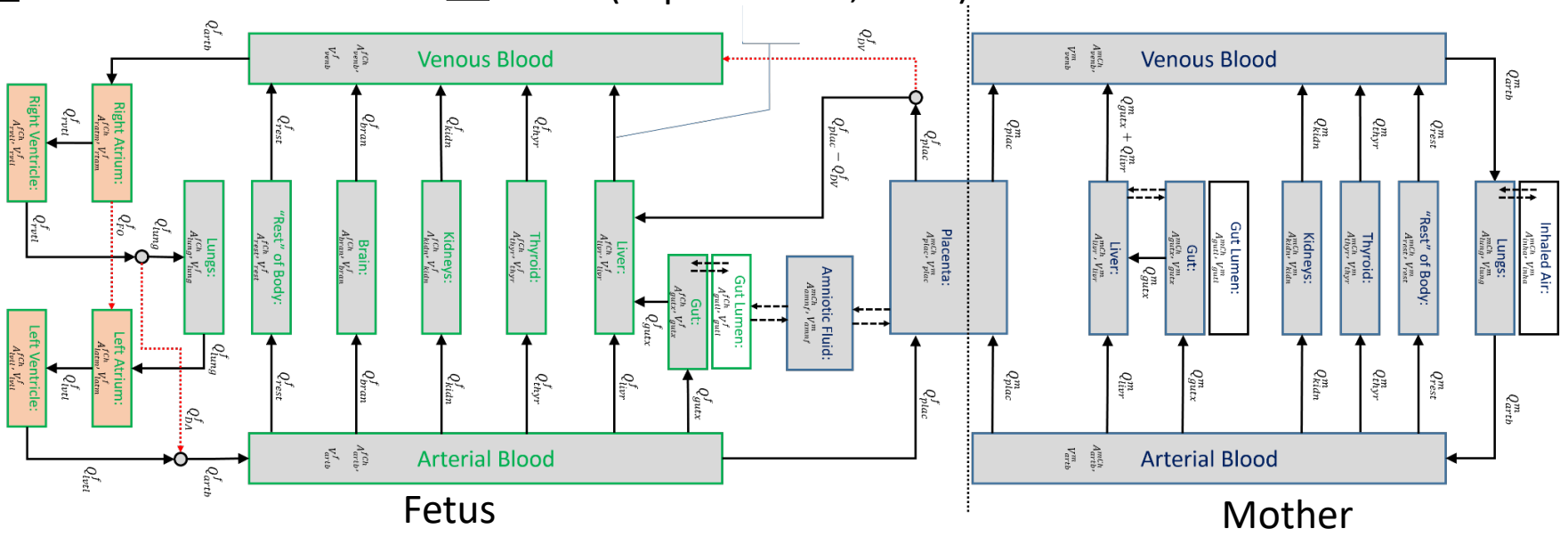
(Linakis, in prep.)



Human Gestational Model

EPA, FDA

(Kapraun et al, 2022)



Fetus

Mother

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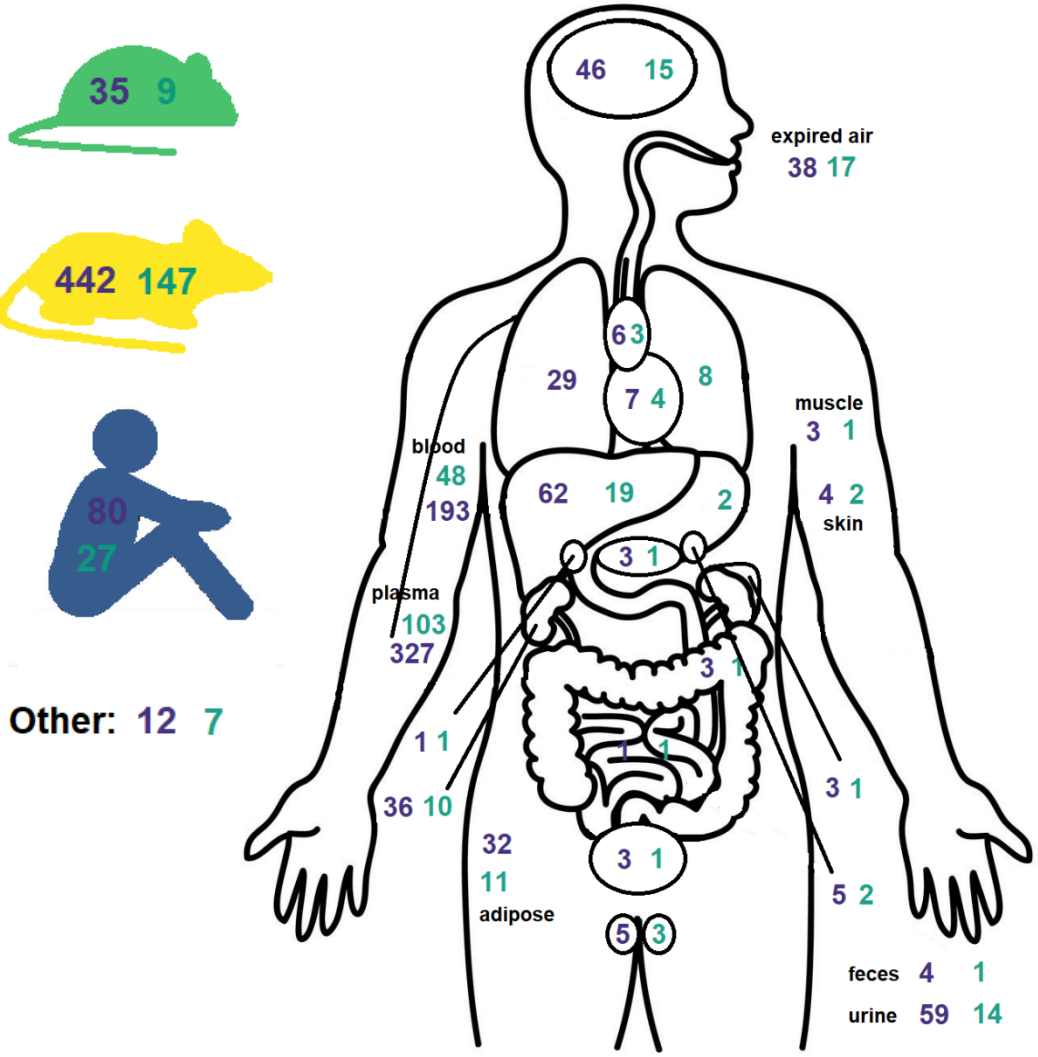
Evaluation with Legacy *In Vivo* Data

