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Toxicokinetic New Approach Methodologies (NAMs)

Generic TK models: Parameterization and Evaluation

John Wambaugh

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.

Office of Research and Development
Center for Computational Toxicology and Exposure

October 29, 2021

EPA United States Environmental Protection Agency

Overview

- Generic vs. bespoke PBTK models
- Models available within R package “httk”
- Model parameterization
  - Physiologic parameters
  - Chemical-specific parameters
- Model evaluation
  - The Concentration vs. Time Database (CvTdb)

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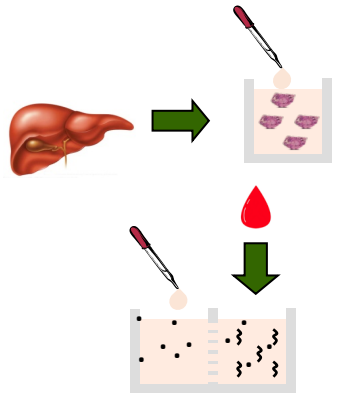
HTTK: A NAM for Exposure

- Toxicokinetics is the predictive description of the absorption, distribution, metabolism, and elimination (ADME) of a chemical compound
- We collect *in vitro*, high throughput toxicokinetic (HTTK) data to provide toxicokinetics for larger numbers of chemicals (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)

High Throughput Toxicokinetics (HTTK)

In vitro toxicokinetic data

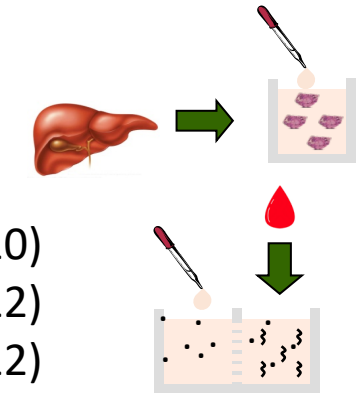


Intrinsic
Hepatic
Clearance

Plasma
Protein
Binding

High Throughput Toxicokinetics (HTTK)

In vitro toxicokinetic data



Intrinsic Hepatic Clearance

Plasma Protein Binding

Rotroff et al. (2010)
Wetmore et al. (2012)
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High Throughput Toxicokinetics (HTTK)

In vitro toxicokinetic data + generic toxicokinetic model

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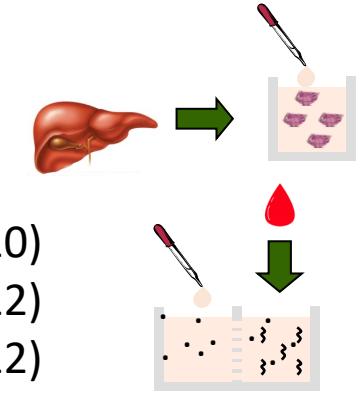

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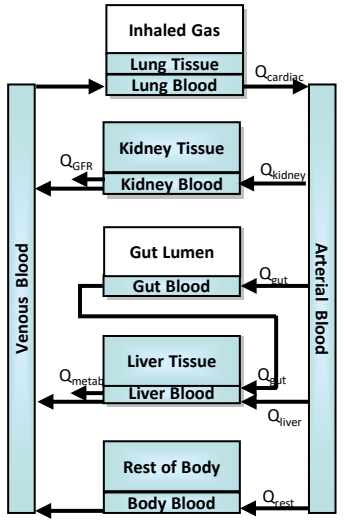

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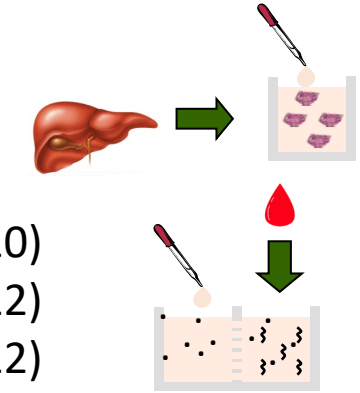

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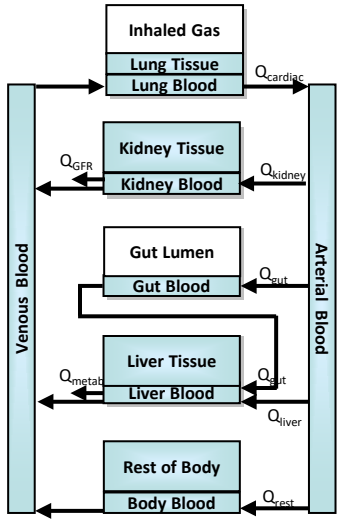

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High Throughput Toxicokinetics (HTTK)

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

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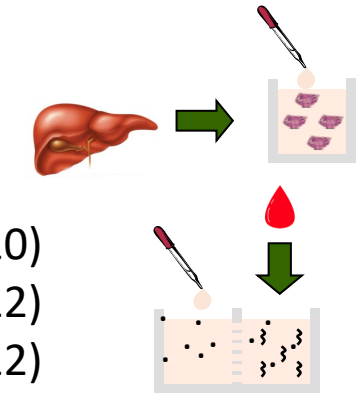

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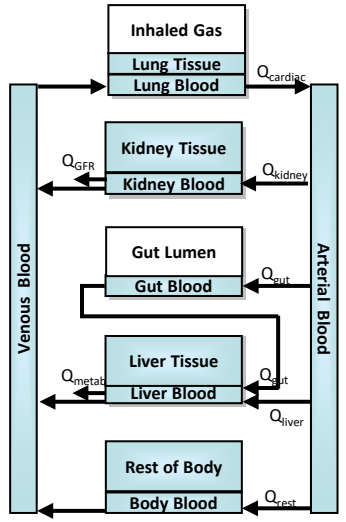

Wambaugh et al. (2015)
Pearce et al. (2017)
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Linakis et al. (2020)

= *httk*

Breen et al. (2021)

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Generic vs. bespoke PBTK models

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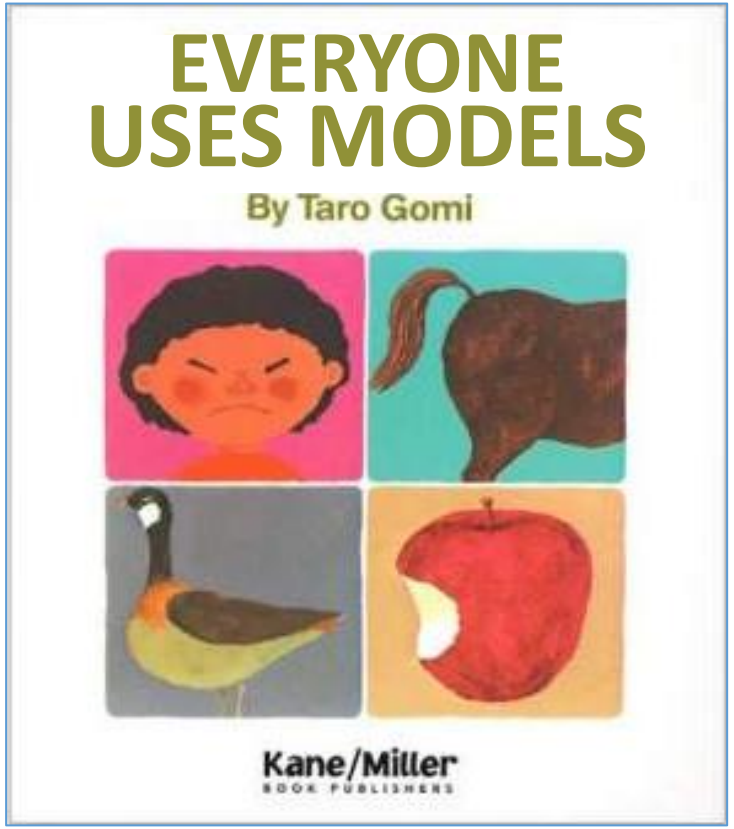
Everyone Uses Models

- Toxicology has long relied upon model animal species
- People rely on mental models every day
  - For example, with repetitive activities like driving home from work
- Mathematical models offer some significant advantages:
  - Reproducible
  - Can (and should) be transparent
- …with some disadvantages:
  - Sometimes reality is complex
  - Sometimes the model doesn't always work well
  - How do we know we can extrapolate?
- …that can be turned into advantages:
  - If we have evaluated confidence/uncertainty and know the "domain of applicability" we can make better use of mathematical models



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Fit for Purpose Models

- A “fit for purpose” model is an abstraction of a complicated problem that allows us to reach a decision.

  “Now it would be very remarkable if any system existing in the real world could be *exactly* represented by any simple model. However, cunningly chosen parsimonious models often do provide remarkably useful approximations… **The only question of interest is ‘Is the model illuminating and useful?’**”
  George Box

- A fit for purpose model is defined as much by what is omitted as what is included in the model.

- We must accept that there will always be areas in need of better data and models – our knowledge will always be incomplete, and thus we wish to extrapolate.

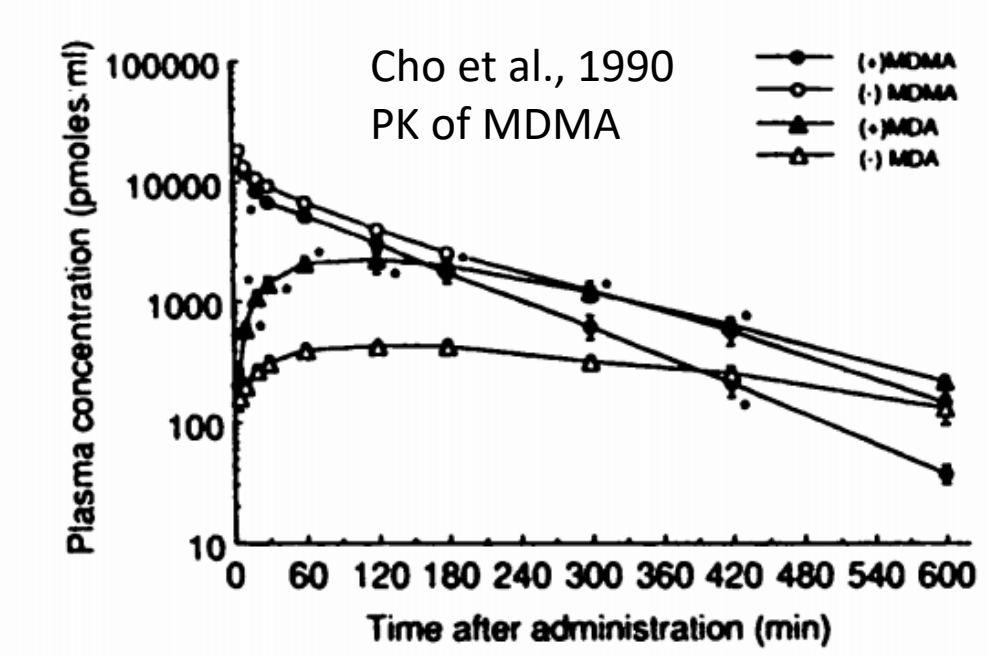
  - How do I drive to a place I’ve never been before?

Complexity should match the data…

"Since all models are wrong the scientist cannot obtain a 'correct' one by excessive elaboration. On the contrary, following William of Occam, they should seek an economical description of natural phenomena."

George Box

We choose to make the complexity of the model and the number of physiological processes appropriate given the data and the decision context



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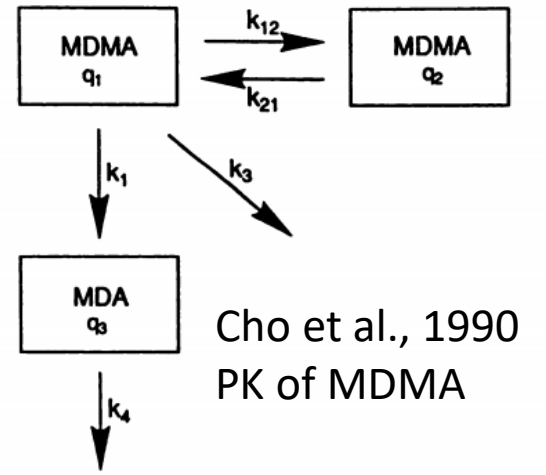
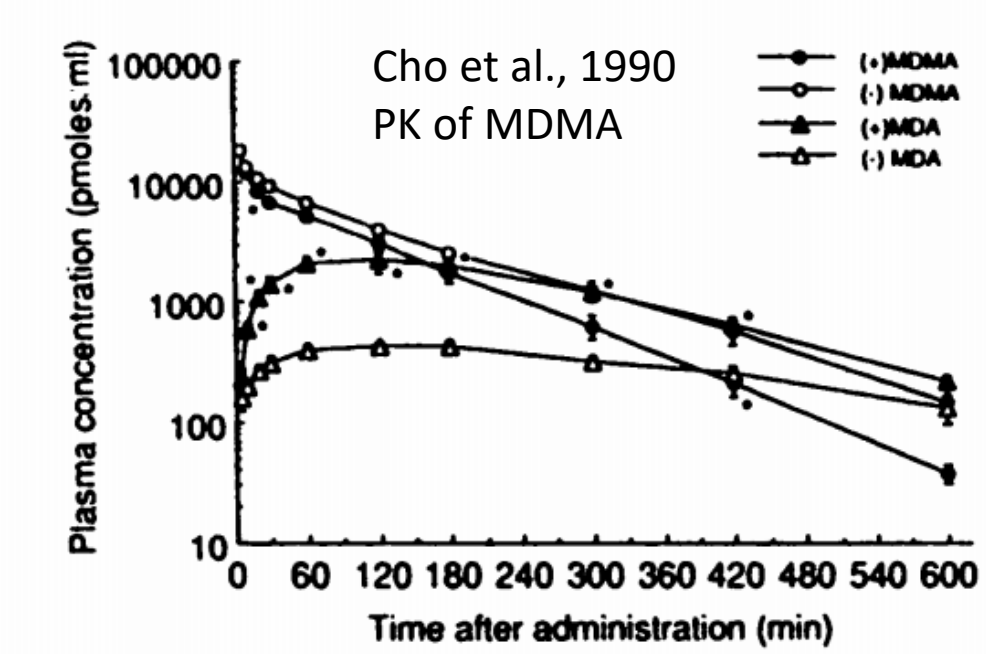


Cho et al., 1990
PK of MDMA



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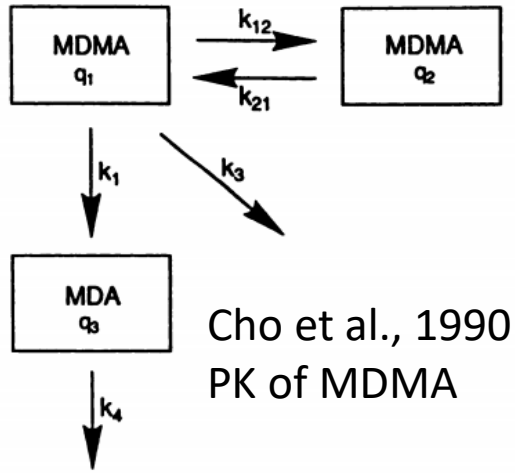

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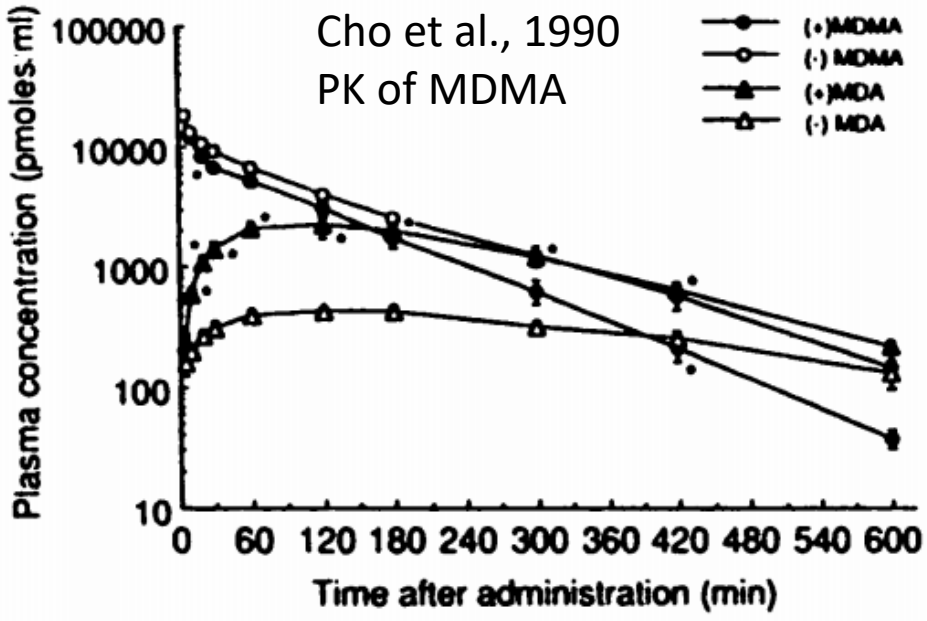


Jones et al., 2012
PK of Statins

In this case they had transporter-specific data

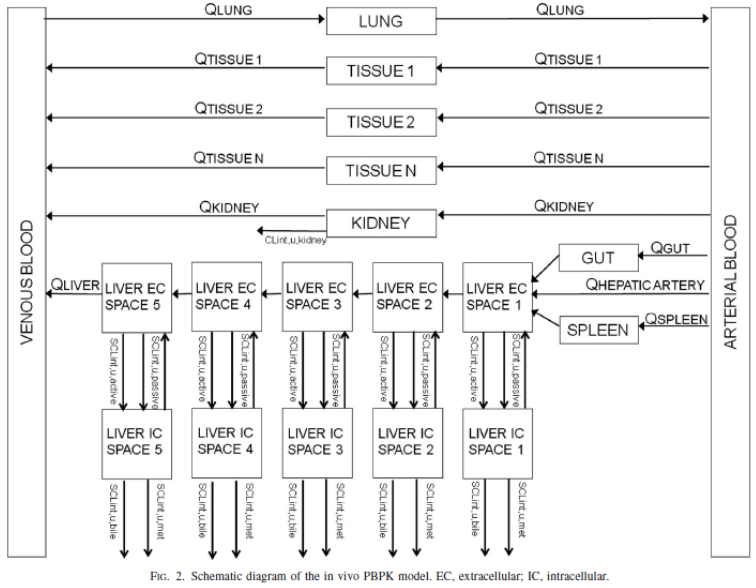


FIG. 2. Schematic diagram of the in vivo PBPK model. EC, extracellular; IC, intracellular.

Lex Parsimoniae
"Law of Parsimony"

"Among competing hypotheses, the one with the fewest assumptions should be selected."
William of Occam

"While Occam's razor is a useful tool in the physical sciences, it can be a very dangerous implement in biology. It is thus very rash to use simplicity and elegance as a guide in biological research. "
Francis Crick

"With four parameters I can fit an elephant, and with five I can make him wiggle his trunk."
John von Neumann

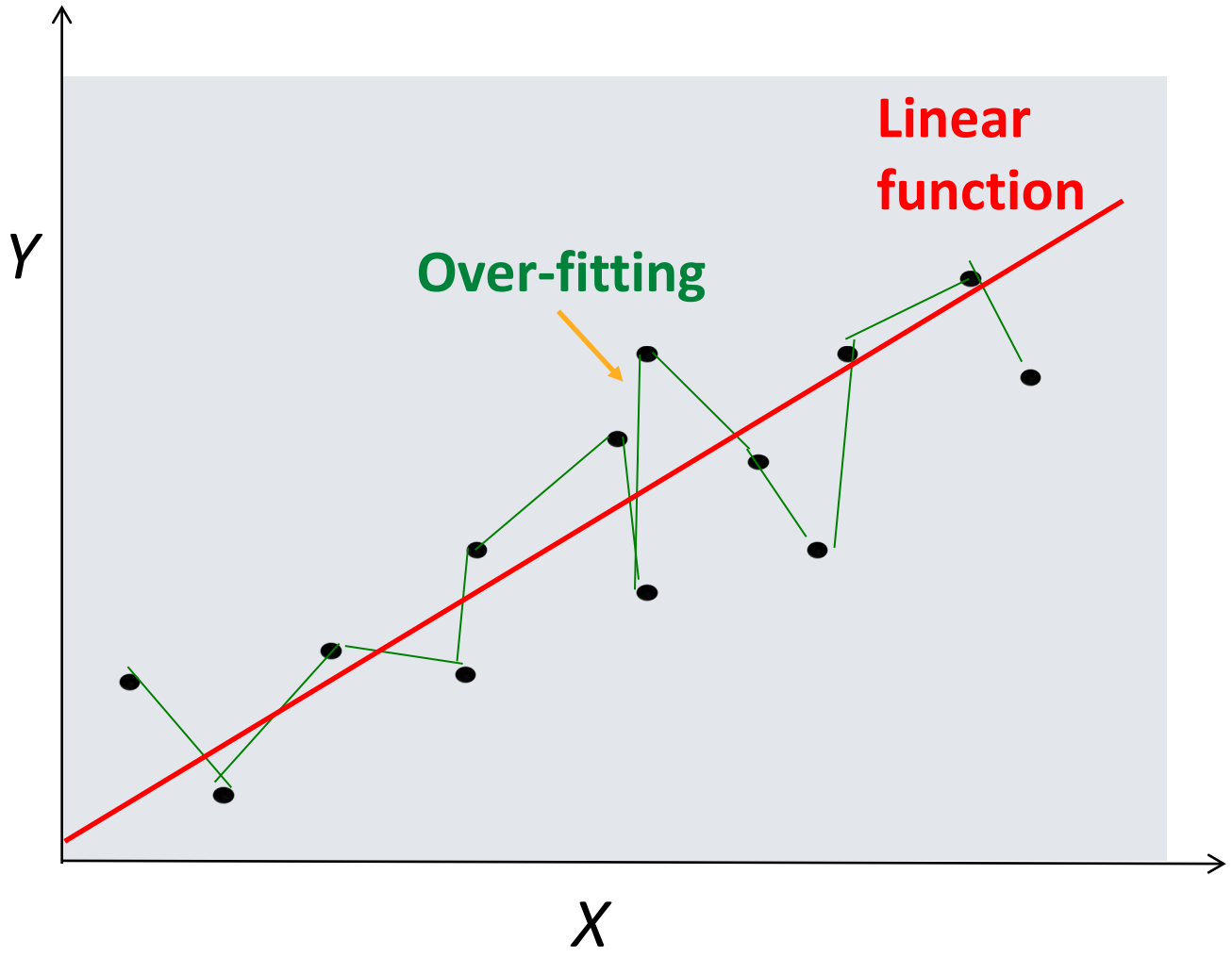
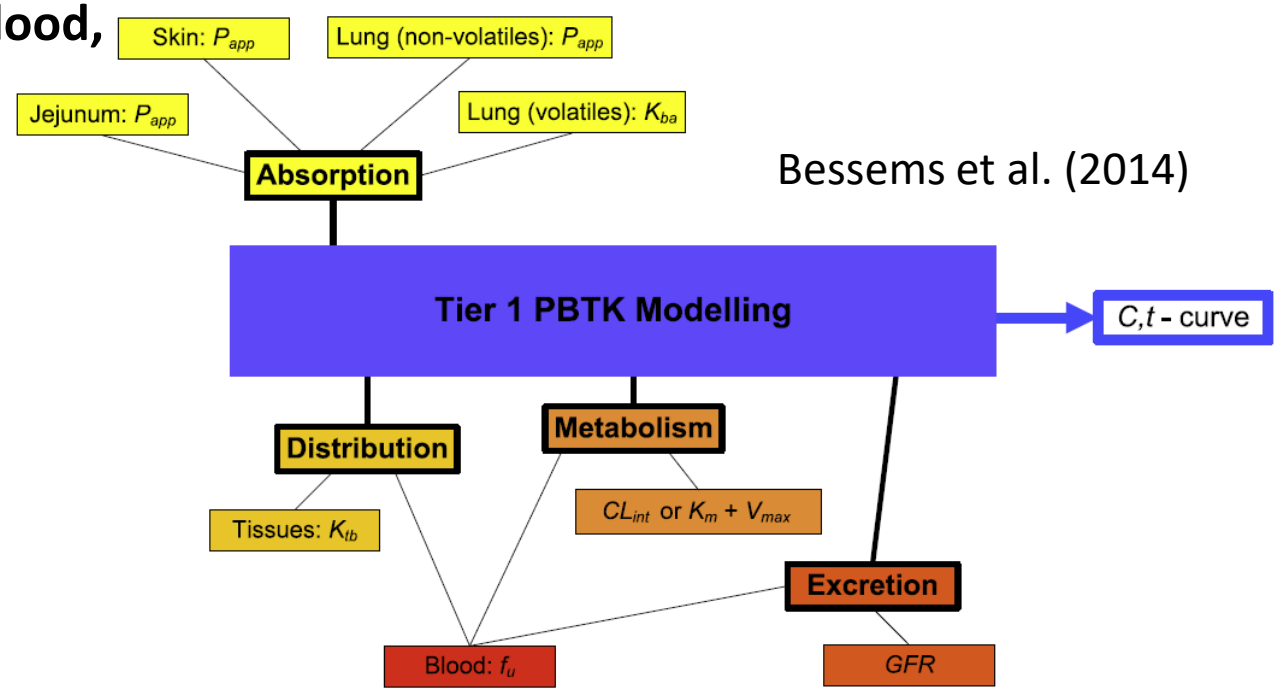


Figure from Anran Wang

Fit for Purpose Toxicokinetics

- Chiu et al. (2007) "…[P]arsimony in selecting [toxicokinetic] model structures is an important and guiding principle in developing models for use in risk assessments."
  - **Complexity is constrained by the limited data** available to calibrate and test TK models and the need to justify both the model assumptions and predictions

- Bessems et al. (2014): **We need "a first, relatively quick ('Tier 1'), estimate" of concentration vs. time in blood, plasma, or cell**
  - At the time they suggested that we might neglect active metabolism. Thanks to *in vitro* measurements we can now do better
  - We still neglect transport and other protein-specific phenomena



Bessems et al. (2014)

Bespoke vs. Generic

Bespoke, Tailored, Custom…
Requires specific measurements

Generic, Off-the-Shelf/Rack, One-Size-Fits-Most
Approximately fits certain categories

Why Use Generic Models?

- Each of the models provided by the R package "httk" is a generic model
  - Each model is designed to used standardized chemical-specific *in vitro* measurements (fraction unbound in plasma, intrinsic hepatic clearance)

high(er) throughput toxicokinetics =

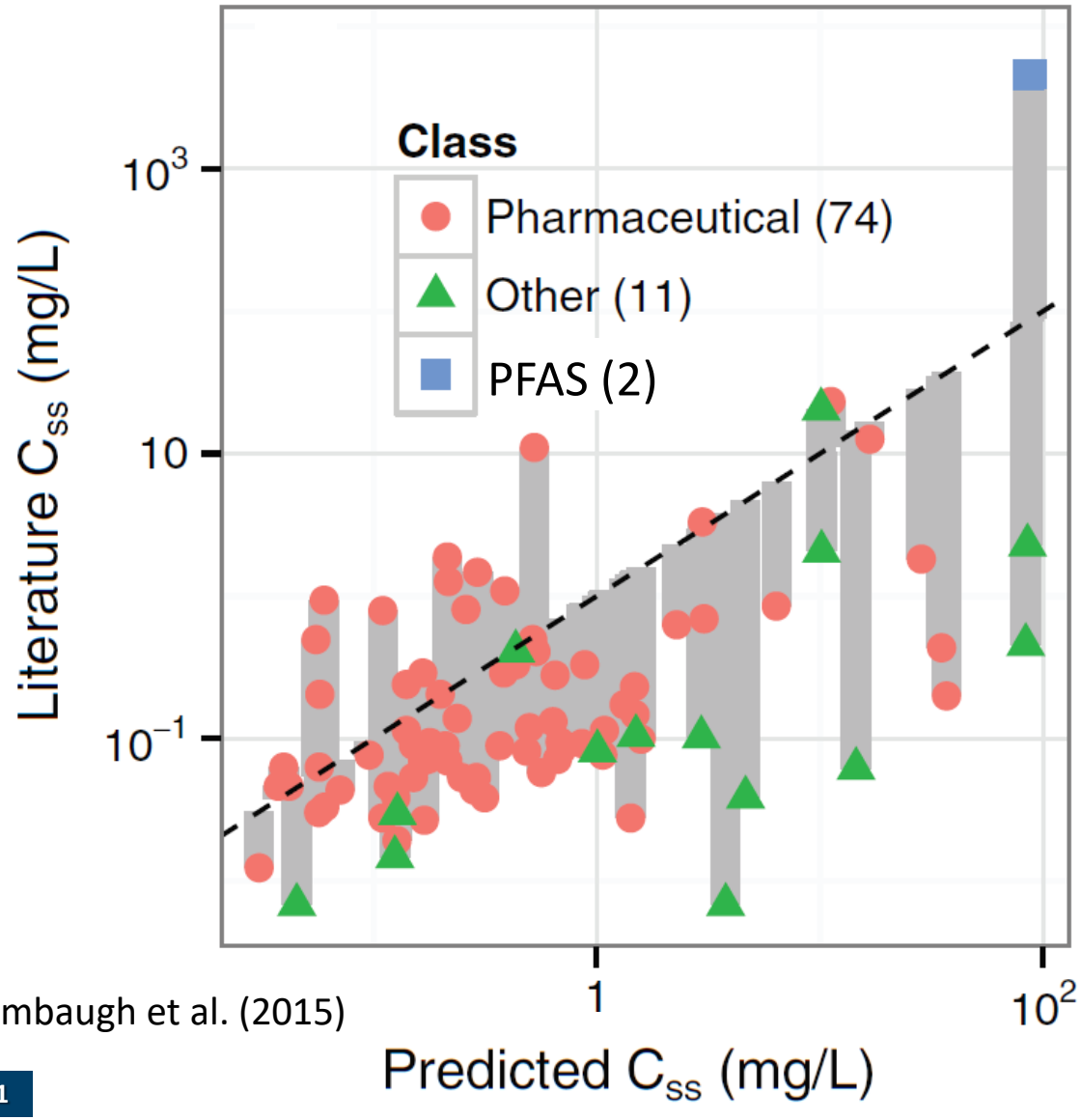
***In vitro* toxicokinetic data + generic toxicokinetic model**

- 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

- The generic model is a hypothesis
  - If we have evaluation data then we can check if we need to elaborate the model (for example, create a bespoke model)

- We can estimate the accuracy of a generic model for a new chemical using performance across multiple chemicals where data happen to exist

Generic Models as a Hypothesis

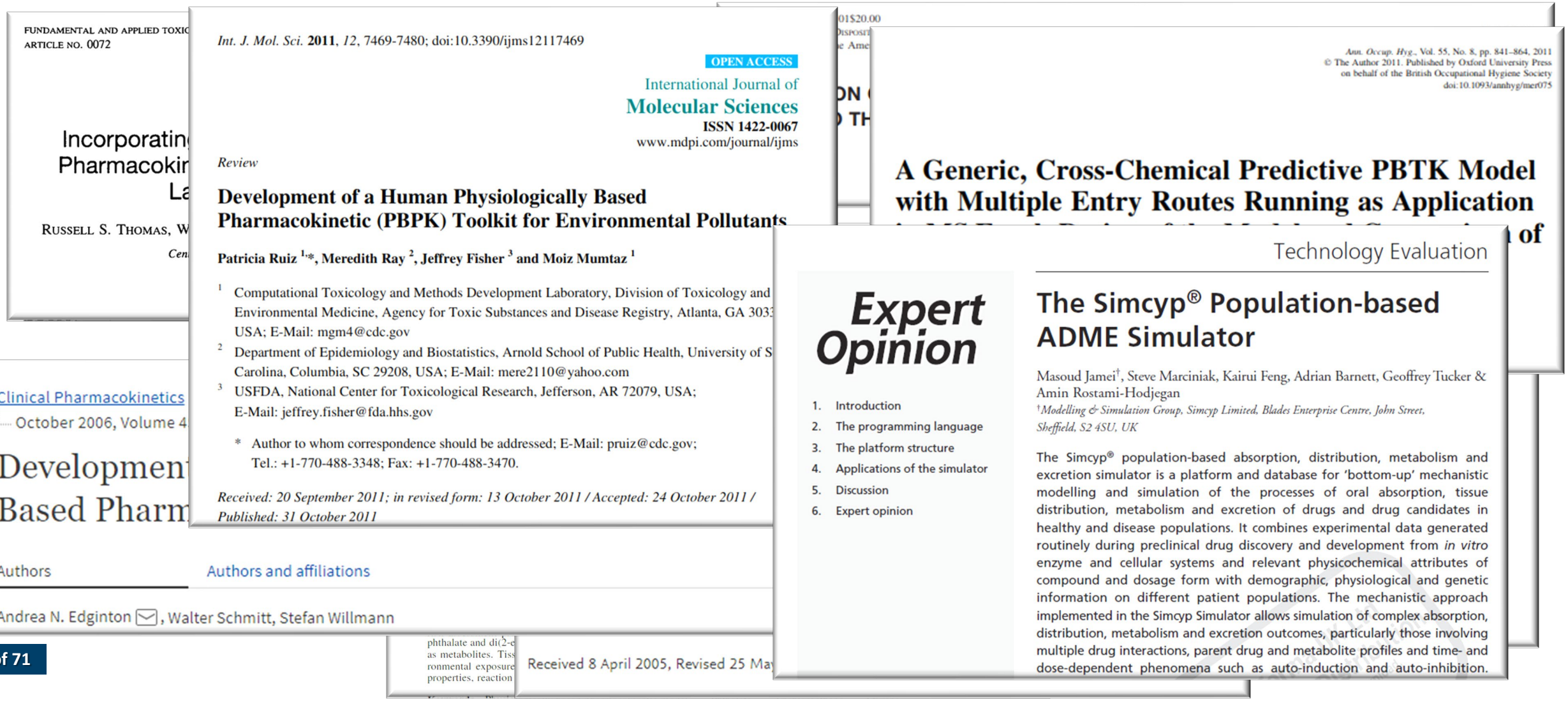


Wambaugh et al. (2015)

- For pharmaceuticals, *in vitro* data plus a model including hepatic metabolism and passive glomerular filtration (kidney) are often enough to make predictions within a factor of 3 of in vivo data (Wang, 2010)
- For other chemicals there may be complications, for example there is thought to be (Andersen et al. 2006) active transport of some per- and poly-fluorinated alkyl substances (PFAS) in the kidney
- We could add a renal resorption process to HTTK (that is, add a new generic model) only if there was some way to parameterize the process for most chemicals – otherwise we are back to tailoring the model to a chemical

Generic PBTK Models

The idea of generic PBTK has been out there for a while…



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Int. J. Mol. Sci. **2011**, *12*, 7469-7480; doi:10.3390/ijms12117469

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Review

Development of a Human Physiologically Based Pharmacokinetic (PBPK) Toolkit for Environmental Pollutants

Patricia Ruiz [1,*], Meredith Ray [2], Jeffrey Fisher [3] and Moiz Mumtaz [1]

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[2] Department of Epidemiology and Biostatistics, Arnold School of Public Health, University of S… Carolina, Columbia, SC 29208, USA; E-Mail: mere2110@yahoo.com
[3] USFDA, National Center for Toxicological Research, Jefferson, AR 72079, USA; E-Mail: jeffrey.fisher@fda.hhs.gov

* Author to whom correspondence should be addressed; E-Mail: pruiz@cdc.gov; Tel.: +1-770-488-3348; Fax: +1-770-488-3470.

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doi:10.1093/annhyg/mer075

A Generic, Cross-Chemical Predictive PBTK Model with Multiple Entry Routes Running as Application

Clinical Pharmacokinetics
October 2006, Volume 4

Developmen
Based Pharm

Authors | Authors and affiliations

Andrea N. Edginton, Walter Schmitt, Stefan Willmann

phthalate and di(2-e…
as metabolites. Tiss…
ronmental exposure…
properties, reaction…

Received 8 April 2005, Revised 25 May…

Technology Evaluation

Expert Opinion

1. Introduction
2. The programming language
3. The platform structure
4. Applications of the simulator
5. Discussion
6. Expert opinion

The Simcyp® Population-based ADME Simulator

Masoud Jamei†, Steve Marciniak, Kairui Feng, Adrian Barnett, Geoffrey Tucker & Amin Rostami-Hodjegan

†*Modelling & Simulation Group, Simcyp Limited, Blades Enterprise Centre, John Street, Sheffield, S2 4SU, UK*

The Simcyp® population-based absorption, distribution, metabolism and excretion simulator is a platform and database for 'bottom-up' mechanistic modelling and simulation of the processes of oral absorption, tissue distribution, metabolism and excretion of drugs and drug candidates in healthy and disease populations. It combines experimental data generated routinely during preclinical drug discovery and development from *in vitro* enzyme and cellular systems and relevant physicochemical attributes of compound and dosage form with demographic, physiological and genetic information on different patient populations. The mechanistic approach implemented in the Simcyp Simulator allows simulation of complex absorption, distribution, metabolism and excretion outcomes, particularly those involving multiple drug interactions, parent drug and metabolite profiles and time- and dose-dependent phenomena such as auto-induction and auto-inhibition.

