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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

August 13, 2021

EPA United States Environmental Protection Agency

Overview

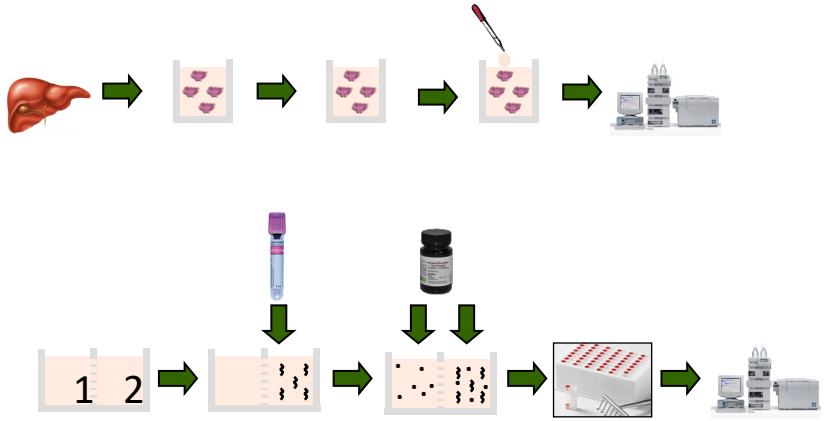
- Generic vs. bespoke PBTK models
- Models available within R package “httk”
- Model parameterization
  - Physiologic parameters
  - Chemical-specific parameters
- Model evaluation
  - The Concentration vs. Time Database (CvTdb)

HTTK: A NAM for Exposure

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

High Throughput Toxicokinetics (HTTK)

In vitro toxicokinetic data



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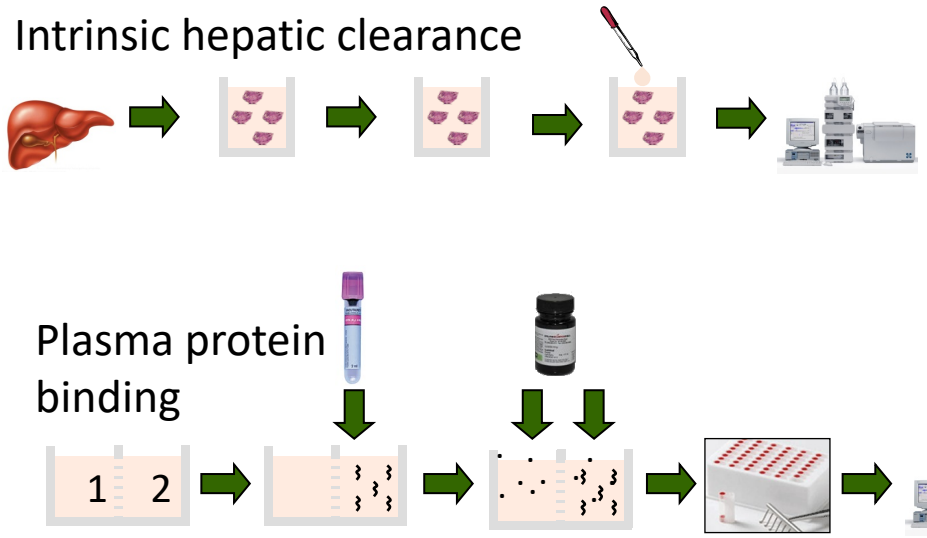

Rotroff et al. (2010)
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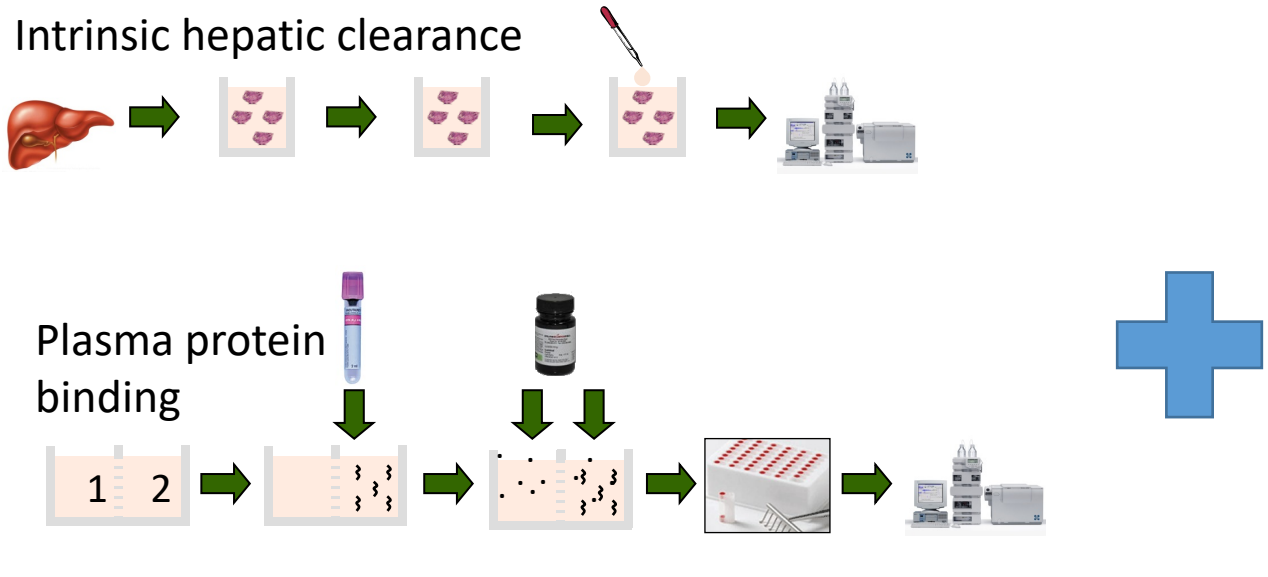
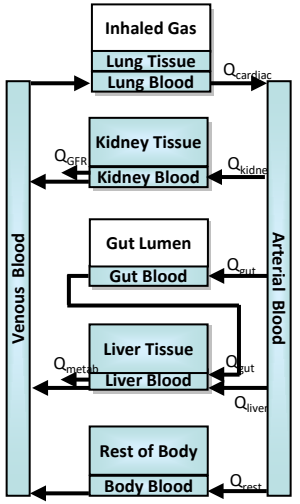
High Throughput Toxicokinetics (HTTK)

In vitro toxicokinetic data + generic toxicokinetic model





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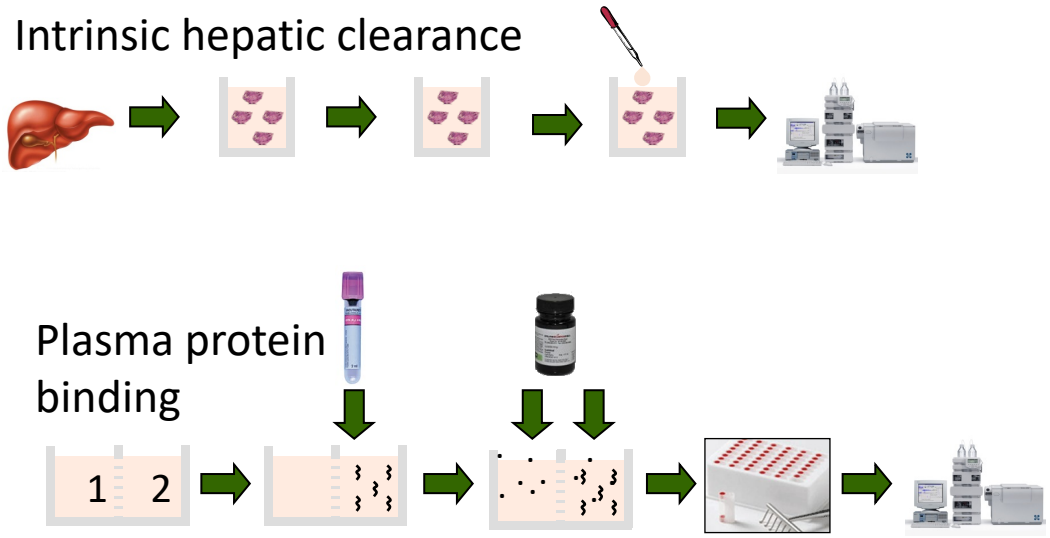

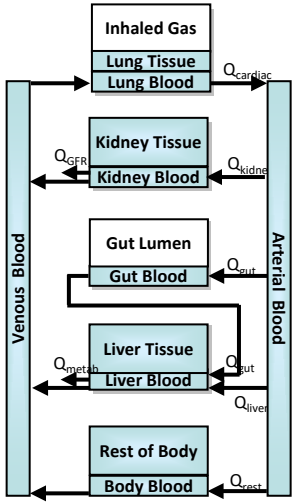

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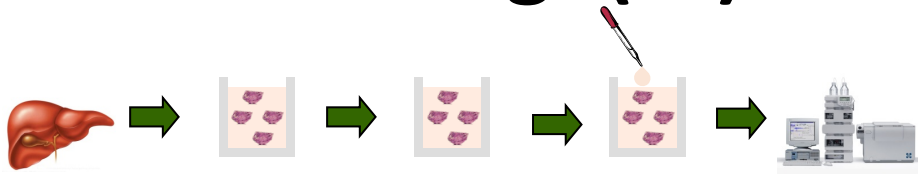
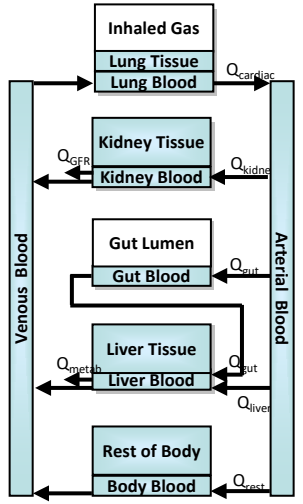
High Throughput Toxicokinetics (HTTK)

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





= *httk*

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Generic vs. bespoke PBTK models

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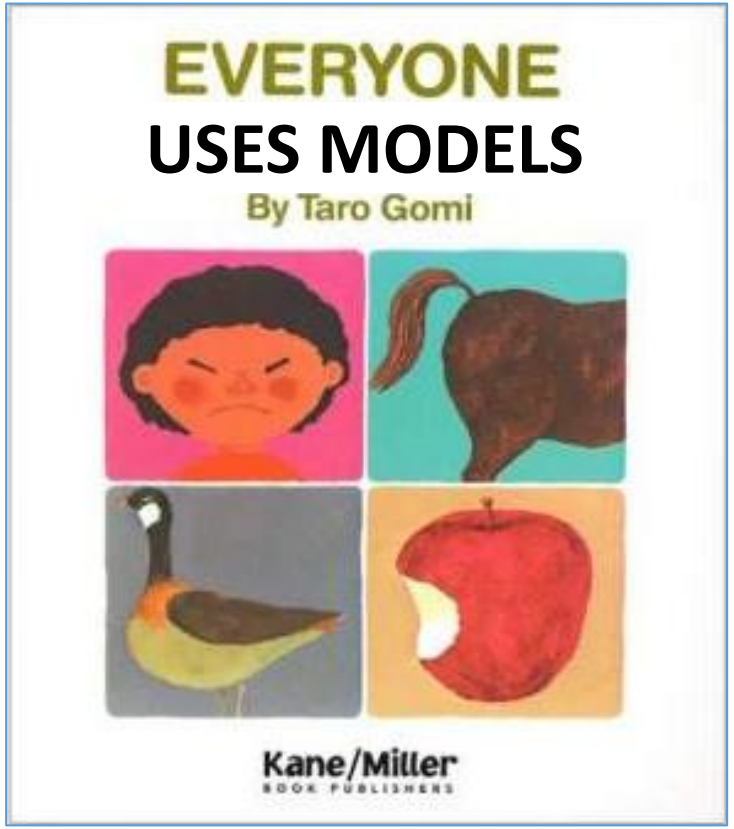
Everyone Uses Models

- 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.

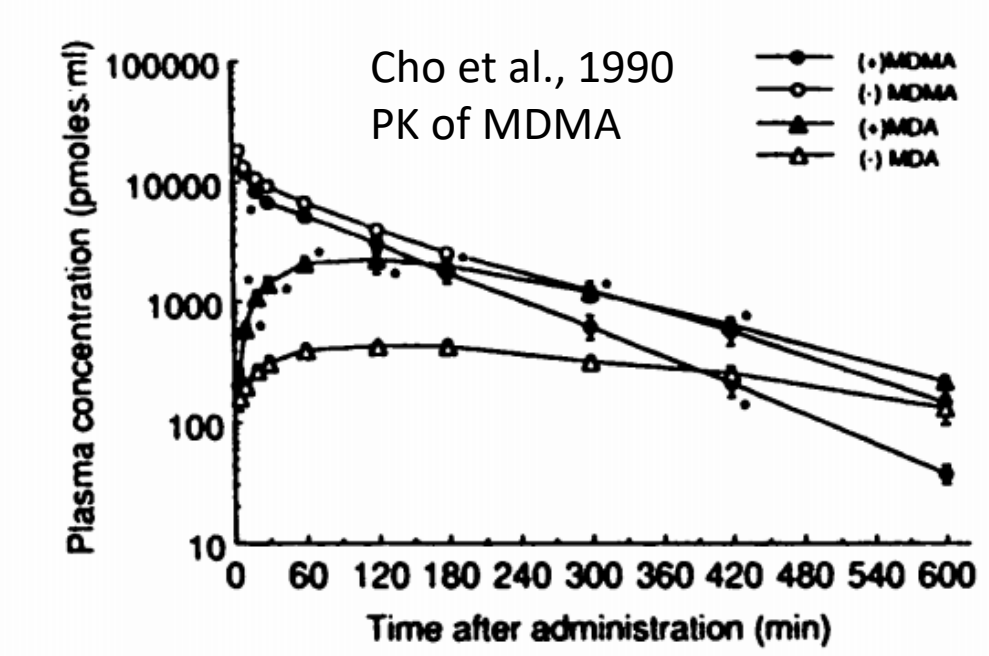
  - How do I drive to a place I’ve never been before?