CvtDB: An In Vivo TK Database

https://github.com/USEPA/CompTox-PK-CvTdb

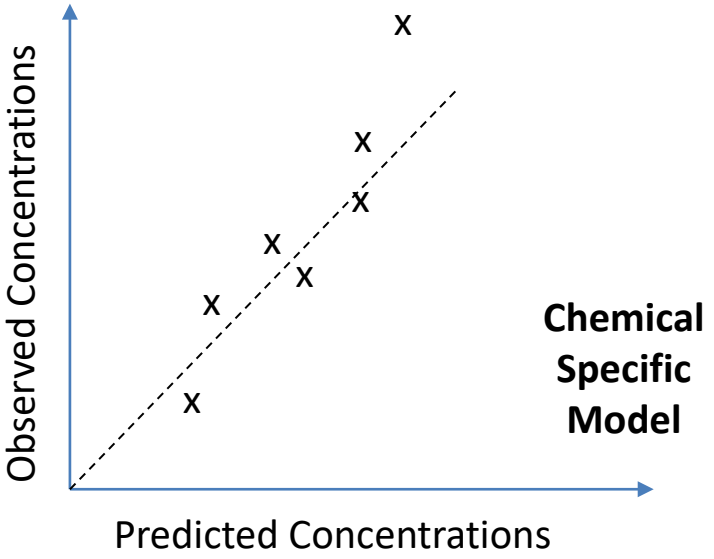
- The most important thing for evaluating PBK/PBPK/PBTK is evaluation data
- EPA has developed a **public database** of **concentration vs. time data** for building, calibrating, and evaluating TK models
- Curation and development is ongoing, but to date includes:
  - >200 analytes (EPA, National Toxicology Program, Showa Pharmaceutical University, literature)
  - Routes: Intravenous, dermal, oral, sub-cutaneous, and inhalation exposure
- Efforts led at EPA by Risa Sayre and Taylor Wall



Sayre et al. (2020)

Building Confidence in TK Models

- To evaluate a **chemical-specific TK model** for "chemical x" you can compare the predictions to *in vivo* measured data
  - Can estimate bias
  - Can estimate uncertainty
  - Can consider using model to extrapolate to other situations (dose, route, physiology) where you have no data



Cohen Hubal et al. (2019)

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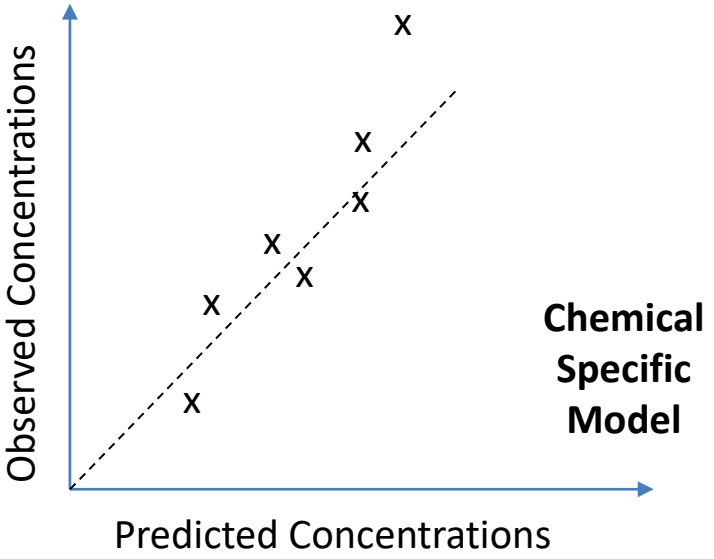

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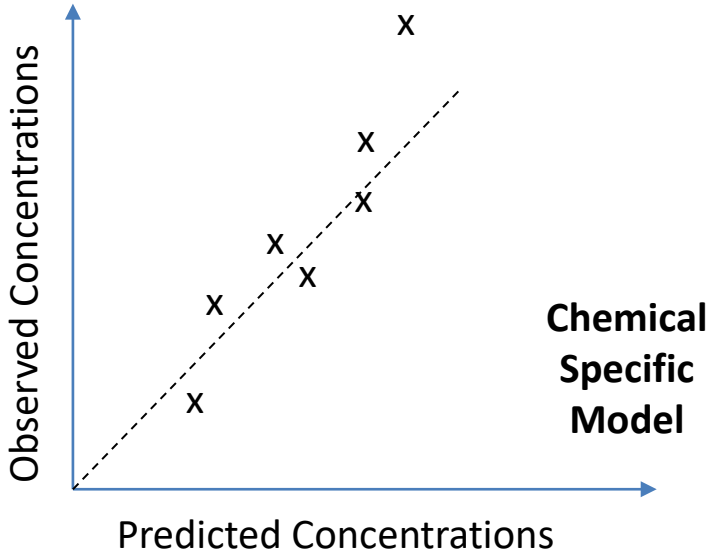