Why Build Another Generic PBTK Tool?

from Breen et al. (2021)

	SimCYP	ADMET Predictor / GastroPlus	PK-Sim	IndusChem Fate	pbktool	G-PBTK	httk
References	Jamei (2009)	Parrott (2009)	Eissing (2011)	Jongeneelen (2011)	Punt (2020)	Armitage (2021)	Pearce (2017)
Availability	License, but inexpensive for research	License, but inexpensive for research	Free	Free	Free	Free	Free
Open Source	No	No	GitHub	No	GitHub	Planned Release	CRAN and GitHub
Default PBTK Structure	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Population Variability	Yes	Yes	Yes	No	No	No	Yes
Data Needs	High/Low	High/Low	High	High	Low	Low	Low
Typical Use Case	Drug Discovery	Drug Discovery	Drug Discovery	Environmental Assessment	Food and Drug Safety Evaluation	Environmental Assessment	Screening
Batch Mode	Yes	Yes	Yes	No	No	No	Yes
Graphical User Interface	Yes	Yes	Yes	Excel	No	Excel	No
Built-in Chemical-Specific Library	Many Clinical Drugs	No	Many pharmaceutical-specific models available	15 Environmental Compounds	No	No	Pharmaceuticals and ToxCast: 998 human, 226 rat
Oral Bioavailability Modeling	Yes	Yes	No	No	No	No	No (Will be available in the future version)
In Vitro Distribution	SIVA VIVD	No	No	No	No	No	Armitage Model
Exposure Route	Oral, IV	Oral, IV	Oral, IV	Oral, Gas Inhalation, Dermal	Oral	Oral, IV, Inhalation	Oral, IV, Gas Inhalation (Dermal, Aerosol, and Fetal forthcoming)
Ionizable Compounds	Yes	Yes	Yes	No	No	Yes	Yes
Export Function	No	No	Matlab and R	No	No	No	SBML and Jarnac
R Integration	No	No	Yes (2017)	No	Yes	Yes	Yes
Reverse Dosimetry	Yes	Yes	Yes	No	No	No	Yes

*Both **PLETHEM** (Pendse et al., 2020) and **Web-ICE** (Bell et al., 2020) provide GUI's to HTTK and other models

Pre-computed HTTK results are also available at https://comptox.epa.gov/dashboard

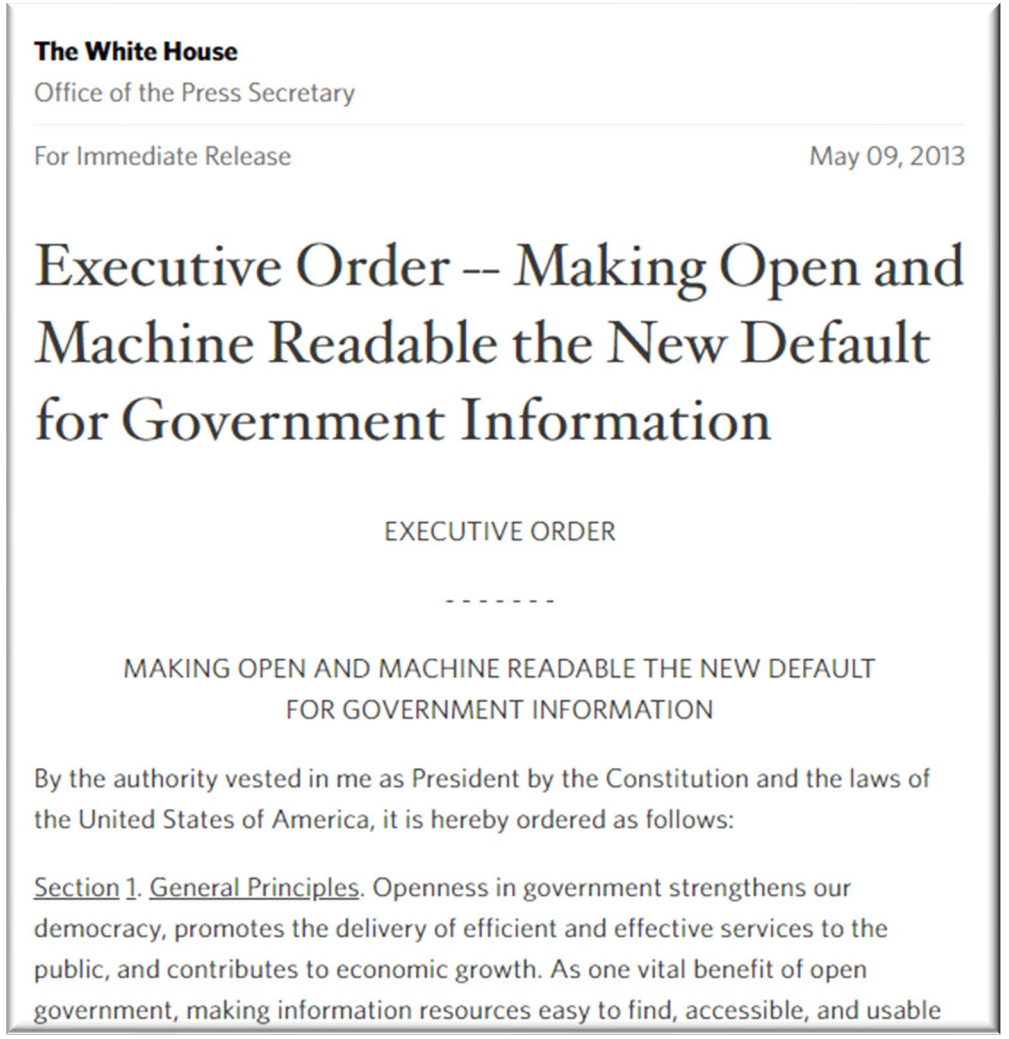
Regulatory Acceptance

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]"



The White House
Office of the Press Secretary

For Immediate Release May 09, 2013

Executive Order -- Making Open and Machine Readable the New Default for Government Information

EXECUTIVE ORDER

- - - - - - -

MAKING OPEN AND MACHINE READABLE THE NEW DEFAULT FOR GOVERNMENT INFORMATION

By the authority vested in me as President by the Constitution and the laws of the United States of America, it is hereby ordered as follows:

Section 1. General Principles. Openness in government strengthens our democracy, promotes the delivery of efficient and effective services to the public, and contributes to economic growth. As one vital benefit of open government, making information resources easy to find, accessible, and usable

"…the default state of new and modernized Government information resources shall be open and machine readable."

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*Both **PLETHEM** (Pendse et al., 2020) and **Web-ICE** (Bell et al., 2020) provide GUI's to HTTK and other models

Pre-computed HTTK results are also available at https://comptox.epa.gov/dashboard

Exquisite Systems

"Although NASA has always partnered with industry, the nature of that relationship is changing. **Historically, NASA would design an exquisite system or spacecraft,** select a commercial contractor to build it, oversee its construction in detail while sometimes changing its requirements, then own and operate the result. The government was the sole buyer/owner."

After retirement of the Space Shuttle, NASA began working with multiple contractors who may provide their services to multiple customers. Once "…certified, the manufacturers would deliver cargo for NASA—and any other customer the company could engage in the growing LEO commercial marketplace. **Rather than building, owning, and operating a luxury sedan, NASA now essentially hails a taxi**."

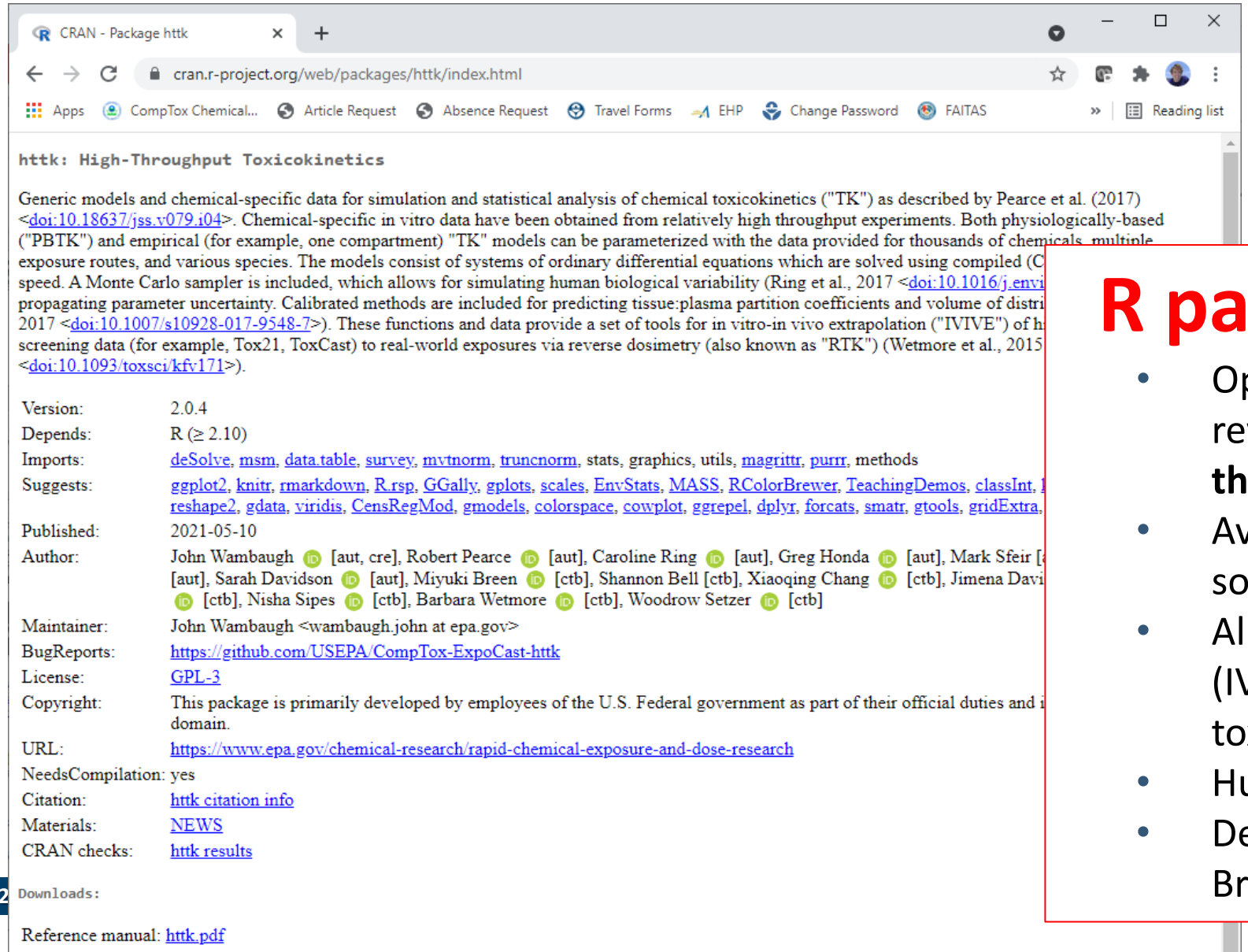
From:
https://www.nasa.gov/directorates/heo/scan/services/nasas_commercial_communications_services

EPA United States Environmental Protection Agency

Models available within R package "httk"

Open Source Tools and Data for HTTK

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

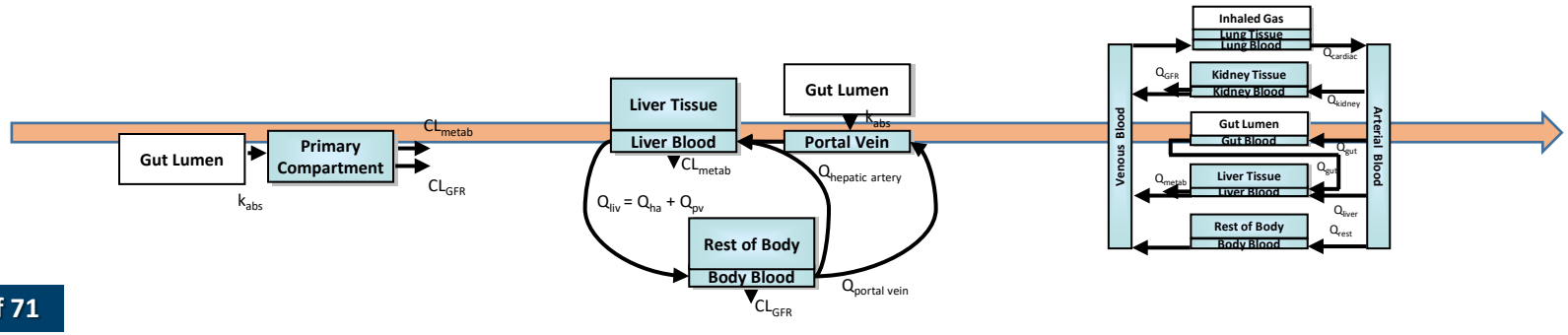


R package "httk"

- 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 998 chemicals
- Described in Pearce et al. (2017a) and Breen et al. (2020)

HTTK Models Range in Complexity

Model	Hepatic clearance	Partition coefficients	Fraction unbound	Hematocrit	Molecular weight	Ratio of blood to plasma	Elimination rate[1]	Volume of distribution[2]	Dynamic prediction	Steady state prediction
pbtk	Yes	Yes	Yes	Yes	Yes	Yes	**No**	**No**	Yes	Yes
Gas_pbtk	Yes	Yes	Yes	Yes	Yes	Yes	**No**	**No**	Yes	Coming Soon
1compartment	**No**	**No**	**No**	**No**	Yes	**No**	Yes	Yes	Yes	Yes
3compartment	Yes	Yes	Yes	Yes	Yes	Yes	**No**	**No**	Yes	Yes
3compartmentss	Yes	**No**	Yes	**No**	Yes	**No**	**No**	**No**	**No**	Yes



[1]Partition coefficients are needed in calculating V_{dist}
[2]Clearances and *fup* are needed in calculating k_{elim}

Adapted from Pearce et al. (2017a)

EPA United States Environmental Protection Agency

HTTK Models Range in Complexity

Model	Hepatic clearance	Partition coefficients	Fraction unbound	Hematocrit	Molecular weight	Ratio of blood to plasma	Elimination rate[1]	Volume of distribution[2]	Dynamic prediction	Steady state prediction
pbtk	Yes	Yes	Yes	Yes	Yes	Yes	**No**	**No**	Yes	Yes
Gas_pbtk	Yes	Yes	Yes	Yes	Yes	Yes	**No**	**No**	Yes	Coming Soon
1compartment	**No**	**No**	**No**	**No**	Yes	**No**	Yes	Yes	Yes	Yes
3compartment	Yes	Yes	Yes	Yes	Yes	Yes	**No**	**No**	Yes	Yes
3compartmentss	Yes	**No**	Yes	**No**	Yes	**No**	**No**	**No**	**No**	Yes

- The simplest models often allow predictions with a single equation
- More complex models often require numerical solvers to determine the solution to a system of differential equations as a function of exposure (dose) and time

Adapted from Pearce et al. (2017a)

EPA United States Environmental Protection Agency

HTTK Models Range in Complexity

Model	Hepatic clearance	Partition coefficients	Fraction unbound	Hematocrit	Molecular weight	Ratio of blood to plasma	Elimination rate[1]	Volume of distribution[2]	Dynamic prediction	Steady state prediction
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Gas_pbtk	Yes	Yes	Yes	Yes	Yes	Yes	**No**	**No**	Yes	Coming Soon
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3compartment	Yes	Yes	Yes	Yes	Yes	Yes	**No**	**No**	Yes	Yes
3compartmentss	Yes	**No**	Yes	**No**	Yes	**No**	**No**	**No**	**No**	Yes

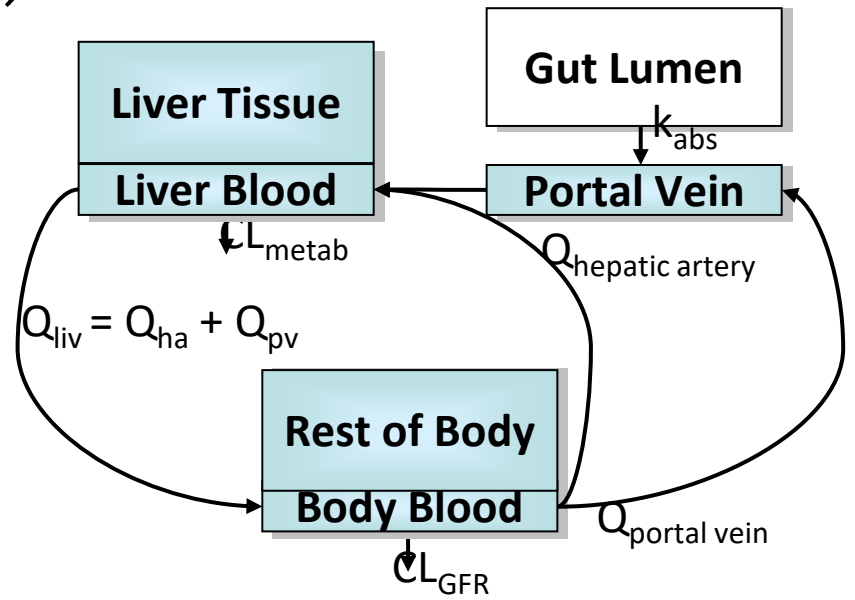
- At steady-state all compartments are at equilibrium and the concentrations can be predicted with a single equation, **but**:
  - The exposure (dose) must be constant
  - Enough time must pass to reach equilibrium

Adapted from Pearce et al. (2017a)

Simple Model for Steady-State Plasma Concentration (C_{ss})

- This equation is the steady-state solution for a three-compartment model (3compartmentss):

$$C_{ss} = \frac{oral\ dose\ rate\ * F_{hepfirstpass}}{(GFR * f_{up}) + \left(Q_l * f_{up} * \frac{Cl_{hepatic}}{Q_l + f_{up} * Cl_{hepatic}}\right)}$$



Wilkinson and Shand (1975)

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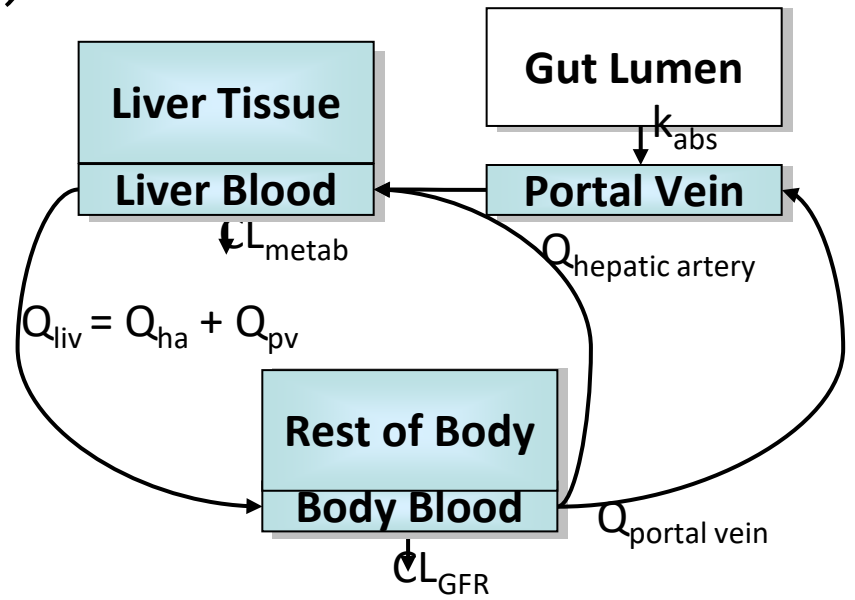
Estimated fraction not metabolized in first pass through liver before systemic circulation



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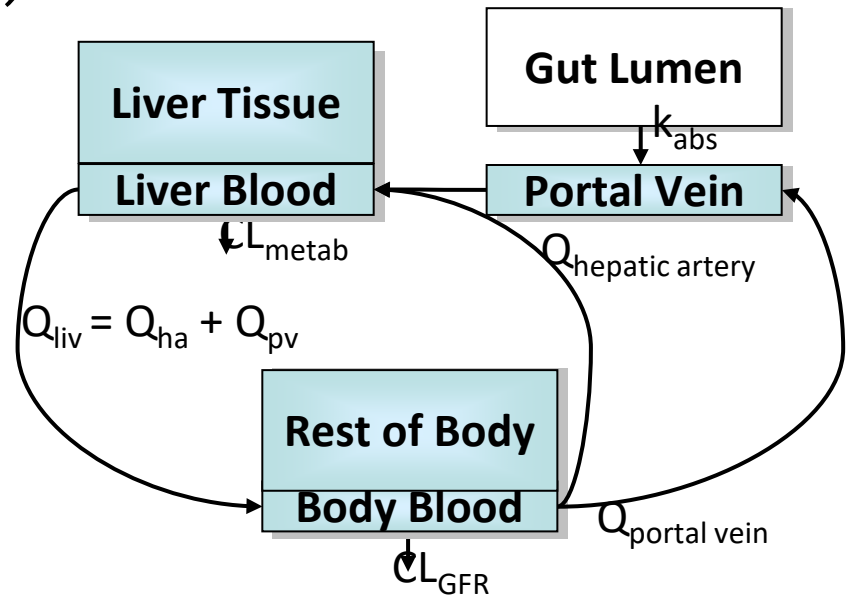
Passive Renal Clearance
(GFR: Glomerular filtration rate
f_{up}: fraction unbound in plasma)



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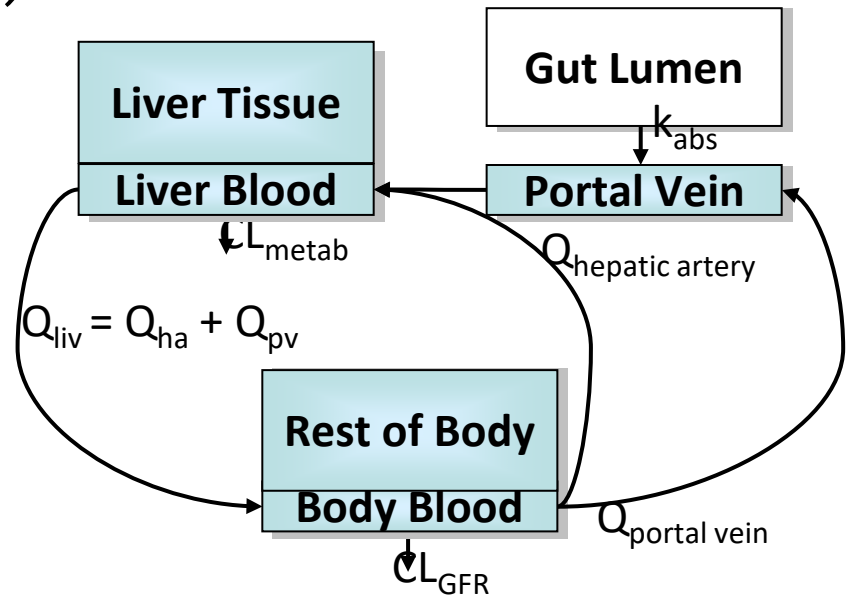
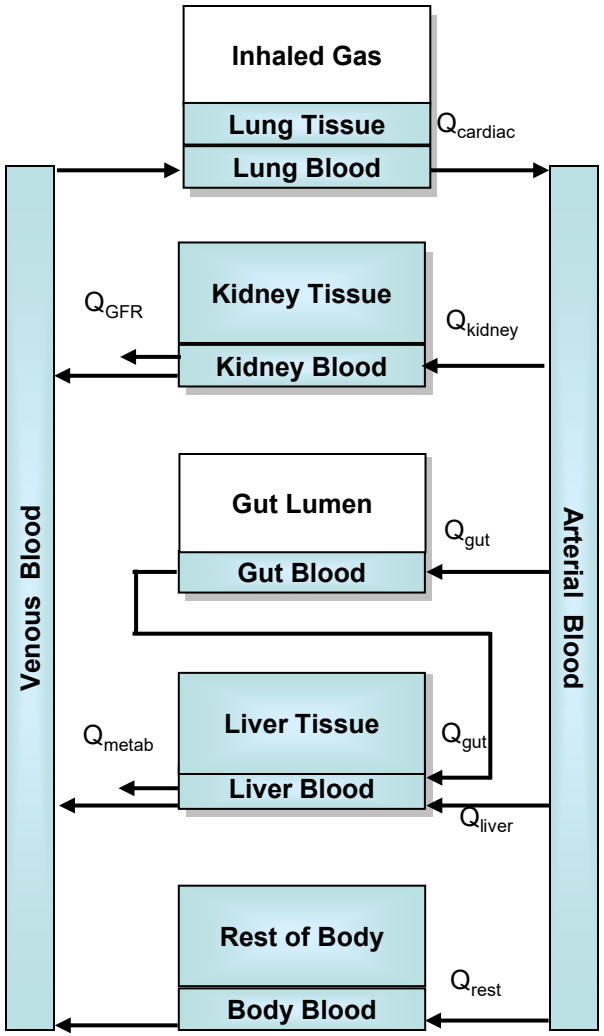
Hepatic Metabolism
($Cl_{hepatic}$: Scaled hepatic clearance
Q_l: Blood flow to liver)



Wilkinson and Shand (1975)

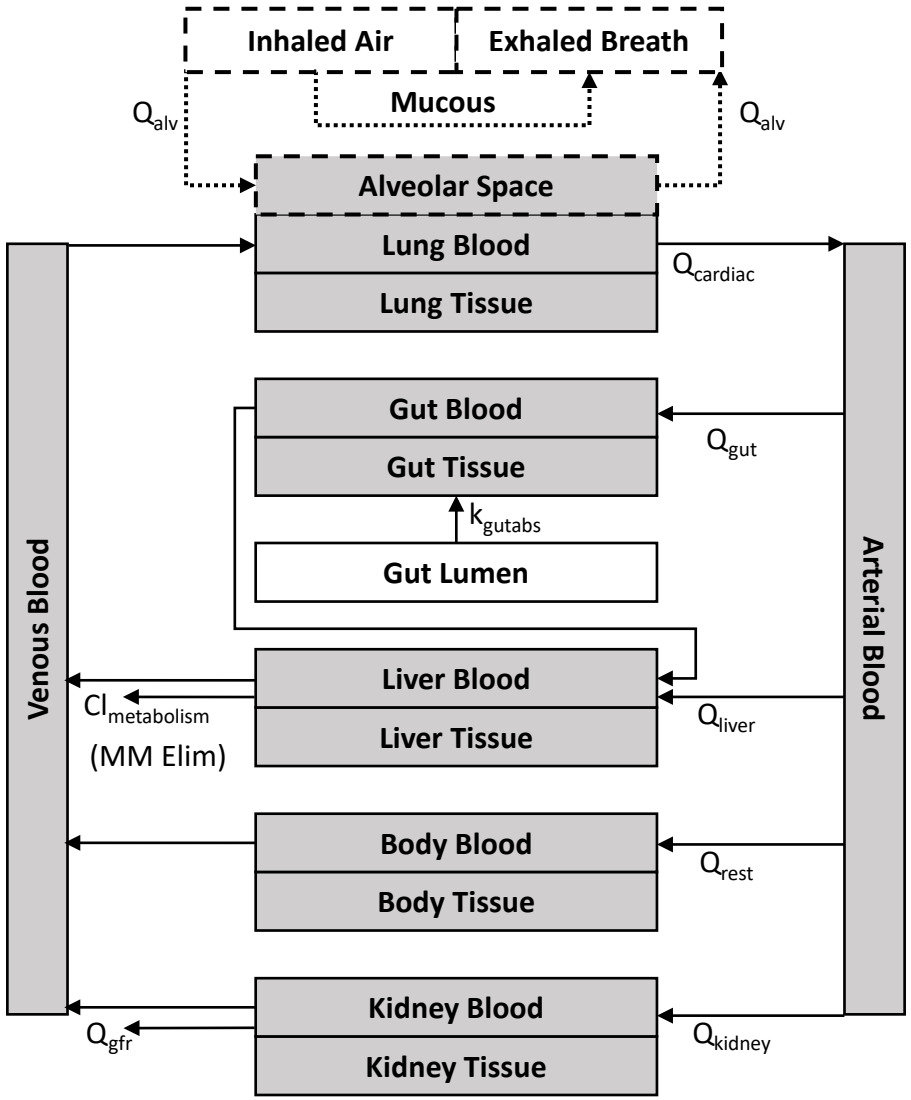
The "httk" General Physiologically-based Toxicokinetic (PBTK) Model



Pearce et al. (2017a)

- Tissues are modeled by compartments:
  - Some tissues (for example, arterial blood) are simple compartments
  - Others (for example, kidney) are compound compartments consisting of separate blood and tissue sections with constant partitioning (that is, tissue specific tissue:plasma partition coefficients)
  - Remaining tissues (for example, fat, brain, bones) are lumped into the "Rest of Body" compartment
- Clearance from the body depends on two processes:
  - Metabolism in the liver (estimated from *in vitro* clearance and binding)
  - Excretion by glomerular filtration in the kidney (estimated from *in vitro* binding)
- Model parameters are either:
  - **Physiological:** determined by species and potentially varied via Monte Carlo (including HTTK-pop, Ring et al. 2017)
  - **Chemical-specific**: physico-chemical properties (Mansouri et al., 2018) and equilibrium partition coefficients plus plasma binding and metabolism rates that are determined from *in vitro* measurements or potentially predicted from structure

Generic Gas Inhalation Model



Linakis et al. (2020)

- Inhalation is an important route of exposure, particularly for occupational settings
- The structure of the inhalation model was developed from two previously published physiologically-based models from Jongeneelen *et al.* (2011) and Clewell *et al*. (2001)
- The model can be parameterized with chemical-specific *in vitro* data from the HTTK package for 917 chemicals in human and 181 chemicals in rat
- Model was made publicly available with the release of httk v2.0.0 in February 2020

Model parameterization

Key Physiological Parameters for *In Vitro-In Vivo* Extrapolation

Model parameters are either:

Physiological: determined by species and potentially varied via Monte Carlo (including HTTK-pop, Ring et al. 2017)

Chemical-specific: physico-chemical properties (Mansouri et al., 2018) and equilibrium partition coefficients plus plasma binding and metabolism rates that are determined from *in vitro* measurements or potentially predicted from structure

Parameter	Definition	Value (Mean)	Units	Reference
Q_{liverc}	Total blood flow to liver (arterial, gut)	3.6	1/h/kg BW	Davies and Morris (1993)
Q_{GFR}	Flow to glomerulus (glomerular filtration rate)	0.32	1/h/kg BW	Davies and Morris (1993)
$n_{cell\_density}$	Hepatocellularity	110	Millions of cells / g Liver	Carlile et al. (1997)
V_{liverc}	Liver volume (scaled to kg body weight)	0.0245	1/kg BW	Davies and Morris (1993)
d_{liver}	Liver density	1.05	g/ml	International Commission on Radiological Protection (1975)
Hematocrit	Fraction of blood that is red blood cells	0.43	Unitless	Davies and Morris (1993)

$$Cl_{hepatic} = n_{cell\ density} \times V_{liverc} \times d_{liver} \times Cl_{int}$$

Breen et al. (2021)

Species-Specific Physiological Parameters for Physiologically-Based Toxicokinetics

- Rates, volumes, and tissue-specific information (not shown) are needed for a species
  - Users can choose to add new species to HTTK by providing this information

Parameter	Units	Mouse	Rat	Dog	Human	Rabbit	Monkey
Total Body Water	ml/kg	725.000	668.000	603.600	600.000	40.812	693.000
Plasma Volume	ml/kg	50.000	31.200	51.500	42.857	110.000	44.800
Cardiac Output	ml/min/kg^(3/4)	150.424	209.304	213.394	231.401	266.576	324.790
Average BW	kg	0.020	0.250	10.000	70.000	2.500	5.000
Total Plasma Protein	g/ml	0.062	0.067	0.090	0.074	0.057	0.088
Plasma albumin	g/ml	0.033	0.032	0.026	0.042	0.039	0.049
Plasma a-1-AGP	g/ml	0.013	0.018	0.004	0.002	0.001	0.002
Hematocrit	fraction	0.450	0.460	0.420	0.440	0.360	0.410
Urine Flow	ml/min/kg^(3/4)	0.013	0.098	0.037	0.040	0.042	0.151
Bile Flow	ml/min/kg^(3/4)	0.026	0.044	0.015	0.010	0.083	0.004
GFR	ml/min/kg^(3/4)	5.265	3.705	10.901	5.165	3.120	2.080
Average Body Temperature	C	37.000	38.700	38.900	37.000	39.350	38.000
Plasma Effective Neutral Lipid Volume Fraction	unitless	0.004	0.002	0.001	0.007	0.002	0.007
Plasma Protein Volume Fraction	unitless	0.060	0.059	0.090	0.070	0.057	0.070
Pulmonary Ventilation Rate	l/h/kg^(3/4)	24.750	24.750	24.750	27.750	24.750	27.750
Alveolar Dead Space Fraction	unitless	0.330	0.330	0.330	0.330	0.330	0.330

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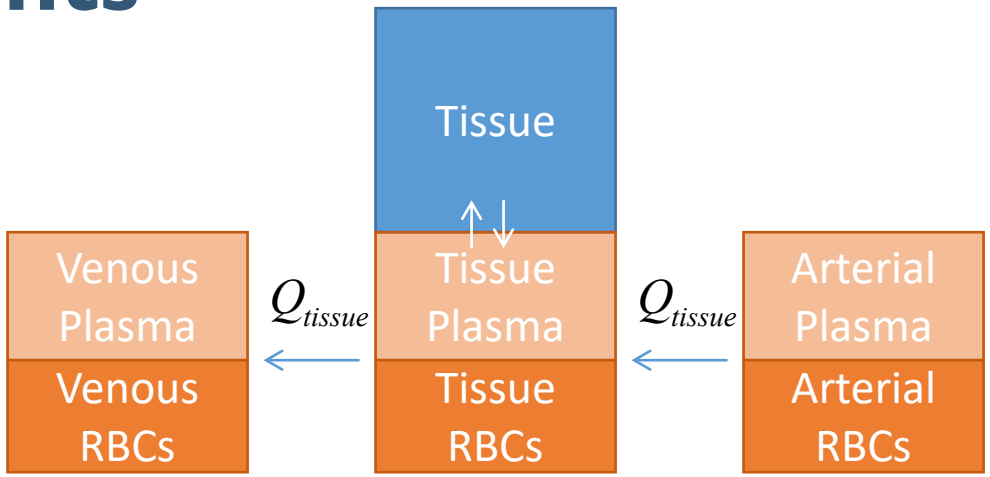
PBTK Partition Coefficients

- Although in our model there are really three separate concentrations (C) that describe a tissue, we assume that they are related to each other by constants
- We assume that the ratio between the blood and plasma ($R_{blood:plasma}$) is a uniform constant throughout the body



$$C_{compartment,blood} = R_{blood:plasma} C_{compartment,plasma}$$

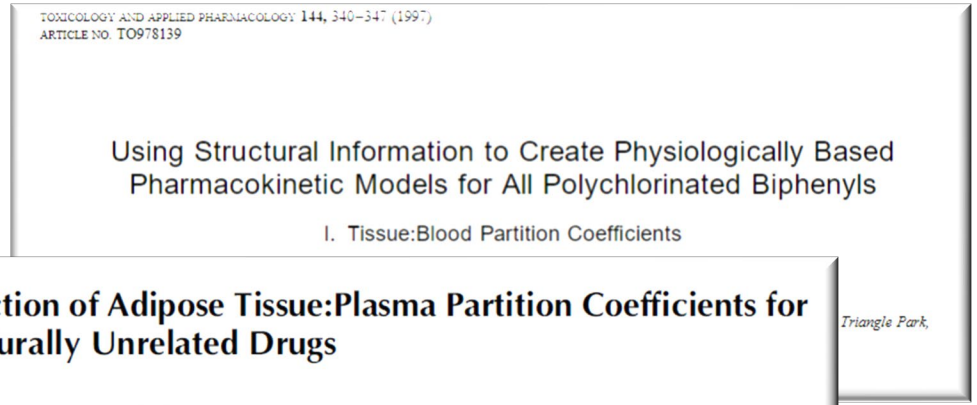
- We assume that all the tissues are "perfusion limited", which means that the tissue concentration instantly comes to equilibrium with the free fraction in plasma (concentration is limited by flow to the tissue)

$$C_{compartment,tissue} = K_{tissue:plasma} * f_{up} * C_{compartment,plasma}$$

$K_{tissue:plasma}$ is the tissue partition coefficient which we either measure experimentally or predict *in silico* (*for example* Schmitt's method)

Tools for Chemical-Specific PBTK Parameters

Physiological parameters depend on species, but we must also make chemical-specific estimates of tissue partitioning…


TOXICOLOGY AND APPLIED PHARMACOLOGY 144, 340–347 (1997)
ARTICLE NO. TO978139

Using Structural Information to Create Physiologically Based Pharmacokinetic Models for All Polychlorinated Biphenyls

I. Tissue:Blood Partition Coefficients

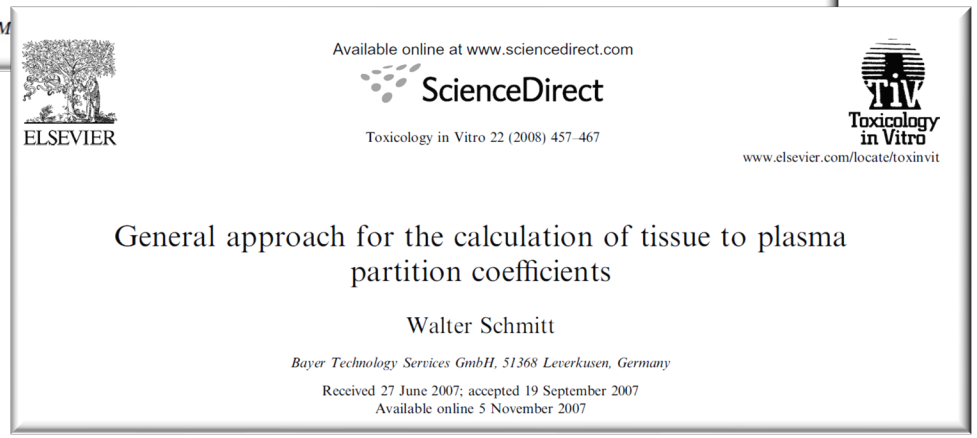

Prediction of Adipose Tissue:Plasma Partition Coefficients for Structurally Unrelated Drugs

PATRICK POULIN, KERSTIN SCHOENLEIN, FRANK-PETER THEIL

F. Hoffmann-La Roche, Ltd., Pharmaceuticals Division, Non-Clinical Development—Drug Safety, CH-4070 Basel, Switzerland

Received 4 M

Triangle Park,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Toxicology in Vitro 22 (2008) 457–467

Toxicology in Vitro

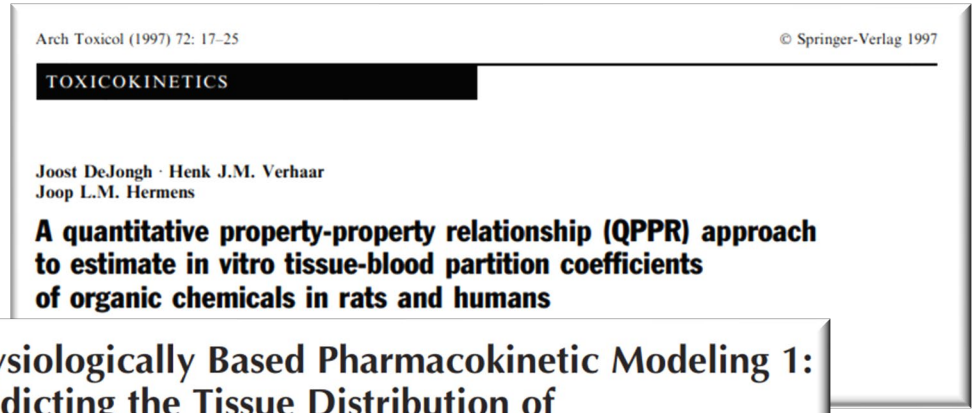
www.elsevier.com/locate/toxinvit

General approach for the calculation of tissue to plasma partition coefficients

Walter Schmitt

Bayer Technology Services GmbH, 51368 Leverkusen, Germany

Received 27 June 2007; accepted 19 September 2007
Available online 5 November 2007


Arch Toxicol (1997) 72: 17–25 © Springer-Verlag 1997

TOXICOKINETICS

Joost DeJongh · Henk J.M. Verhaar
Joop L.M. Hermens

A quantitative property-property relationship (QPPR) approach to estimate in vitro tissue-blood partition coefficients of organic chemicals in rats and humans


Physiologically Based Pharmacokinetic Modeling 1: Predicting the Tissue Distribution of Moderate-to-Strong Bases