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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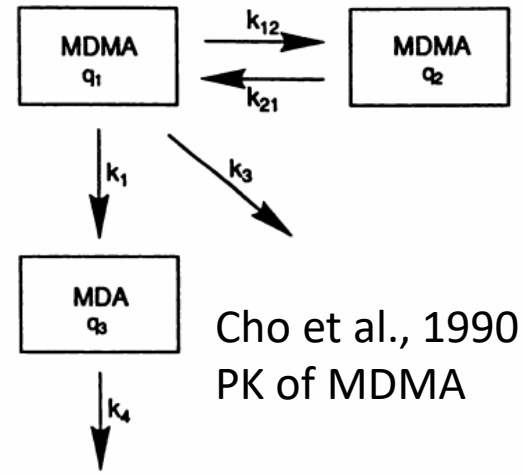
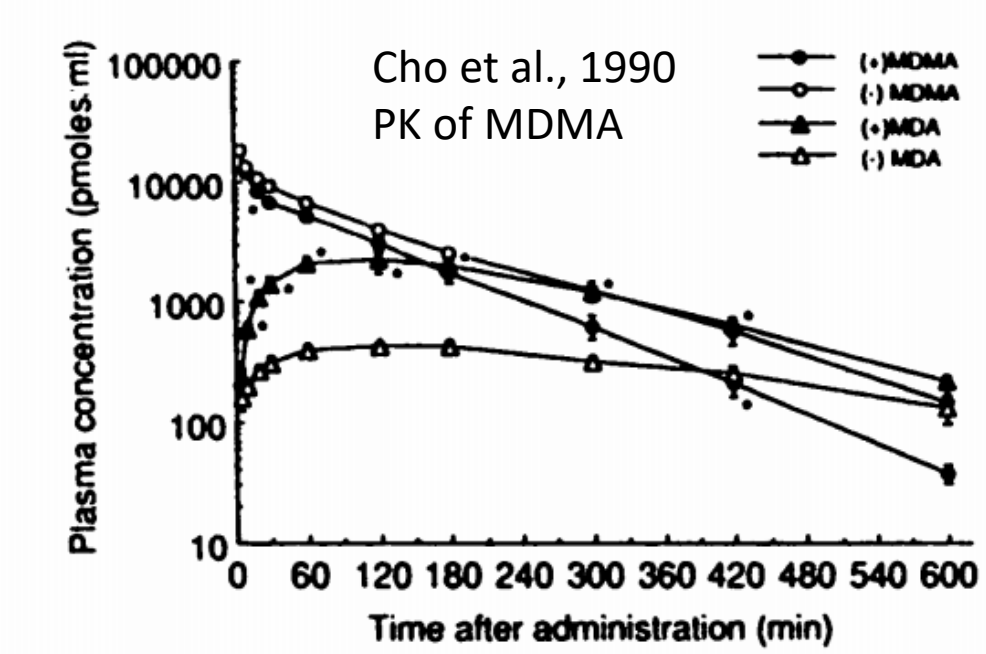


Cho et al., 1990
PK of MDMA



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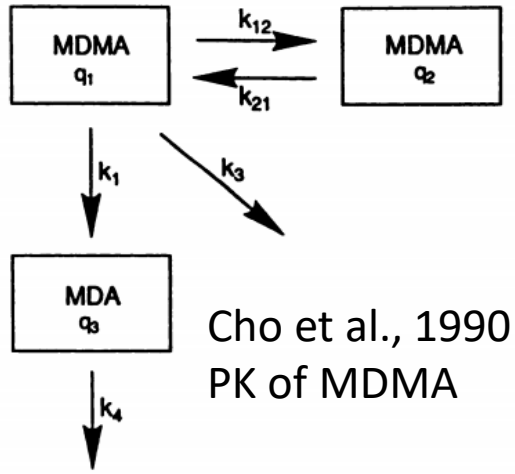

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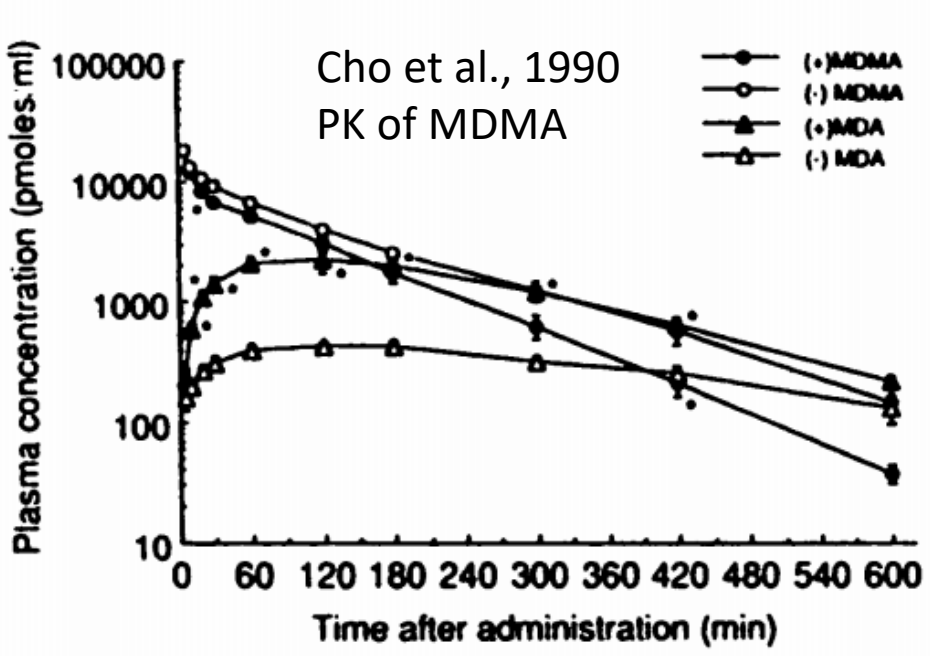


Jones et al., 2012
PK of Statins

In this case they had transporter-specific data

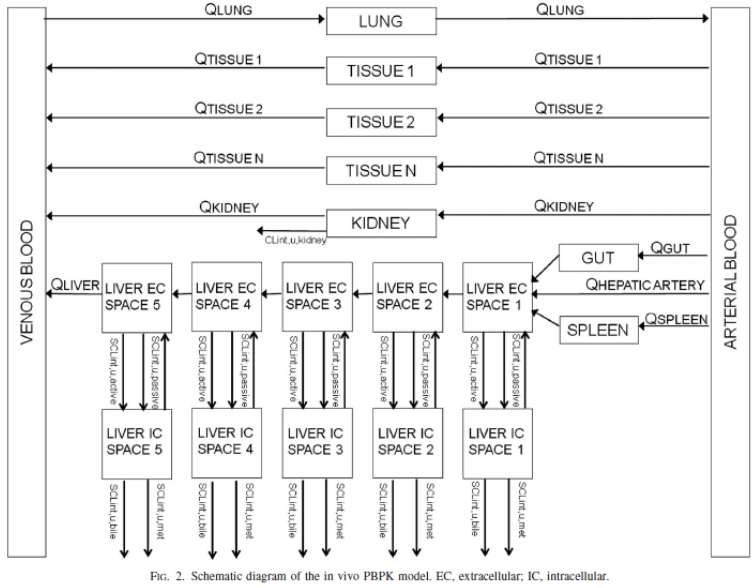


FIG. 2. Schematic diagram of the in vivo PBPK model. EC, extracellular; IC, intracellular.

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

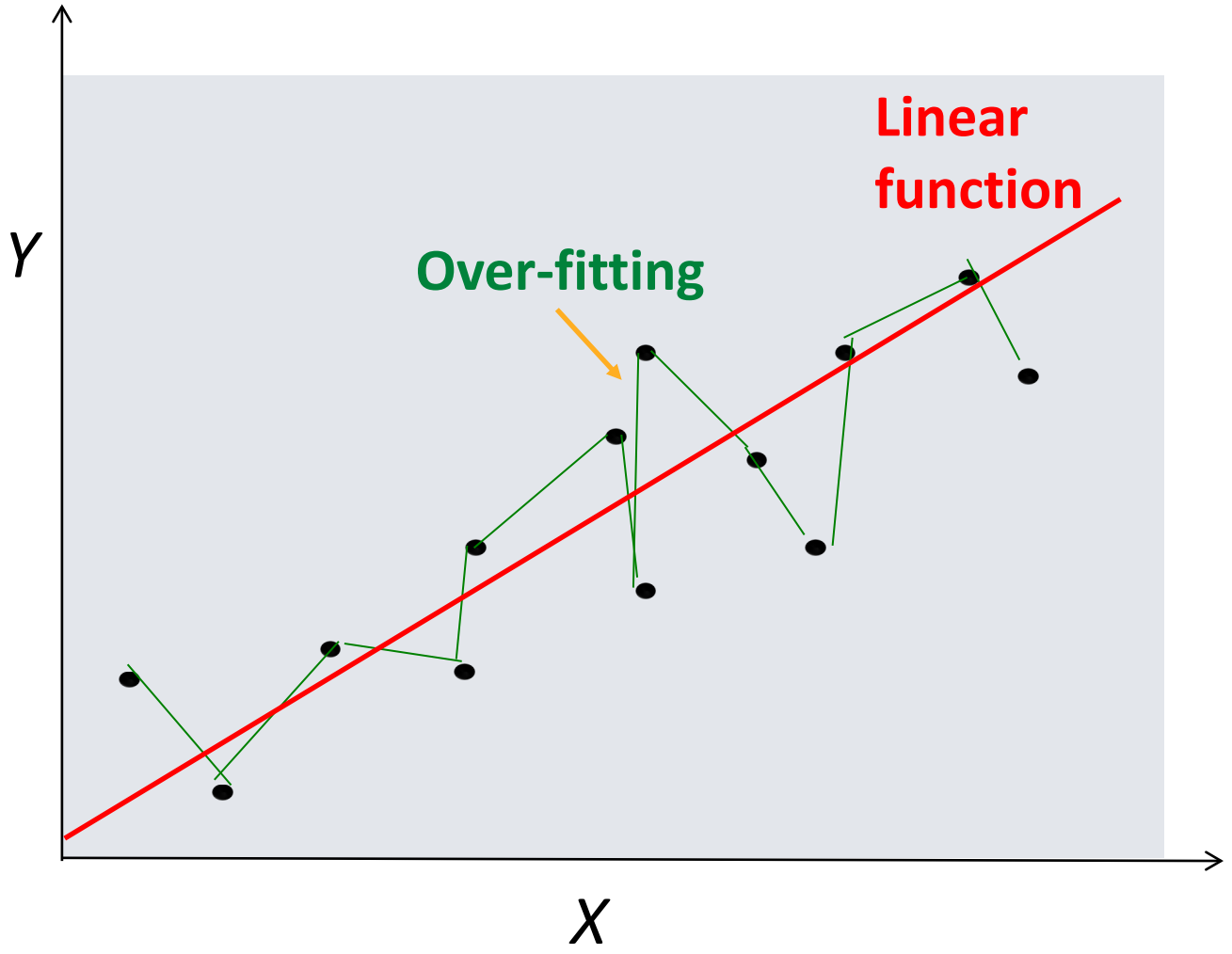
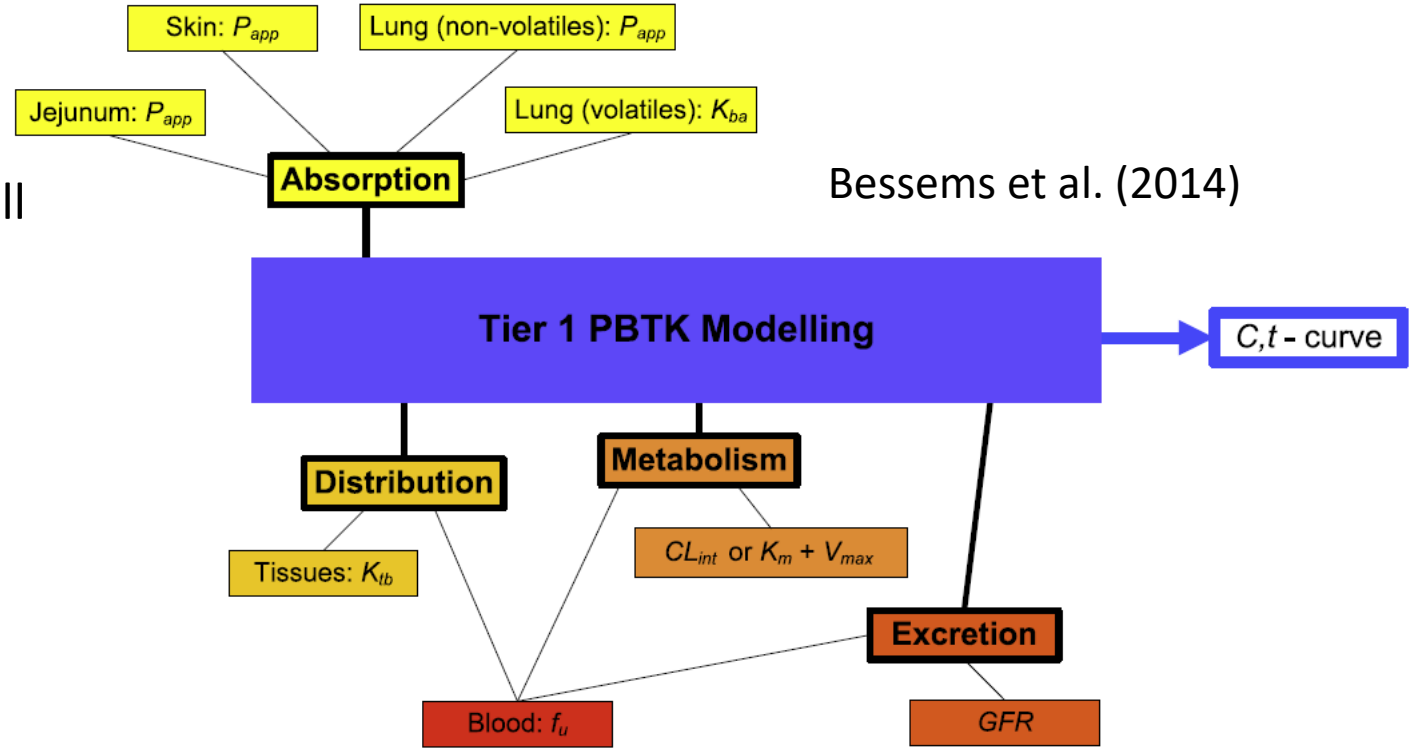