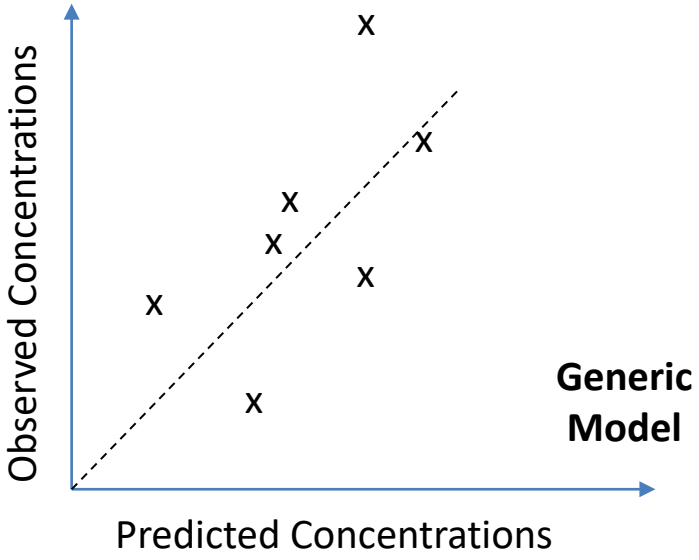

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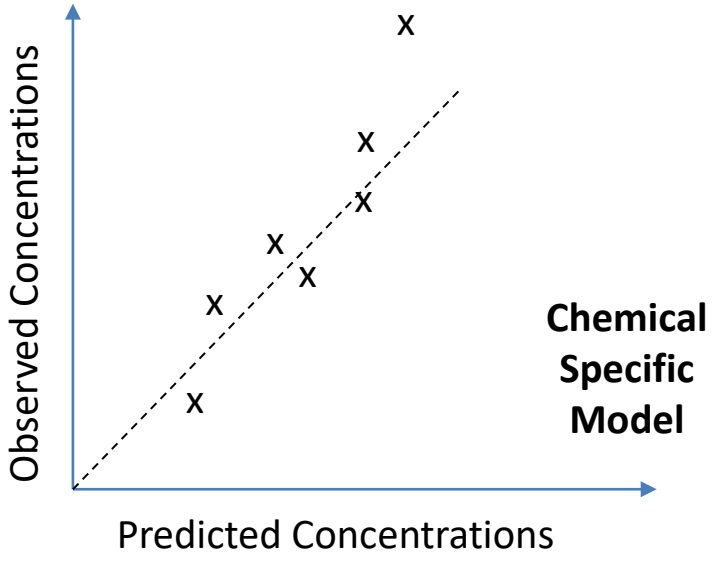

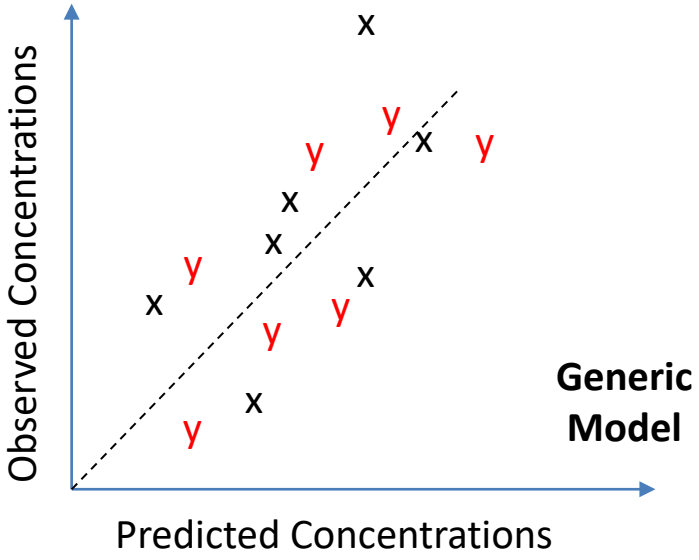

Cohen Hubal et al. (2019)

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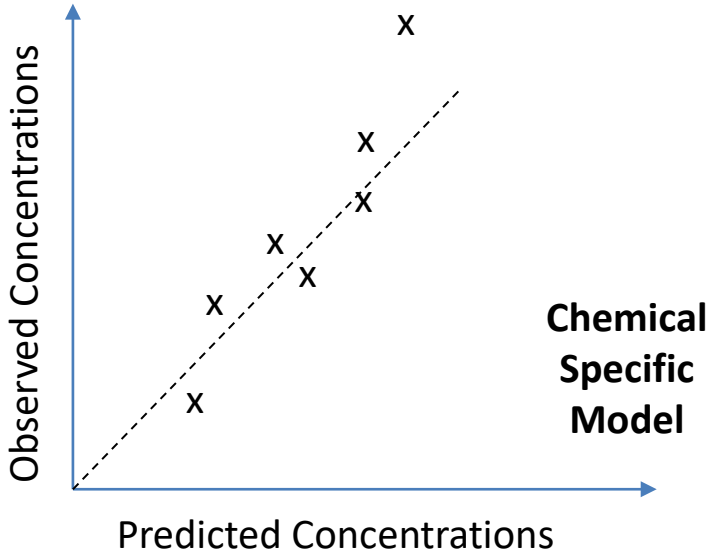