TRUDY RODGERS,[1] DAVID LEAHY,[2] MALCOLM ROWLAND[1]

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A unified algorithm for predicting partition coefficients for PBPK modeling of drugs and environmental chemicals

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Schmitt's Method (2008)

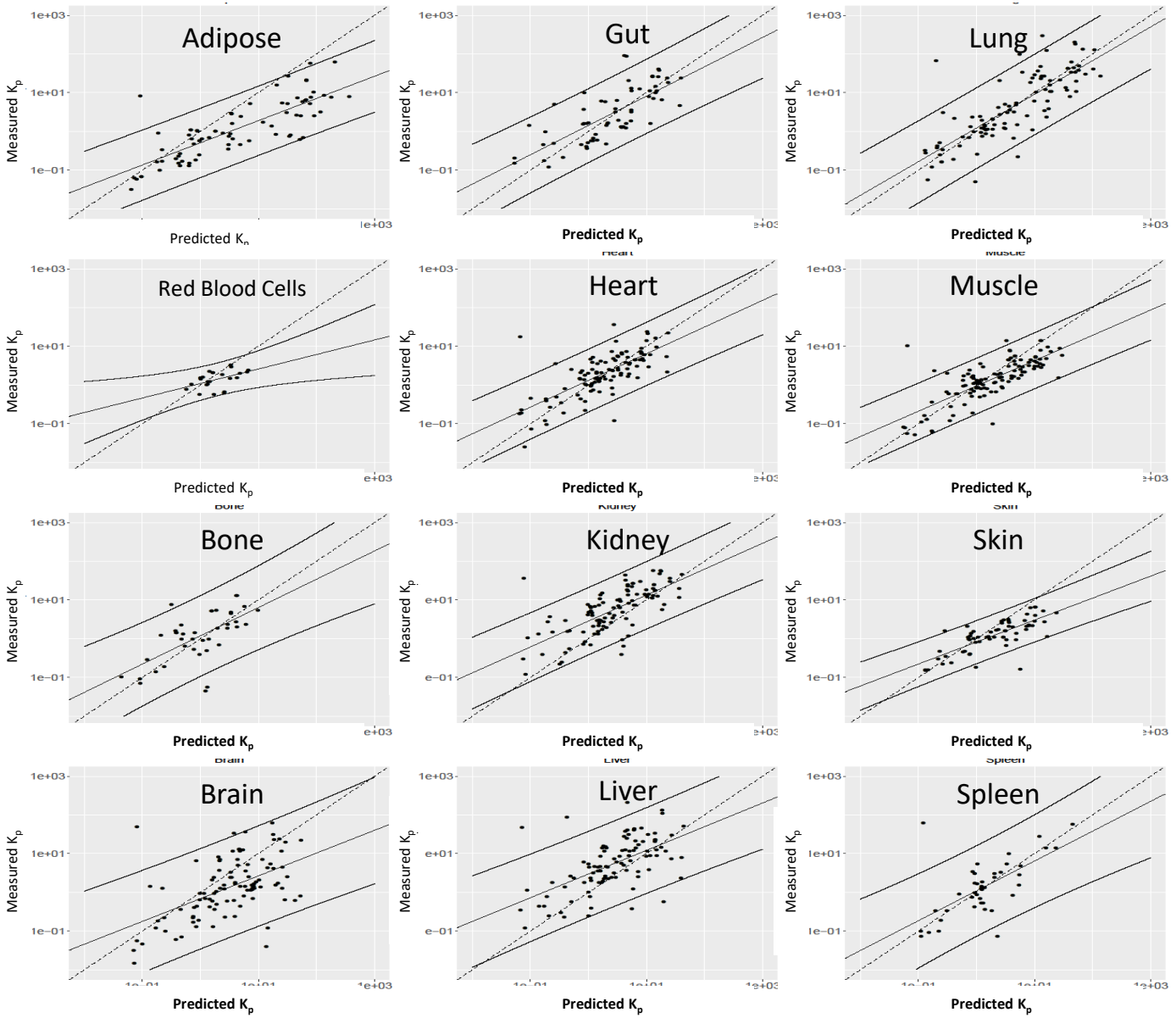
- Depending on its structure a chemical partitions differently into water, fats, and charged materials
- Schmitt's method predicts chemical affinity based on the composition of a tissue
  - Users can choose to add new tissue to HTTK by providing this information

	Fraction of total volume		Fraction of cell volume			Fraction of total lipid			
Tissue	Cells	Interstitium	Water	Lipid	Protein	Neutral Lipid	Neutral Phospholipid	Acidic Phospholipid	pH
Adipose	0.86	0.14	0.02	0.93	0.05	0.94	0.06	0.01	7.10
Bone	0.90	0.10	0.26	0.02	0.21	0.85	0.11	0.04	7.00
Brain	1.00	0.01	0.80	0.11	0.08	0.37	0.46	0.17	7.10
Gut	0.90	0.10	0.78	0.07	0.15	0.69	0.26	0.05	7.00
Heart	0.75	0.25	0.70	0.14	0.17	0.89	0.08	0.03	7.10
Kidney	0.84	0.17	0.77	0.06	0.17	0.64	0.29	0.07	7.22
Liver	0.77	0.23	0.72	0.09	0.18	0.72	0.23	0.05	7.23
Lung	0.80	0.20	0.80	0.01	0.18	0.30	0.56	0.14	6.60
Muscle	0.85	0.15	0.80	0.02	0.18	0.54	0.38	0.08	6.81
Skin	0.40	0.60	0.43	0.28	0.29	0.36	0.50	0.14	7.00
Spleen	0.75	0.26	0.77	0.04	0.19	0.53	0.39	0.07	7.00
Red blood cells	1.00	0.00	0.66	0.01	0.33	0.40	0.50	0.10	7.20

HTTK Partition Coefficients

- We use a modified Schmitt (2008) method with elements of Peyret et al. (2010)
- Pearce et al. (2017b) analyzed literature measurements of chemical-specific partition coefficients (PC) in rat
  - 945 tissue-specific PC
  - 137 unique chemicals
  - Mostly pharmaceuticals
- We use tissue-specific calibrations for the *in silico* predictors
- Pearce et al. (2017b) evaluated with human measured volumes of distribution for 498 chemicals from Obach (2008) – root mean squared error was 0.48

Pearce et al. (2017b)



Review: HTTK model parameters

Chemical-specific parameters	
Intrinsic hepatic clearance rate (CL_{int})	Measured in HT *in vitro* assays (Rotroff *et al.* 2010; Wetmore *et al.* 2012, 2014, 2015; Wambaugh *et al.* 2019) or predicted *in silico* (Sipes *et al.* 2017)
Fraction unbound to plasma protein (F_{up})	
Tissue:blood partition coefficients (for compartmental models)	Predict from phys-chem properties and tissue properties (Pearce et al., 2017)
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	

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Model evaluation

Verifying PBTK Models

Process for the Evaluation of PBPK Models

1. Assessment of Model Purpose
2. Assessment of Model Structure and Biological Characterizations
3. Assessment of Mathematical Descriptions
4. Assessment of Computer Implementation
5. Parameter Analysis and Assessment of Model Fitness
6. Assessment of any Specialized Analyses

Clark et al. (2004)

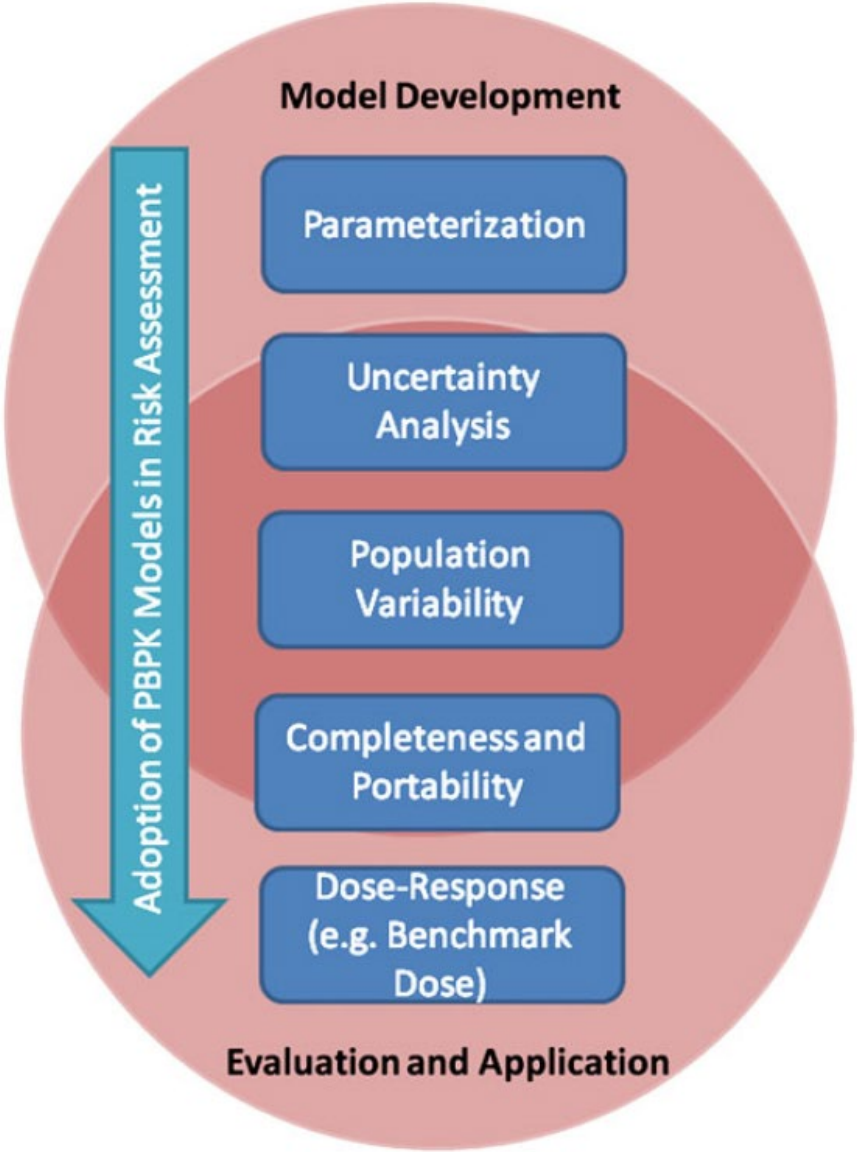


FIG. 1. This figure shows examples of key considerations during model development, evaluation, and application that are necessary before a PBPK model may be adopted for use in a HHRA.

McLanahan et al. (2012)

Why Build Another Generic PBTK Tool?

from Breen et al. (2021)

	SimCYP	ADMET Predictor / GastroPlus	PK-Sim	IndusChem Fate	pbktool	G-PBTK	httk
References	Jamei (2009)	Parrott (2009)	Eissing (2011)	Jongeneelen (2011)	Punt (2020)	Armitage (2021)	Pearce (2017)
Availability	License, but inexpensive for research	License, but inexpensive for research	Free	Free	Free	Free	Free
Open Source	No	No	GitHub	No	GitHub	Planned Release	CRAN and GitHub
Default PBTK Structure	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Population Variability	Yes	Yes	Yes	No	No	No	Yes
Data Needs	High/Low	High/Low	High	High	Low	Low	Low
Typical Use Case	Drug Discovery	Drug Discovery	Drug Discovery	Environmental Assessment	Food and Drug Safety Evaluation	Environmental Assessment	Screening
Batch Mode	Yes	Yes	Yes	No	No	No	Yes
Graphical User Interface	Yes	Yes	Yes	Excel	No	Excel	No
Built-in Chemical-Specific Library	Many Clinical Drugs	No	Many pharmaceutical-specific models available	15 Environmental Compounds	No	No	Pharmaceuticals and ToxCast: 998 human, 226 rat
Oral Bioavailability Modeling	Yes	Yes	No	No	No	No	No (Will be available in the future version)
In Vitro Distribution	SIVA VIVD	No	No	No	No	No	Armitage Model
Exposure Route	Oral, IV	Oral, IV	Oral, IV	Oral, Gas Inhalation, Dermal	Oral	Oral, IV, Inhalation	Oral, IV, Gas Inhalation (Dermal, Aerosol, and Fetal forthcoming)
Ionizable Compounds	Yes	Yes	Yes	No	No	Yes	Yes
Export Function	No	No	Matlab and R	No	No	No	SBML and Jarnac
R Integration	No	No	Yes (2017)	No	Yes	Yes	Yes
Reverse Dosimetry	Yes	Yes	Yes	No	No	No	Yes

*Both **PLETHEM** (Pendse et al., 2020) and **Web-ICE** (Bell et al., 2020) provide GUI's to HTTK and other models

Pre-computed HTTK results are also available at https://comptox.epa.gov/dashboard

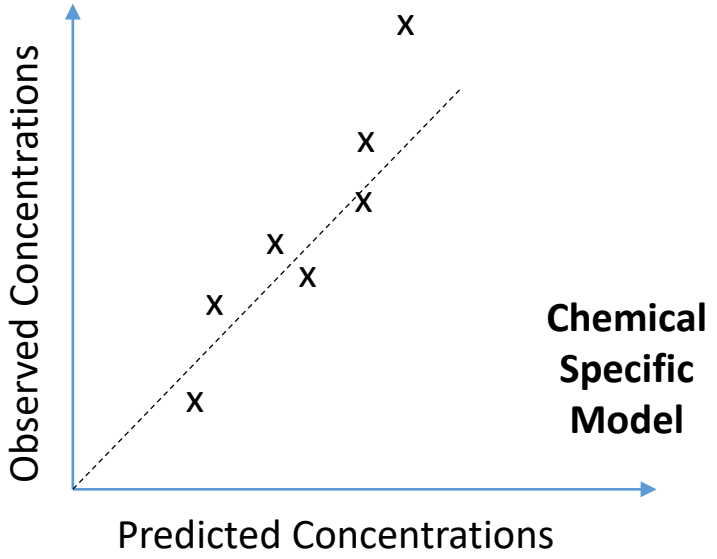
EPA United States Environmental Protection Agency

Statistical Analysis with HTTK

- If we are to use HTTK, then we need confidence in its predictive ability
- In drug development, HTTK methods estimate therapeutic doses for clinical studies – predicted concentrations are typically on the order of values measured in clinical trials (Wang, 2010)
  - For most compounds in the environment there will be no clinical trials
- Uncertainty must be well characterized
  - We compare to *in vivo* data to get **empirical estimates of HTTK uncertainty**
  - ORD has both compiled existing (literature) TK data (Wambaugh *et al*., 2015) and conducted new experiments in rats on chemicals with HTTK *in vitro* data (Wambaugh *et al*., 2018)
  - Any approximations, omissions, or mistakes should work to increase the estimated uncertainty when evaluated systematically across chemicals

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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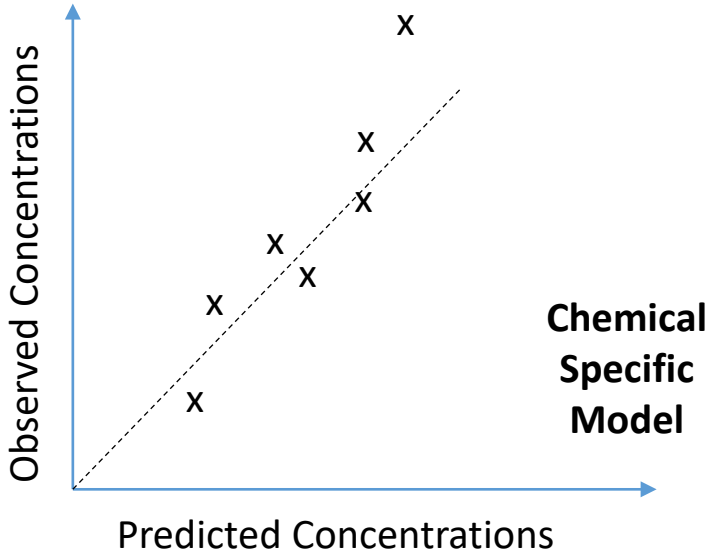
- However, we do not typically have TK data



Cohen Hubal et al. (2019)

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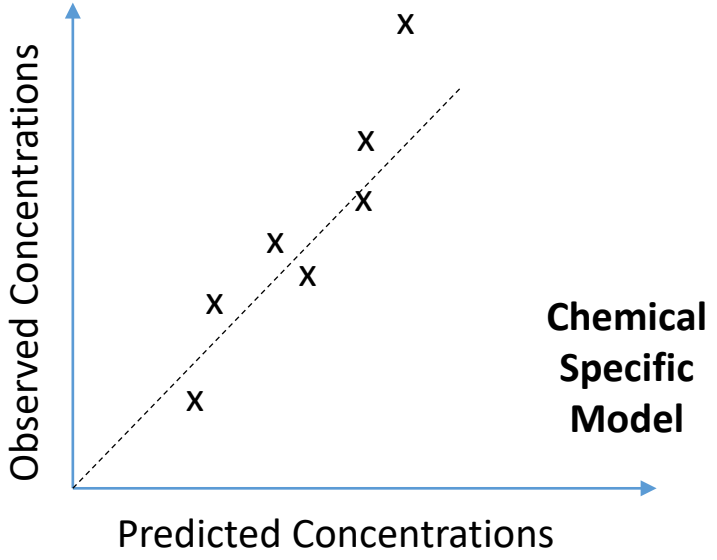
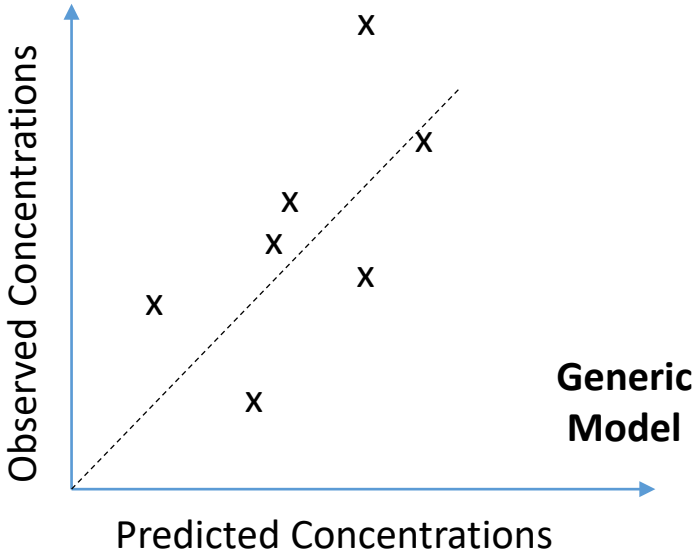
  - Can estimate bias
  - Can estimate uncertainty
  - Can consider using model to extrapolate to other situations (dose, route, physiology) where you have no data

- However, we do not typically have TK data

- We can parameterize a **generic TK model**, and evaluate that model for as many chemicals as we do have data
  - We do expect larger uncertainty, but also greater confidence in model implementation
  - Estimate bias and uncertainty, and try to correlate with chemical-specific properties





Cohen Hubal et al. (2019)

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

- However, we do not typically have TK data

- We can parameterize a **generic TK model**, and evaluate that model for as many chemicals as we do have data
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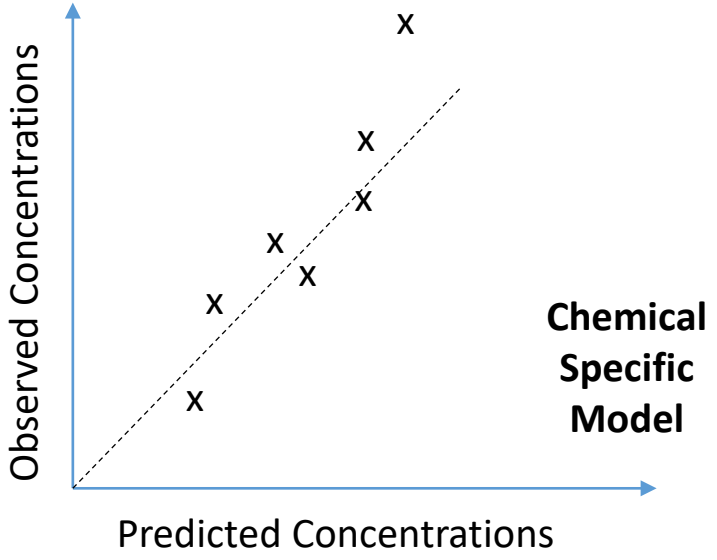
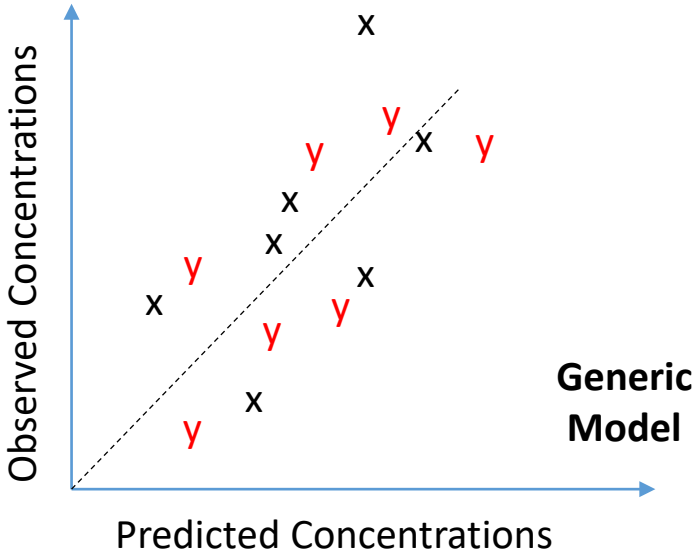
  - Can consider using model to extrapolate to other situations (chemicals without *in vivo* data)





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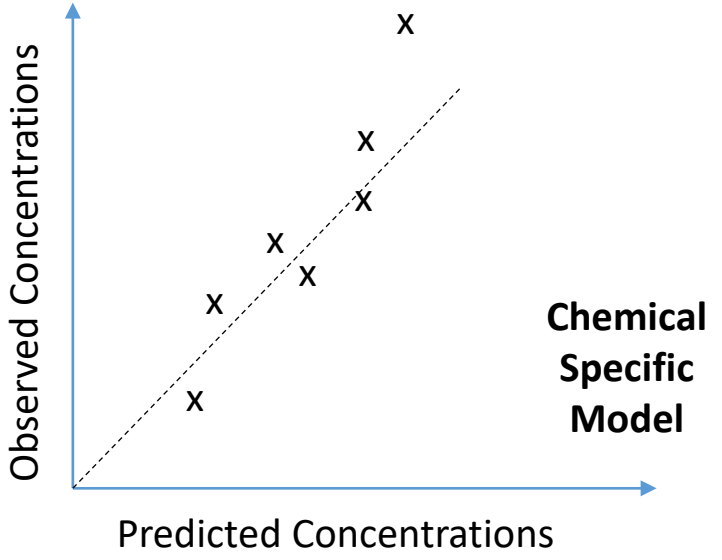
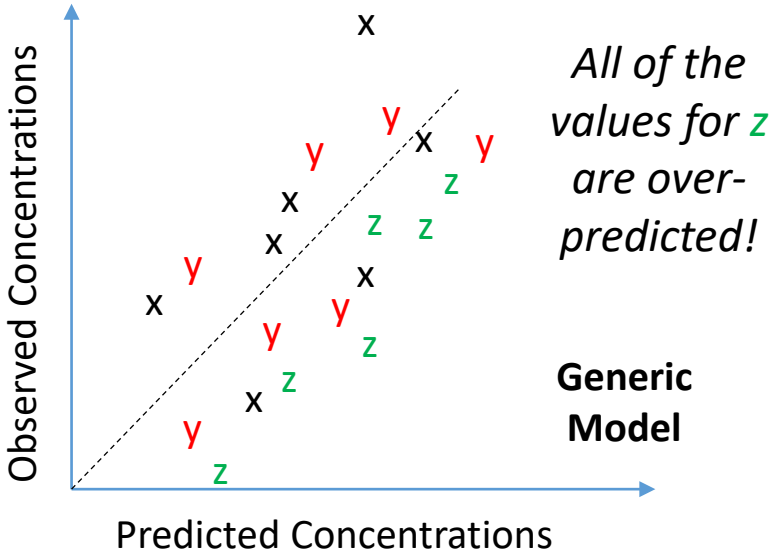
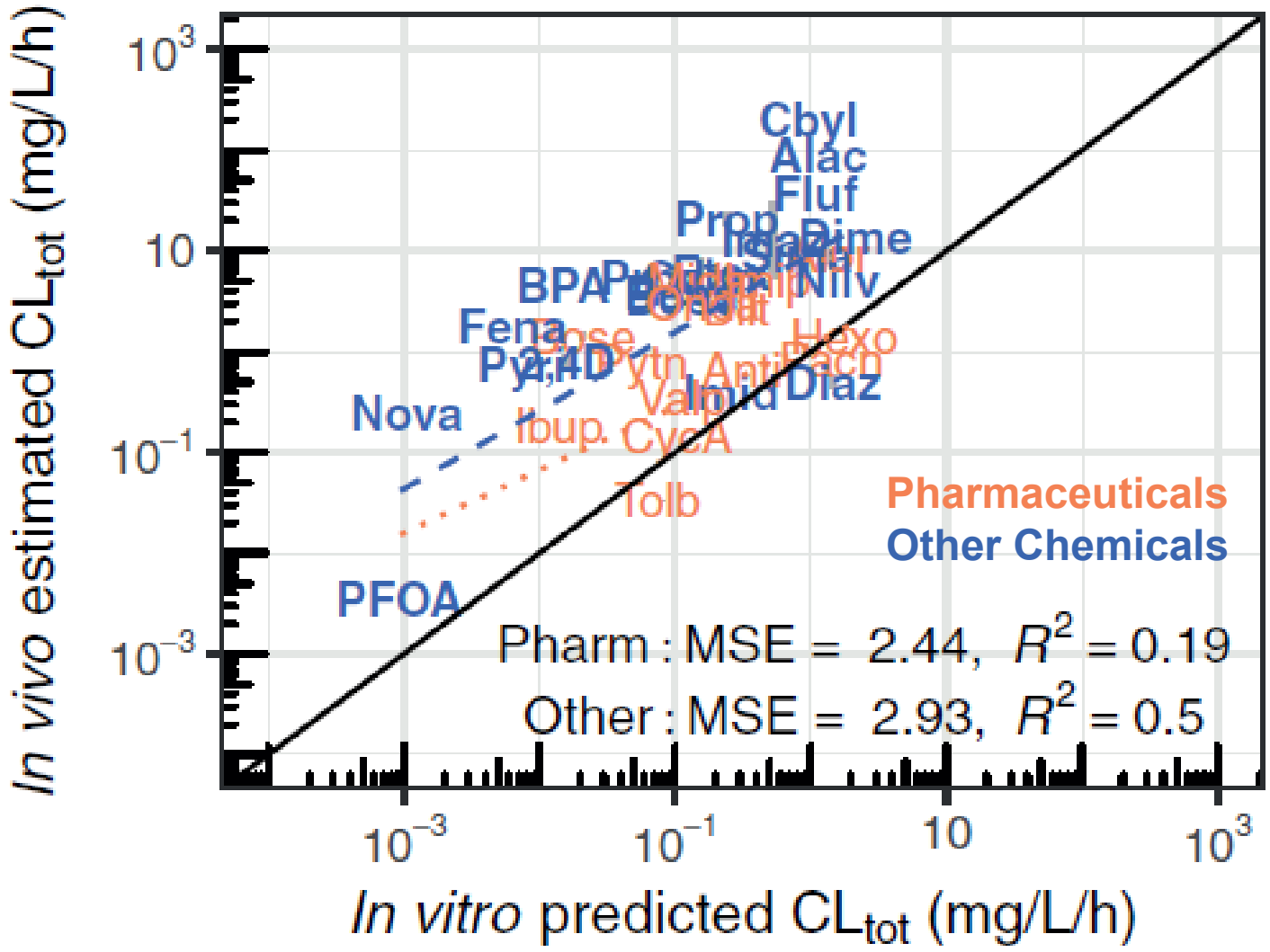




Cohen Hubal et al. (2019)

Evaluation Example: Observed Total Clearance

- We estimate clearance from two processes – hepatic metabolism (liver) and passive glomerular filtration (kidney)
- This appears to work better for pharmaceuticals than other chemicals:
  - ToxCast chemicals are overestimated
- Non-pharmaceuticals may be subject to extrahepatic metabolism and/or active transport



Wambaugh et al. (2018)

CvTdb: An *In Vivo* TK Database

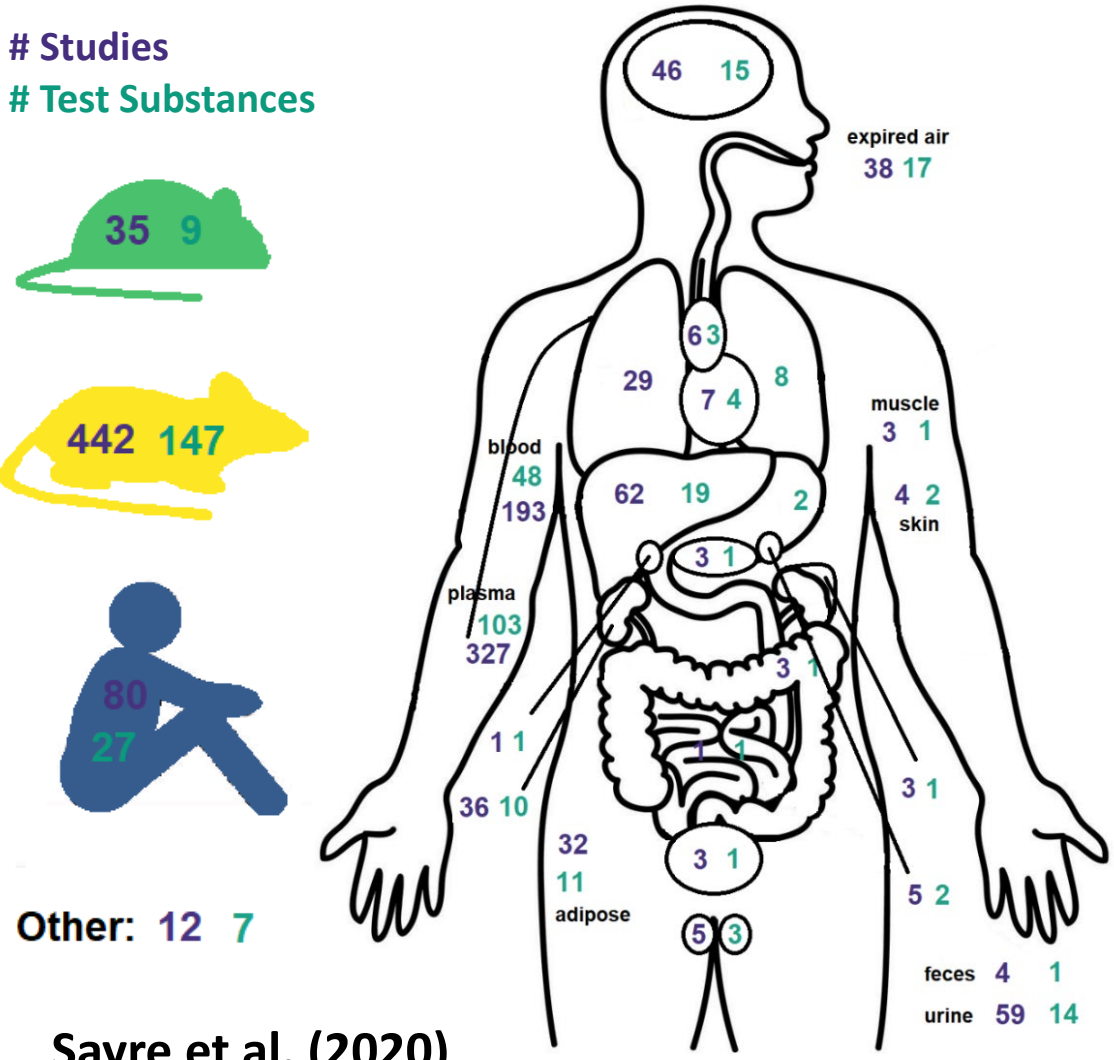
https://github.com/USEPA/CompTox-PK-CvTdb

- 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:
  - 198 analytes (EPA, National Toxicology Program, open literature)
  - Routes: Intravenous, dermal, oral, sub-cutaneous, and inhalation exposure

- Standardized, open-source curve fitting software invivoPKfit used to calibrate models to all data:

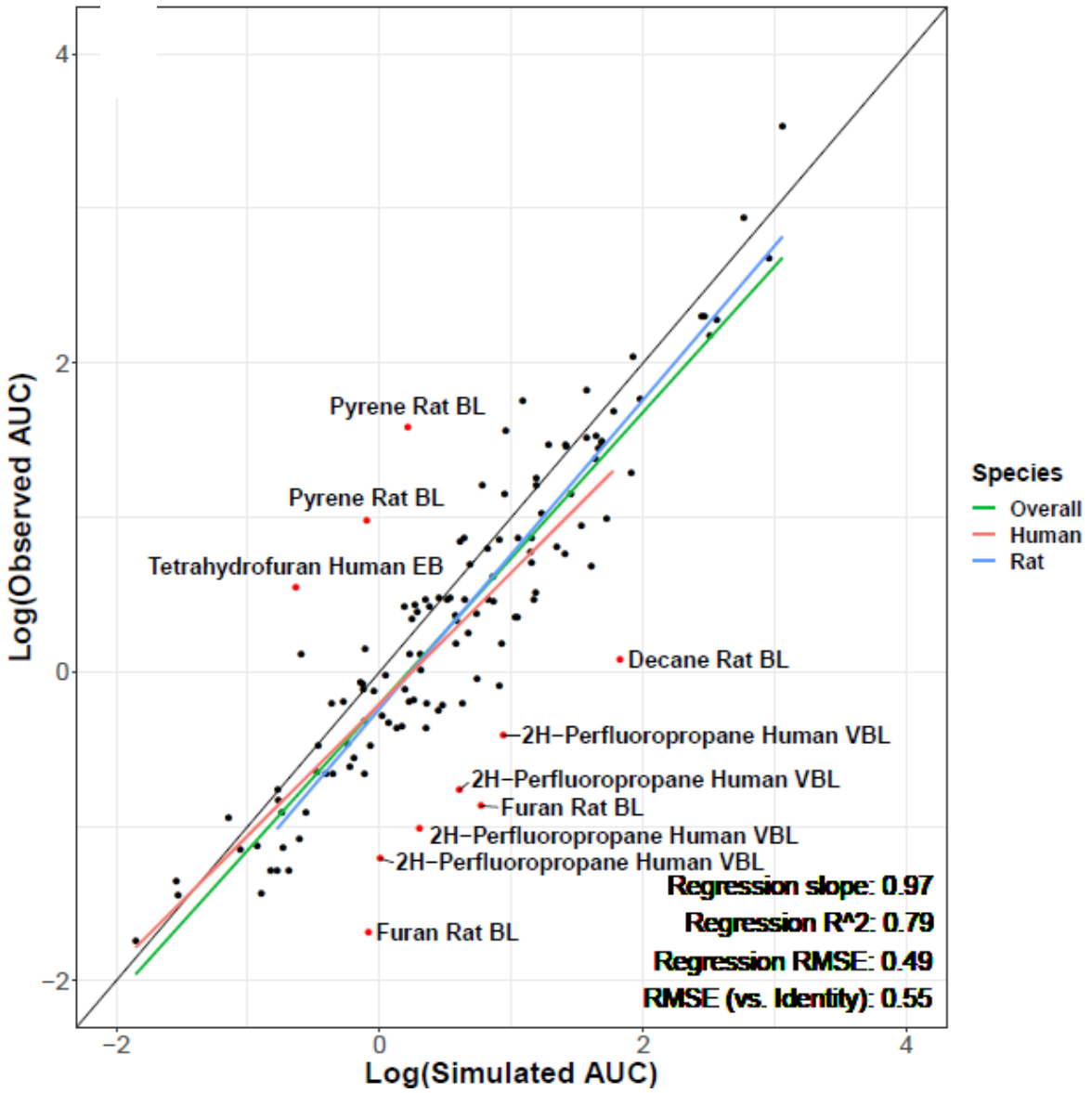
https://github.com/USEPA/CompTox-ExpoCast-invivoPKfit



Sayre et al. (2020)

Developing Models with the CvT Database

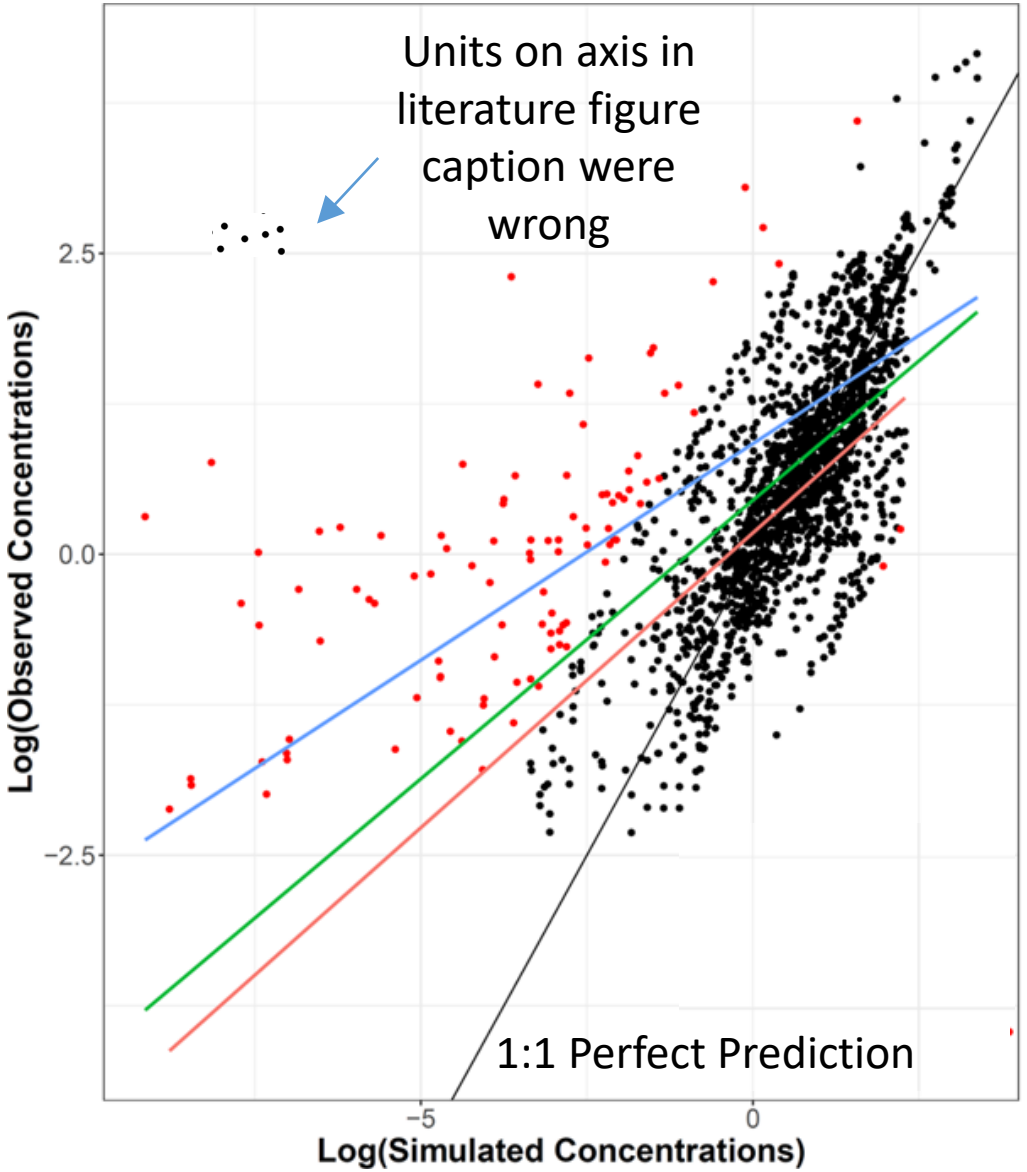
- USAF and EPA developed generic gas inhalation physiologically-based toxicokinetic (PBTK) model
- Evaluated HTTK with CvTdb: 142 exposure scenarios across 41 volatile organic chemicals were modeled and compared to published *in vivo* data for humans and rat
- R^2 was 0.69 for predicting peak concentration
- R^2 was 0.79 for predicting time integrated plasma concentration (Area Under the Curve, AUC)