Figure from Anran Wang

Fit for Purpose Toxicokinetics

- Chiu et al. (2007) "…[P]arsimony in selecting model structures is an important and guiding principle in developing models for use in risk assessments."
  - Complexity is constrained by limited data available to calibrate and test the model 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
  - They suggested that we neglect active metabolism. But 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 =

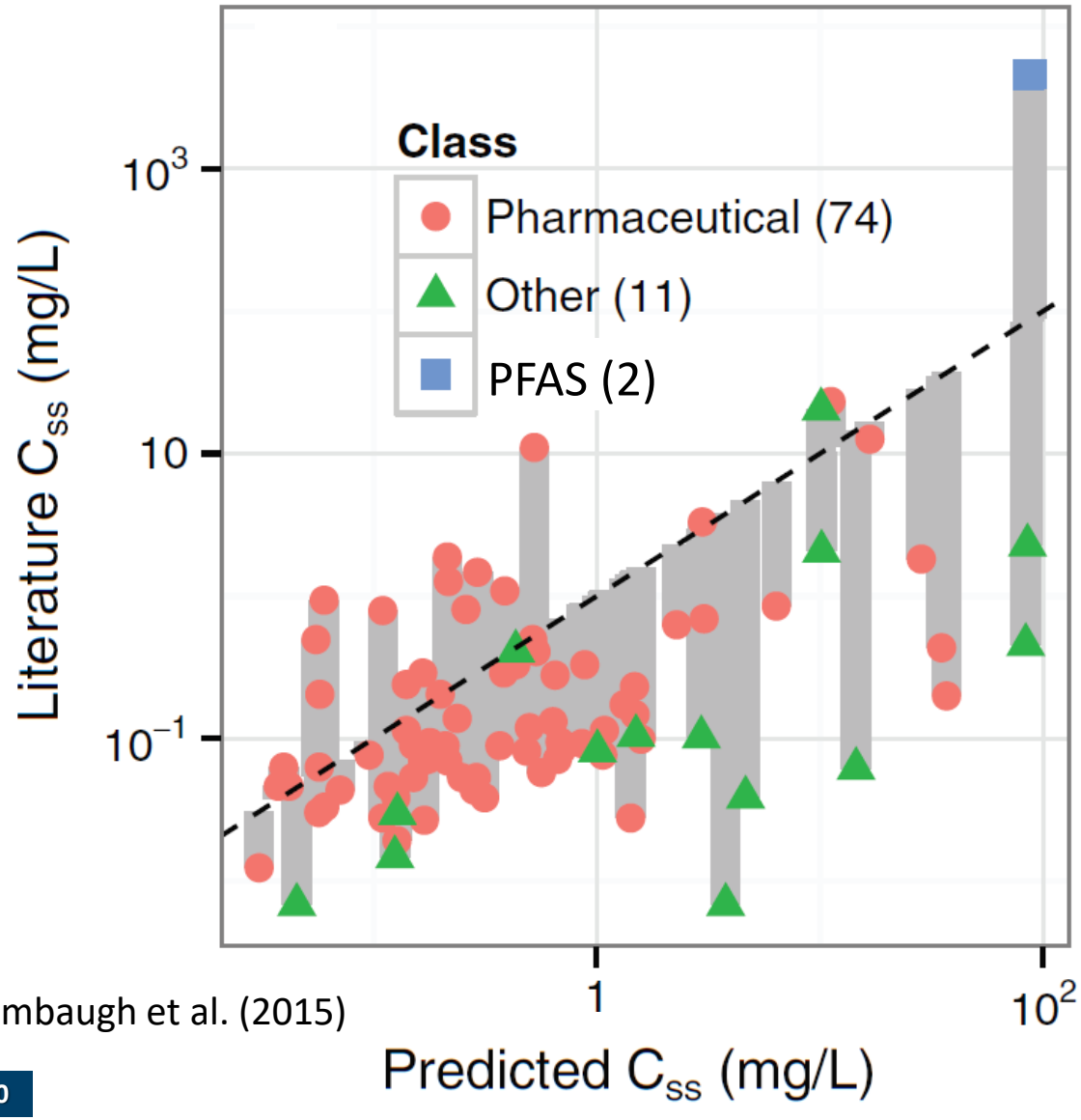
***In vitro* toxicokinetic data + generic toxicokinetic model**

- 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

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Ann. Occup. Hyg., Vol. 55, No. 8, pp. 841–864, 2011

doi:10.1093/annhyg/mer075

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

of

Clinical Pharmacokinetics
October 2006, Volume 4

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Authors | Authors and affiliations

Andrea N. Edginton, Walter Schmitt, Stefan Willmann

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Received 8 April 2005, Revised 25 Ma

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)	Lukacova (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 [154]	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 [155]	No	No	No	No	No	Armitage Model [21,22]
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

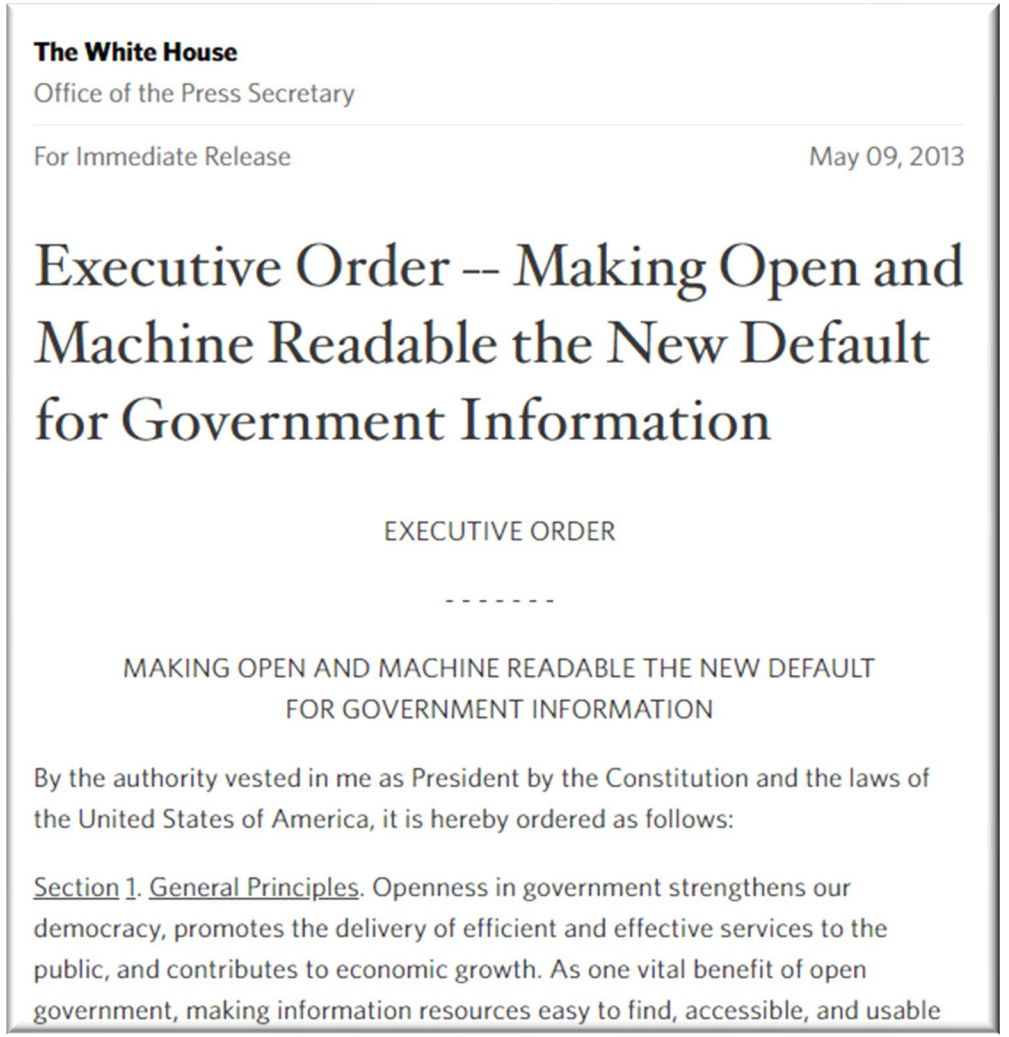
Regulatory Acceptance

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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Reverse Dosimetry	Yes	Yes	Yes	No	No	No	Yes

When HTTK was first developed only PK-Sim was open source, and it focuses on bespoke models

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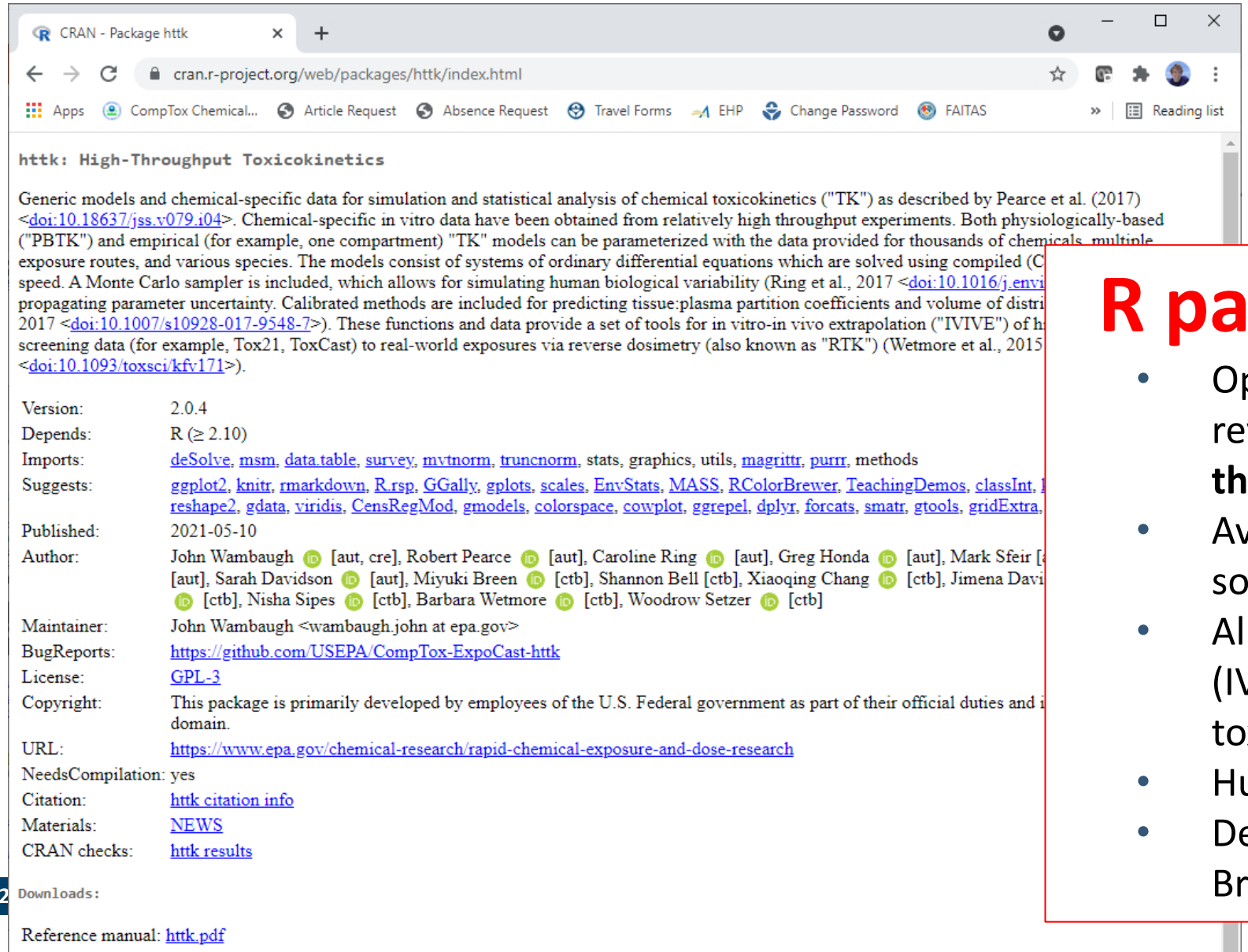
Models available within R package "httk"