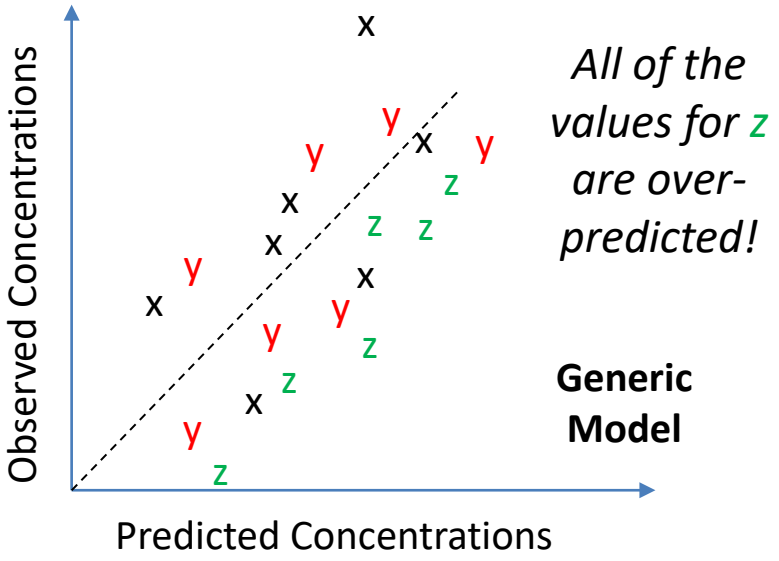

Cohen Hubal et al. (2019)

Evaluating the Confidence in HTTK

- WHO recommends TK model predictions generally be within a factor of 2, on average

- For HTTK, summary statistics such as peak concentration and time-integrated ("area under the curve" or AUC) concentration:
  - Wang (2010): For 54 pharmaceutical clinical trials the predicted AUC differed from observed by 2.3x
  - Linakis et al. (2020): RMSE = 0.46 or 2.9x for peak concentration and RMSE = 0.5 or 3.2x for AUC
  - Wambaugh et al. (2018): For 45 chemicals of both pharmaceutical and non-pharmaceutical nature, RMSE of 2.2x for peak and 1.64x for AUC
  - Pearce et al. (2017b):The calibrated method for predicting tissue partitioning that is included in httk predicted human volume of distribution with a RMSE of 0.48 (3x)

Breen et al. (2021)