Linakis et al. (2020)

Developing Models with the CvT Database

- Access to *in vivo* concentration vs. time data made it easier to identify coding and other modeling errors
- **Access to *in vivo* concentration vs. time data also made it easier to find fault with specific data sets**



Linakis et al. (2020)

Review of HTTK Evaluations

- World Health Organization (2010): PBTK models are "adequate" when predictions "are, on average, **within a factor of 2** of the experimental data"

- Predictions of full concentration vs. time curve (that is, all time points for all chemicals):
  - Linakis et al. (2020): For forty volatile, non-pharmaceutical chemicals root mean squared error (RMSE) of 1.11 (on a log10 scale, therefore **a factor of 13x**) and a coefficient of determination (R^2) of 0.47

- Prediction of TK 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 similarly predicted human volume of distribution with a RMSE of 0.48 **(3x**)

Breen et al. (2020)

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Conclusions

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Verifying the HTTK R Package

	Clark et al. (2004) Process for the Evaluation of PBPK Models	Evaluation of HTTK R Package
	Assessment of Model Purpose	
	Assessment of Model Structure and Biology	
	Assessment of Mathematical Descriptions	
	Assessment of Computer Implementation	
	Parameter Analysis and Assessment of Model Fitness	
	Assessment of any Specialized Analyses	

Verifying the HTTK R Package

	Clark et al. (2004) Process for the Evaluation of PBPK Models	Evaluation of HTTK R Package
√	Assessment of Model Purpose	Rapidly parameterized *in vitro-in vivo* extrapolation
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	Parameter Analysis and Assessment of Model Fitness	
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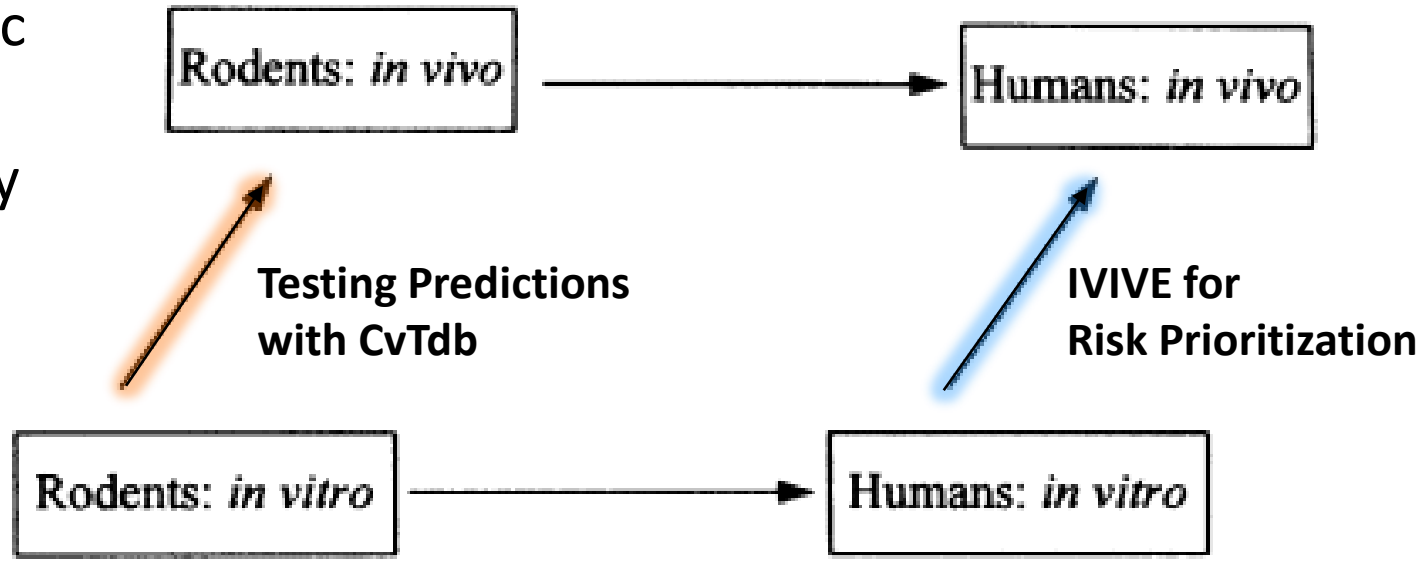
√	Parameter Analysis and Assessment of Model Fitness	Model fitness quantified through comparison with CvTdb
√	Assessment of any Specialized Analyses	Population variability simulator httk-pop has been published (Ring et al., 2017) and is being revised with most recent NHANES biometrics (Breen et al., in prep.)

Conclusions

- The *in vitro*-measured chemical specific parameters may be used to build a variety of models ranging in complexity from steady-state to full PBTK
- Chemical-independent information on physiology and tissue composition allow predictions of chemical distribution



- Generic models allow for verification of model implementation
- Comparing model predictions for chemicals with *in vivo* data allows estimation of confidence in predictions for chemicals without *in vivo* data

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There is time for questions now followed by a BREAK

Talk Three will begin at 2:00 PM EST

Feel free to contact me at: wambaugh.john@epa.gov

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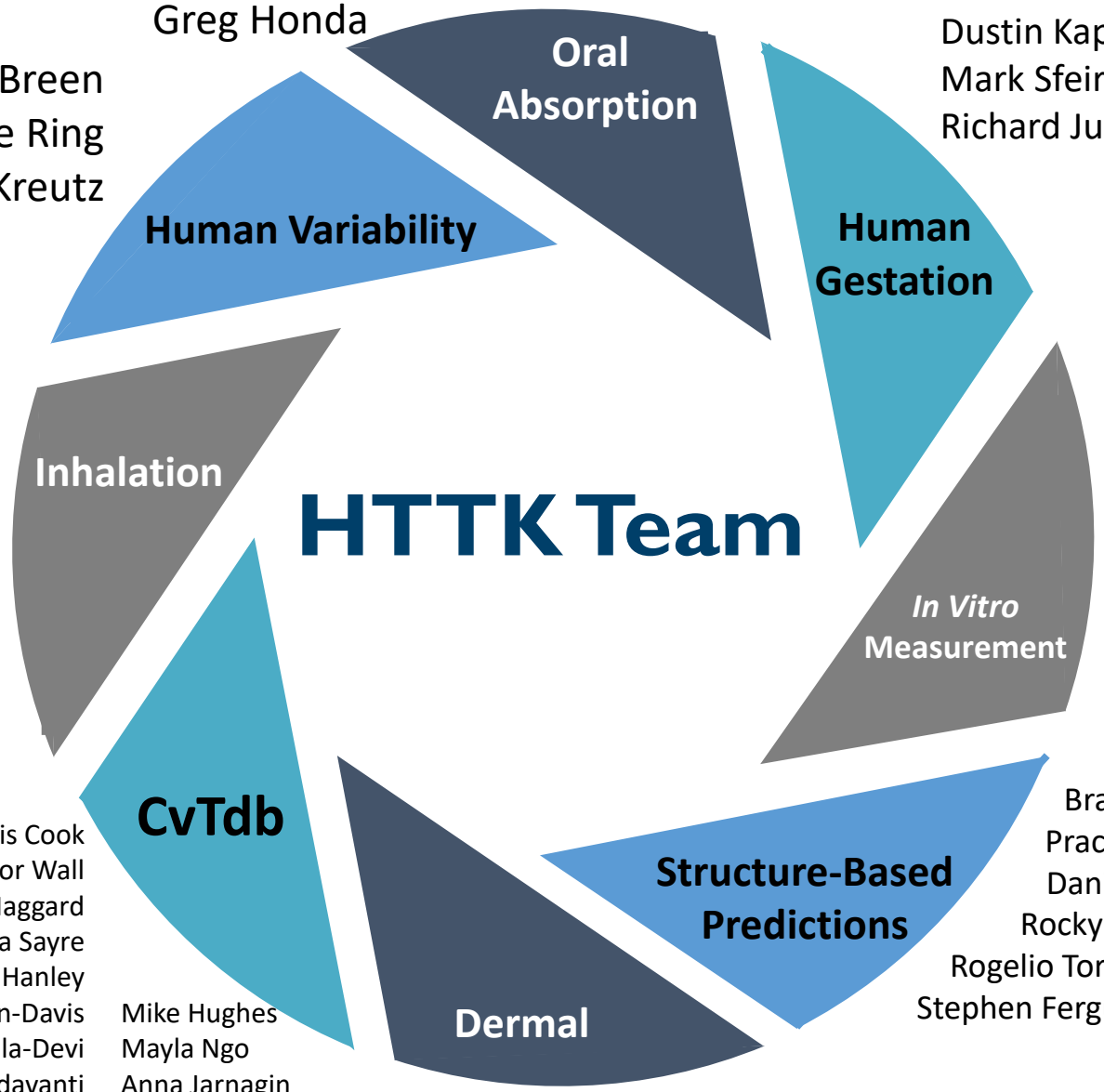
HTTK Team



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Monte Carlo for variability simulation and uncertainty

Caroline L. Ring

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.

Office of Research and Development
Center for Computational Toxicology and Exposure

October 29, 2021

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Overview

- Uncertainty vs. Variability in HTTK model parameters
- Characterizing key uncertainty in chemical-specific TK parameters
  - Fraction unbound in plasma protein (Fup)
  - Intrinsic hepatic clearance rate (Clint)
- Characterizing variability: HTTK-Pop for human TK variability
- Relative contributions of uncertainty and variability to TK model predictions
- Simulating sensitive subpopulations

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Uncertainty vs. variability in HTTK model parameters

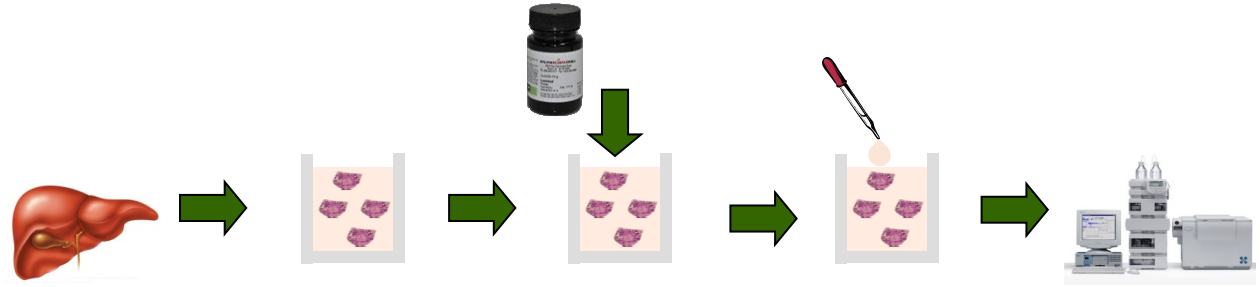
Review: HTTK model parameters

Chemical-specific parameters	
Intrinsic hepatic clearance rate (CLint)	Measured in HT *in vitro* assays (Rotroff *et al.* 2010; Wetmore *et al.* 2012, 2014, 2015; Wambaugh *et al.* 2019) or predicted *in silico* (Sipes *et al.* 2017)
Fraction unbound to plasma protein (Fup)	
Tissue:blood partition coefficients (for compartmental models)	Predict from phys-chem properties and tissue properties (Pearce et al., 2017)
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	

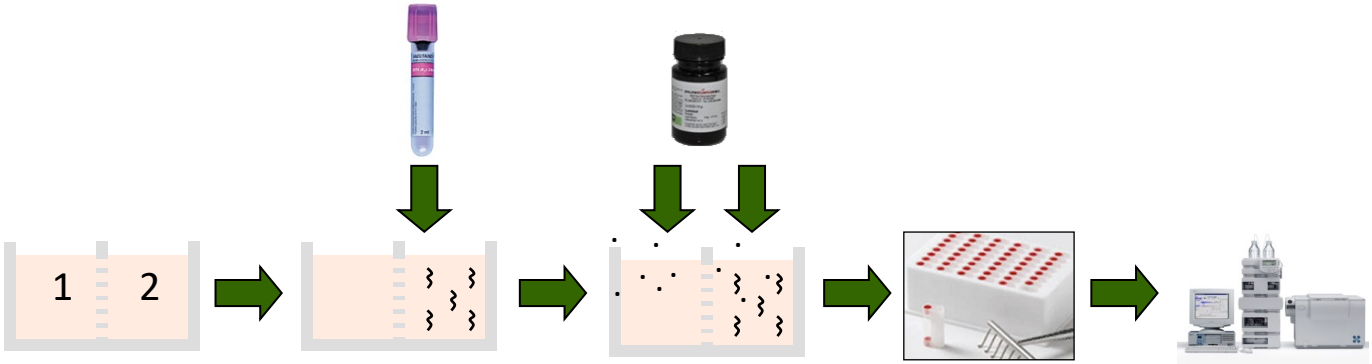
Chemical-specific parameters measured *in vitro* carry measurement uncertainty

Chemical-specific parameters	
Intrinsic hepatic clearance rate (CLint)	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 (Fup)	

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



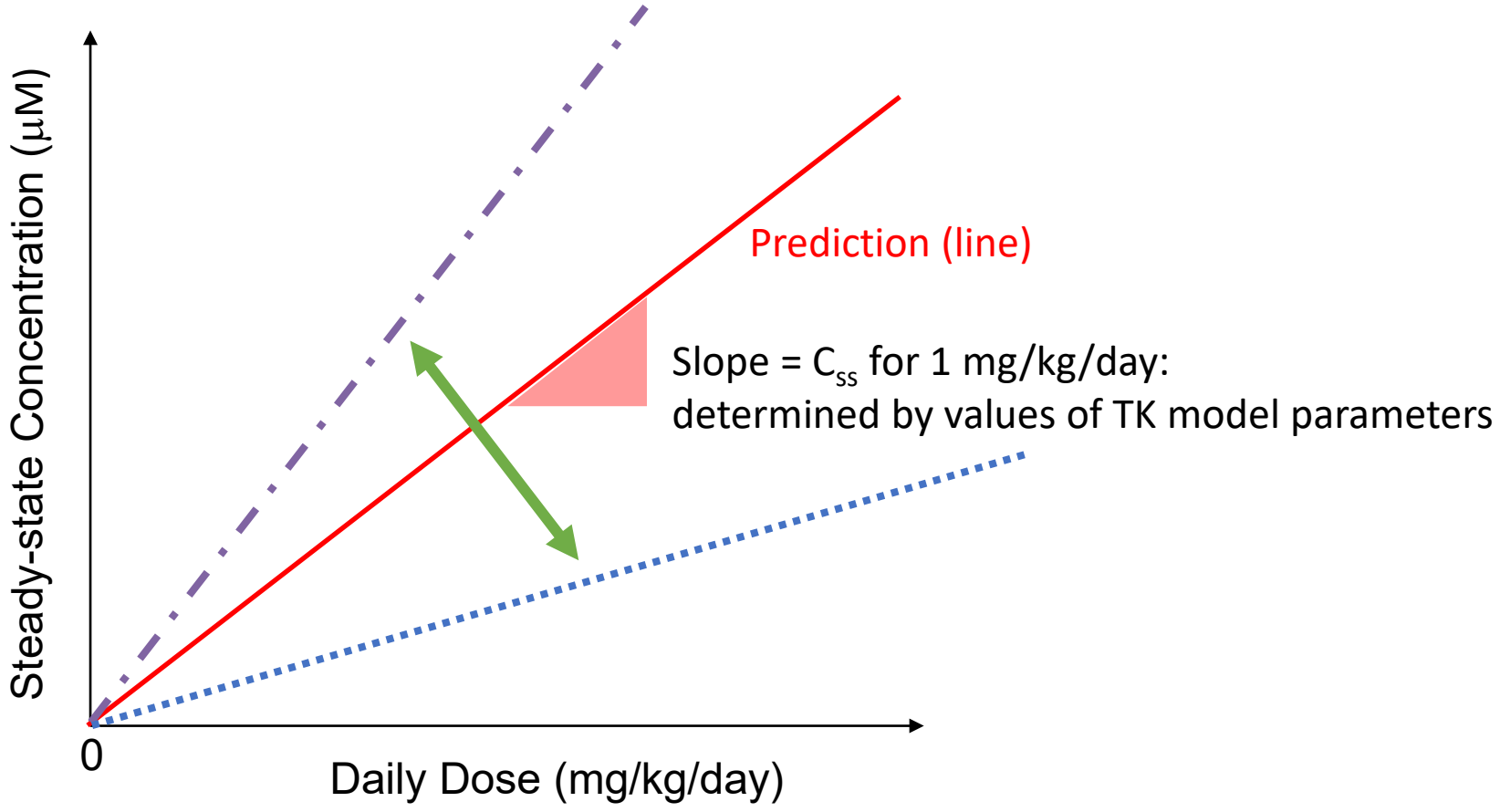
Fup: Rapid Equilibrium Dialysis (RED)
Waters *et al.* (2008)



Parameters represent biology — so they have population variability

Chemical-specific parameters	
Intrinsic hepatic clearance rate (CLint)	Represent chemical-body interactions — vary with individual genetics, environmental factors, age, etc.
Fraction unbound to plasma protein (Fup)	
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	

HTTK model parameters determine the slope relating Css to daily dose – need to propagate both uncertainty & variability



Wambaugh et al. (2019)

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Approach to uncertainty & variability: Monte Carlo

- Characterize uncertainty in chemical-specific parameters Fup and Clint in terms of probability distributions
- Characterize population variability in physiological parameters in terms of (correlated) probability distributions
- Draw samples from distributions: “simulated population”
- Evaluate HTTK model for each “simulated individual” in the “simulated population”
- Describe resulting distribution of HTTK model predictions

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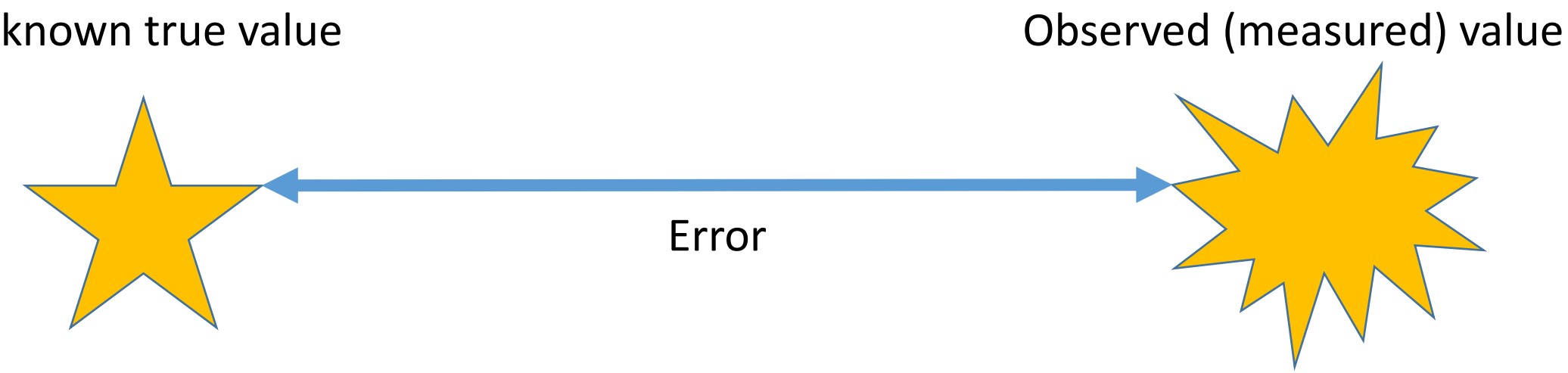
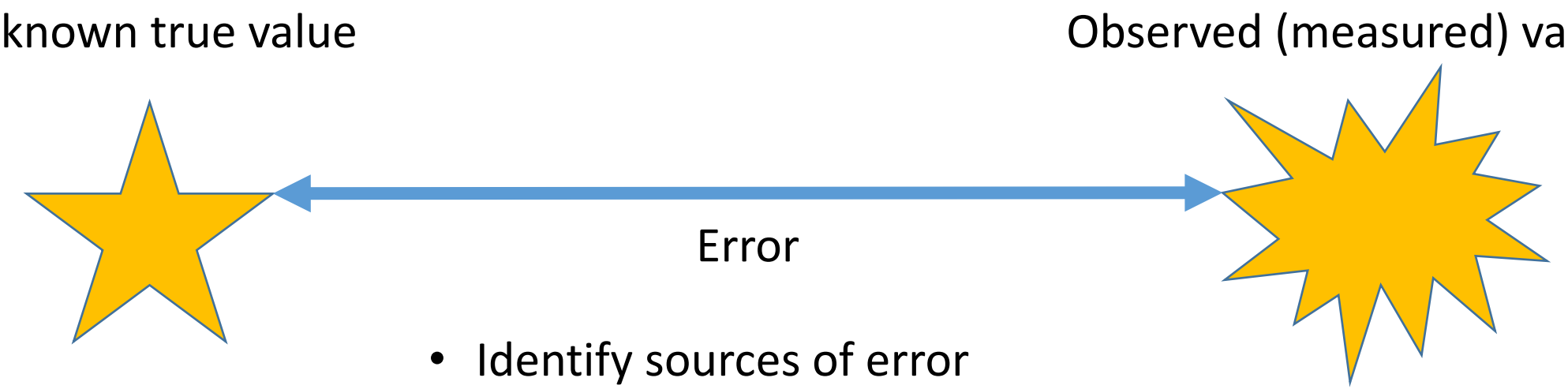
Characterizing key uncertainty in chemical-specific TK parameters

General approach to uncertainty quantification



General approach to uncertainty quantification

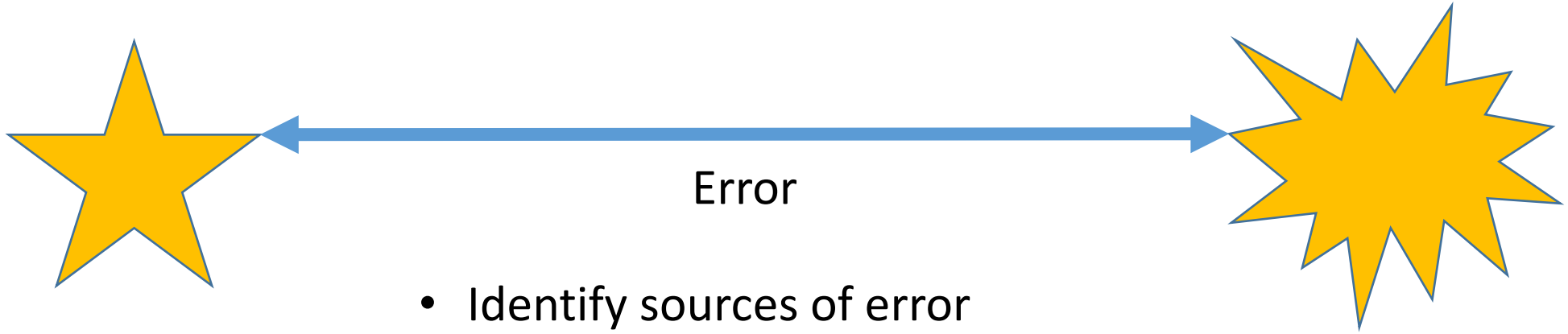


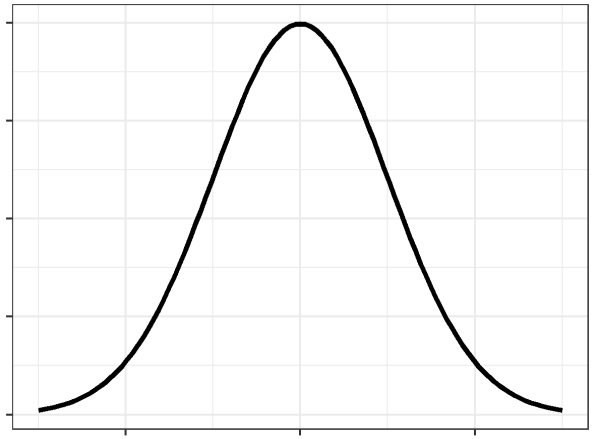
- Identify sources of error
- Develop mathematical model of error

General approach to uncertainty quantification

Unknown true value

Observed (measured) value





- Identify sources of error
- Develop mathematical model of error

Bayesian inference:
Find a *distribution* of possible true values compatible with the observed values, under this error model

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Uncertainty in Fup

Understanding sources of error in Fup: How to measure *in vitro* using Rapid Equilibrium Dialysis (RED)

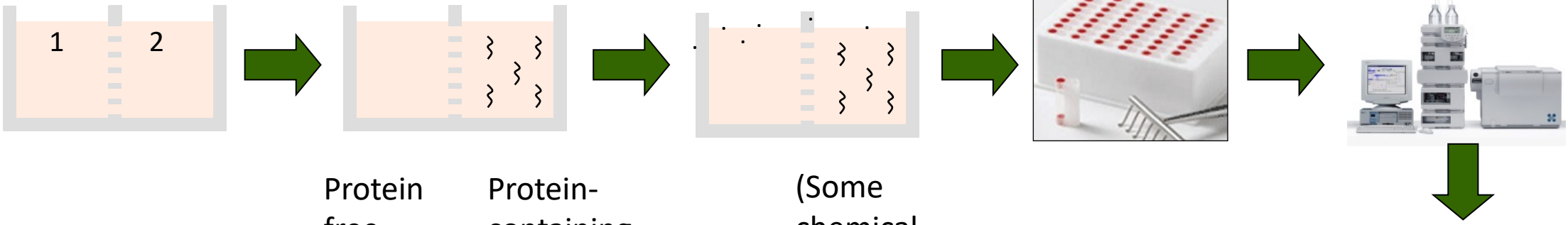
Semi-permeable membrane (chemical can pass, but not protein)

Add plasma to one side

Add chemical

Let equilibrate (until unbound conc. equal on both sides)

Use mass spectrometry to measure chemical conc. from each side



Protein free side

Protein-containing side

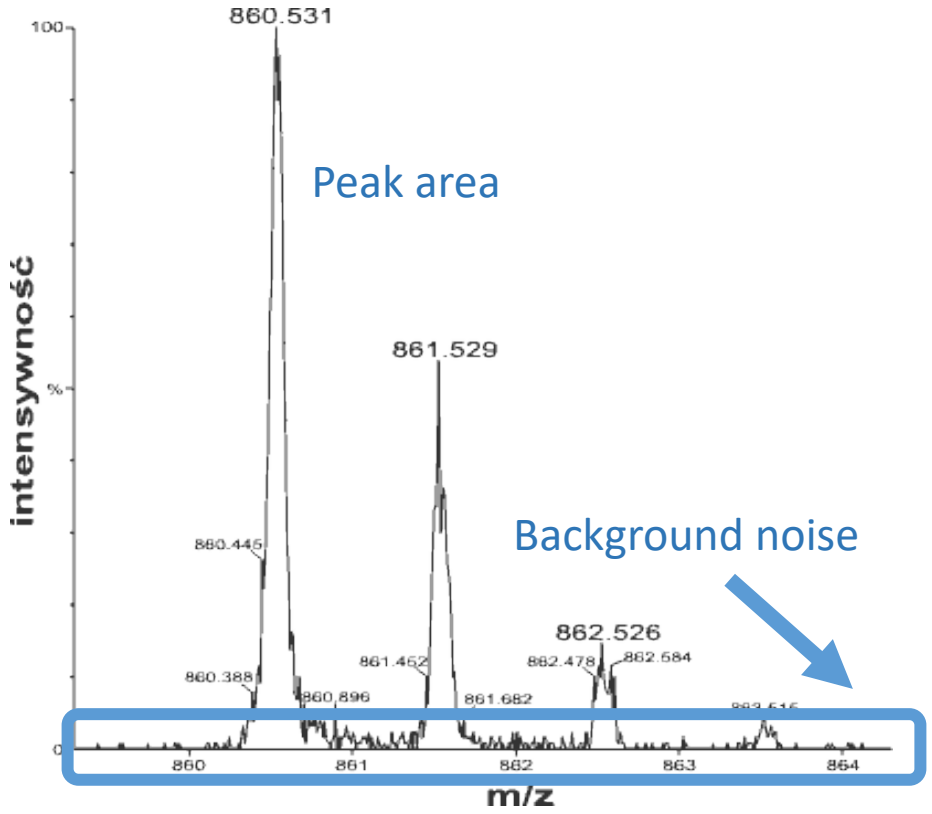
(Some chemical will bind to protein)

$$F_{up} = \frac{\text{Protein−free conc.}}{\text{Protein−containing conc.}}$$

Waters et al. (2008); Rotroff et al. (2010); Wambaugh et al. (2019)

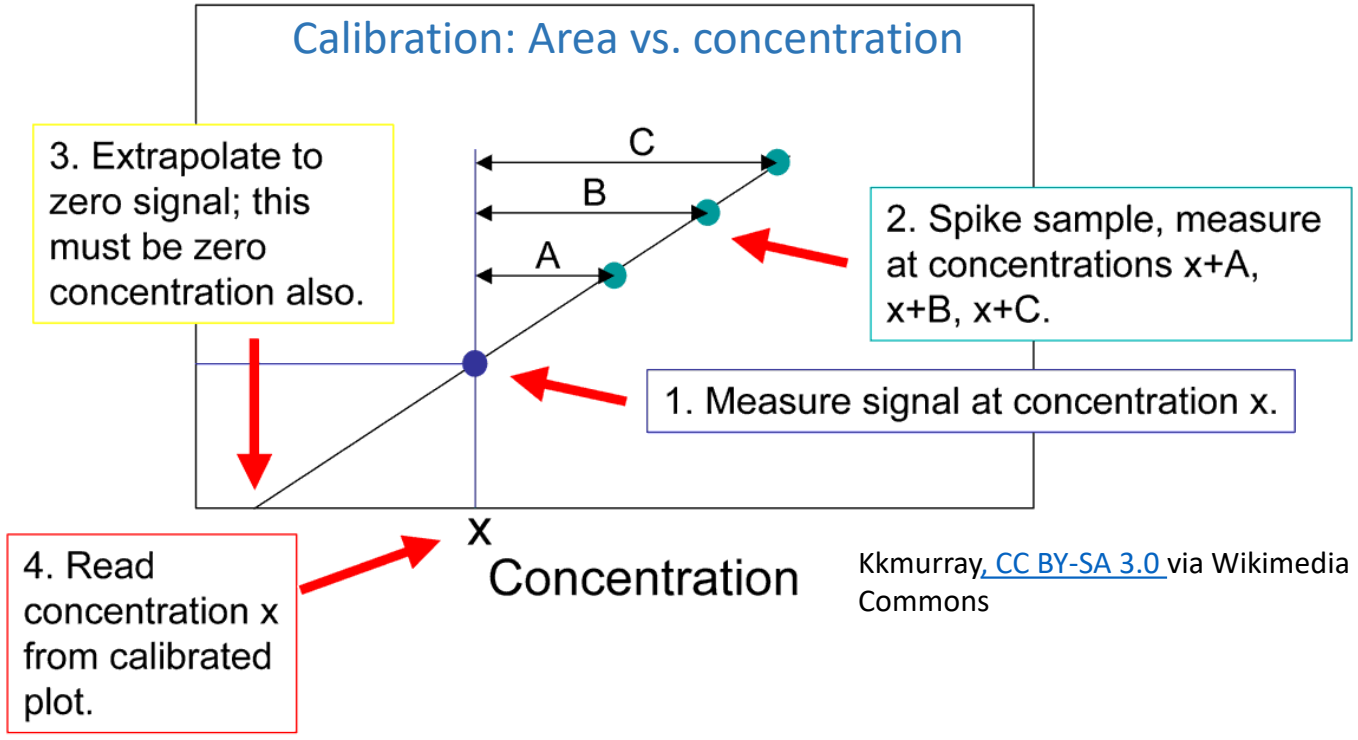
Sources of measurement uncertainty: Mass spectrometry

- Instrument noise
- Limit of quantification (LOQ)
- Instrument calibration



https://commons.wikimedia.org/wiki/File:ObwiedniaPeptydu.gif (GPL)

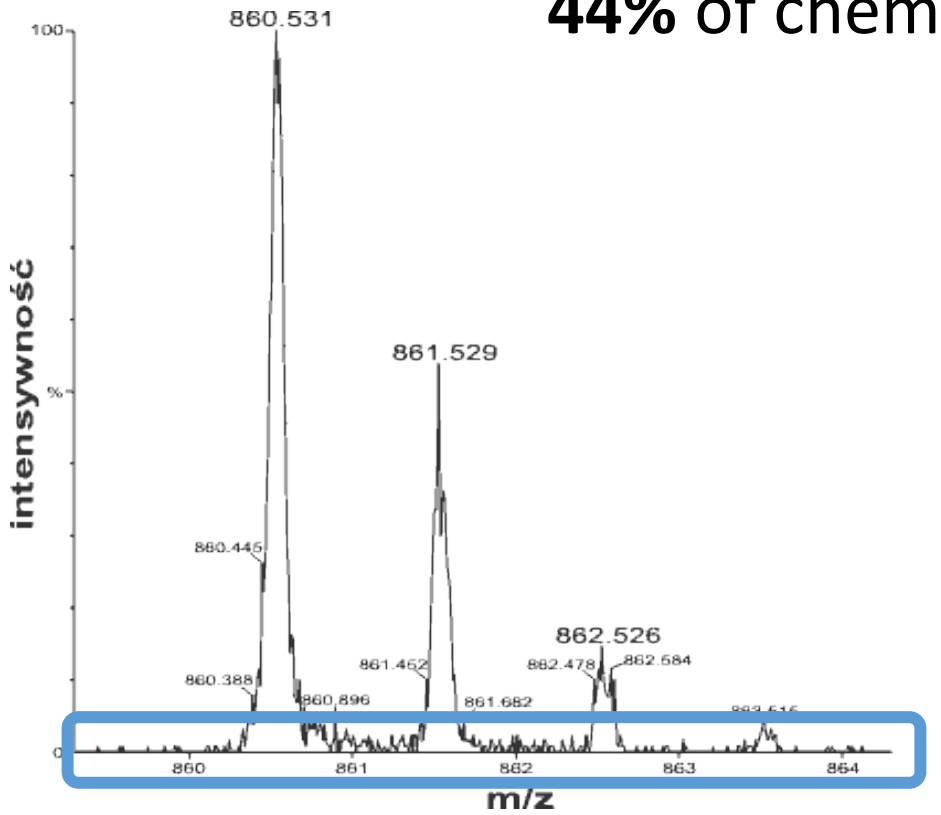
Wambaugh et al. (2019)



Kkmurray, CC BY-SA 3.0 via Wikimedia Commons

LOQ is a problem in the RED assay for highly-bound chemicals

44% of chemicals in Wambaugh et al. (2019)



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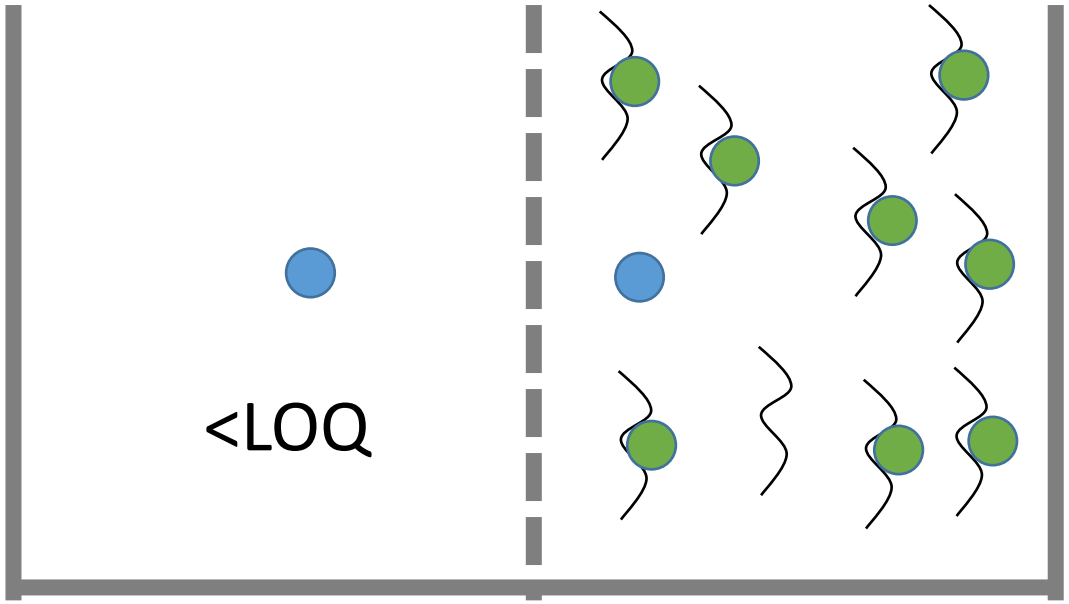
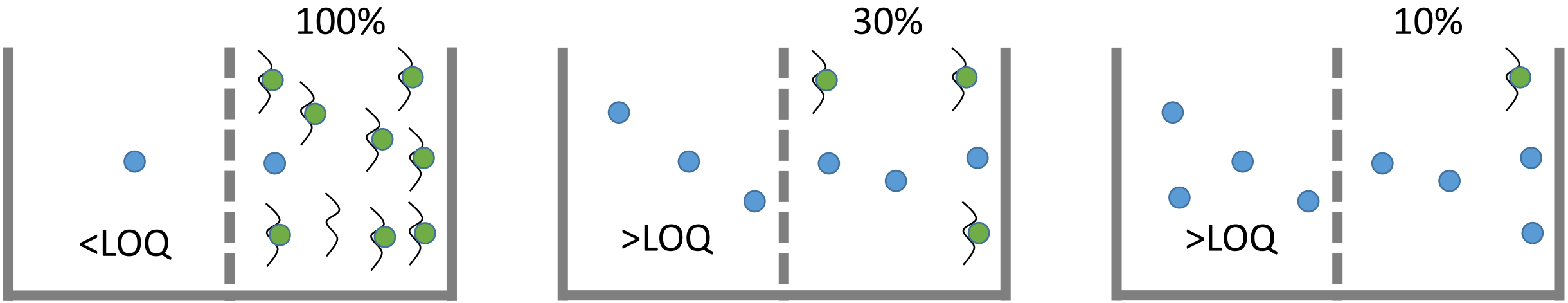