Open Source Tools and Data for HTTK

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

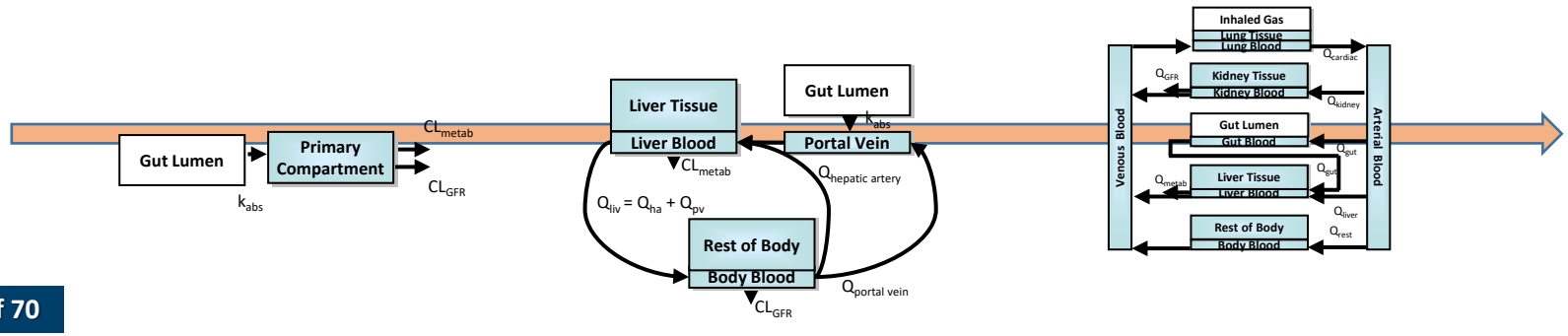


R package "httk"

- 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)

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)

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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

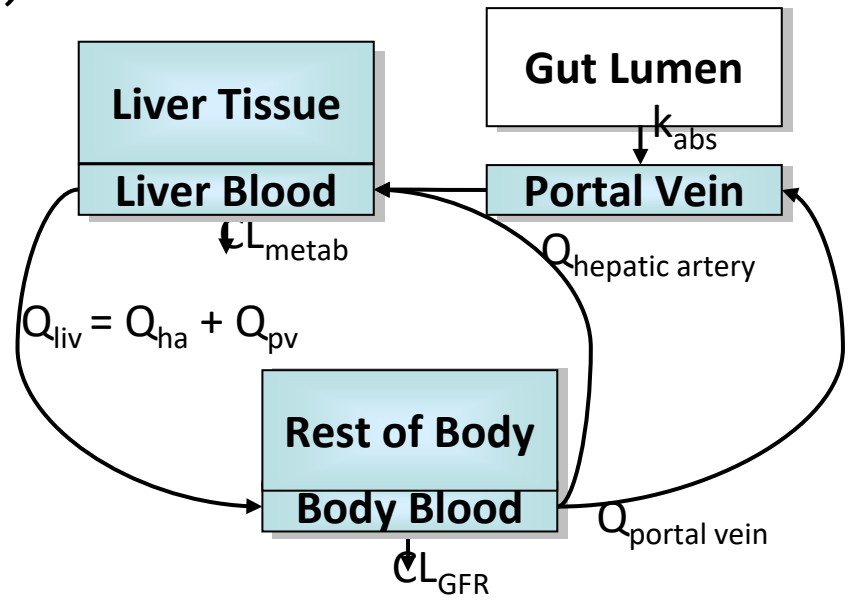
- At steady-state all compartments are at equilibrium and the concentrations can be predicted with a single equation, **but**:
  - The exposure (dose) must be constant
  - Enough time must pass to reach equilibrium

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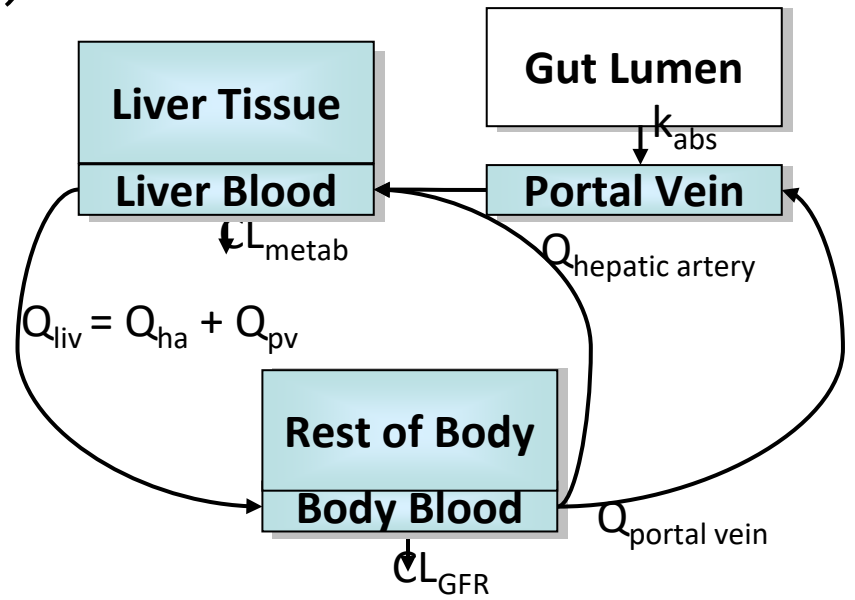
Estimated fraction not metabolized in first pass through liver before systemic circulation



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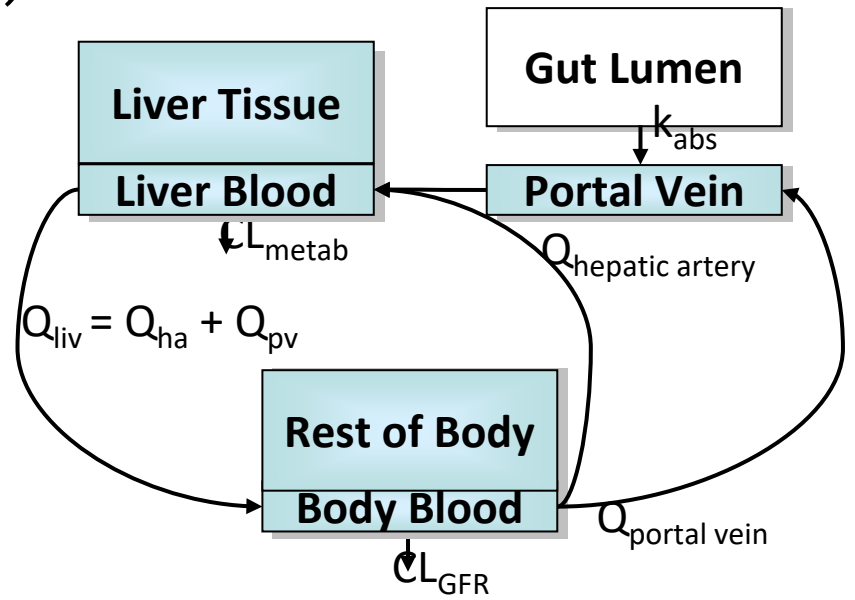
Passive Renal Clearance
(GFR: Glomerular filtration rate
f_{up}: fraction unbound in plasma)



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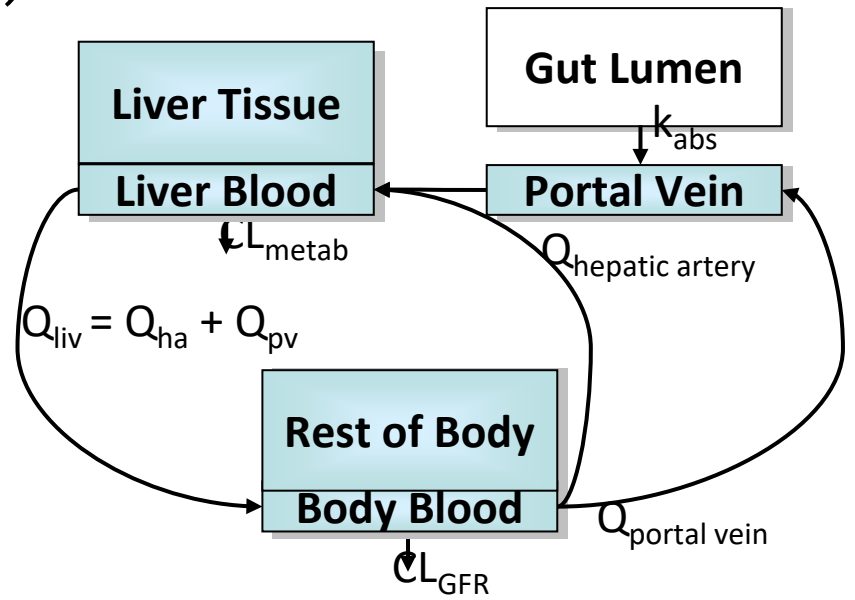
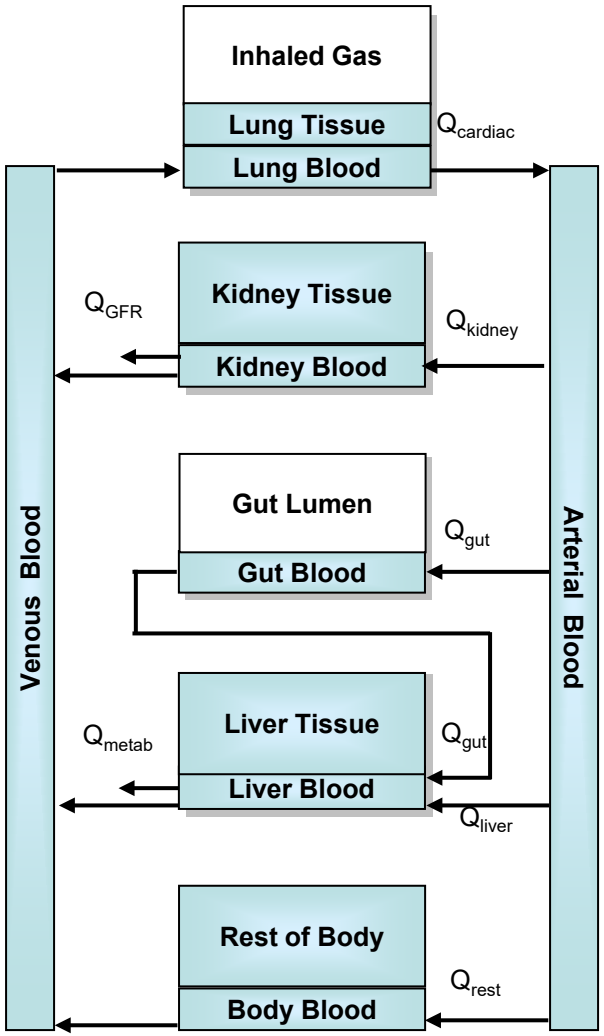
Hepatic Metabolism
($Cl_{hepatic}$: Scaled hepatic clearance
Q_l: Blood flow to liver)



Wilkinson and Shand (1975)