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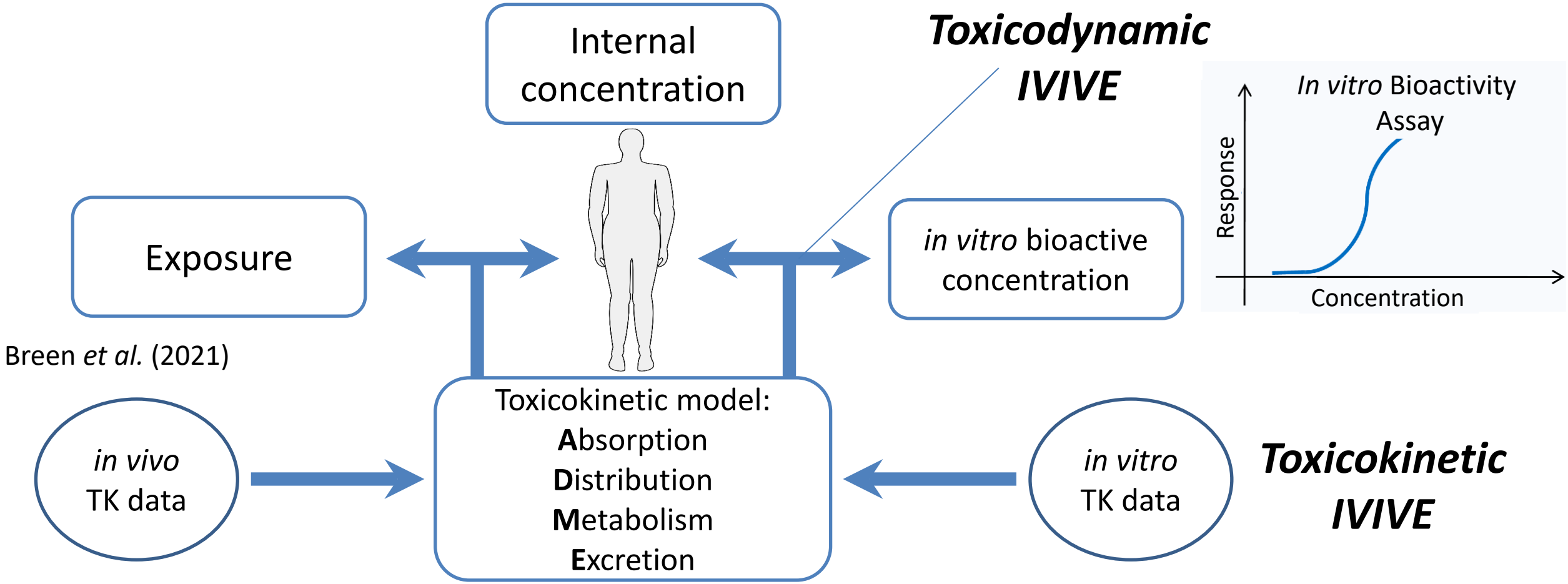
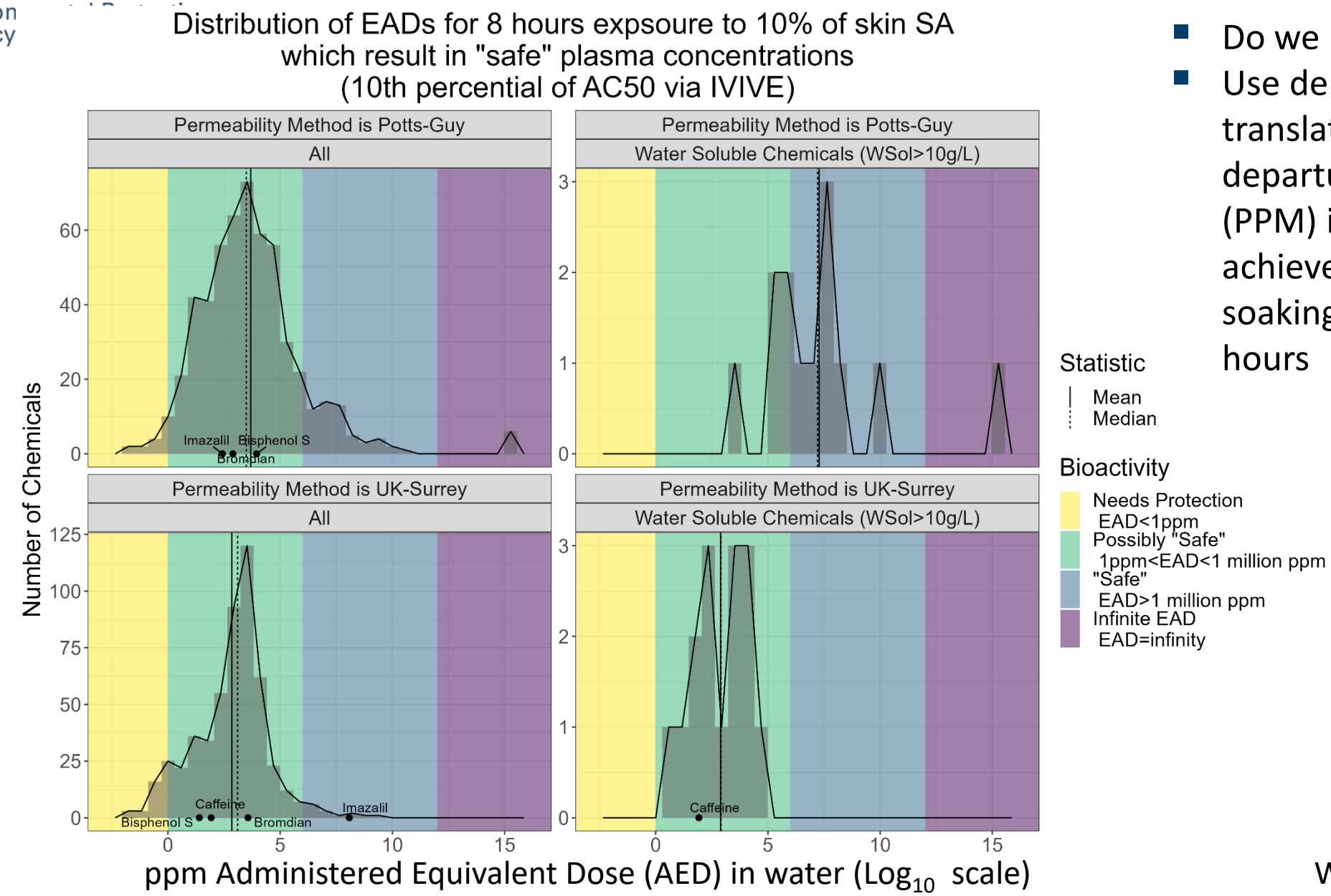
Key Exposure Routes

High Throughput Translation of *In Vitro* PODs

- The combination of *in vitro* TK measurements or structure-based predictions with a high throughput PBTK model allows *in vitro* points of departure to be translated into *in vivo* equivalent doses