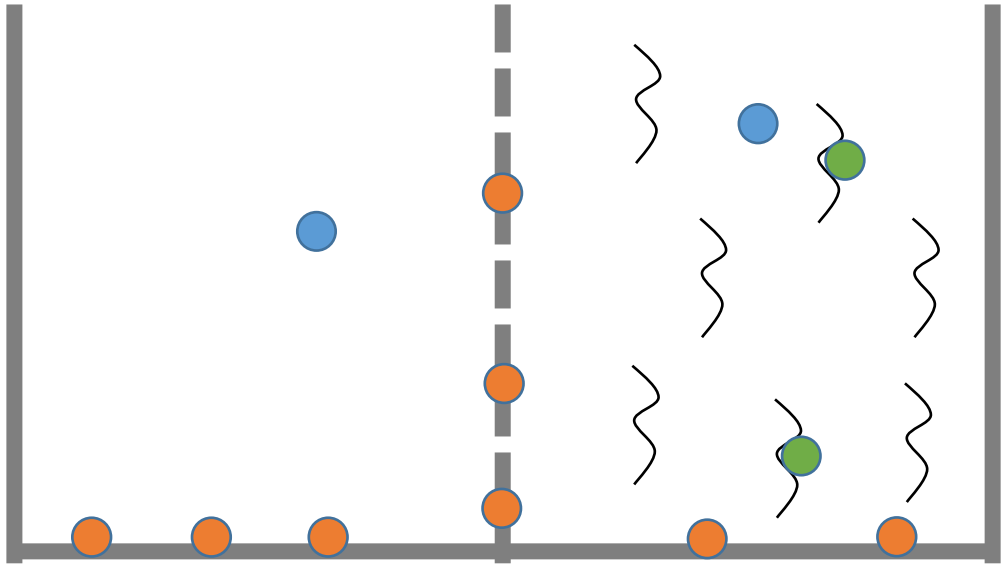
Approach to <LOQ problem:
Repeat RED assay with varying amounts of protein



Estimate dissociation constant K_d
(strength of binding affinity between chemical and protein)

Wambaugh et al. (2019)

Additional source of uncertainty: Non-specific chemical binding to membrane or walls

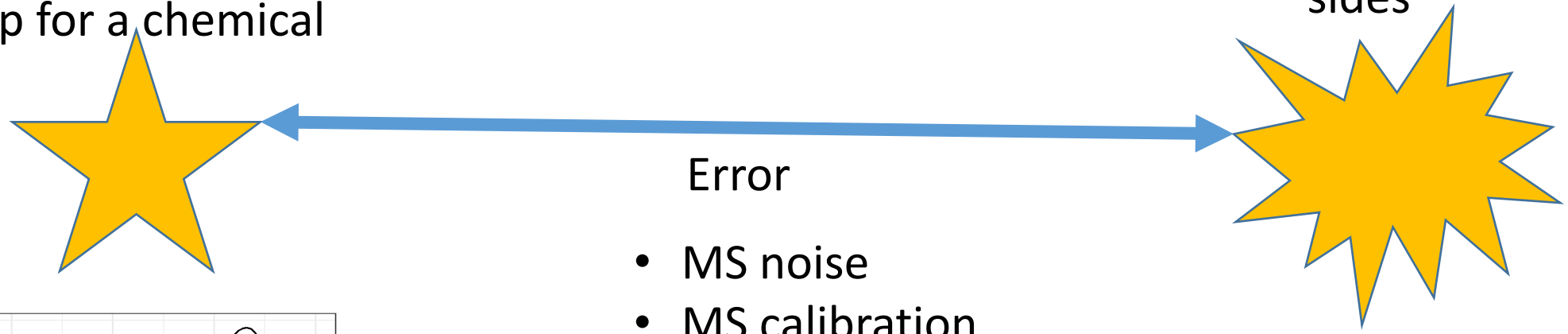


Wambaugh et al. (2019)

Bayesian inference model for Fup uncertainty

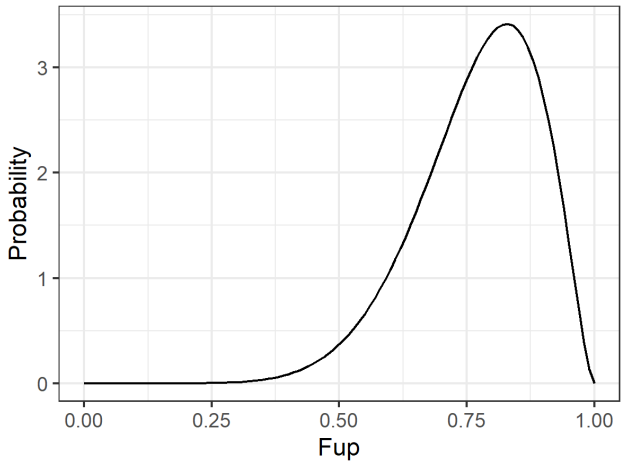
Unknown true value:
Fup for a chemical

Observed (measured) value:
MS peak areas for protein-free and protein-containing sides



Error

- MS noise
- MS calibration
- LOQ
- Non-specific binding



Wambaugh et al. (2019)

Result: *Distribution* of Fup values for a chemical

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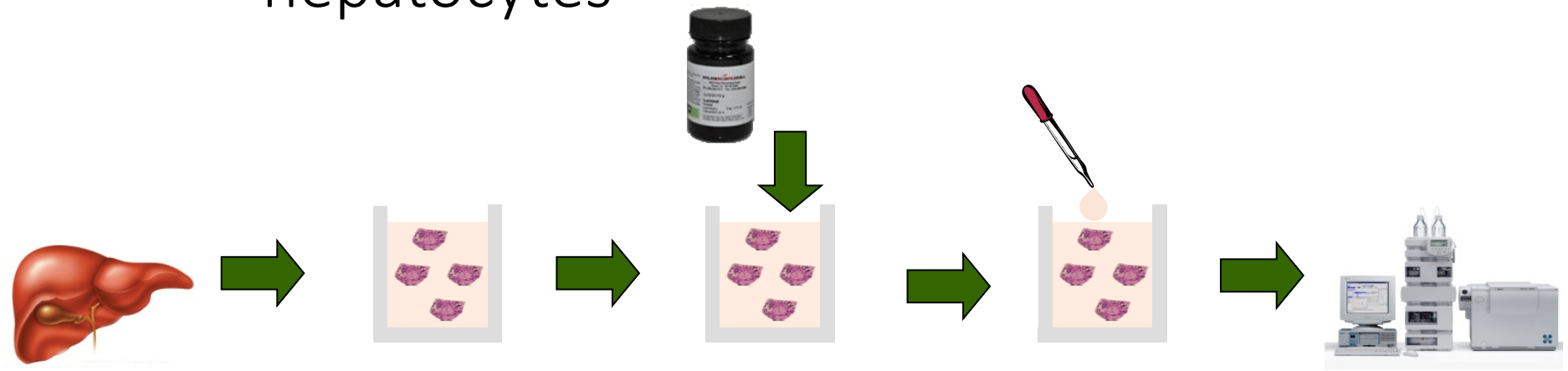
Uncertainty in CLint

CLint: How to measure *in vitro* using pooled human hepatocytes

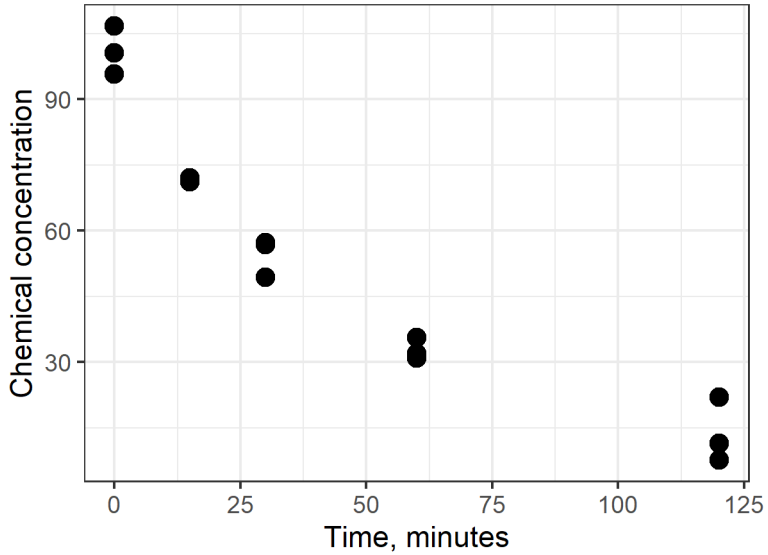


Culture donated human hepatocytes from 10 adult volunteers

Add known amount of chemical

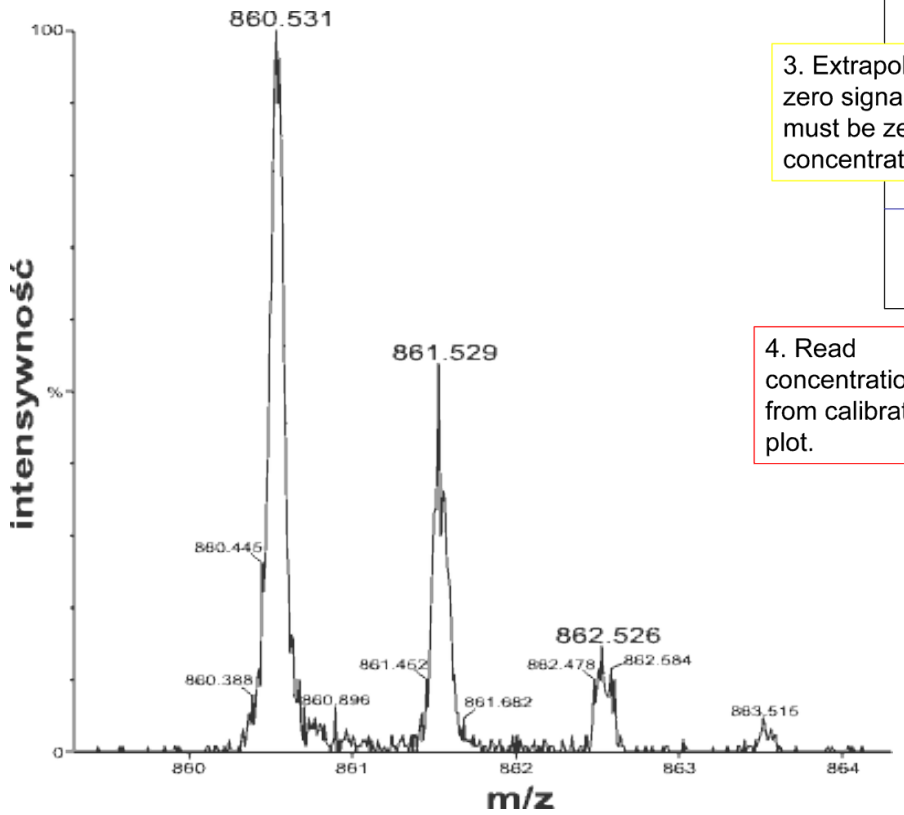
Measure chemical concentration remaining at 0, 15, 30, 60, and 120 minutes

CLint can be estimated from fitting a decaying exponential



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

Mass spec uncertainties also apply to CLint



https://commons.wikimedia.org/wiki/File:ObwiedniaPeptydu.gif
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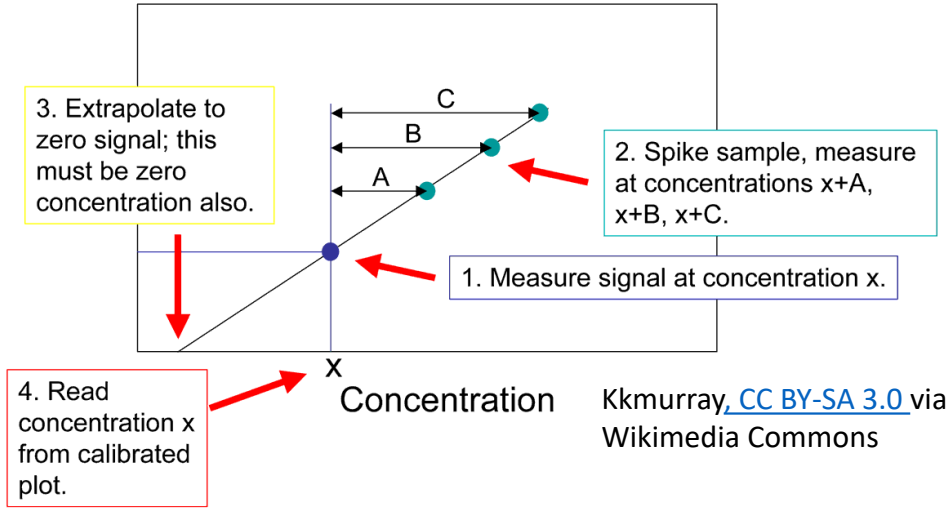

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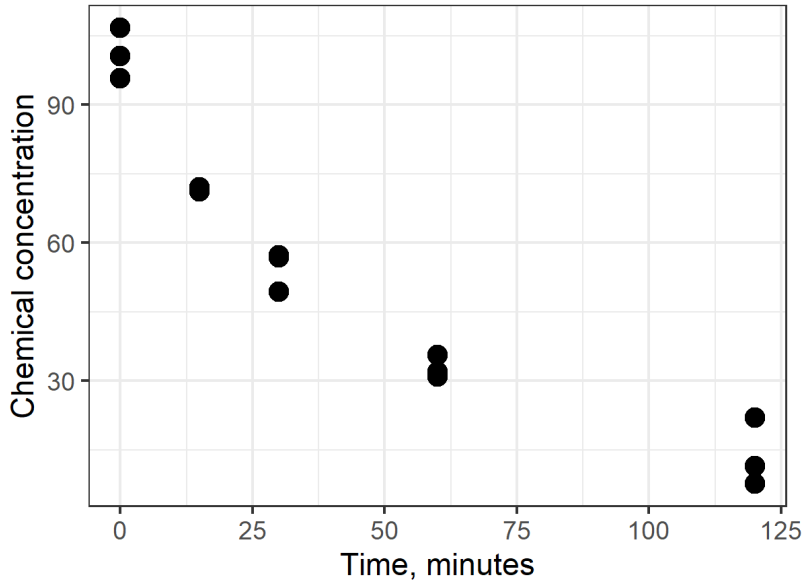
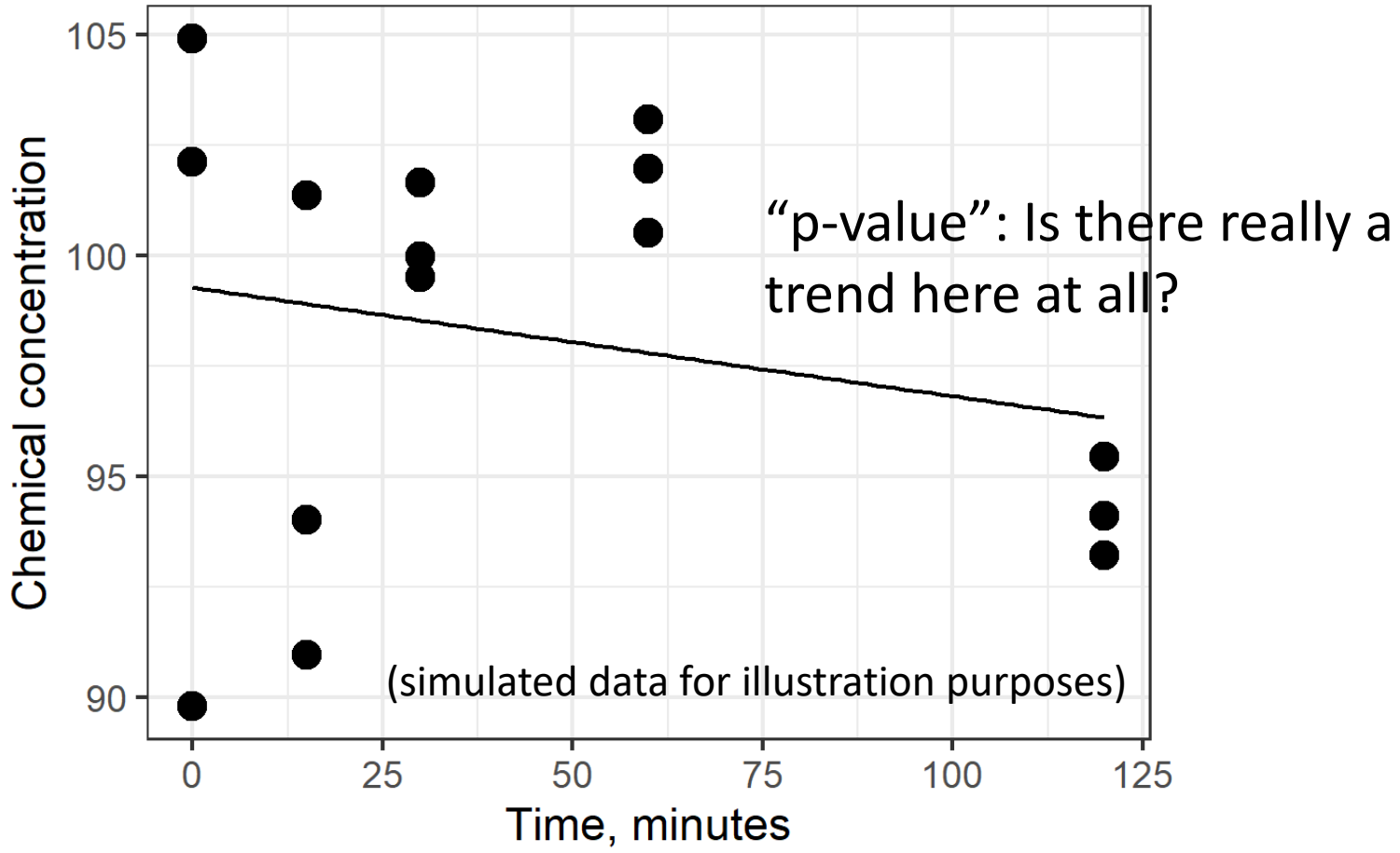
- Uncertainty in peak area
- LOQ
- Calibration curve



Wambaugh et al. (2019)

Additional uncertainty source: Is chemical really metabolized at all?



Wambaugh et al. (2019)

Additional uncertainty source: Saturable metabolism

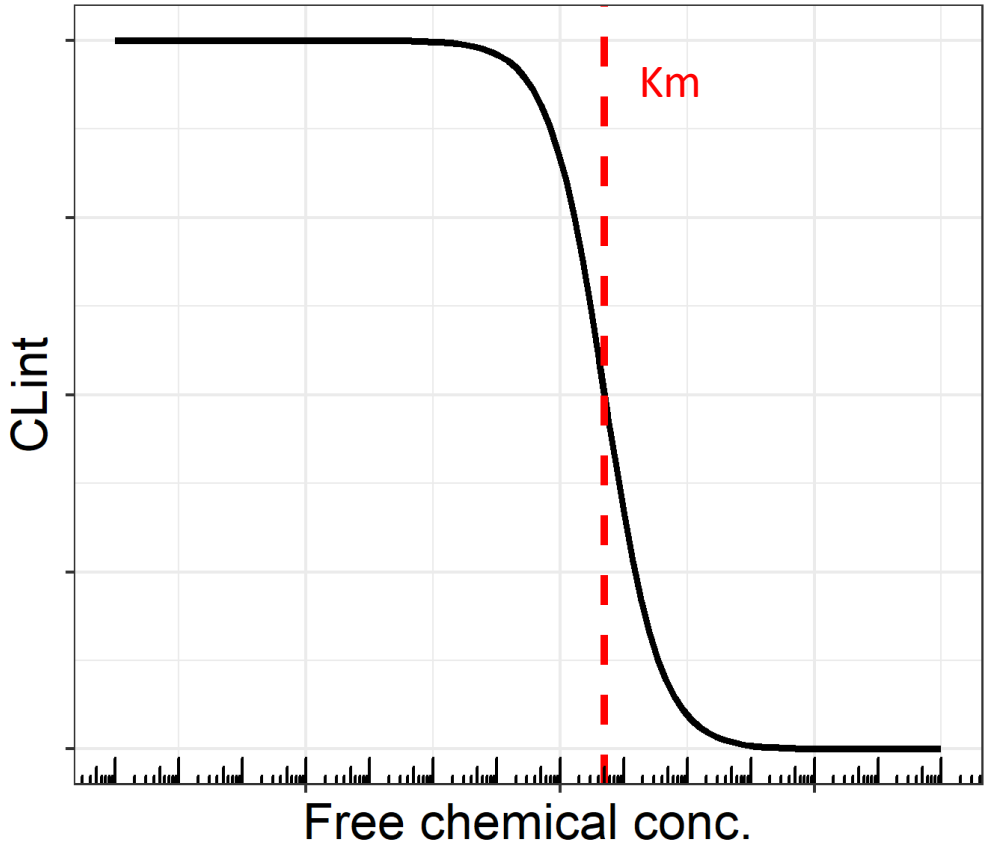
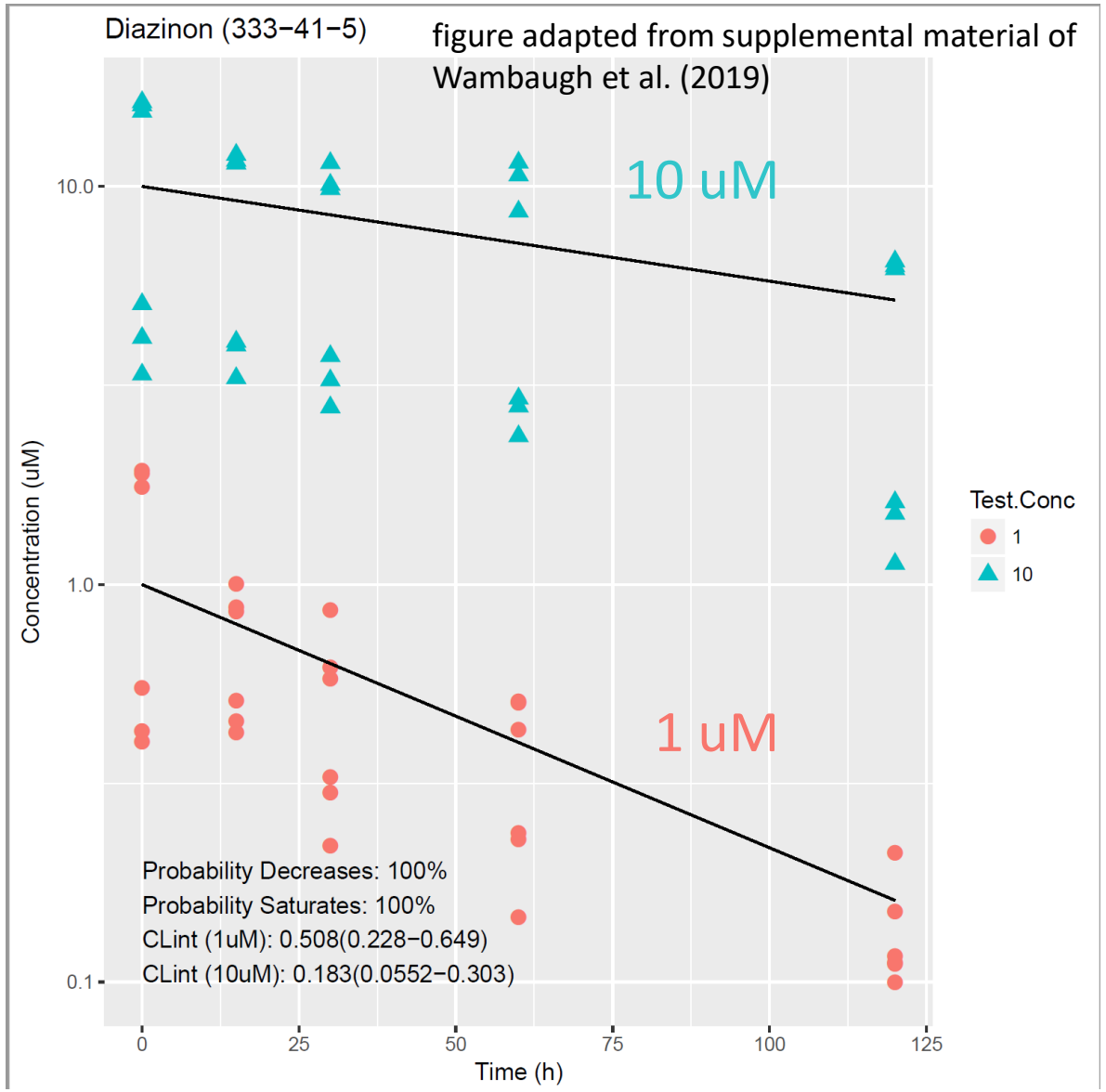


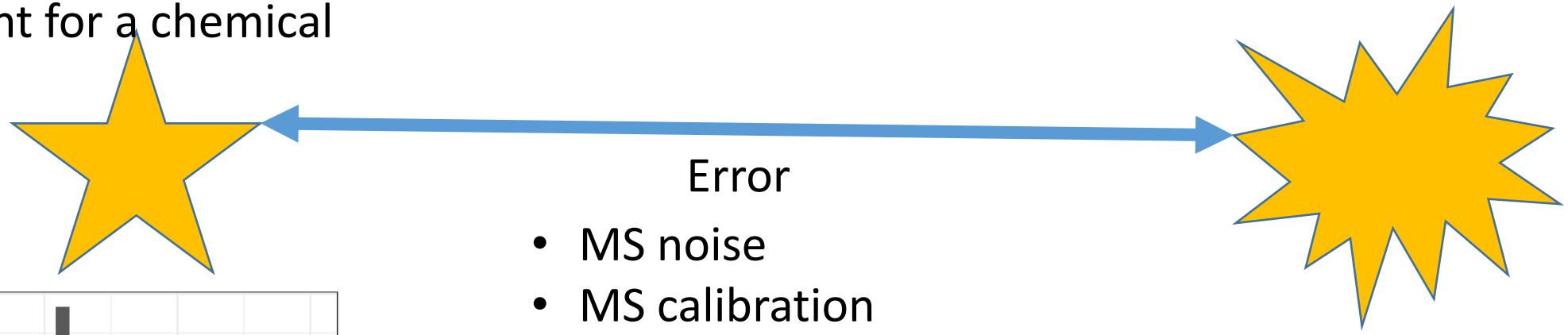
figure adapted from supplemental material of Wambaugh et al. (2019)



Bayesian inference model for Clint uncertainty

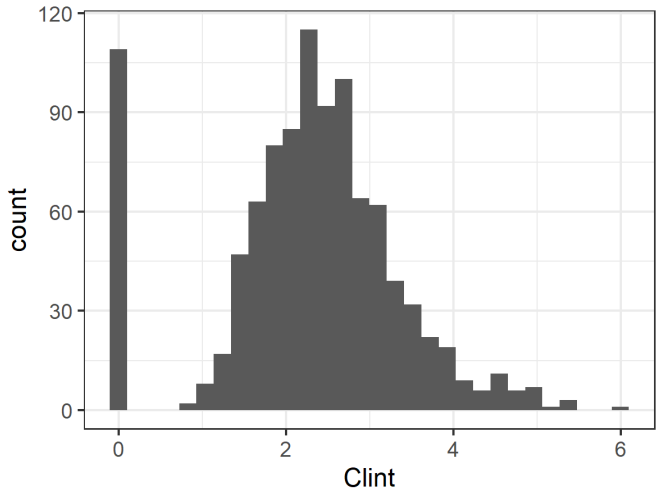
Observed (measured) value:
MS peak areas at 5 time points

Unknown true value:
Clint for a chemical



Error

- MS noise
- MS calibration
- LOQ
- Probability of no metabolism
- Probability of saturation



Wambaugh et al. (2019)

Result: *Distribution* of Clint values for a chemical

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Characterizing variability: HTTK-Pop for human TK variability

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HTTK physiological parameters

Physiological parameters
Tissue masses (including body weight)
Tissue blood flows
Glomerular filtration rate (passive renal clearance)
Hepatocellularity

Data source for population physiology: CDC NHANES

CDC NHANES = Centers for Disease Control National Health and Nutrition Examination Survey

Large, representative, ongoing survey of US population: demographics, body measures, medical examination data….

NHANES does measure:

Sex
Age
Height
Weight
Serum creatinine

NHANES does not measure:

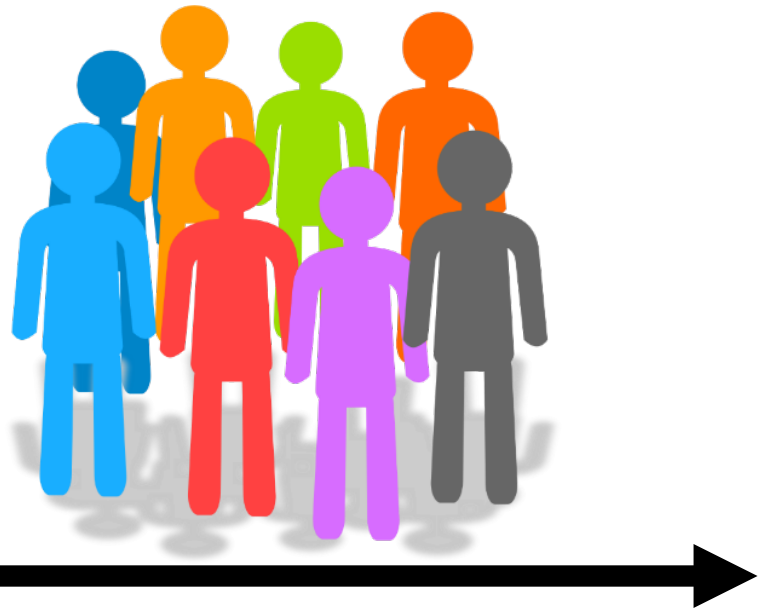
Tissue masses
Tissue blood flows
GFR (kidney function)
Hepatocellularity

Ring et al. (2017)

Correlated Monte Carlo approach to simulating population variability in physiology: HTTK-Pop

Sample NHANES measured quantities for actual NHANES individuals (capturing covariance):

- Sex
- Age
- Height
- Weight
- Serum creatinine



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

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

- 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.)

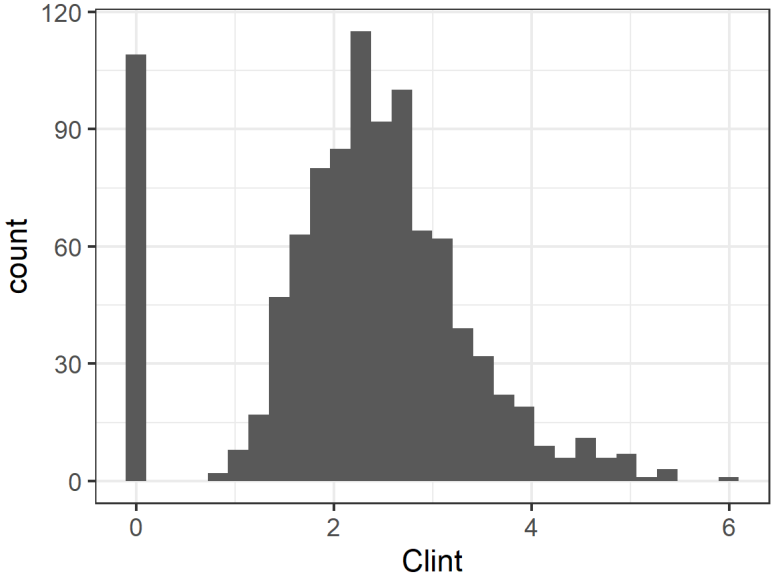
Ring *et al*. (2017)

Chemical-specific parameters have both uncertainty and variability

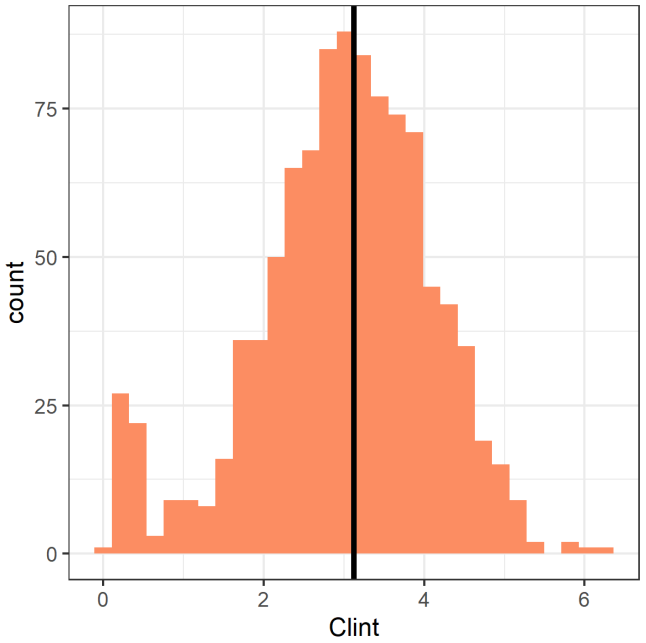
Chemical-specific parameters	
Intrinsic hepatic clearance rate (CLint)	Carry uncertainty from *in vitro* measurements **Also** have population variability: represent chemical-body interactions — vary with individual genetics, environmental factors, age, etc.
Fraction unbound to plasma protein (Fup)	

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

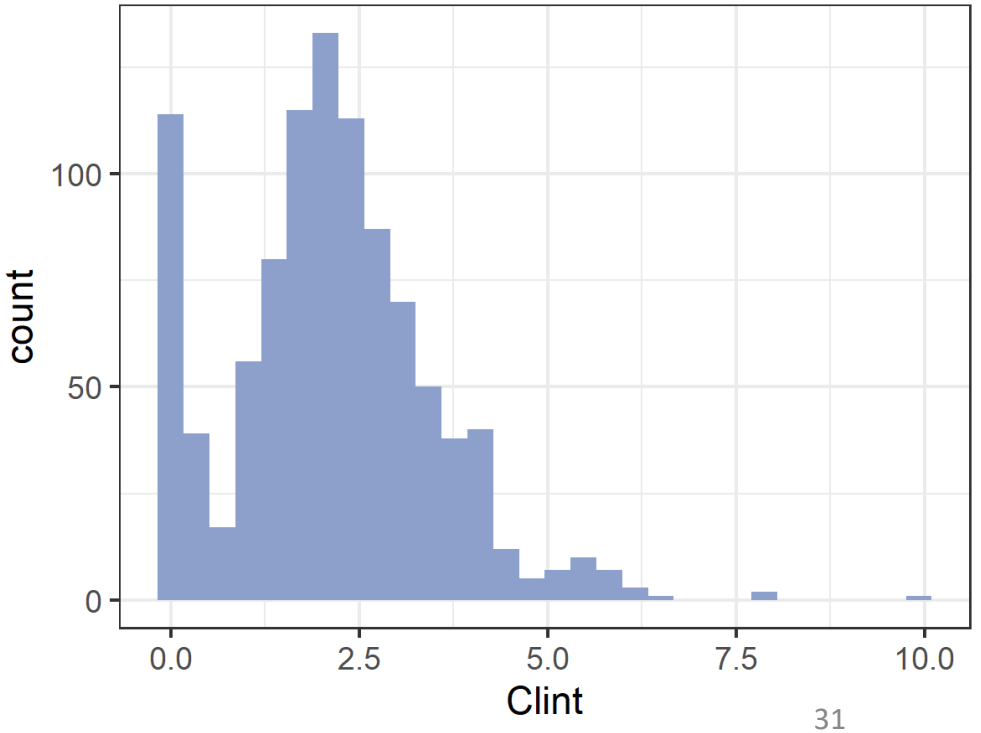


Step 2: Assume population variability (30% CV) around the sampled "population average" value from Step 1, and draw 1 sample



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"