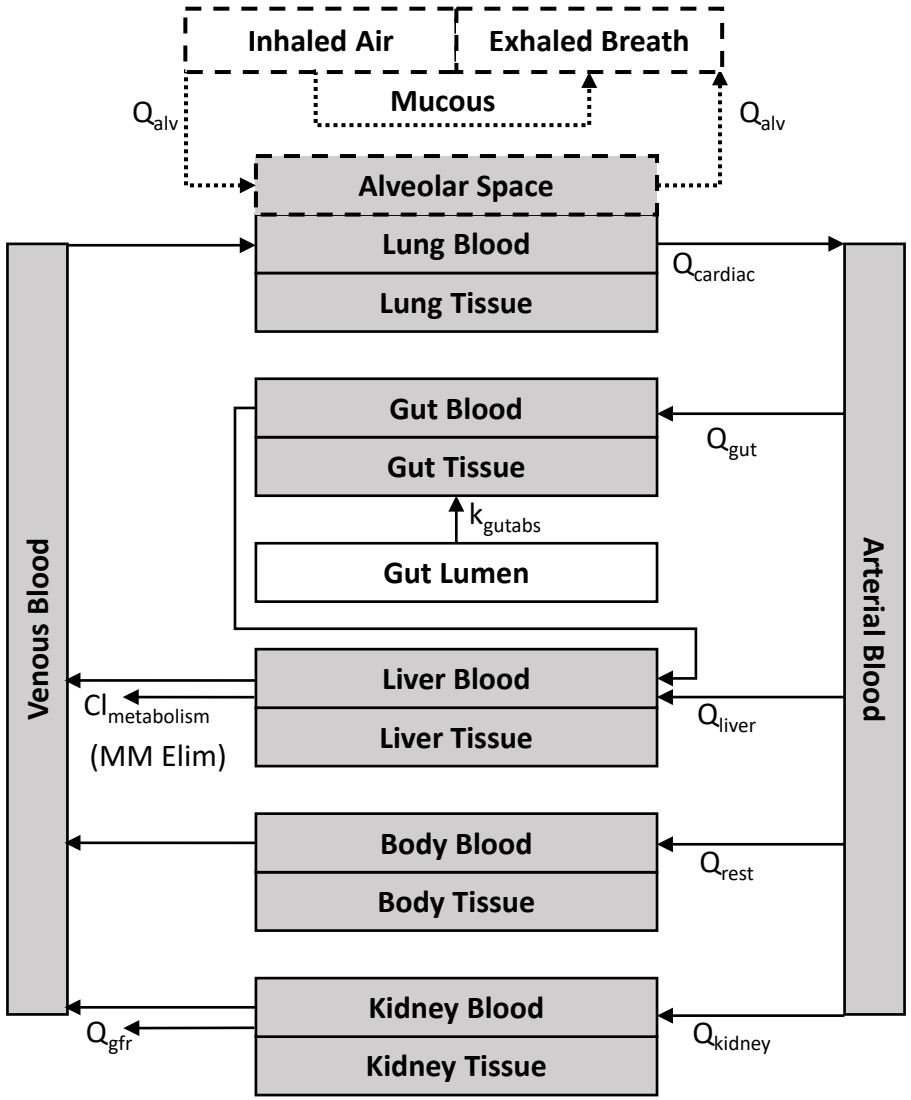
The "httk" General Physiologically-based Toxicokinetic (PBTK) Model



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	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)
$C_{protein}$	Concentration of protein used in f_{up} assay	5	µM	Wambaugh et al. (2019)

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

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

- Davies, Brian, and Tim Morris. "Physiological parameters in laboratory animals and humans." Pharmaceutical research 10.7 (1993): 1093-1095.
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- Stammers, Arthur Dighton. "The blood count and body temperature in normal rats." The Journal of physiology 61.3 (1926): 329.
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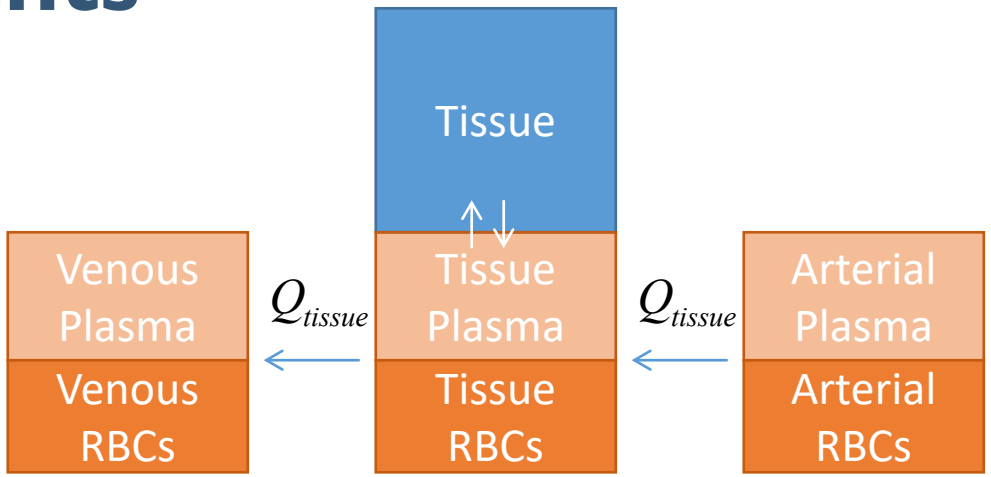
- Gauvin, David V. "Electrocardiogram, hemodynamics, and core body temperatures of the normal freely moving cynomolgus monkey by remote radiotelemetry", Journal of Pharmacological and Toxicological Methods

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



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

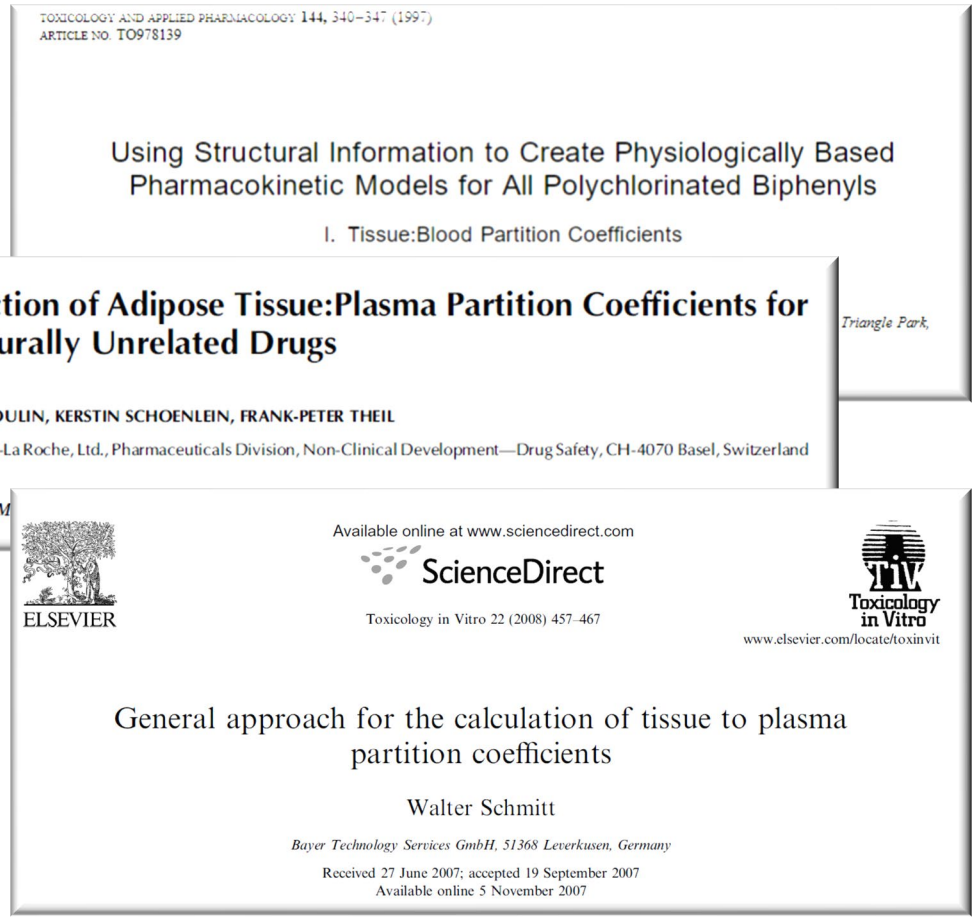
- We assume that all the tissues are "perfusion limited", which means that the tissue concentration instantly comes to equilibrium with the free fraction in plasma (concentration is limited by flow to the tissue)

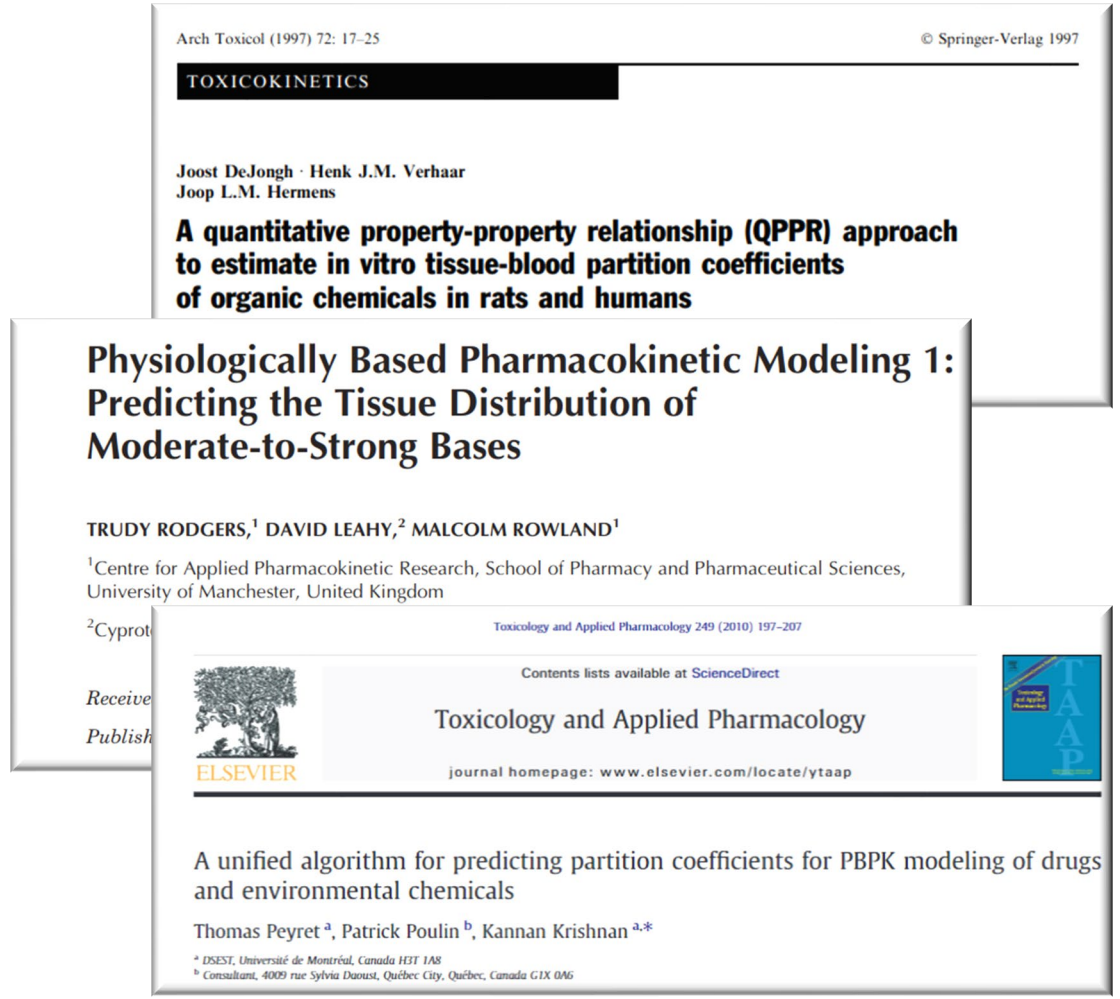
$$C_{compartment,tissue} = K_{tissue:plasma} * f_{up} * C_{compartment,plasma}$$

$K_{tissue:plasma}$ 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…





Schmitt's Method (2008)