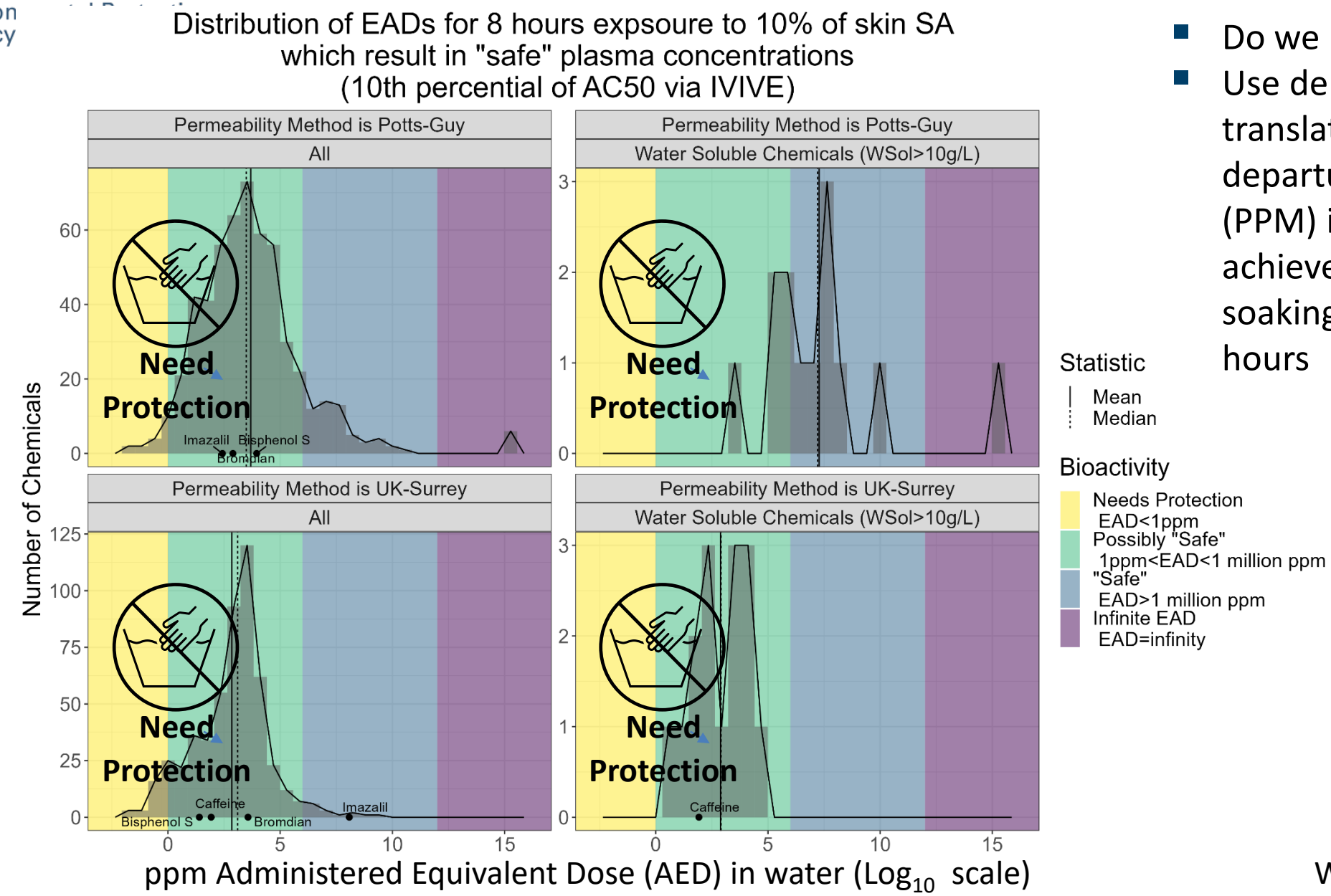
IVIVE with Dermal HTTK Model



- Do we need to wear gloves?
- Use dermal HTTK to translate *in vitro* points of departure to concentration (PPM) in solution needed to achieve POD in blood after soaking hands for eight hours

Work by Annabel Meade

IVIVE with Dermal HTTK Model



- Do we need to wear gloves?
- Use dermal HTTK to translate *in vitro* points of departure to concentration (PPM) in solution needed to achieve POD in blood after soaking hands for eight hours

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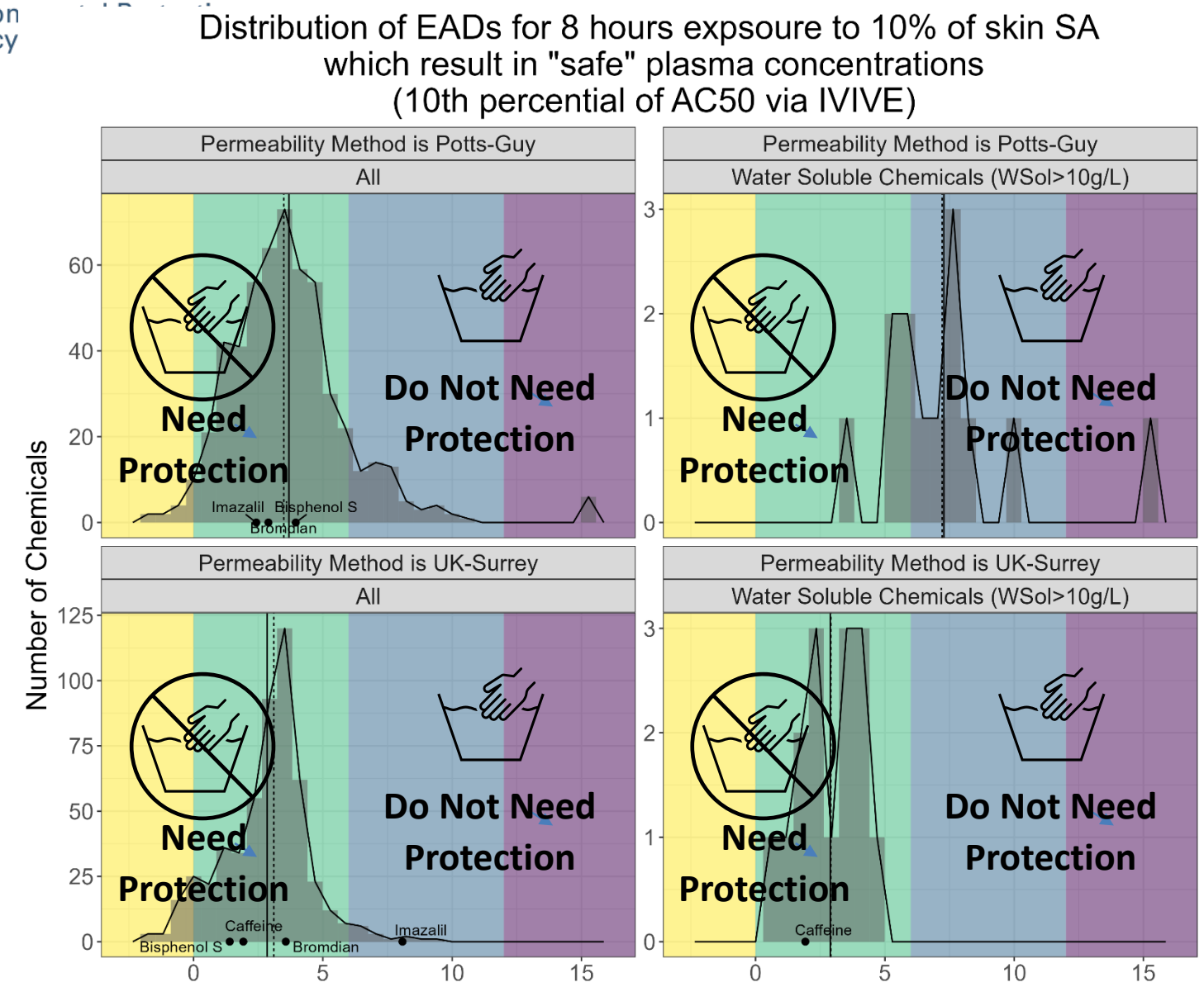