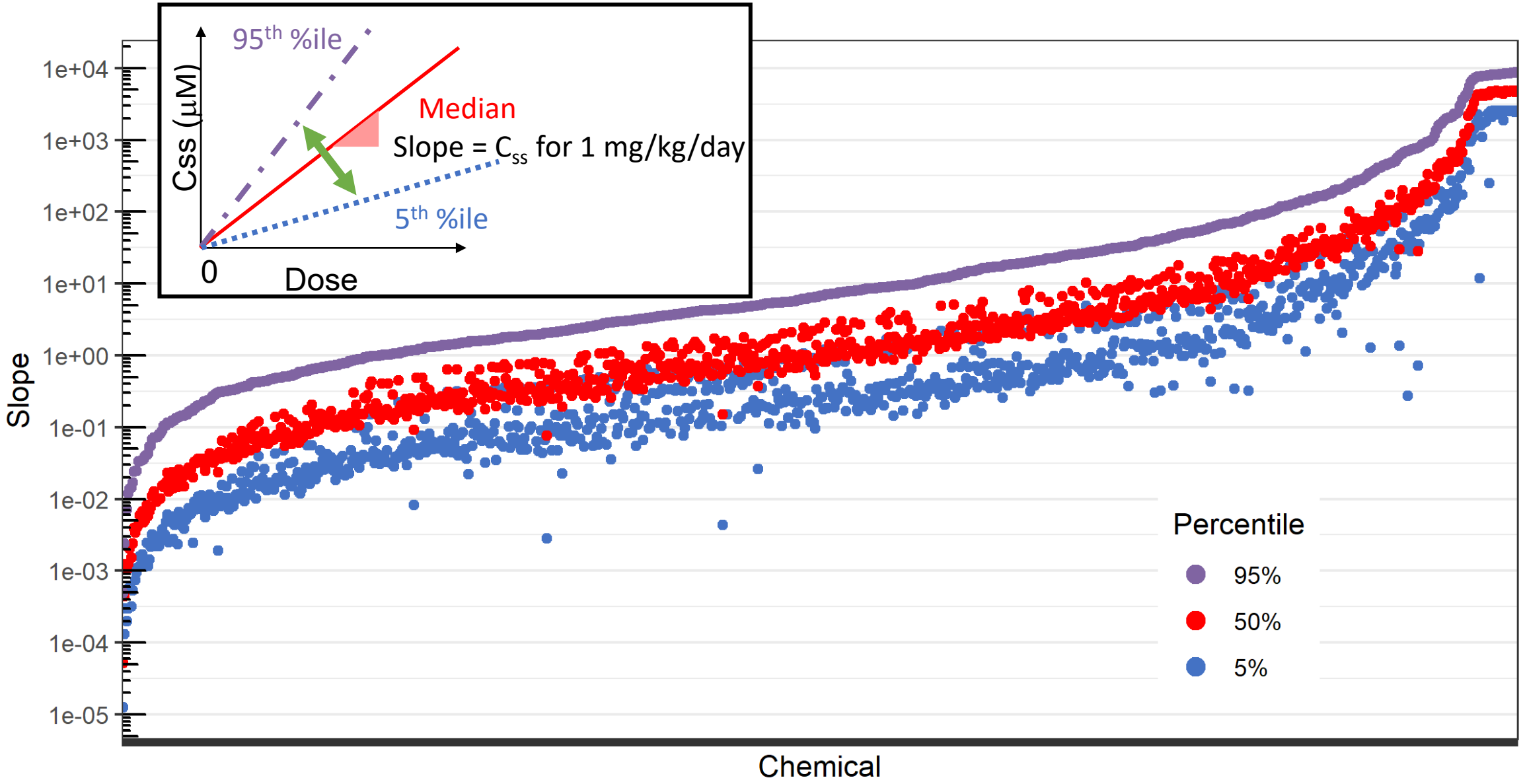
SEQN	Demographics		Body measures		Tissue volumes	Blood flows	GFR	Hepatocellularity	Fup	Clint
	Sex	Age	Ht	Wt						
67184	M	42	171	55	[…]	[…]	[…]	[…]	[…]	[…]
52034	M	0.5	73	9	[…]	[…]	[…]	[…]	[…]	[…]
64847	F	11	154	47	[…]	[…]	[…]	[…]	[…]	[…]
51787	F	22	166	87	[…]	[…]	[…]	[…]	[…]	[…]
49889	M	9	147	50	[…]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

httk R package automates this Monte Carlo sampling & model evaluation process

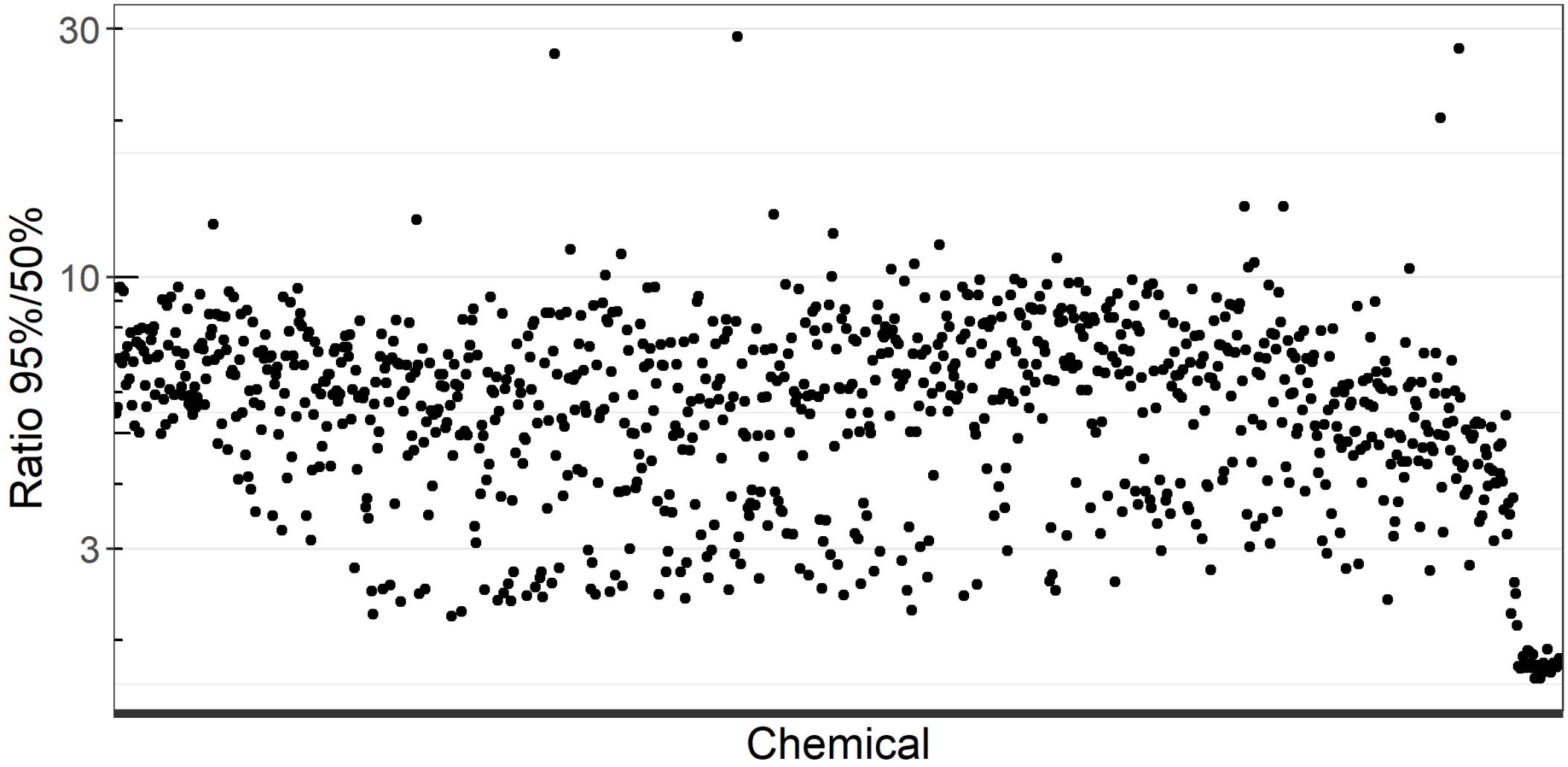
```
> library(httk)
> set.seed(42)
> #Css for 1 mg/kg/day = slope
 calc_mc_css(chem.name="benzo(a)pyrene",
                which.quantile = c(0.95, 0.5, 0.05))

Human plasma concentration returned in mg/L units
for 0.95 0.5 0.05 quantile.
   95%    50%     5%
68.510 13.070  3.742
```

Result: Percentiles of predicted Css vs. dose slope



Another way to visualize: ratio of 95th percentile to median (roughly, how wide is the Css slope distribution?)



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Relative contributions of variability & uncertainty

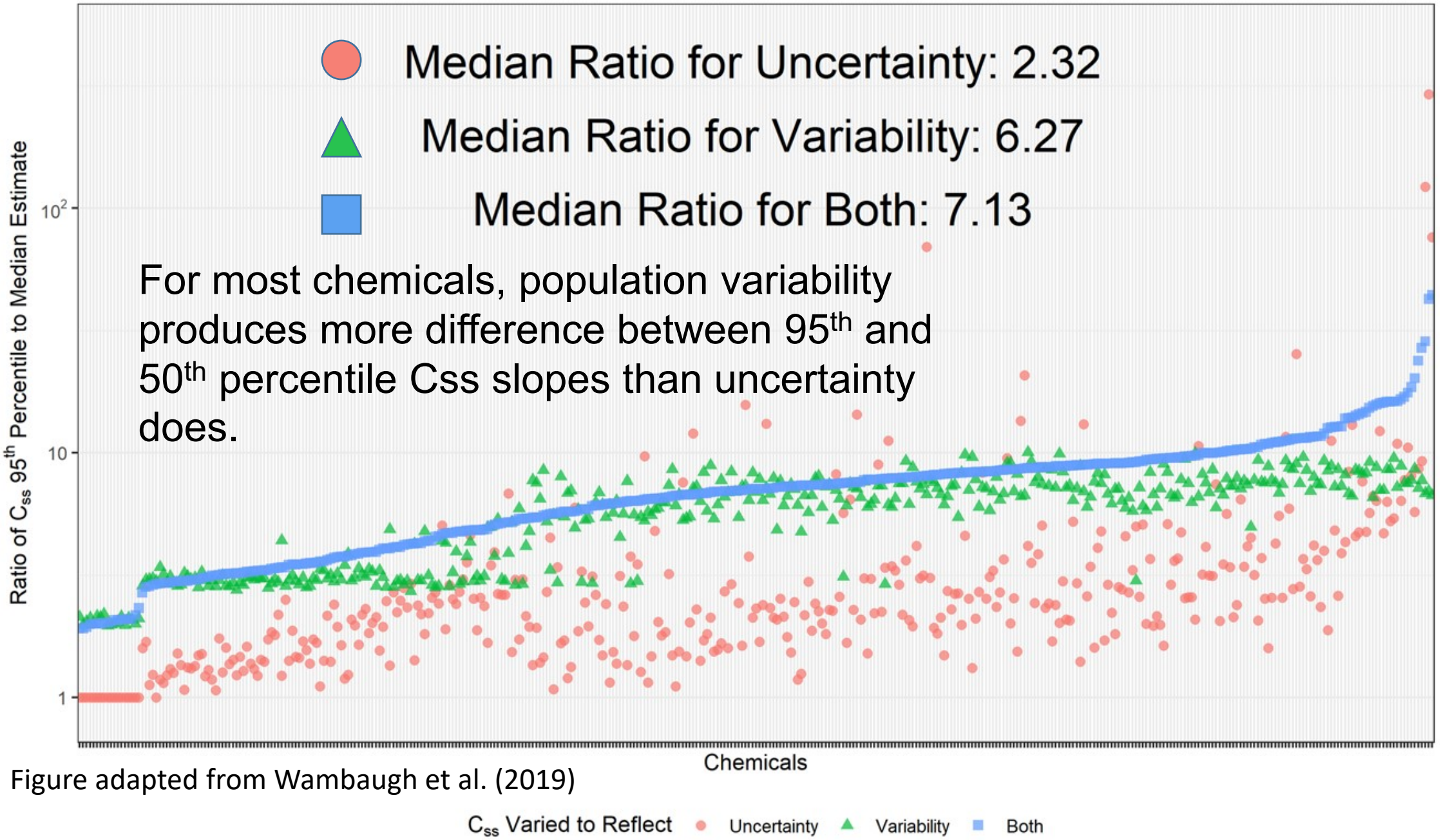

Figure adapted from Wambaugh et al. (2019)

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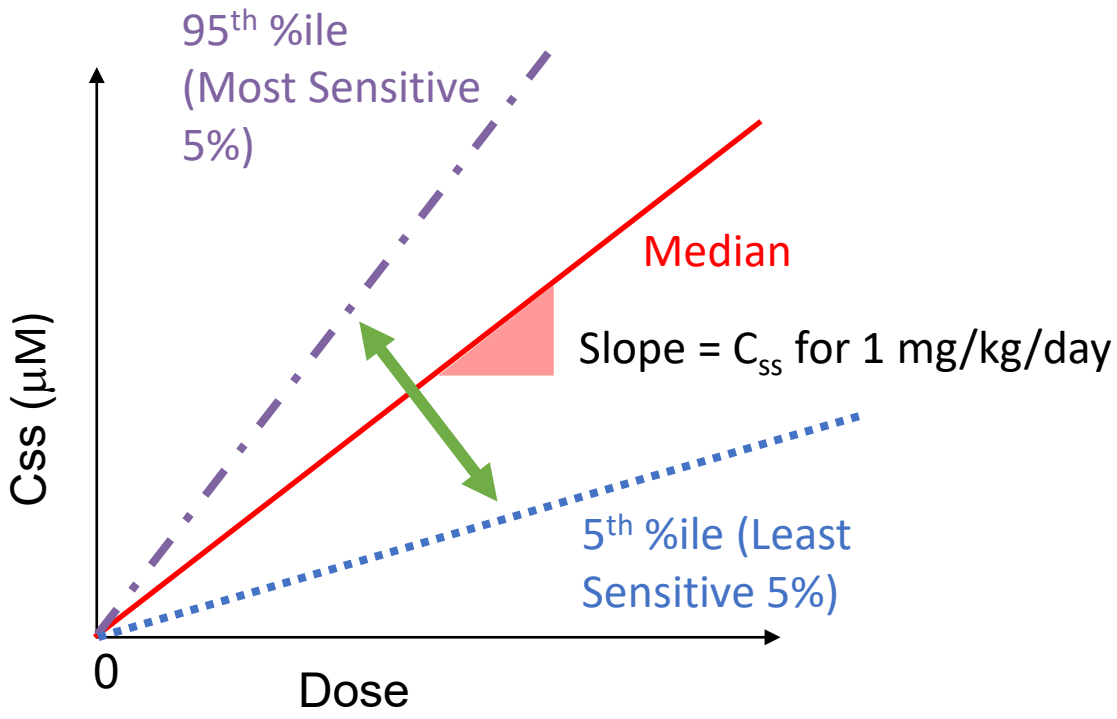
Simulating sensitive subpopulations

Identifying potentially sensitive sub-populations



Who is in the most sensitive portion of the population?

What does this slope distribution look like for kids, for example?

Or people over 65?

To answer this question: Need to model TK variability for specified sub-populations

Ring et al. (2017)

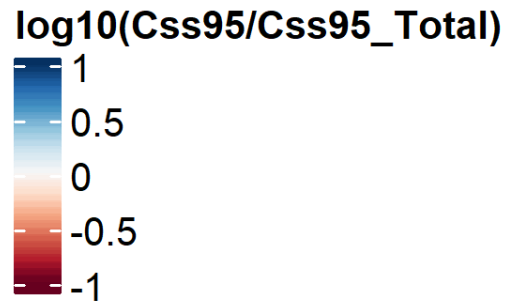
HTTK-Pop can generate simulated subpopulations with user-specified demographics

Use `httkpop.generate.args` argument to `calc_mc_css()` function: Takes a named list of arguments

Name of list element	User can specify…	Example		Default if not specified
`agelim_years`	Age limits in years	`c(6,11)`	Ages 6-11 years	All NHANES (0-79 years)
`agelim_months`	Age limits in months	`c(0,36)`	Ages 0-36 months	All NHANES (0-79 years)
`gendernum`	# of males and females	`list(Male = 1000, Female = 0)`	1000 males, 0 females	Randomly selected from NHANES
`weight_category`	BMI category	`c('Overweight', 'Obese')`	BMI > 25 (overweight & obese)	c('Underweight', 'Normal', 'Overweight', 'Obese')

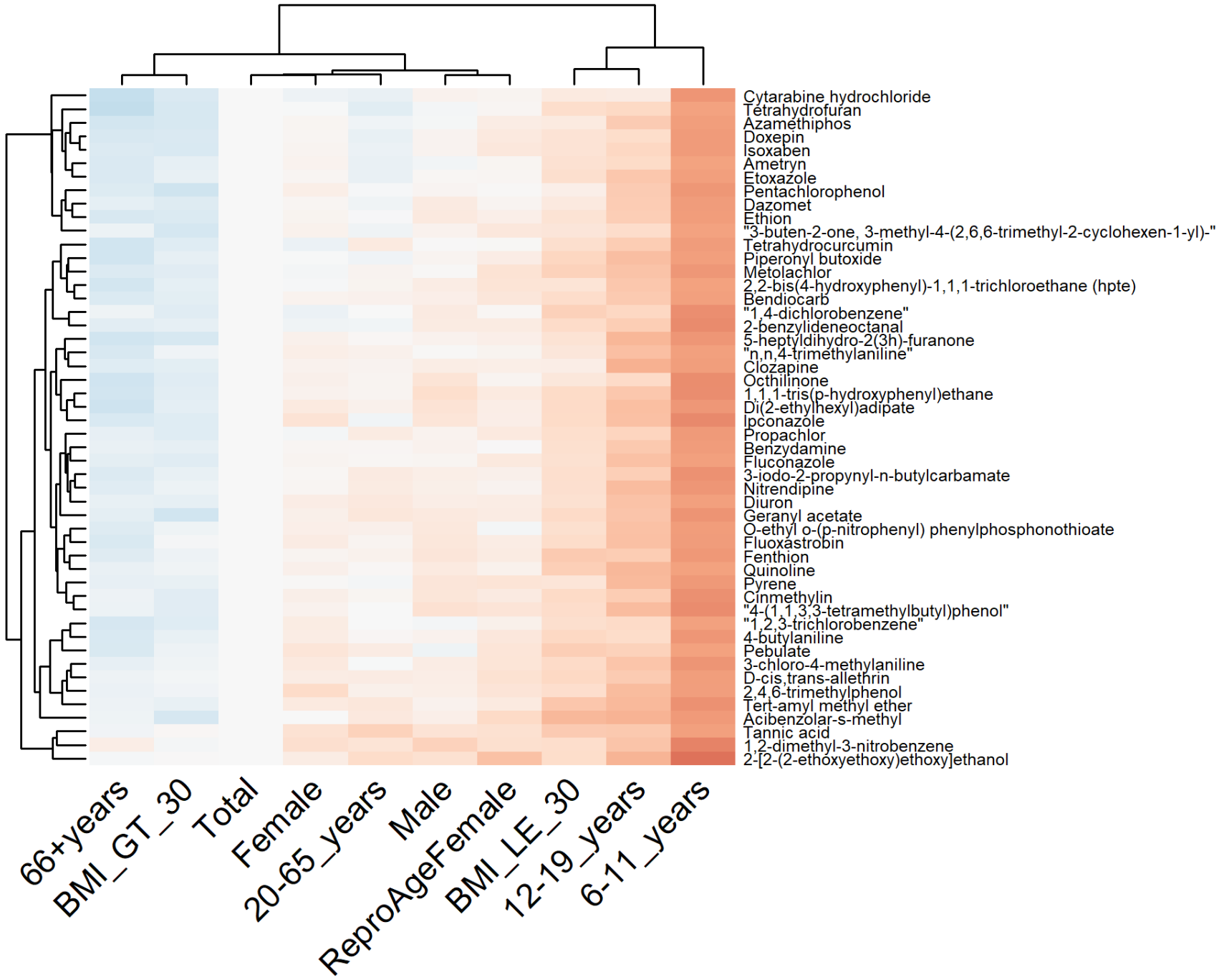
HTTK-Pop generates physiology based on NHANES respondents in the specified demographic groups

Example of Css95 differences by subpopulation



10 subgroups of interest

Heatmap: Css95 difference (subgroup vs. Total population) for 50 chemicals with largest Css95 difference in *any* subgroup



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Conclusions

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Conclusions

- Uncertainty vs. Variability in TK model parameters
  - Measurement uncertainty: Chemical-specific parameters measured *in vitro*
  - Population variability: Physiological & chemical-specific parameters
- Characterizing key uncertainty in chemical-specific TK parameters using Bayesian inference
  - Fraction unbound in plasma protein (Fup)
  - Intrinsic hepatic clearance rate (Clint)
- Characterizing variability: HTTK-Pop for human TK variability
  - Correlated Monte Carlo approach based on CDC NHANES data
- Relative contributions of uncertainty and variability to TK model predictions
  - For most chemicals, population variability has larger effect
- Simulating sensitive subpopulations
  - HTTK-Pop can simulate populations with user-specified demographics

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Thank you!

Questions?

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In vitro to *in vivo* extrapolation for decision-making

Katie Paul Friedman, PhD

August 13, 2021

Presented to the SETAC NA 2021 Continuing Education Course:
Toxicokinetic New Approach Methodologies

Overview

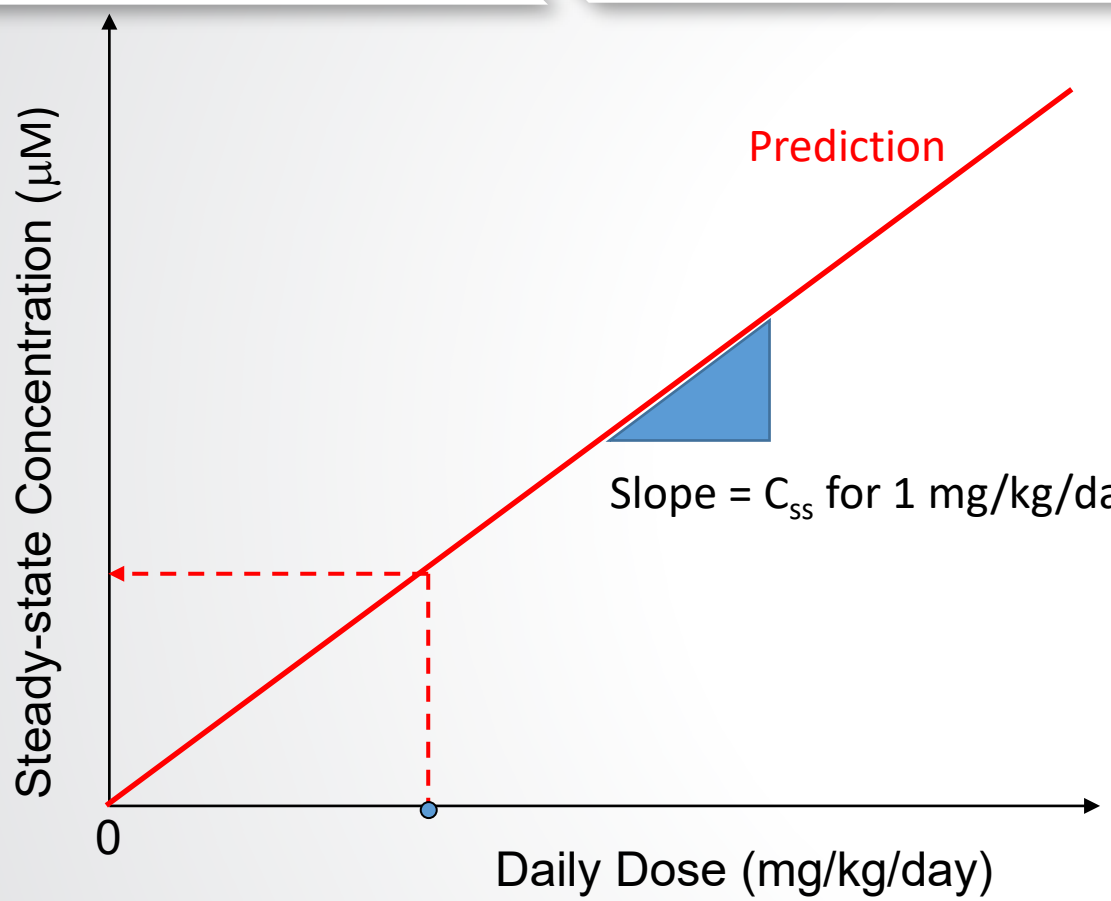
- Reverse dosimetry for in vitro to in vivo extrapolation (IVIVE)
  - Key assumptions
  - Operationalizing library(httk)

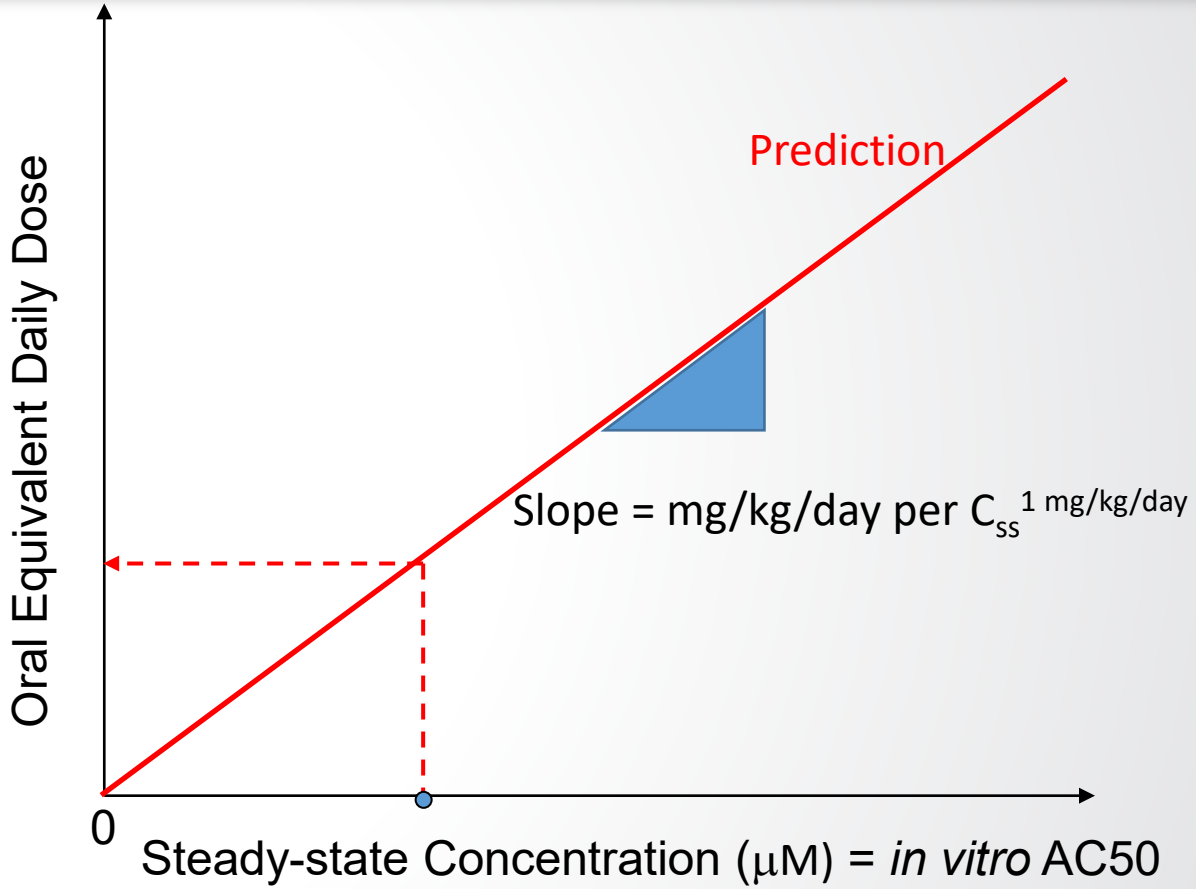
- Impacts of choices made in IVIVE on a NAM-based point of departure (POD_{NAM})
  - What are the key choices to be made in using library(httk)
  - Continuing uncertainties

- Case studies using the bioactivity:exposure ratio (BER)

Reverse dosimetry for in vitro to in vivo extrapolation (IVIVE)

Steady state in vitro-in vivo extrapolation assumption: blood::tissue partitioning ≈ cells::medium partitioning





$$C_{ss} = \frac{\text{oral dose rate}}{(GFR * F_{ub}) + \left(Q_l * F_{ub} * \frac{Cl_{int}}{Q_l + F_{ub} * Cl_{int}}\right)}$$

Wetmore *et al*. (2012)

- Swap the axes (this is the "reverse" part of reverse dosimetry)
- Can divide bioactive concentration by C_{ss} for for a 1 mg/kg/day dose to get oral equivalent dose

Derivation of PODs from NAMs: IVIVE that employs toxicokinetic extrapolation of dose

High-throughput toxicokinetic (HTTK) approaches make it possible to predict doses corresponding to *in vitro* bioactivity for thousands of chemicals.


TOXICOLOGICAL SCIENCES 125(1), 157–174 (2012)
doi:10.1093/toxsci/kfr254
Advance Access publication September 26, 2011

2012

Integration of Dosimetry, Exposure, and High-Throughput Screening Data in Chemical Toxicity Assessment

Barbara A. Wetmore,* John F. Wambaugh,† Stephen S. Ferguson,‡ Mark A. …
Kimberly Freeman,‡ Harvey J. Clewell, III,* David J. Dix,† Melvin E. Andersen,
Richard S. Judson,† Reetu Singh,* Robert J. Kavlock,† Ann M. Richard,

*The Hamner Institutes for Health Sciences, Research Triangle Park, North Carolina 27709-2137; †United …
Research and Development, National Center for Computational Toxicology, Research Triangle Park, North …
Durham, North Carolina 27703; and §Department of Environmental Sciences and Engineering, U…

Environmental Science & Technology

2017

An Intuitive Approach for Predicting … with the Tox21 10k Library

Nisha S. Sipes,*,† John F. Wambaugh,‡ Robert Pearce,‡
Jui-Hua Hsieh,# Andrew J. Shapiro,† Daniel Svoboda,§ Mi…

†National Toxicology Program, National Institute of Environmental Heal…
Park, North Carolina 27709, United States
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Triangle Park, North Carolina 27711, United States
§Sciome, Research Triangle Park, 2 Davis Drive, North Carolina 27709, United States
#Kelly Government Solutions, 111 T.W. Alexander Drive, Research Triangle Park, North Carolina 27709, United States
⊥National Exposure Research Laboratory, U.S. Environmental Protection Agency, 109 T.W. Alexander Drive, Research Triangle Park, North Carolina 27711, United States


OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 142(1), 2014, 210–224
doi: 10.1093/toxsci/kfu169
Advance Access Publication Date: August 21, 2014

2014

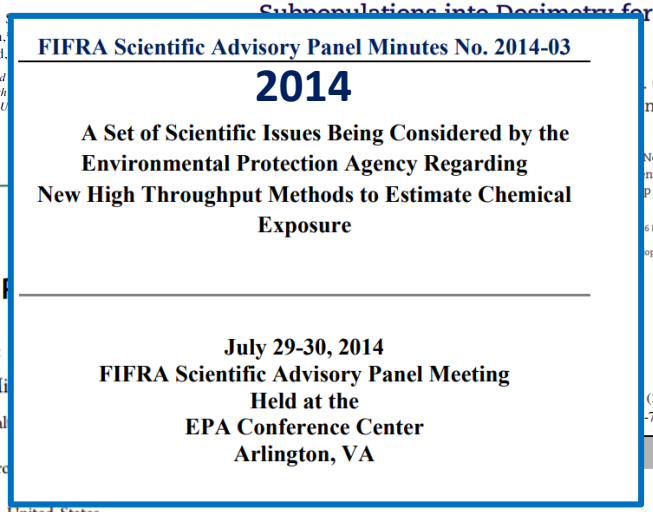
Incorporating Population Variability and Susceptible … for High-Throughput

… Clewell, III*,
…mond†, Mark A. Sochaski*,

…North Carolina 27709-2137, †United States
…nt, National Center for Computational
…p Limited (a Certara company), Blades

…6 Davis Drive, PO Box 12137, Research Triangle Park, NC

…opment, National Center for Computational Toxicology,


FIFRA Scientific Advisory Panel Minutes No. 2014-03

2014

A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding New High Throughput Methods to Estimate Chemical Exposure

July 29-30, 2014
FIFRA Scientific Advisory Panel Meeting
Held at the
EPA Conference Center
Arlington, VA


OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 147(1), 2015, 55–67
doi: 10.1093/toxsci/kfv118
Advance Access Publication Date: June 16, 2015
Research Article

2015

Toxicokinetic Triage for Environmental Chemicals

John F. Wambaugh*,†, Barbara A. Wetmore†, Robert Pearce*, Cory Strope*,‡, Rocky Goldsmith§, James P. Sluka¶, Alexander Sedykh‖, Alex Tropsha‖, Sieto Bosgra‖, Imran Shah*, Richard Judson*, Russell S. Thomas*, R. Woodrow Setzer*

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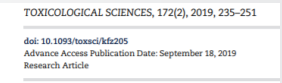
Organisation for Applied Scientific Research (TNO), 3700 AJ Ze…

†To whom correspondence should be addressed at National Center for Computatio…
Alexander Dr., Research Triangle Park, North Carolina 27711. Fax: (919) 541-1194. E-m…
Disclaimer: The views expressed in this publication are those of the authors and do …
Environmental Protection Agency. Reference to commercial products or services doe…


OXFORD SOT Society of Toxicology academic.oup.com/toxsci


TOXICOLOGICAL SCIENCES, 172(2), 2019, 235–251
doi: 10.1093/toxsci/kfz205
Advance Access Publication Date: September 18, 2019
Research Article

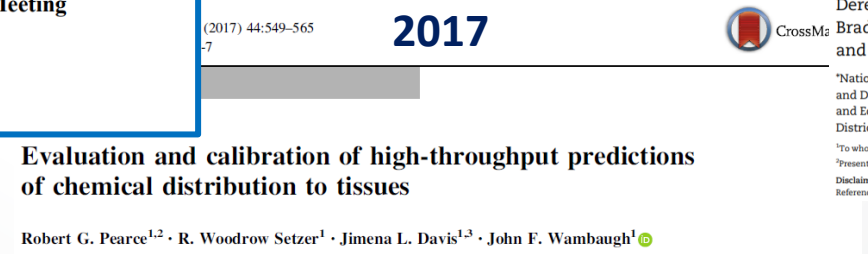
2019

Assessing Toxicokinetic Uncertainty and Variability in Risk Prioritization

John F. Wambaugh ,*,1 Barbara A. Wetmore,† Caroline L. Ring ,*,‡,2 Chantel I. Nicolas,*,‡,§ Robert G. Pearce,*,‡ Gregory S. Honda,*,‡ Roger Dinallo,¶ Derek Angus,¶ Jon Gilbert,¶ Teresa Sierra,¶ Akshay Badrinarayanan,¶ Bradley Snodgrass,¶ Adam Brockman,¶ Chris Strock,¶ R. Woodrow Setzer,* and Russell S. Thomas *

*National Center for Computational Toxicology; †National Exposure Research Laboratory, Office of Research and Development, U.S. EPA, Research Triangle Park, North Carolina 27711; ‡Oak Ridge Institute for Science and Education, Oak Ridge, Tennessee 37831; §Office of Pollution Prevention and Toxics, U.S. EPA, Washington, District of Columbia 20460; and ¶Cyprotex US, LLC, Watertown, Massachusetts 02472

1To whom correspondence should be addressed at 109 T.W. Alexander Dr./, NC 27711. Fax: (919) 541-1194. E-mail: wambaugh.john@epa.gov.
2Present address: ToxStrategies, Austin, TX 78759.
Disclaimer: The views expressed in this publication are those of the authors and do not necessarily represent the views or policies of the U.S. EPA. Reference to commercial products or services does not constitute endorsement.


(2017) 44:549–565
-7

CrossMa

2017

Evaluation and calibration of high-throughput predictions of chemical distribution to tissues

Robert G. Pearce[1,2] · R. Woodrow Setzer[1] · Jimena L. Davis[1,3] · John F. Wambaugh[1]

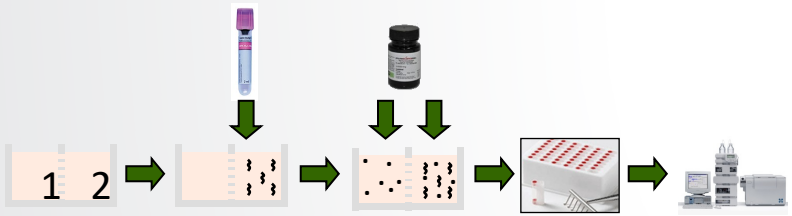
A subset of the papers describing the development of a high-throughput toxicokinetic approach

Reverse dosimetry can be leveraged in IVIVE to estimate the exposure that would produce the plasma concentration corresponding to bioactivity

High throughput toxicokinetics (HTTK)

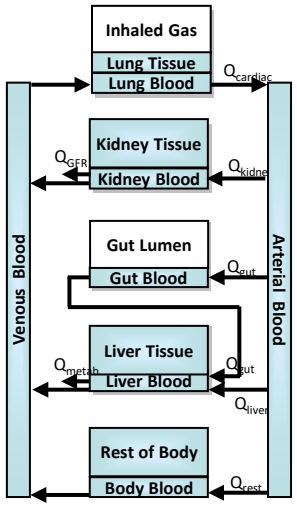
in vitro toxicokinetic data

Hepatic clearance from suspended hepatocytes



Plasma protein binding

Generic toxicokinetic models



httk

Some high-level assumptions commonly employed:

(1) bioactive nominal *in vitro* assay concentration ~ *in vivo* plasma concentration that would correspond to a similar effect;

(2) external exposures (in mg/kg/day units) that may have resulted in that plasma concentration can be constructed using estimates of species-specific physiology and Phase I and Phase II enzyme-driven hepatic clearance; and,

(3) Often, we expect that plasma concentration can be approximated by steady-state kinetics (unless we have enough information to use other dose metrics).

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

Simplifying assumptions for a steady-state model

- 100% bioavailability (all of an oral dose is received by the liver through the portal vein);
- No extrahepatic metabolism: the liver is the only source of chemical clearance from the body by metabolism;
- Hepatic metabolism is first order (proportional to concentration) and does not saturate;
- Renal clearance is proportional to fraction unbound in plasma and glomerular filtration rate (i.e., no active transport); and,
- No biliary excretion or enterohepatic recirculation occurs.

With these assumptions, HTTK models have demonstrated reasonable accuracy in predicting relevant TK endpoints, for example plasma concentrations over time (AUC) (R^2 = 0.62) and maximum plasma concentrations (Cmax) (R^2 = 0.48) (Wambaugh et al., 2018).

AED values in mg/kg/day units were calculated using the following equation:

$$Eq.2: \quad AED_{50}\left(\frac{\frac{mg}{kg}}{day}\right) = AC_{50}(\mu M) * \frac{1\frac{\frac{mg}{kg}}{day}}{Css_{50}}$$

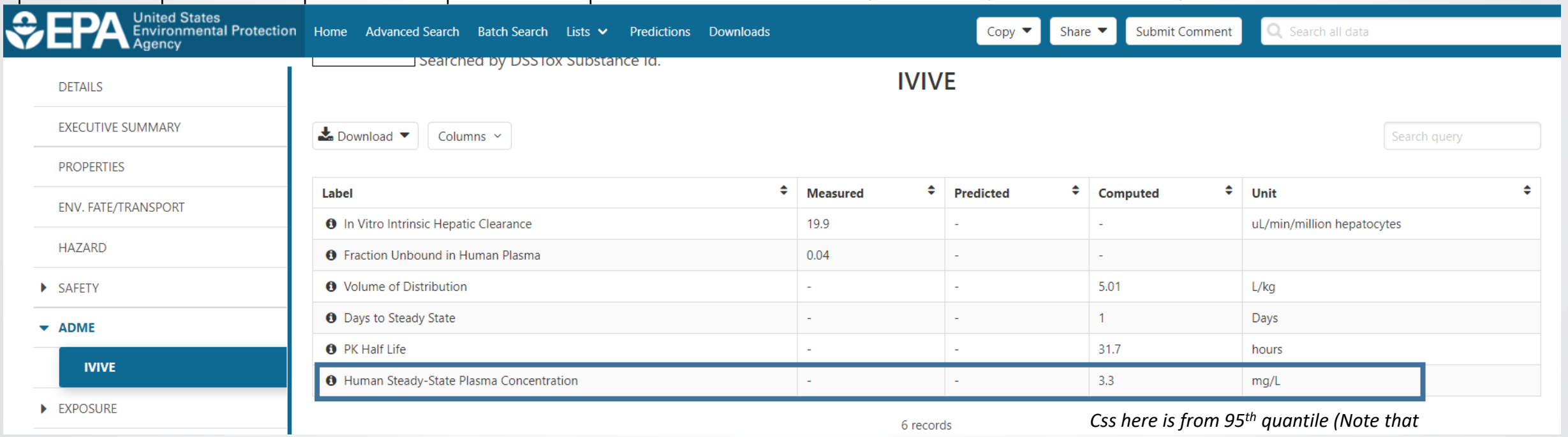
Where the Css (steady-state concentration) values for the median individual based on Monte Carlo simulation of species-specific physiological parameters (Css_{50}) (Pearce et al. 2017) were generated using the 3-compartment steady state model.