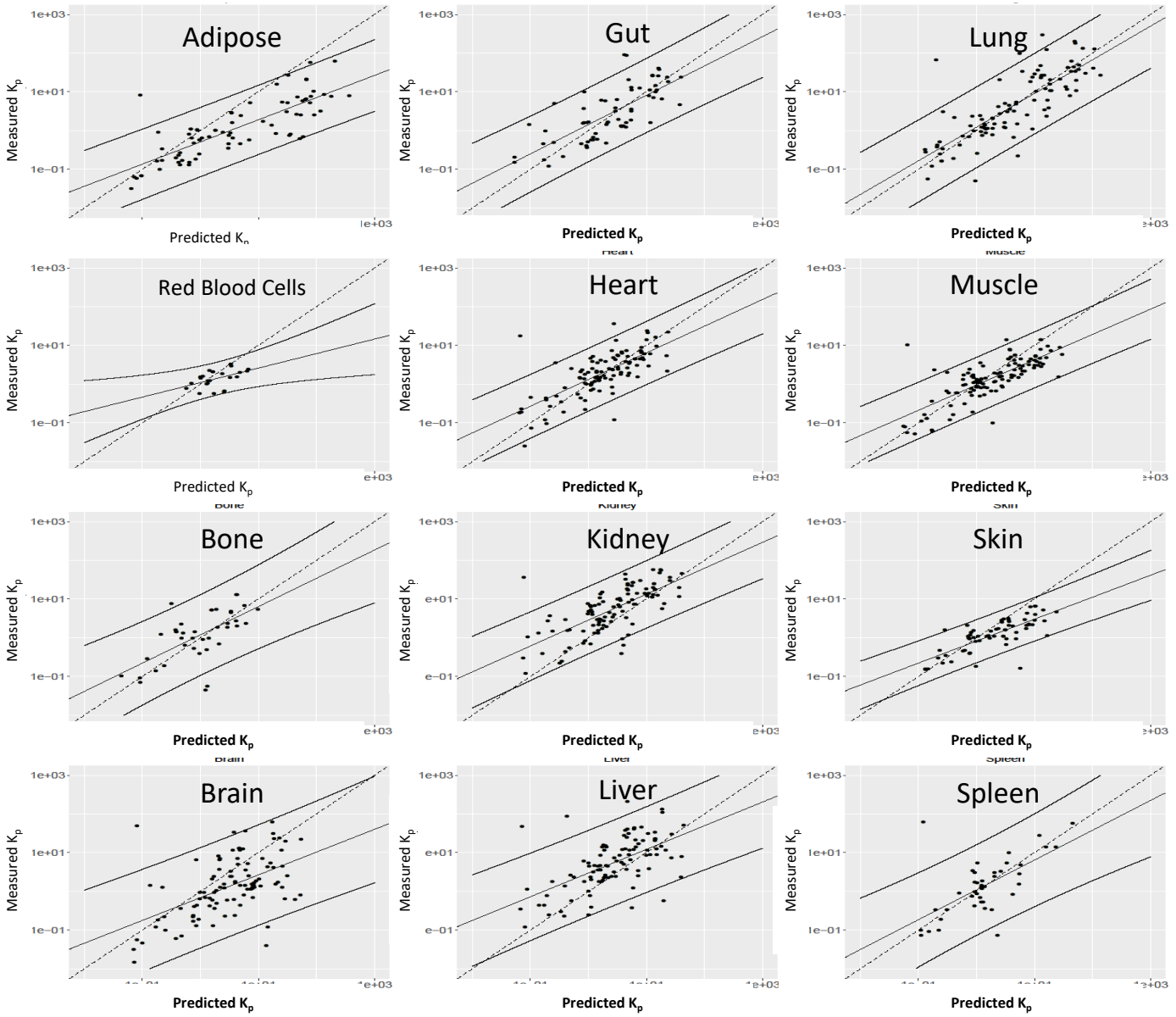
- Depending on its structure a chemical partitions differently into water, fats, and charged materials
- Schmitt's method predicts chemical affinity based on the composition of a tissue
  - Users can choose to add new tissue to HTTK by providing this information

	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)



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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)

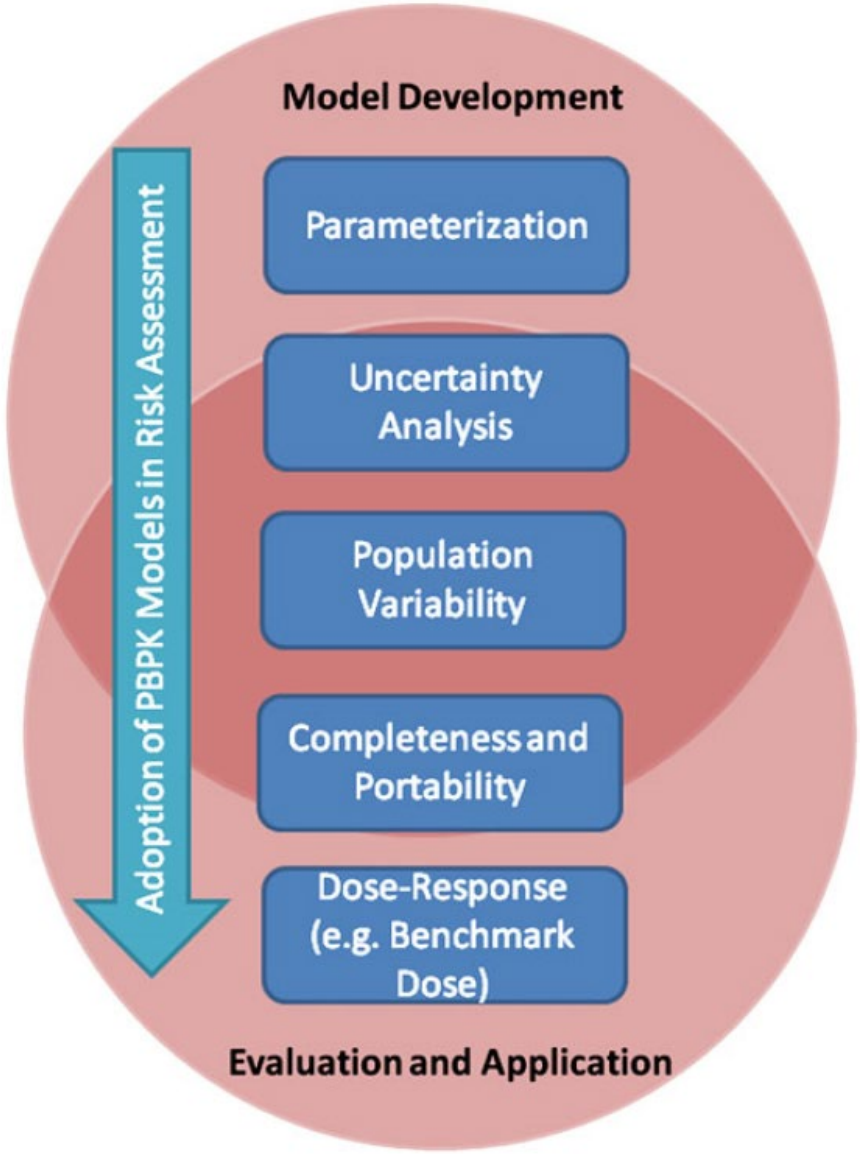

FIG. 1. This figure shows examples of key considerations during model development, evaluation, and application that are necessary before a PBPK model may be adopted for use in a HHRA.

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)	Lukacova (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 [154]	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 [155]	No	No	No	No	No	Armitage Model [21,22]
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

PK-Sim added integration with the R statistical analysis language after HTTK was developed

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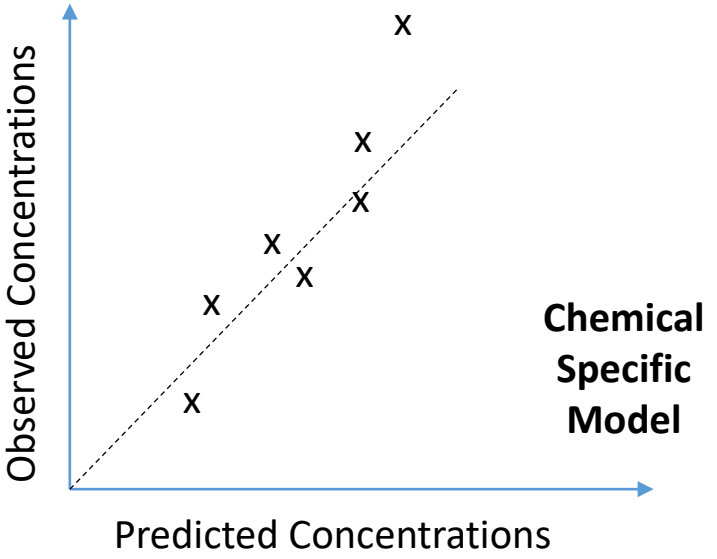
Statistical Analysis with HTTK

- 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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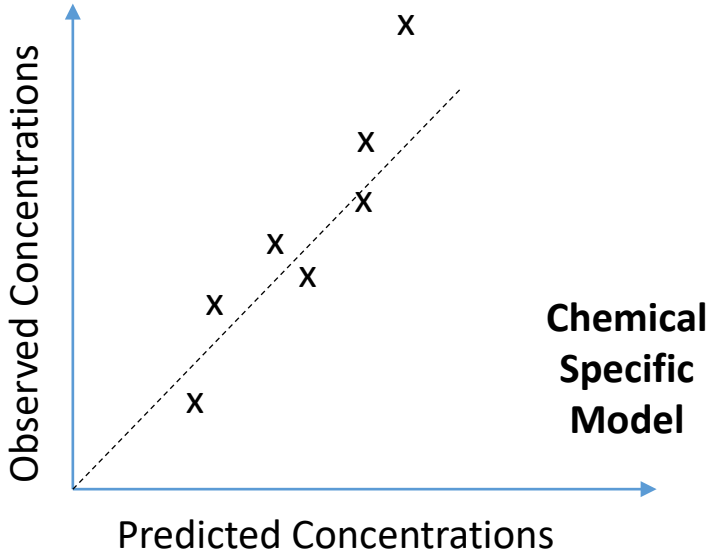
- However, we do not typically have TK data



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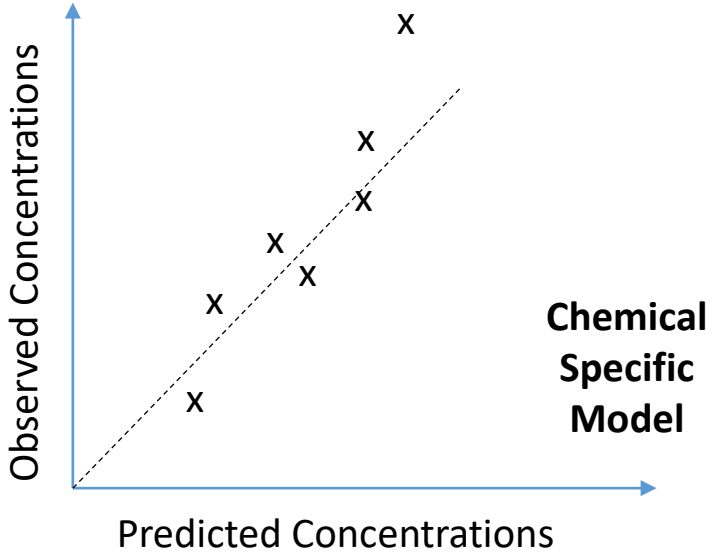
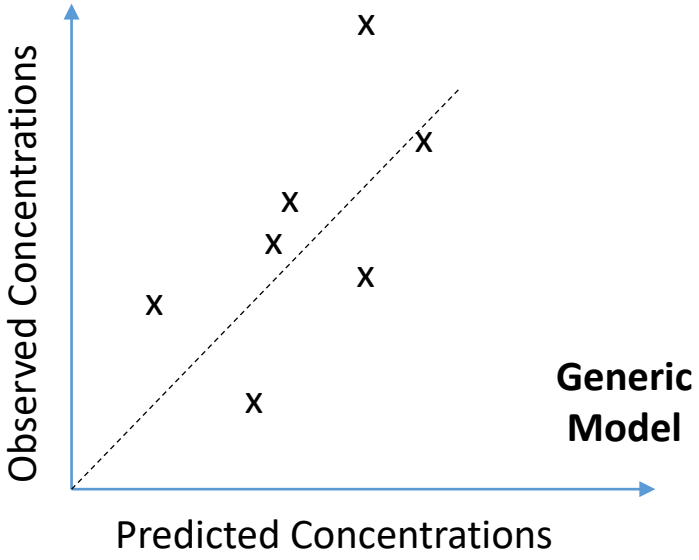
- We can parameterize a **generic TK model**, and evaluate that model for as many chemicals as we do have data
  - We do expect larger uncertainty, but also greater confidence in model implementation
  - Estimate bias and uncertainty, and try to correlate with chemical-specific properties





Cohen Hubal et al. (2019)

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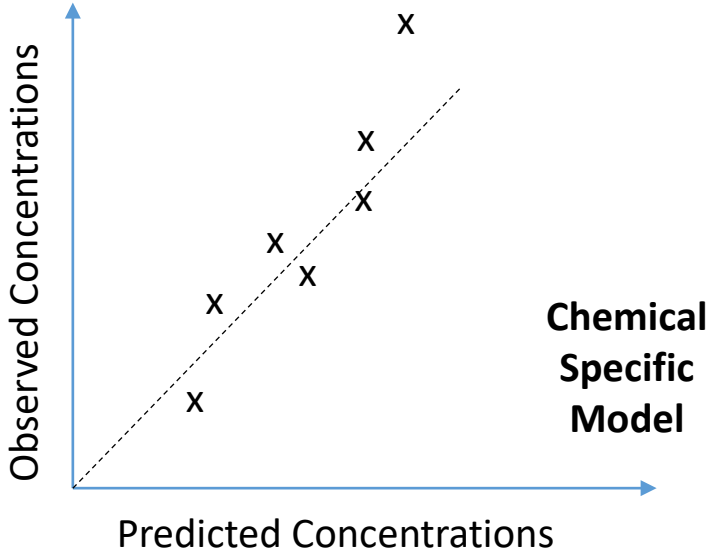