- Do we need to wear gloves?
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Statistic
- Mean
- Median

Bioactivity
- Needs Protection EAD<1ppm
- Possibly "Safe" 1ppm<EAD<1 million ppm
- "Safe" EAD>1 million ppm
- Infinite EAD EAD=infinity

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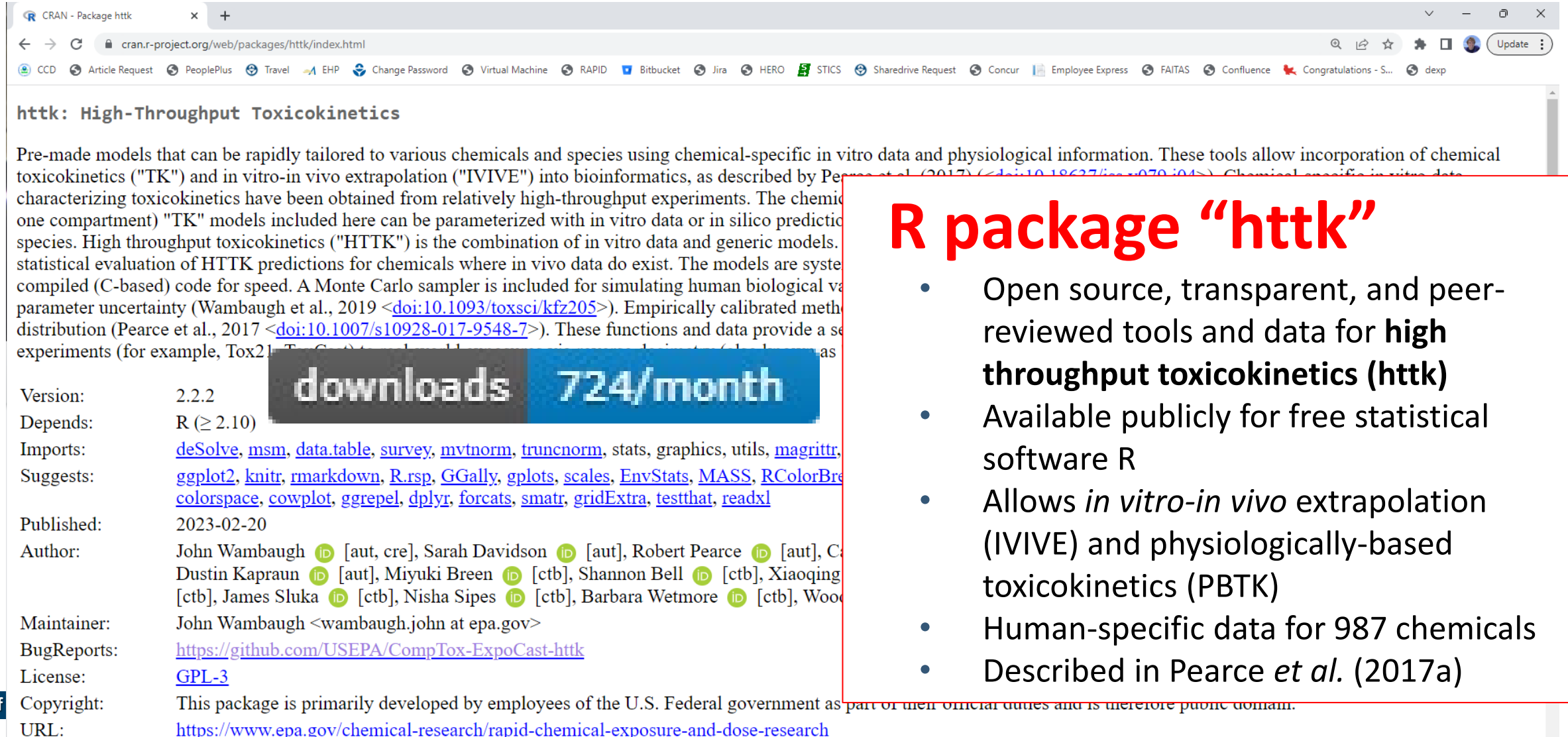
EPA
United States
Environmental Protection
Agency