A simple approach for using the CompTox Chemicals Dashboard to estimate a POD_{NAM}

- Operationally, the httk R package (v 2.0.4) can be downloaded from CRAN or GitHub for reproducible generation of administered equivalent doses (AEDs).
- AC50 or LEC (micromolar) * (1 mg/kg/day/Css (micromolar)) = AED prediction
- Httk package optionally implements multiple models that can have increasing complexity based on data available (e.g., using pbtk model or including interindividual toxicokinetic variability).

$$\frac{3.3\ \cancel{mg}}{L} \times \frac{\cancel{g}}{1000\ \cancel{mg}} \times \frac{\cancel{mol}}{228.291\ \cancel{g}} \times \frac{1e6\ \mu mol}{\cancel{mol}} = 14.45523\ \mu mol/L = \mu M$$

$$\frac{0.1\ \cancel{\mu M}}{} \times \frac{1\ mg/kg/day}{14.45523\ \cancel{\mu M}} = \mathbf{0.007\ mg/kg/day = AED95}$$



Css here is from 95th quantile (Note that 95th concentration quantile is the same population as the 5th dose quantile).

A simple operational use of library(httk)

Default micromolar concentration; this is the in vitro point of departure you want to use

Which quantile from Monte Carlo steady-state simulation (for Css). 95th concentration quantile produces the 5th dose quantile.

Which generic toxicokinetic model to use?

```
> set.seed(12345)
> library(httk)
> calc_mc_oral_equiv(0.1, dtxsid='DTXSID7020182', species = 'Human', which.quantile = c(0.5), output.units = 'mgpkgpday', restrictive.clearance = TRUE, model = '3compartmentss')
uM concentration converted to mgpkgpday dose for 0.5 quantile.
    50%
0.04836
```

'Rat', 'Rabbit', 'Dog', 'Mouse' or default 'Human'

Restrictive clearance indicates that chemical bound to protein is relatively unavailable for hepatic metabolism or renal excretion (whereas non-restrictive clearance assumes that chemical bound to protein rapidly disassociates from that protein for metabolism and excretion).

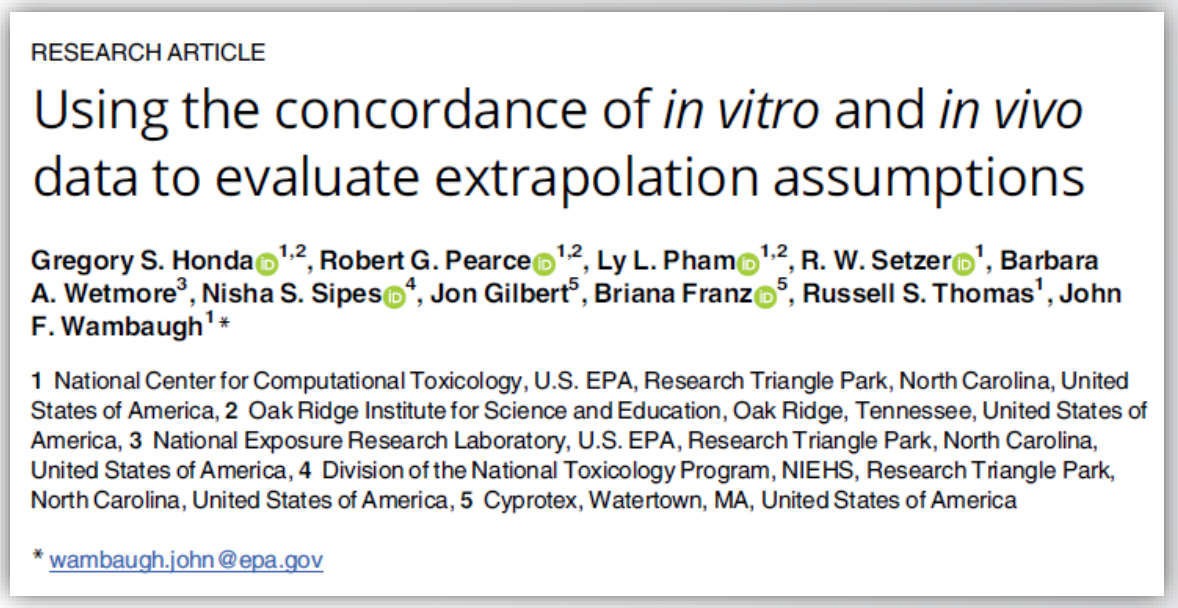
Impacts of choices in the IVIVE approach to POD_{NAM}

Some key choices

- What species physiology should be considered for the application?
- Which generic HTTK model is fit-for-purpose?
- How should interindividual variability be considered?
- What assumptions should be made about restrictive clearance and bioavailability of a chemical for bioactivity?
- To what extent will our predictions of POD be inaccurate because of differential *in vitro* partitioning of the chemical?

On selection of the species for the physiology

- Does the application require comparison to animal-based PODs or human exposure predictions or both?
- How much *in vitro* toxicokinetic data is available for the species in question/how many chemicals can IVIVE be performed?
- Another approach: is allometric scaling (based on body surface area) useful for converting human administered equivalent doses to other species?



RESEARCH ARTICLE

Using the concordance of *in vitro* and *in vivo* data to evaluate extrapolation assumptions

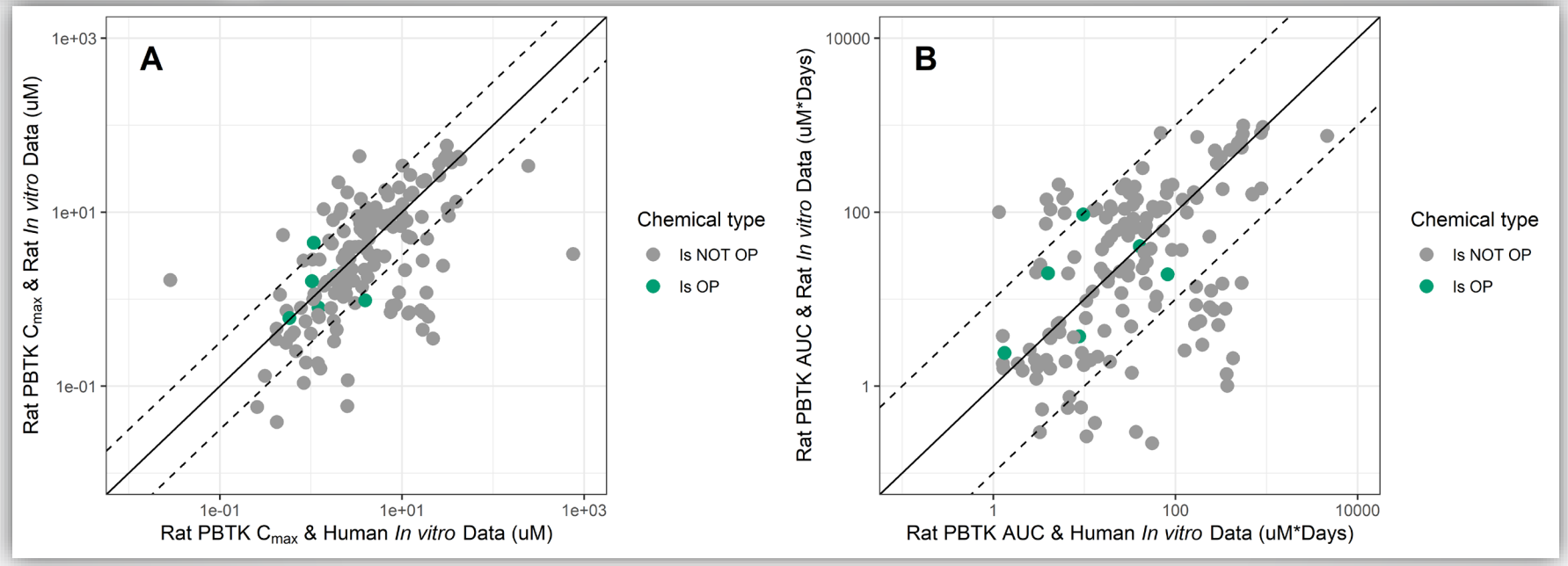
Gregory S. Honda[1,2], Robert G. Pearce[1,2], Ly L. Pham[1,2], R. W. Setzer[1], Barbara A. Wetmore[3], Nisha S. Sipes[4], Jon Gilbert[5], Briana Franz[5], Russell S. Thomas[1], John F. Wambaugh[1]*

1 National Center for Computational Toxicology, U.S. EPA, Research Triangle Park, North Carolina, United States of America, 2 Oak Ridge Institute for Science and Education, Oak Ridge, Tennessee, United States of America, 3 National Exposure Research Laboratory, U.S. EPA, Research Triangle Park, North Carolina, United States of America, 4 Division of the National Toxicology Program, NIEHS, Research Triangle Park, North Carolina, United States of America, 5 Cyprotex, Watertown, MA, United States of America

* wambaugh.john@epa.gov

With this paper came the introduction of a larger set of rat intrinsic hepatic clearance and fraction unbound in plasma data, but there is still more data available for humans.

What to do when data is missing by species?



Supplemental Appendix Figure 2, https://www.regulations.gov/docket/EPA-HQ-OPP-2020-0263/document

- In the absence of hepatic clearance values from rat hepatocytes, rat liver microsomes, or rat liver Phase I enzymes, would the use of human hepatocyte-derived hepatic clearance values be a reasonable substitute?

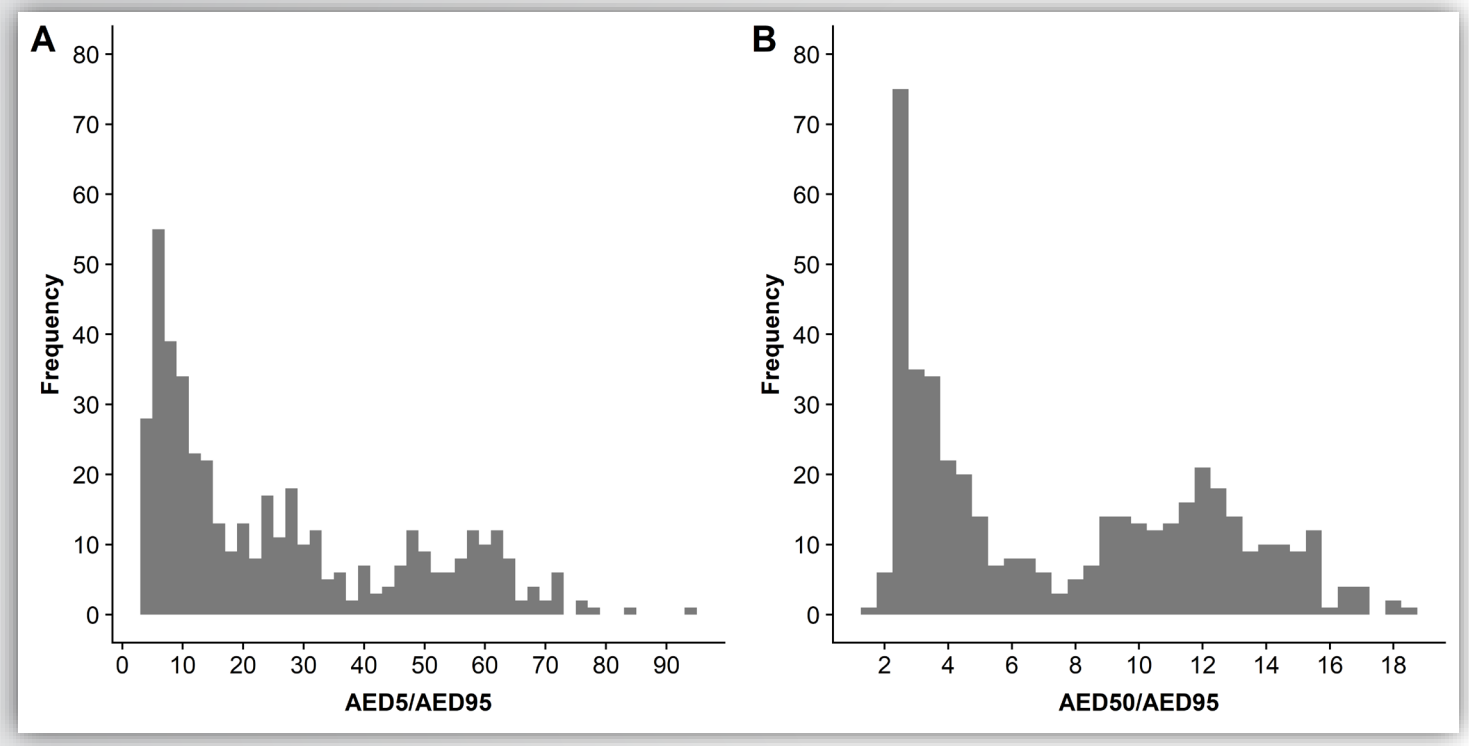
- The Cmax values obtained from the rat PBTK model, using either rat or human HTTK data for Fup and Clint, result in values that are similar (generally within ± 0.5 log10-µM) for the 151 substances compared. Similarly, the plasma AUC values that result from using rat or human HTTK data in a rat PBTK model generally were within ± 1 log10-µM.

On selection of a generic HTTK model

Models:	3-compartment steady state (3compss)	PBTK
Chemical-specific parameters	Clint only	Clint, Fup, logP, pKa
Model inputs	A single oral dose	A single oral dose
Model outputs	Steady-state blood concentrations	Time course of blood concentrations; estimate Cmax, AUC (24 hr), Cmean (AUC/time) from time course simulations
Human interindividual variability	Human physiological parameters (first order hepatic metabolic clearance; plasma protein binding; liver volume, blood flow, and cell density; and glomerular filtration rate) can be varied in a Monte Carlo simulation to estimate the dose required to achieve equivalent blood concentrations for the most to least sensitive individuals.	
Rat interindividual variability	Rat physiological parameters (rat liver volume and glomerular filtration rate) can be varied in a Monte Carlo simulation to estimate the dose required to achieve equivalent blood concentrations for the most to least sensitive individuals.	

- How many chemicals of interest have sufficient data for the model?
- Can *in silico* predictions of Fup or other parameters be used?
- Because the fraction unbound in plasma (Fup) assay fails for highly bound chemicals (Wambaugh et al., 2015), the steady state model can be used with the assumption that plasma protein binding is simply "small," i.e., typically 0.5% (Wetmore et al., 2012).

On consideration of population toxicokinetic variability



Paul Friedman et al., 2020 Supplemental Appendix; 10.1093/toxsci/kfz201

For the 448 chemicals in Paul Friedman et al., 2020, AED50 was typically 2-5 times larger than AED95, though in some cases the differences was much greater.

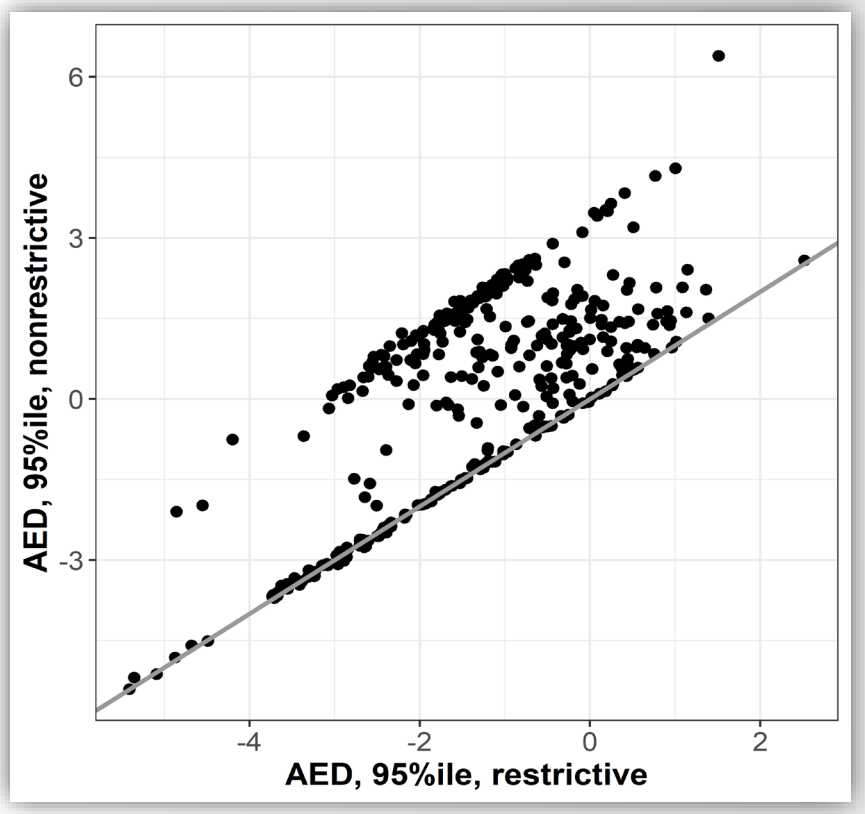
What is the application: screening or assessment?

On consideration of restrictive clearance

The degree to which a protein bound chemical is available for metabolism and excretion is likely chemical specific and a continuous function (i.e., not binary).

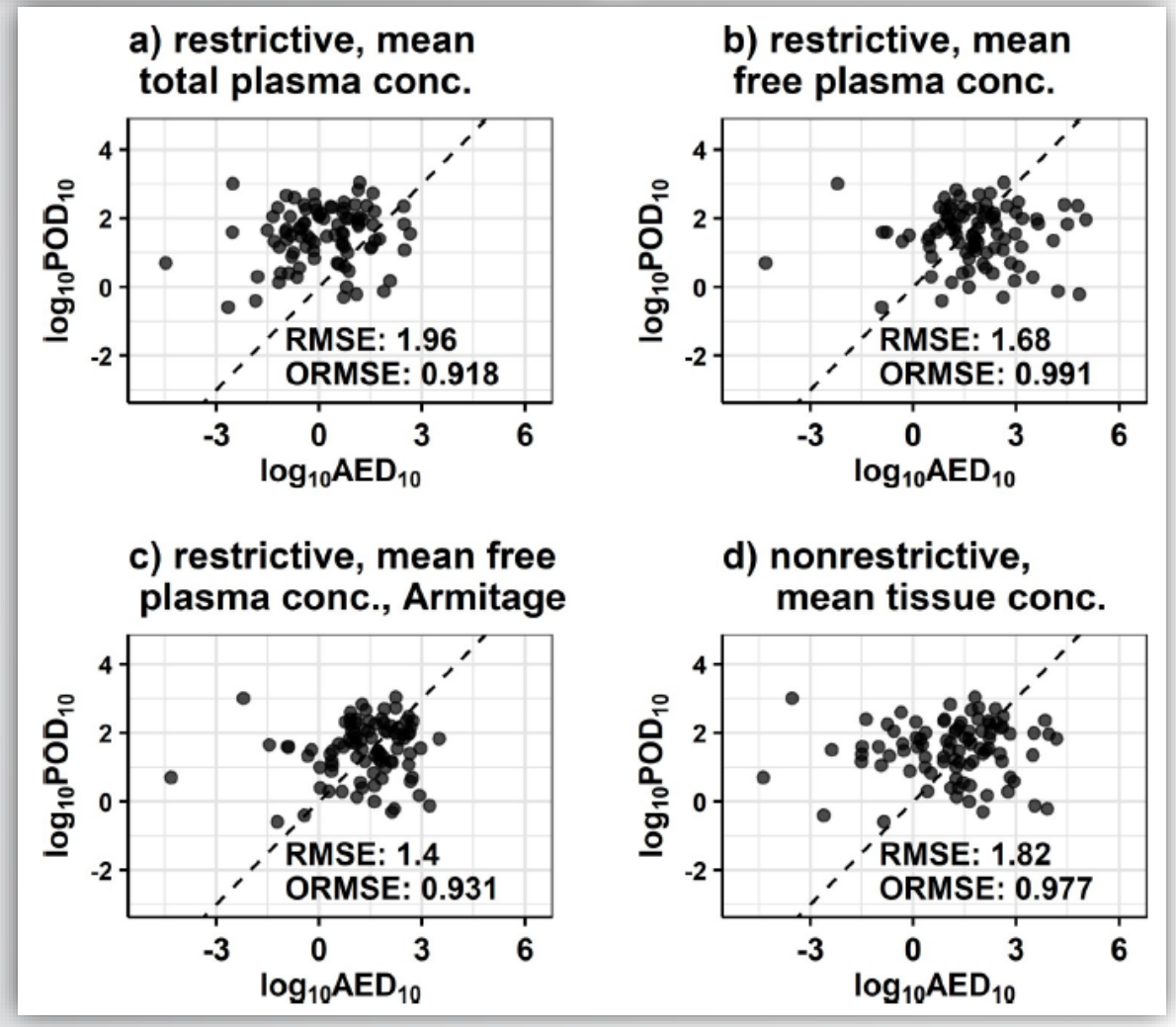
Currently, there is no way to predict or measure this property for a chemical. Restrictive clearance has been used as a conservative assumption.

Because the amount of chemical bound to protein can vary from 0-100%, the AEDs produced using a non-restrictive clearance assumption may be as much as two or three orders of magnitude greater than those produced using a restrictive clearance assumption (on a log10-mg/kg/day scale and based on current measurement ability). The amount of difference observed depends on how much of the chemical is thought to be protein-bound; the more highly protein-bound the chemical, the greater the shift observed.



Paul Friedman et al., 2020 Supplemental Appendix; 10.1093/toxsci/kfz201

Restrictive clearance with the free 'bioactive' fraction in the media may perform best



Honda et al. 2019, Figure 8; 10.1371/journal.pone.0217564

In predicting in vivo PODs, restrictive clearance with the modeled mean free (media) concentration may perform the better.

One would need good curated information and models for *in vitro* disposition of the chemical – here we have ongoing work to apply an existing model (Armitage model) to more data.

The Armitage 2014 model operationalized in Honda et al. 2019 is available in library(httk).

```
# Run the Armitage et al. (2014) model:
out <- armitage_eval(
  casrn.vector = "793-24-8",
  this.FBSf = 0.1,
  this.well_number = 384,
  nomconc = 10)

print(out)
```

What factors really influence in vitro partitioning?

- Armitage et al. (2014) suggest that in vitro partitioning relates strongly to **logK_{ow}** and concentration of **serum** in the medium

- Sorption to plastic played a smaller role in determining the cellular concentration

$$C_W = \frac{M_T}{K_{AW}V_A + V_W + K_{SaW}V_{Sa} + K_{SlW}V_{Sl} + K_{DW}V_D + K_{CW}V_C} \quad (1)$$

Mass-balance model

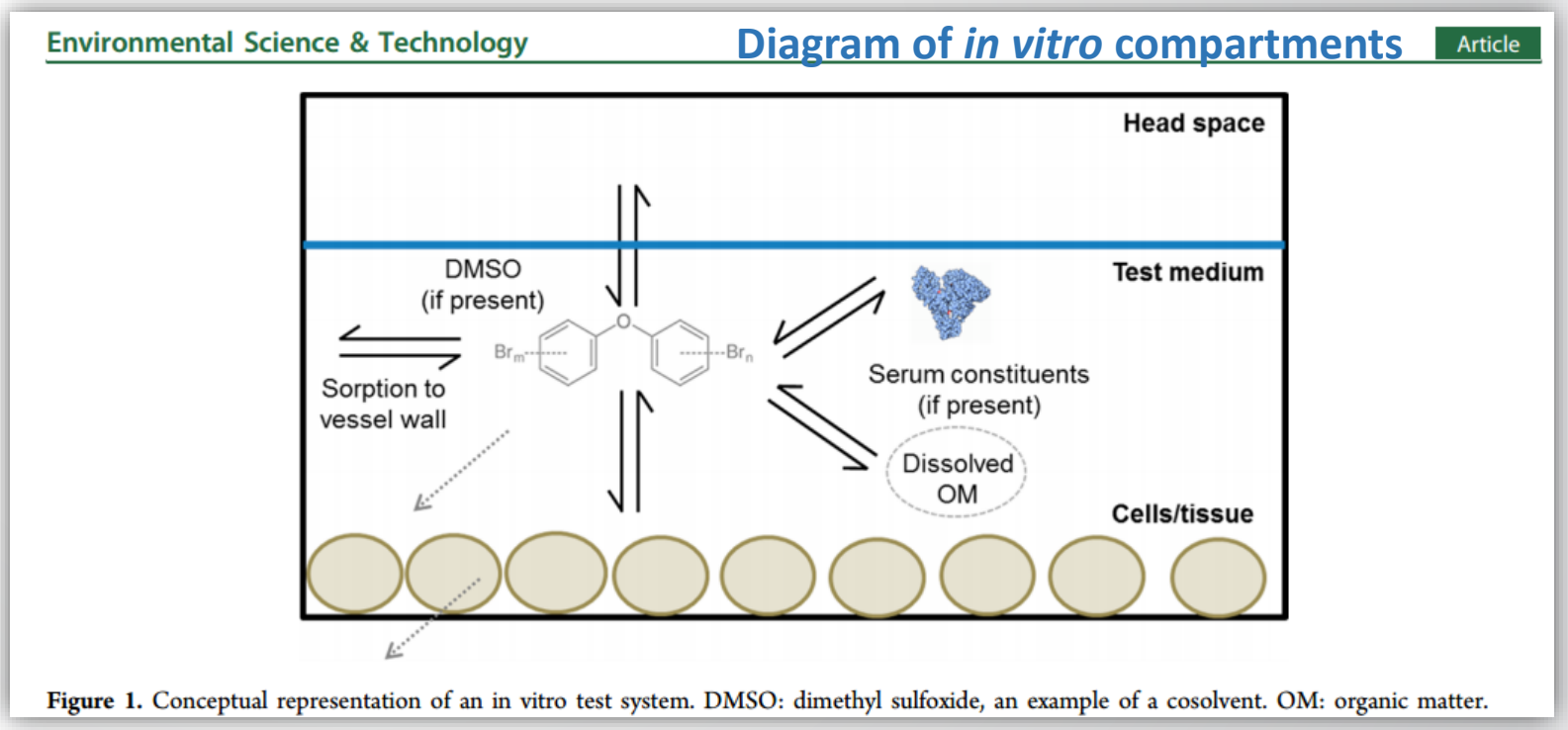


Figure 1. Conceptual representation of an in vitro test system. DMSO: dimethyl sulfoxide, an example of a cosolvent. OM: organic matter.

Armitage et al. 2014; 10.1021/es501955g

Others reinforce that lipid and protein content of media formulations may be an important determinant

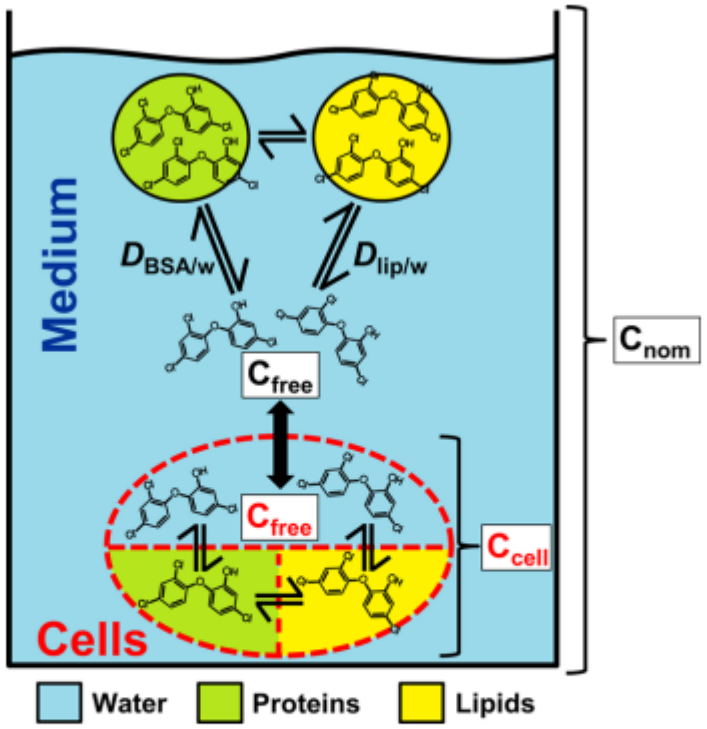


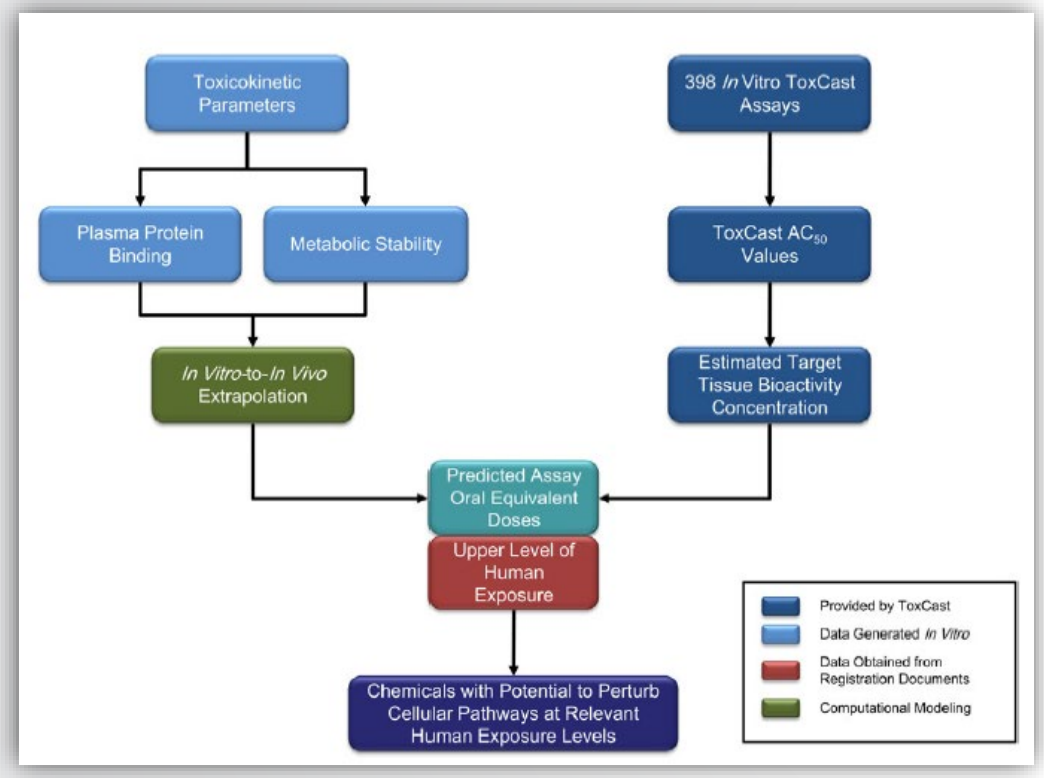
Figure 1. Mass balance model used for this study. The chemical partitioning was calculated from the distribution ratios between medium and cells at a medium pH of 7.4. Both compartments are composed of water, proteins, and lipids. Proteins and lipids are represented by BSA and lip.

- Fischer et al. (2017) suggest that in vitro partitioning relates strongly to medium formulation (lipid and protein content)
- Time may play a role; perhaps equilibrium is not always reached rapidly?
- *What we really need are some additional empirical measures and refinements to models to understand the extent to which differential partitioning is leading to large differences in cellular and media concentrations for the chemical space.*

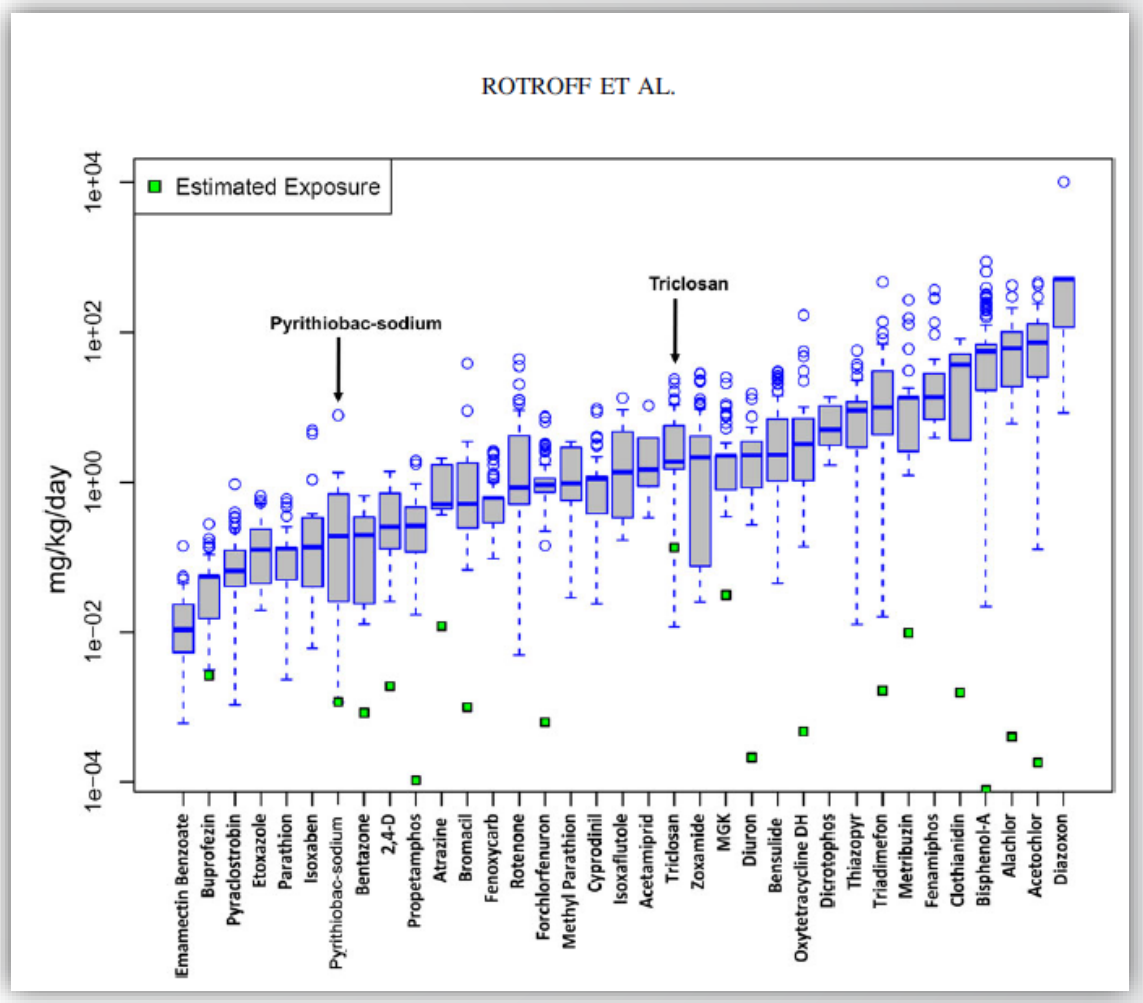
Fischer et al. 2017; Modeling Exposure in the Tox21 in Vitro Bioassays | Chemical Research in Toxicology (acs.org)

Bioactivity:exposure ratios

Bioactivity:exposure ratios are not new



Rotroff et al., 2010 10.1093/toxsci/kfq220



Many works apply HTTK to prioritization and assessment case studies

Chemical Research in Toxicology — PERSPECTIVE — pubs.acs.org/crt — **2011**

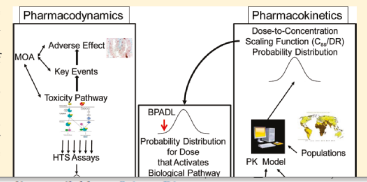
Estimating Toxicity-Related Biological Pathway Altering Doses for High-Throughput Chemical Risk Assessment

Richard S. Judson,*,† Robert J. Kavlock,† R. Woodrow Setzer,† Elaine A. Cohen Hubal,† Matthew T. Martin,† Thomas B. Knudsen,† Keith A. Houck,† Russell S. Thomas,‡ Barbara A. Wetmore,‡ and David J. Dix†

†National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, United States

‡The Hamner Institutes for Health Sciences, Research Triangle Park, North Carolina 27709, United States

ABSTRACT: We describe a framework for estimating the human dose at which a chemical significantly alters a biological pathway *in vivo*, making use of *in vitro* assay data and an *in vitro*-derived pharmacokinetic model, coupled with estimates of population variability and uncertainty. The quantity we calculate, the biological pathway altering dose (BPAD), is analogous to current risk assessment metrics in that it combines dose–response data with analysis of uncertainty and population variability to arrive at conservative exposure limits. The analogy is closest when perturbation of a pathway is a key event in the mode of action (MOA) leading to a specified adverse outcome.



OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org — **2015**

TOXICOLOGICAL SCIENCES, 148(1), 2015, 121–136
doi: 10.1093/toxsci/kfv171
Advance Access Publication Date: August 6, 2015
Research Article

Incorporating High-Throughput Exposure Predictions With Dosimetry-Adjusted *In Vitro* Bioactivity to Inform Chemical Toxicity Testing

Barbara A. Wetmore,*,1 John F. Wambaugh,† Brittany Allen,* Stephen S. Ferguson,†,2 Mark A. Sochaski,* R. Woodrow Setzer,† Keith A. Houck,† Cory L. Strope,* Katherine Cantwell,* Richard S. Judson,† Edward LeCluyse,* Harvey J. Clewell,* Russell S. Thomas,*,†,3 and Melvin E. Andersen*

*The Hamner Institutes for Health Sciences, Institute for Chemical Safety Sciences, Research Triangle Park, North Carolina 27709-2137; †United States Environmental Protection Agency, Office of Research and Development, National Center for Computational Toxicology, Research Triangle Park, North Carolina 27711; and ‡Life Technologies, ADME/Tox Division of the Primary and Stem Cell Systems Business Unit, Durham, North Carolina 27703

Toxicology in Vitro 47 (2018) 213–227
Contents lists available at ScienceDirect
Toxicology in Vitro
journal homepage: www.elsevier.com/locate/toxinvit

2018

Review

In vitro to in vivo extrapolation for high throughput prioritization and decision making

Shannon M. Bell[a], Xiaoqing Chang[a], John F. Wambaugh[b], David G. Allen[a], Mike Bartels[c,1], Kim L.R. Brouwer[d], Warren M. Casey[c], Neepa Choksi[a], Stephen S. Ferguson[f], Grazyna Fraczkiewicz[g], Annie M. Jarabek[b], Alice Ke[h], Annie Lumen[i], Scott G. Lynn[j], Alicia Paini[k], Paul S. Price[b], Caroline Ring[1,2], Ted W. Simon[m], Nisha S. Sipes[f], Catherine S. Sprankle[a], Judy Strickland[a], John Troutman[n], Barbara A. Wetmore[o,3], Nicole C. Kleinstreuer[c,*]

Contents lists available at ScienceDirect
Food and Chemical Toxicology
journal homepage: www.elsevier.com/locate/foodchemtox

2019

Profiling 58 compounds including cosmetic-relevant chemicals using ToxRefDB and ToxCast

Ly L. Pham[a,b], Lisa Truong[a,b,c], Gladys Ouedraogo[d], Sophie Loisel-Joubert[e], Matthew T. Martin[a,f], Katie Paul Friedman[a,*]

[a] National Cent
[b] ORISE Postdo
[c] Currently at O
[d] L'Oréal Safety
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High-throughput screening tools facilitate calculation of a combined exposure-bioactivity index for chemicals with endocrine activity

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TOXICOLOGICAL SCIENCES, 2019, 1–24
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Advance Access Publication Date: September 18, 2019
Research Article

Utility of *In Vitro* Bioactivity as a Lower Bound Estimate of *In Vivo* Adverse Effect Levels and in Risk-Based Prioritization

Katie Paul Friedman ,*,1 Matthew Gagne,† Lit-Hsin Loo,‡ Panagiotis Karamertzanis,§ Tatiana Netzeva,§ Tomasz Sobanski,§ Jill A. Francoeur,¶ Ann M. Richa
Angrish,
Bahadori
Rasenbe

PLOS ONE — **2020**

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2020

The role of fit-for-purpose assays within tiered testing approaches: A case study evaluating prioritized estrogen-active compounds in an in vitro human uterotrophic assay

Tyler Beames[a,*,1], Marjory Moreau[a,1], L. Avery Roberts[b], Kamel Mansouri[b], Saad Haider[a], Marci Smeltz[a], Chantel I. Nicolas[b], Daniel Doheny[b], Martin B. Phillips[a], Miyoung Yoon[b,2], Richard A. Becker[c], Patrick D. McMullen[a], Melvin E. Andersen[a], Rebecca A. Clewell[b,3], Jessica K. Hartman[a,*]

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RESEARCH ARTICLE

Using the concordance of *in vitro* and *in vivo* data to evaluate extrapolation assumptions

Gregory S. Honda[1,2], Robert G. Pearce[1,2], Ly L. Pham[1,2], R. W. Setzer[1], Barbara A. Wetmore[3], Nisha S. Sipes[4], Jon Gilbert[5], Briana Franz[5], Russell S. Thomas[1], John F. Wambaugh[1]*

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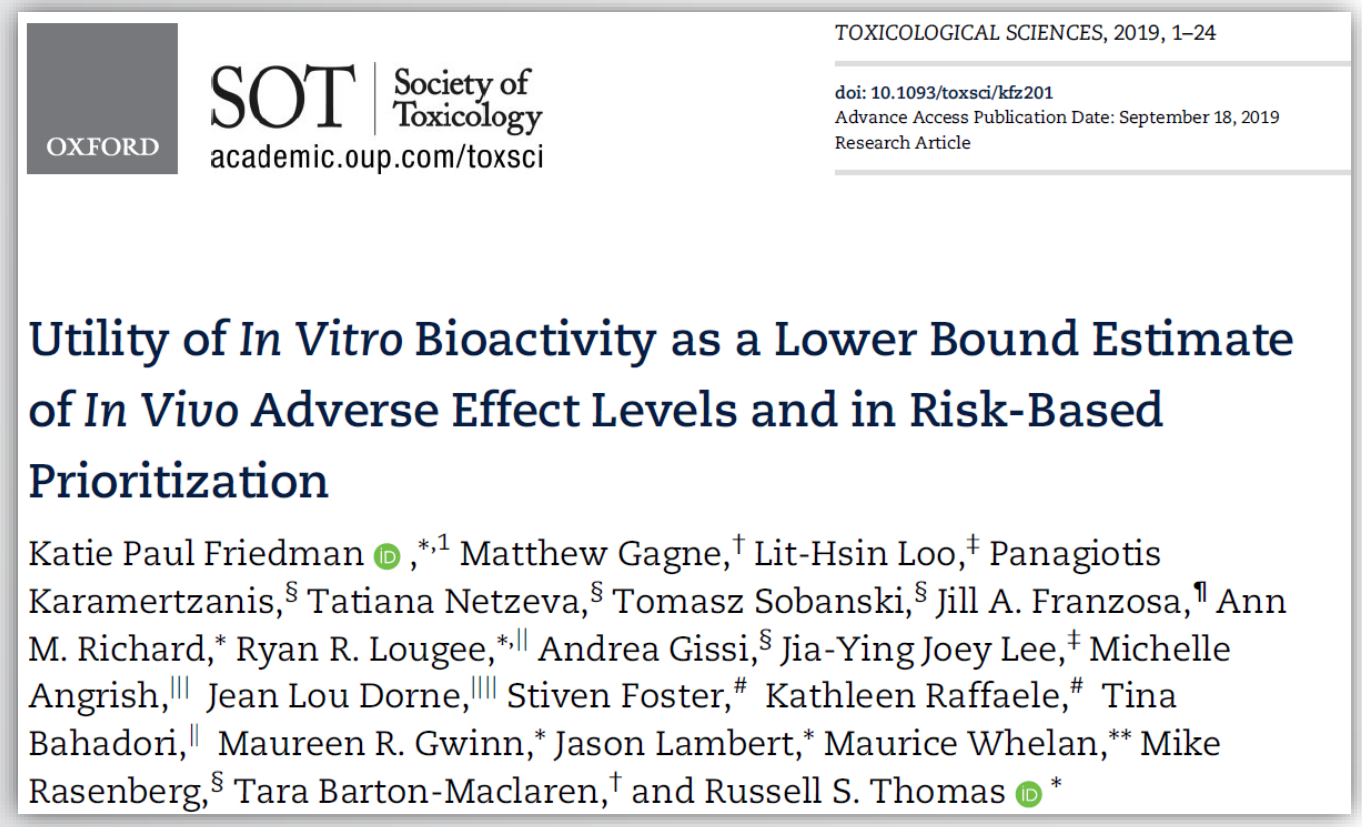
A subset of the papers describing the application of a high-throughput toxicokinetic approach – too many to fit

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



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Utility of *In Vitro* Bioactivity as a Lower Bound Estimate of *In Vivo* Adverse Effect Levels and in Risk-Based Prioritization

Katie Paul Friedman ,*,[1] Matthew Gagne,[†] Lit-Hsin Loo,[‡] Panagiotis Karamertzanis,[§] Tatiana Netzeva,[§] Tomasz Sobanski,[§] Jill A. Franzosa,[¶] Ann M. Richard,[*] Ryan R. Lougee,[*,‖] Andrea Gissi,[§] Jia-Ying Joey Lee,[‡] Michelle Angrish,[‖‖] Jean Lou Dorne,[‖‖] Stiven Foster,[#] Kathleen Raffaele,[#] Tina Bahadori,[‖] Maureen R. Gwinn,[*] Jason Lambert,[*] Maurice Whelan,[**] Mike Rasenberg,[§] Tara Barton-Maclaren,[†] and Russell S. Thomas *

Why is the retrospective case study important?

- Clear need to demonstrate in practical terms, for as many chemicals as possible, how preliminary screening level risk assessment using a new approach methodologies (NAM) based approach would perform when compared to traditional approaches to deriving points-of-departure (PODs).
- Illustrate the current state-of-the-science.
- Evaluate the specific strengths and weaknesses of rapid, screening level risk assessment using NAMs.
- Approach: Take a retrospective look at the traditional and NAM data for as many chemicals as possible (448 at the time).

See the forest for the trees

The big question:

Can *in vitro* bioactivity be used to derive a conservative point-of-departure (POD) for prioritization and screening level risk assessment?

Case study workflow

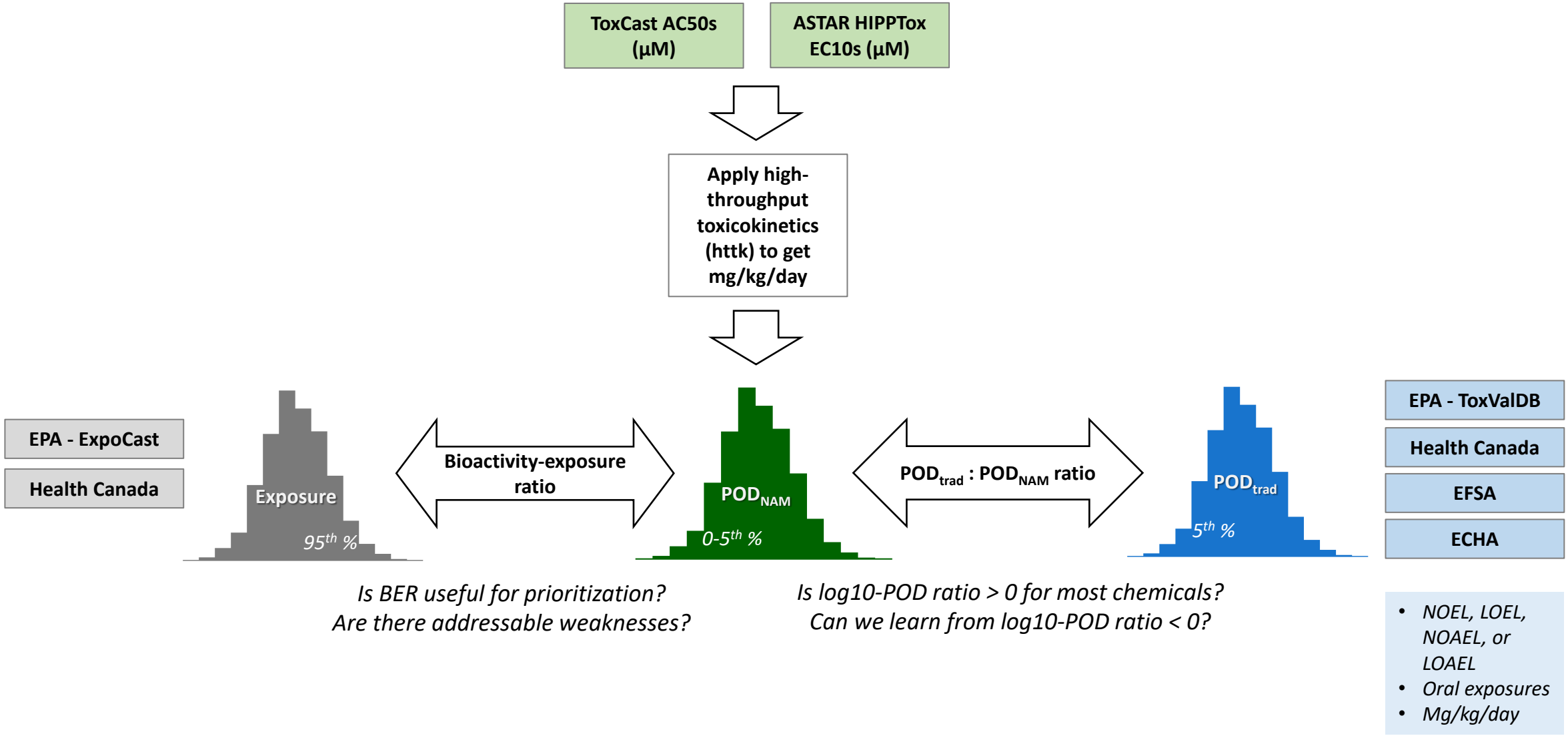
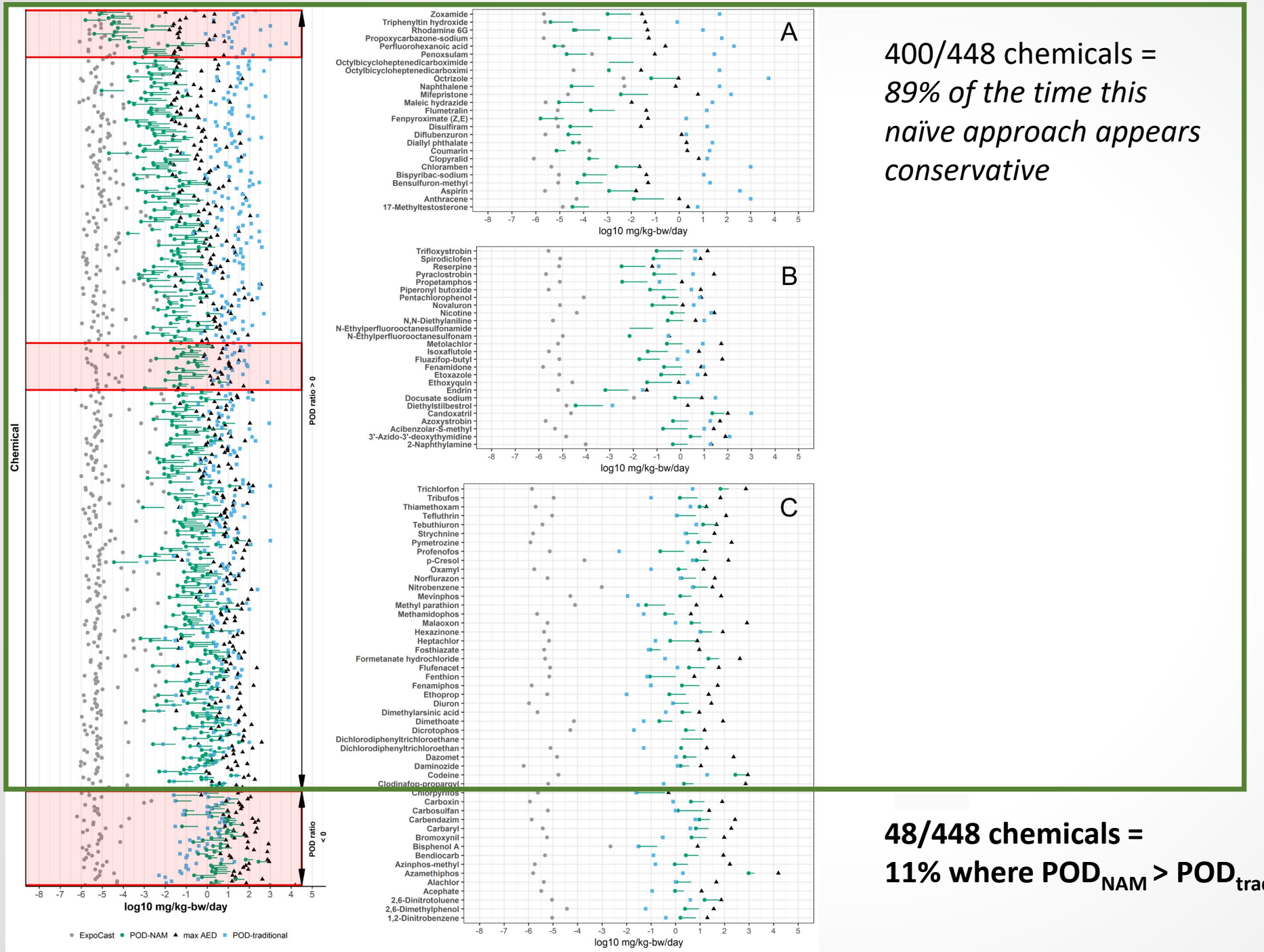


Figure 1, Paul Friedman et al. *2019*



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

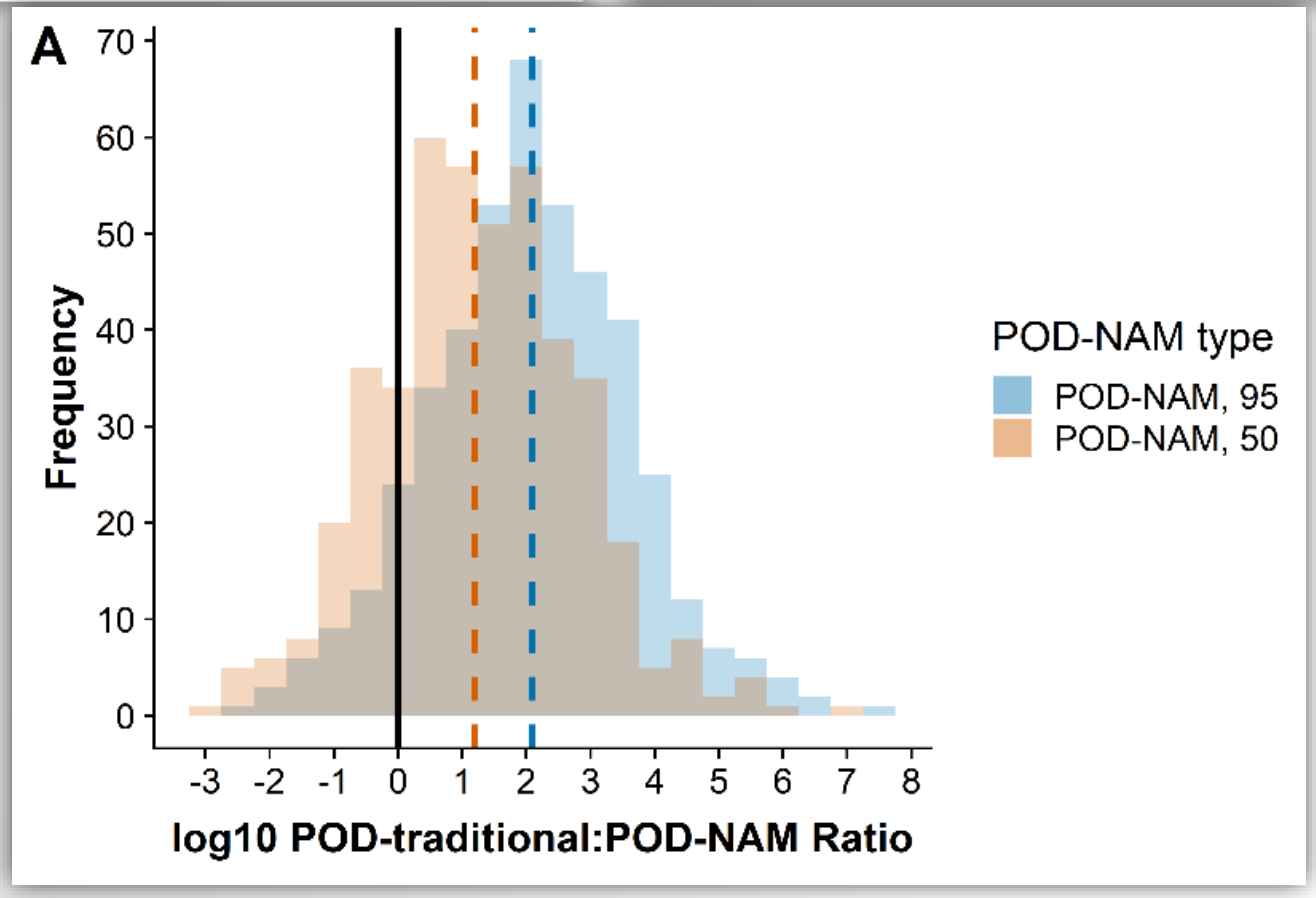
48/448 chemicals =
11% where $POD_{NAM} > POD_{traditional}$

$POD_{NAM} < POD_{traditional}$

(most of the time)

Figure 3, Paul Friedman et al. *2019*

The log10-POD ratio distribution shows POD_{NAM} is generally conservative *and adjustable.*

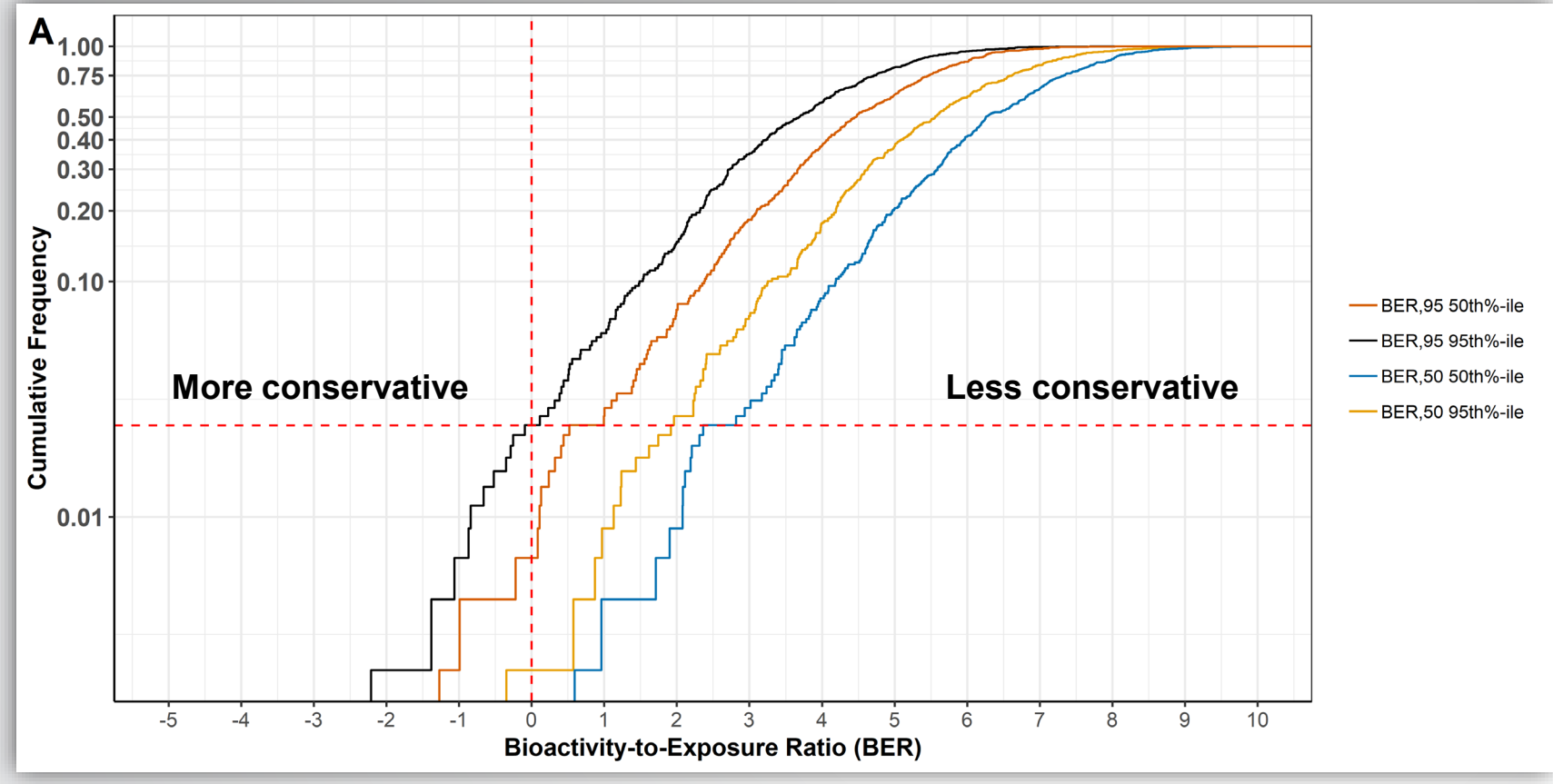


$POD_{NAM,95}$ includes interindividual variability in the in vitro to in vivo extrapolation process to a greater extent and is more often a conservative estimate of $POD_{traditional}$.

This should trigger thinking regarding uncertainty and uncertainty factors/safety factors. In the NAM-based process, we have quantitatively informed uncertainty that can be included explicitly at multiple steps in the screening assessment process.

- log_{10}POD ratio is illustrated for the $POD_{NAM,95}$ and the $POD_{NAM,\ 50}$.
- Using the more conservative (i.e., lower) $POD_{NAM,95}$, 48 of the 448 substances (10.7%) demonstrated a log_{10}POD ratio < 0 (to the left of the solid vertical line), whereas 92 of the 448 substances (20.5%) demonstrated a log10-POD ratio < 0 using the $POD_{NAM,50}$.
- The medians of the log10-POD ratio distributions are indicated by dashed lines for $POD_{NAM,\ 95}$ and $POD_{NAM,\ 50}$ as 2 and 1.2, respectively.

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



- <u>Make choices based on tolerable uncertainty (i.e., based on use case).</u>
- BER_{95} used 95th percentile from the credible interval to predict median total US population exposure (ExpoCast SEEM2);BER_{50} the 50th percentile.
- BER_{95} and BER_{50} values were calculated as the "95th%-ile" and "50th%-ile," using the $POD_{NAM,95}$ and $POD_{NAM,50}$, respectively.

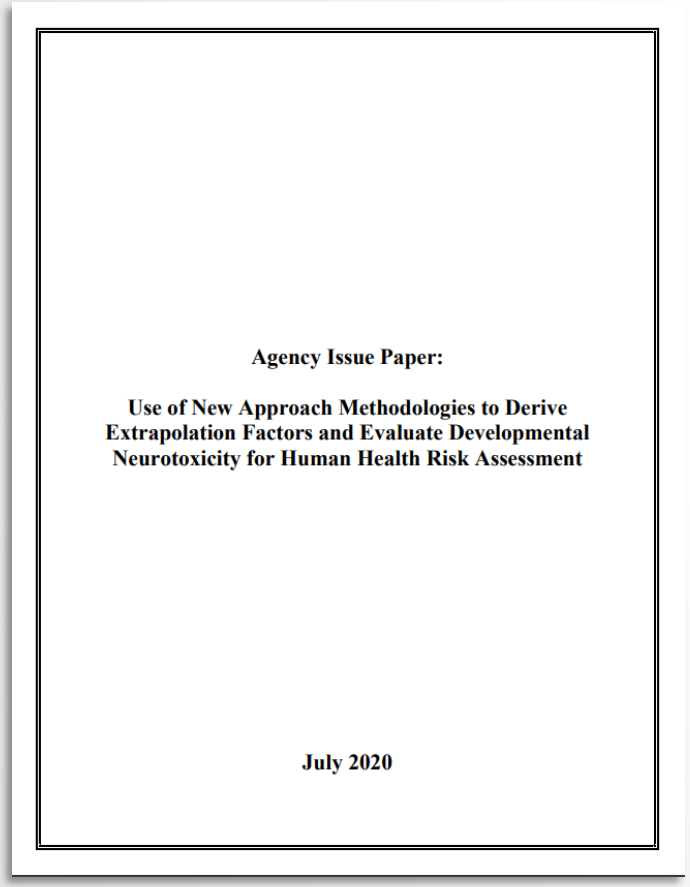
BER_{95} , 95th percentile did not prioritize an unreasonable number of substances; the BER selected reflects the level of conservatism and uncertainty considered within a screening assessment.

Conclusions and limitations

- An approach to using *in vitro* bioactivity data as a POD appears to be a conservative estimate ~ 90% of the time for 448 chemicals.
- POD_{NAM} estimates appear conservative with a margin of ~100-fold.
- POD_{NAM} may provide a refinement of a TTC approach.
- When combined with high-throughput exposure estimates, this approach provides a reasonable basis for risk-based prioritization and screening level risk assessments.

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

Application of hazard-specific NAMs to specific questions about the potential developmental neurotoxicity


Agency Issue Paper:

Use of New Approach Methodologies to Derive Extrapolation Factors and Evaluate Developmental Neurotoxicity for Human Health Risk Assessment

July 2020

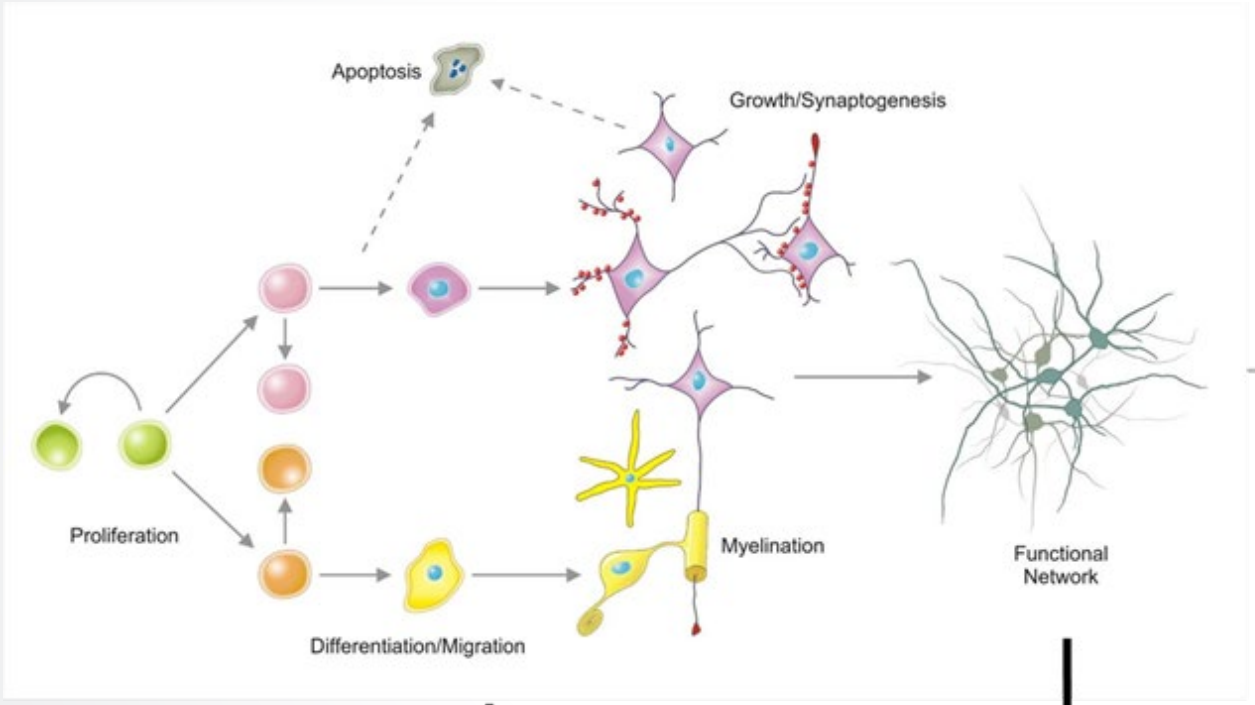
ORD DNT NAMs Team: Josh Harrill, Tim Shafer, Katie Paul Friedman

September 15-18, 2020 Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel met to review this Issue Paper and presentations

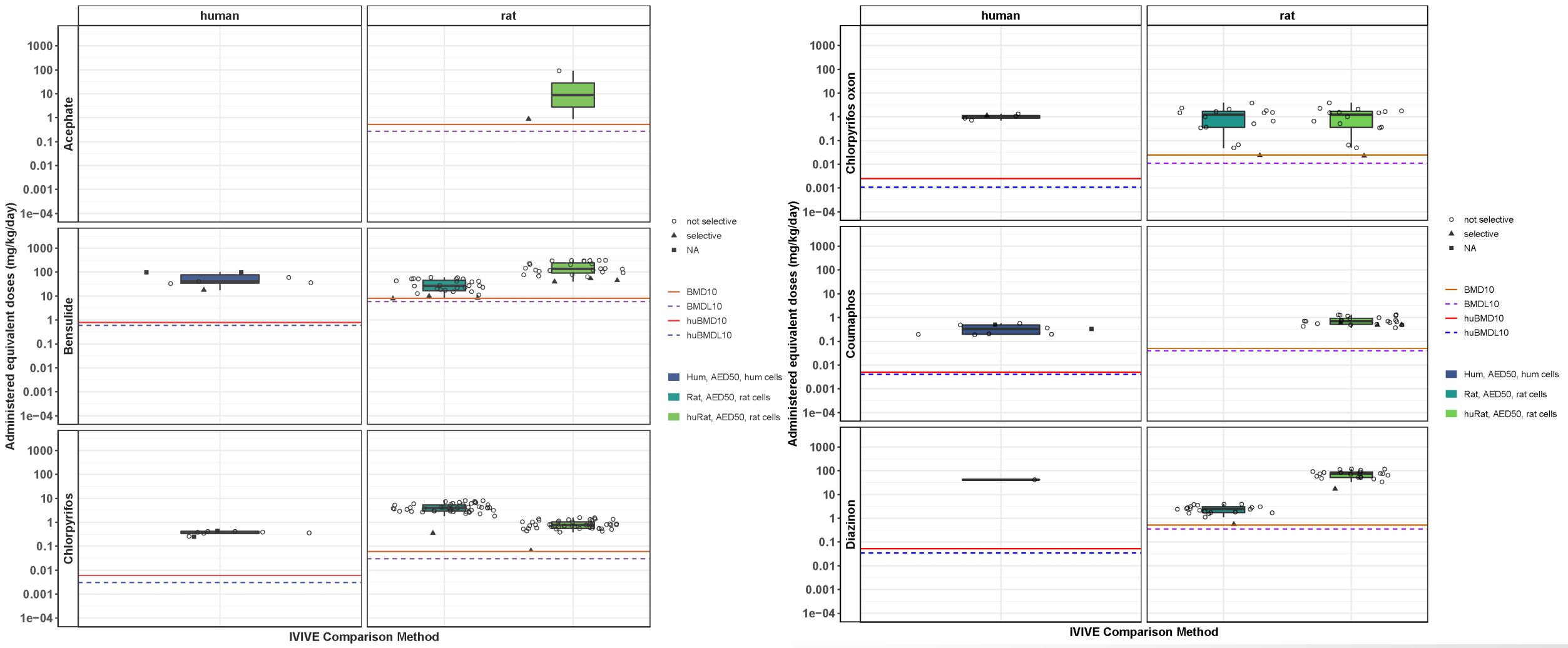
https://beta.regulations.gov/document/EPA-HQ-OPP-2020-0263-0006
Code here: https://www.epa.gov/sap/use-new-approach-methodologies-nams-derive-extrapolation-factors-and-evaluate-developmental

Phenotypic Screening for DNT Hazard

Assays should allow quantitative measurements of key neurodevelopmental events *in vitro*



Example: AED50 to BMD/BMDL10 comparisons



Employing toxicokinetic and toxicodynamic NAMs

EPA New Approach Methods Work Plan: Reducing Use of Animals in Chemical Testing

https://www.epa.gov/chemical-research/epa-new-approach-methods-work-plan-reducing-use-animals-chemical-testing

- How much uncertainty can be tolerated?
- Can BER be informative for the problem?
- Are there specific hazards of interest?
- How should toxicokinetic modeling be tuned?

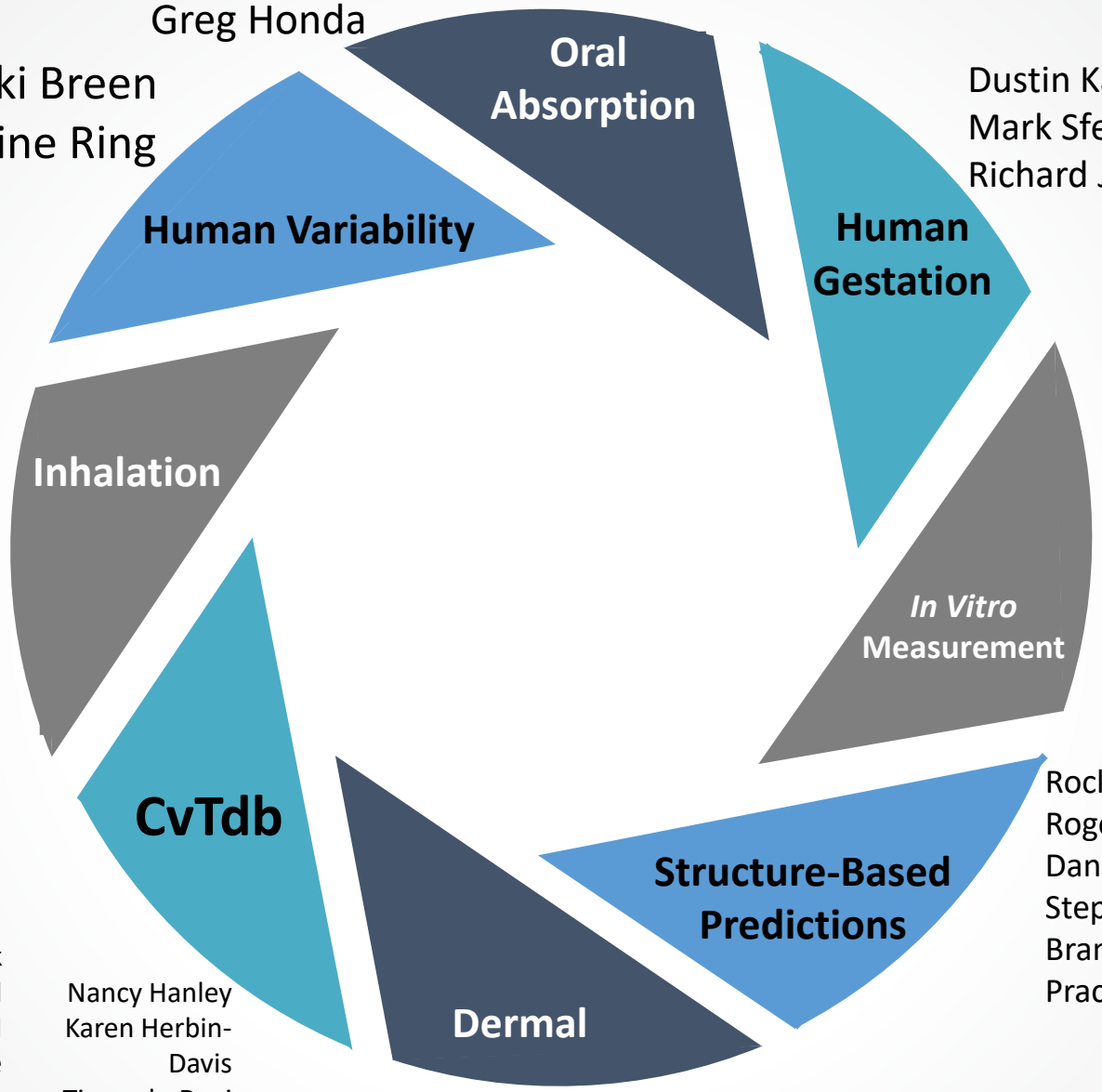
- **Chemical safety assessment with fewer resources is a motivator for rapid data acquisition and model development.**

- **There is a lot more work to do, and case studies will help build confidence and identify gaps to fill.**

Conclusions

- Reverse dosimetry is a powerful tool for deriving NAM-based points-of-departure for different chemical screening and assessment applications.
- The details of the choices made in the IVIVE approach have impacts on the POD_{NAM} derived, and uncertainties and assumptions should be explained.
  - R library(httk) provides a simple way for users to operationalize generic HTTK models and *in vitro* toxicokinetic data to derive POD_{NAM} from *in vitro* bioactivity data such as ToxCast data.
  - For some applications, conservative assumptions can be more tolerated.
  - Ongoing research will further inform sets of decisions for specific chemicals chemical assessment contexts (e.g., improvements and application of *in vitro* chemical disposition modeling).
- Ongoing work to compare POD_{NAM} to existing PODs as well as to values obtained through other PBTK approaches will provide important benchmarks on HTTK approaches to increase the acceptance of POD_{NAM} and BERs.

HTTK Team



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