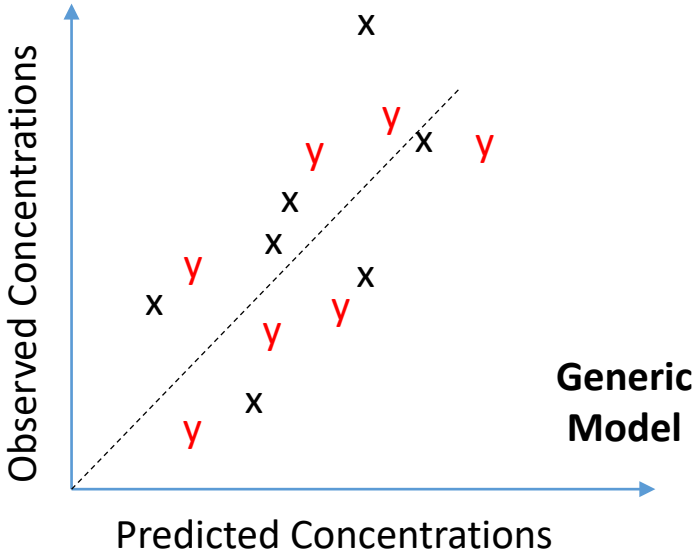

Cohen Hubal et al. (2019)

Building Confidence in TK Models

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  - Can estimate uncertainty
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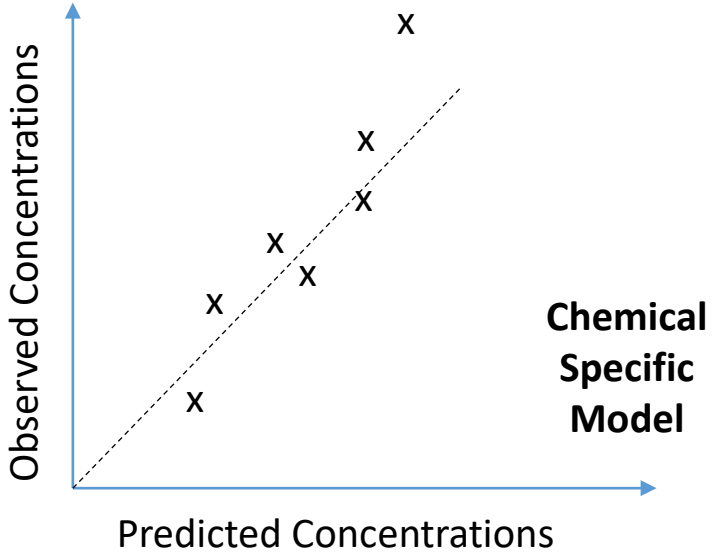
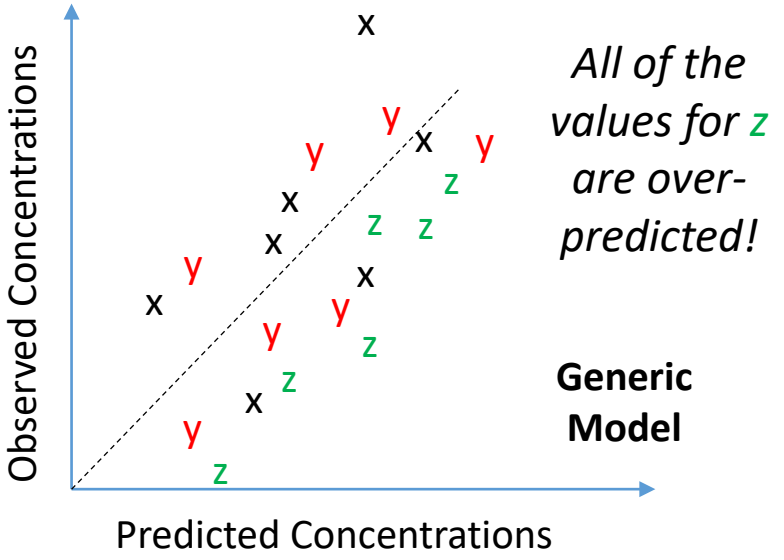
  - Can consider using model to extrapolate to other situations (chemicals without *in vivo* data)

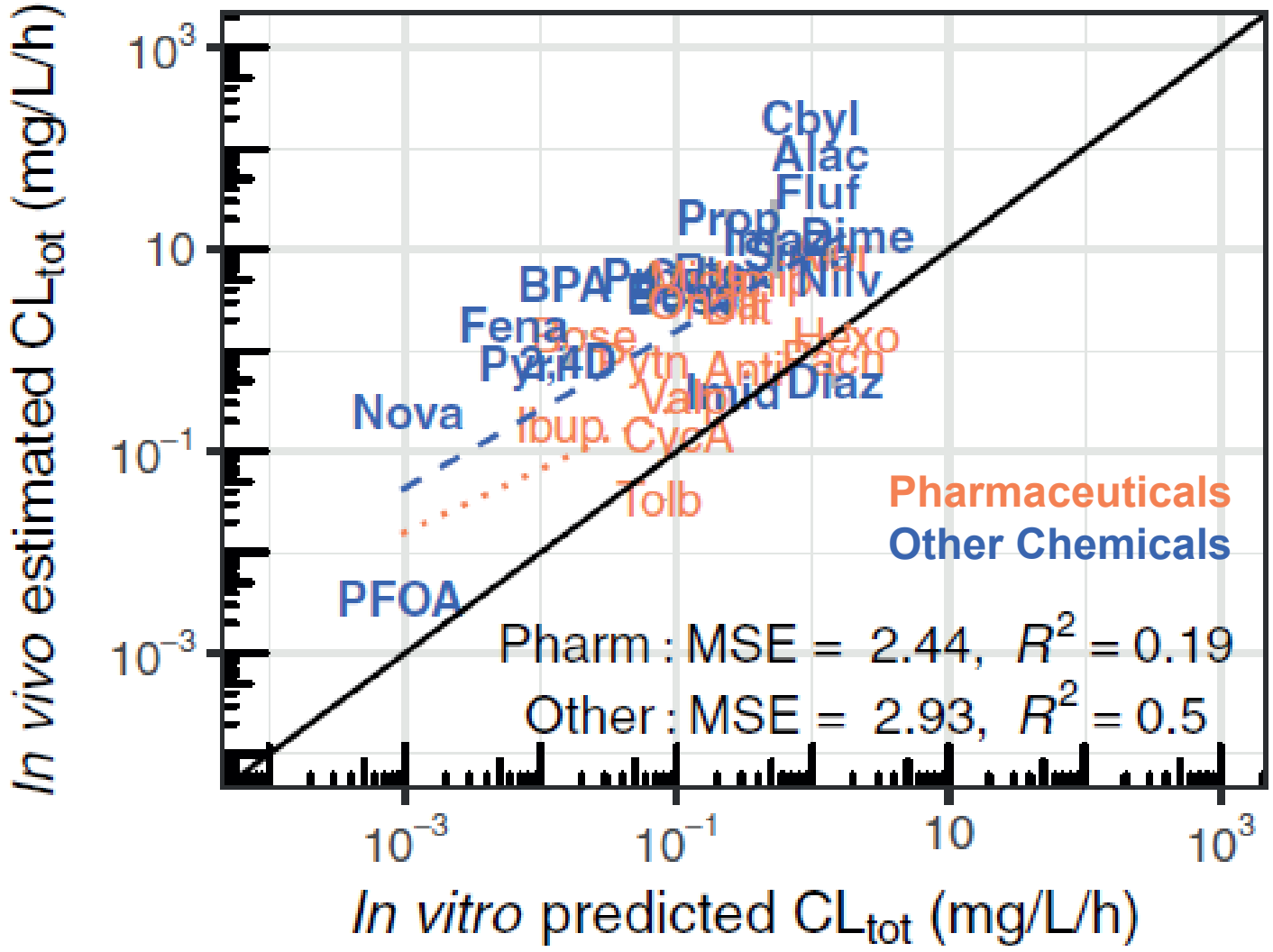




Cohen Hubal et al. (2019)

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

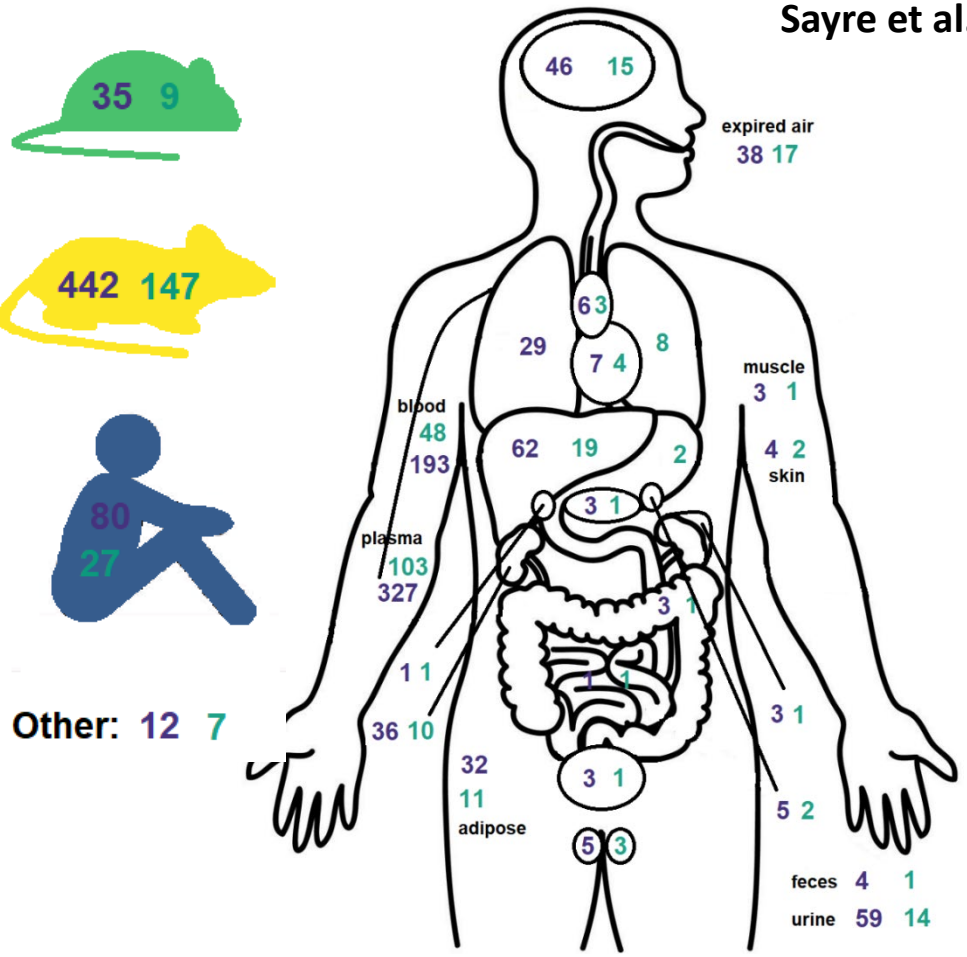
https://github.com/USEPA/CompTox-PK-CvTdb

- 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, literature)
  - Routes: Intravenous, dermal, oral, sub-cutaneous, and inhalation exposure

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

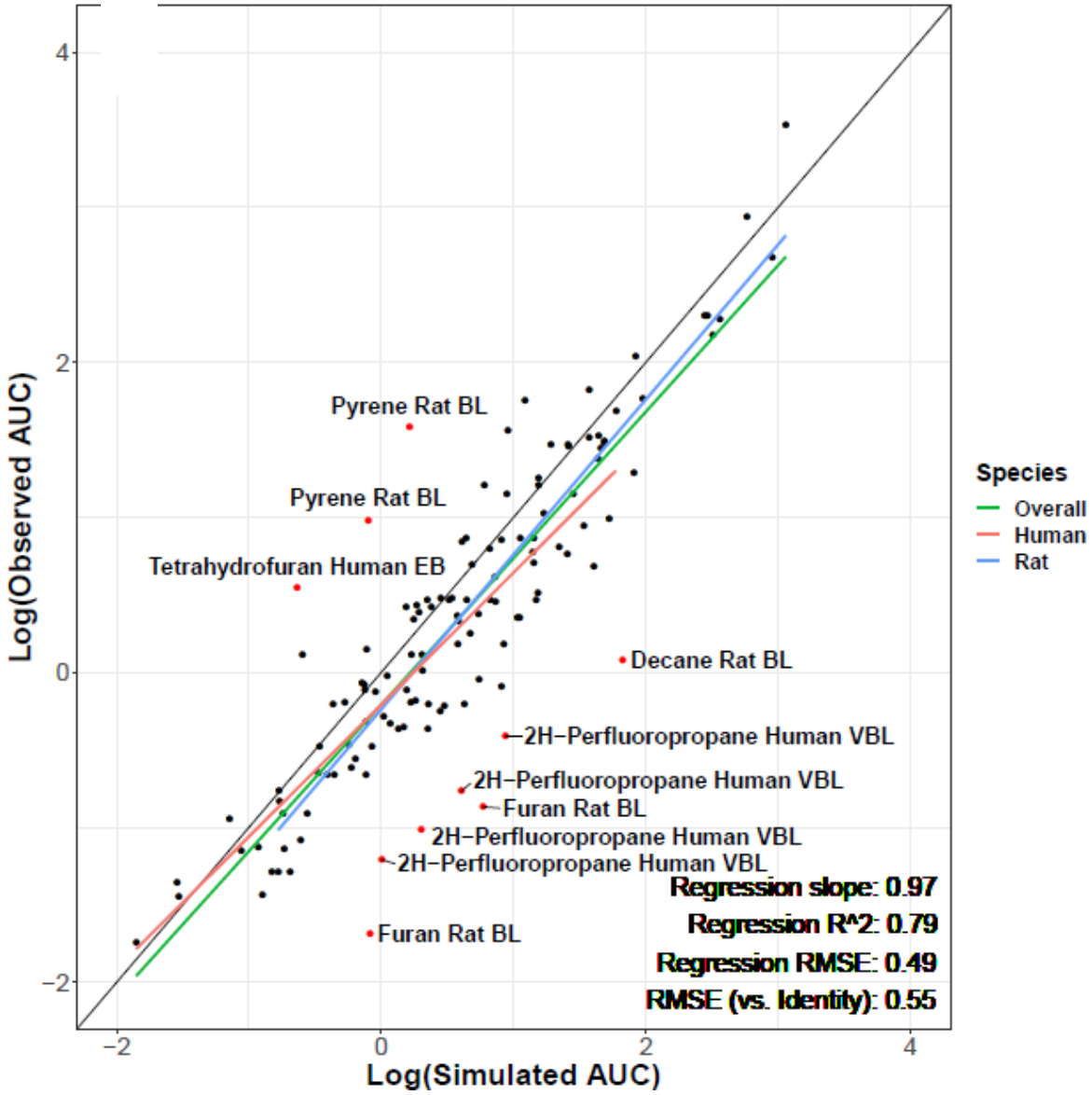
Sayre et al. (2020)