Accessibility

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), Pradeep et al. (2020), Dawson et al. (2021)
Generic toxicokinetic models	One compartment, three compartment, physiologically-based oral, intravenous, and inhalation (PBTK)	Pearce et al. (2017a), Linakis et al. (2020), Kapraun et al. (2022)
Tissue partition coefficient predictors	Modified Schmitt (2008) method	Pearce et al. (2017b)
Variability Simulator	Based on NHANES biometrics	Ring et al. (2017), Breen et al. (2022
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)

Means of Obtaining HTTK

- SimCYP SimRFlow Tool (in use by EU-ToxRisk) (Khalidi et al., 2022)
- NICEATM Web-ICE (in use by NTP) (Bell et al., 2020)
- CompTox Chemicals Dashboard (in use by EPA) (Williams et al., 2017)
- TKPlate (in use by EFSA) (Dorne et al., 2018)
- R package “httk” (general informatics community, including EPA) (Pearce et al., 2017)

- All these tools make use of some or all data/models from R package “httk”

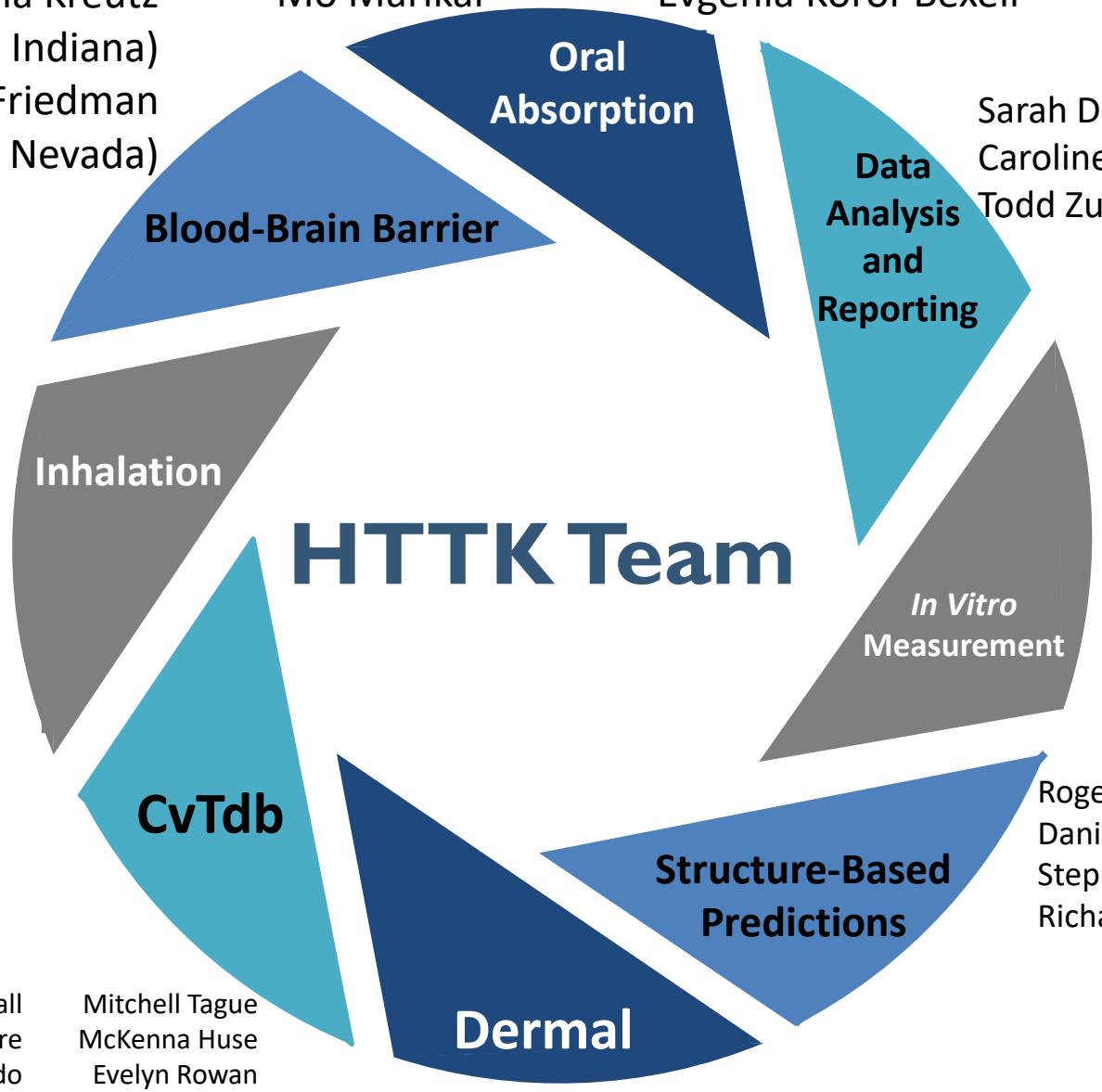
EPA United States Environmental Protection Agency

Summary

Send Questions to:
wambaugh.john@epa.gov

- IVIVE is critical to next generation risk assessment
  - NAMs are often high throughput, producing results for large numbers of chemicals
- HTTK is an approach the allows IVIVE for large numbers of chemicals
- HTTK relies on libraries of accessible, published, chemical-specific data
- Chemical-independent models exist to use these data to make predictions for various important scenarios including occupational and gestational exposures
  - Confidence in these models can be assessed through evaluation with legacy *in vivo* data
- "httk" is an open-source R package developed by EPA for performing HTTK – provides the backbone for many different IVIVE tools

HTTK Team



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References

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