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

Developing Models with the CvT Database

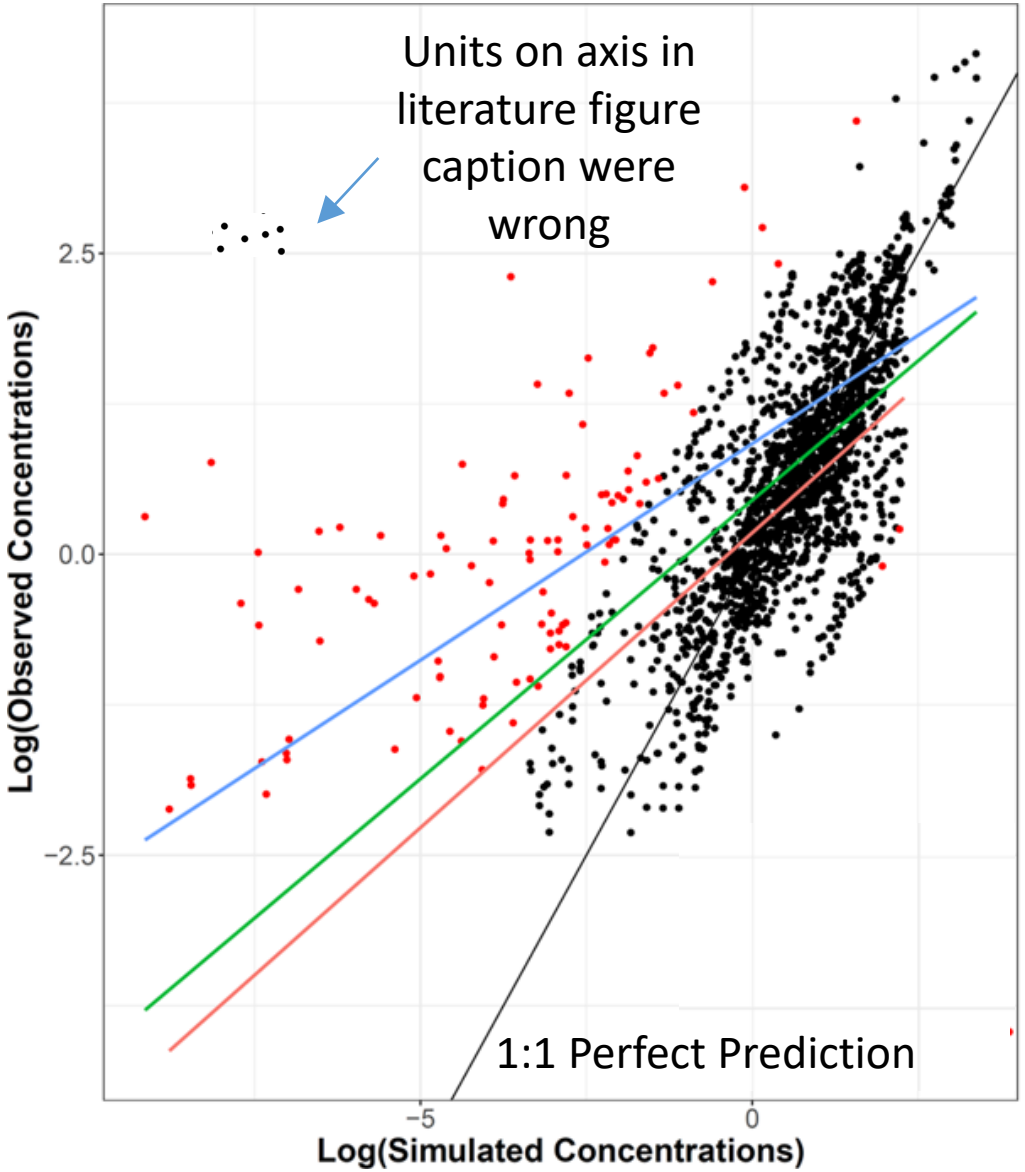
- USAF and EPA developed generic gas inhalation physiologically-based toxicokinetic (PBTK) model
- Evaluated HTTK with CvTdb: 142 exposure scenarios across 41 volatile organic chemicals were modeled and compared to published *in vivo* data for humans and rat
- R^2 was 0.69 for predicting peak concentration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

- Armitage et al. (2021) found that the performance of generic PBTK models in "data poor" situations was both "acceptable in qualitative (that is, shape of concentration versus time (CvT) profiles) and quantitative terms for most of the selected chemicals."
- 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
	Assessment of Model Structure and Biology	
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√	Assessment of Computer Implementation	Open-source code available from GitHub (https://github.com/USEPA/CompTox-ExpoCast-httk) and CRAN (https://CRAN.R-project.org/package=httk) where bugs can be reported and patched
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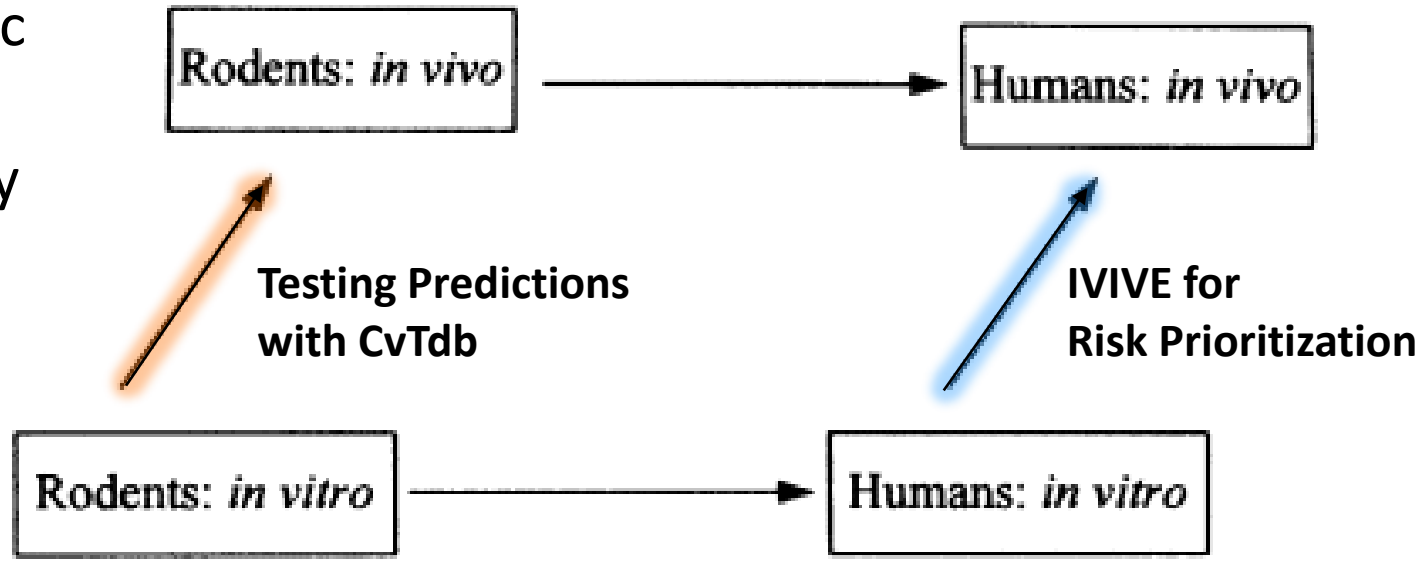
√	Parameter Analysis and Assessment of Model Fitness	Model fitness quantified through comparison with CvTdb
√	Assessment of any Specialized Analyses	Population variability simulator httk-pop has been published (Ring et al., 2017) and is being revised with most recent NHANES biometrics (Breen et al., 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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Questions?

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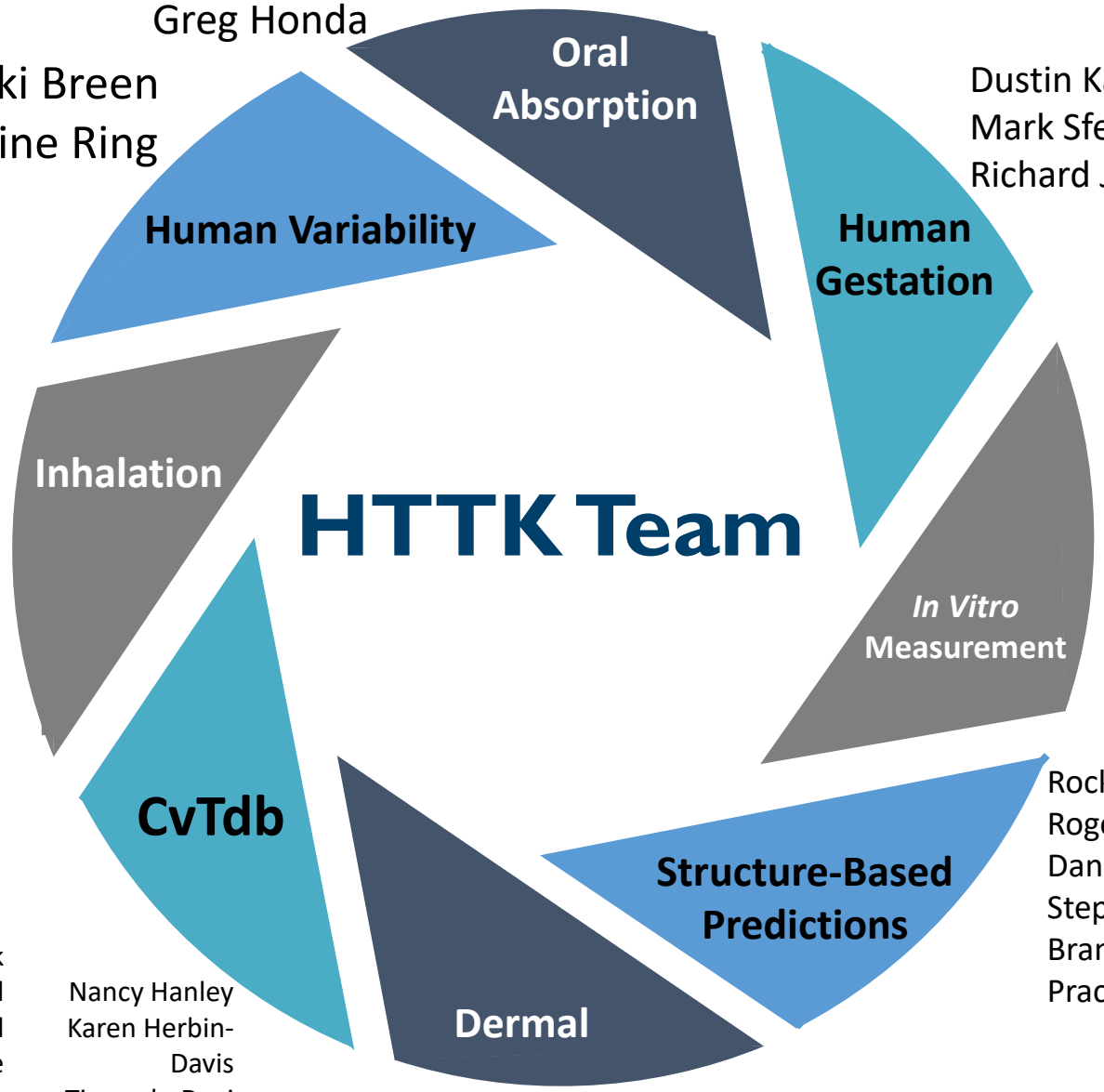

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