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EPA's recently generated Absorption, Distribution, Metabolism, and Excretion (ADME) data and relevance to the Internal Threshold of Toxicological Concern (iTTC)

John Wambaugh
National Center for Computational Toxicology
Office of Research and Development
U.S. Environmental Protection Agency

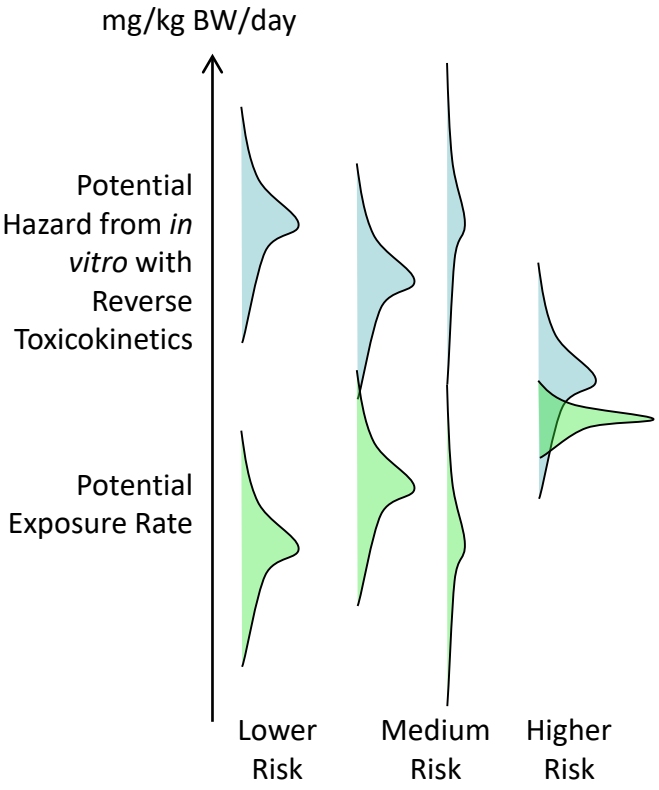
The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA

https://orcid.org/0000-0002-4024-534X

Internal TTC workshop II
26-27 September, 2018

High Throughput Toxicokinetics (HTTK)

- **Most chemicals do not have TK data** – Wetmore et al. (2012…) use *in vitro* methods adapted from pharma to fill gaps
- In order to address greater numbers of chemicals we collect *in vitro*, high throughput toxicokinetic (HTTK) data (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 (*i.e., in vitro-in vivo* extrapolation, or **IVIVE**) (e.g., Wetmore et al., 2015)
- **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) for *In Vitro-In Vivo* Extrapolation (IVIVE)

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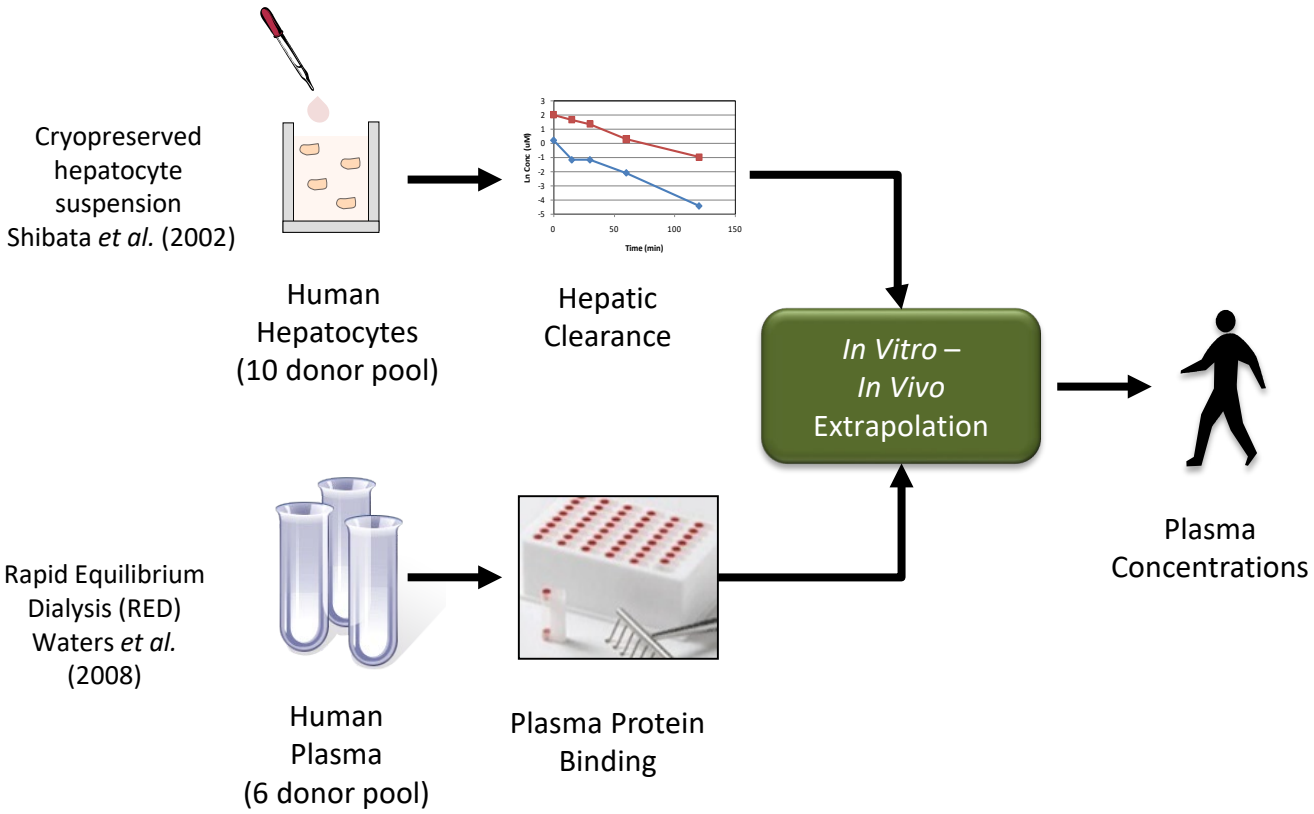

- **Most chemicals do not have TK data** – we use *in vitro* HTTK methods adapted from pharma to fill gaps
- In drug development, HTTK methods allow IVIVE to estimate therapeutic doses for clinical studies – predicted concentrations are typically on the order of values measured in clinical trials (Wang, 2010)

High-Throughput Toxicokinetics (HTTK) for *In Vitro-In Vivo* Extrapolation (IVIVE)

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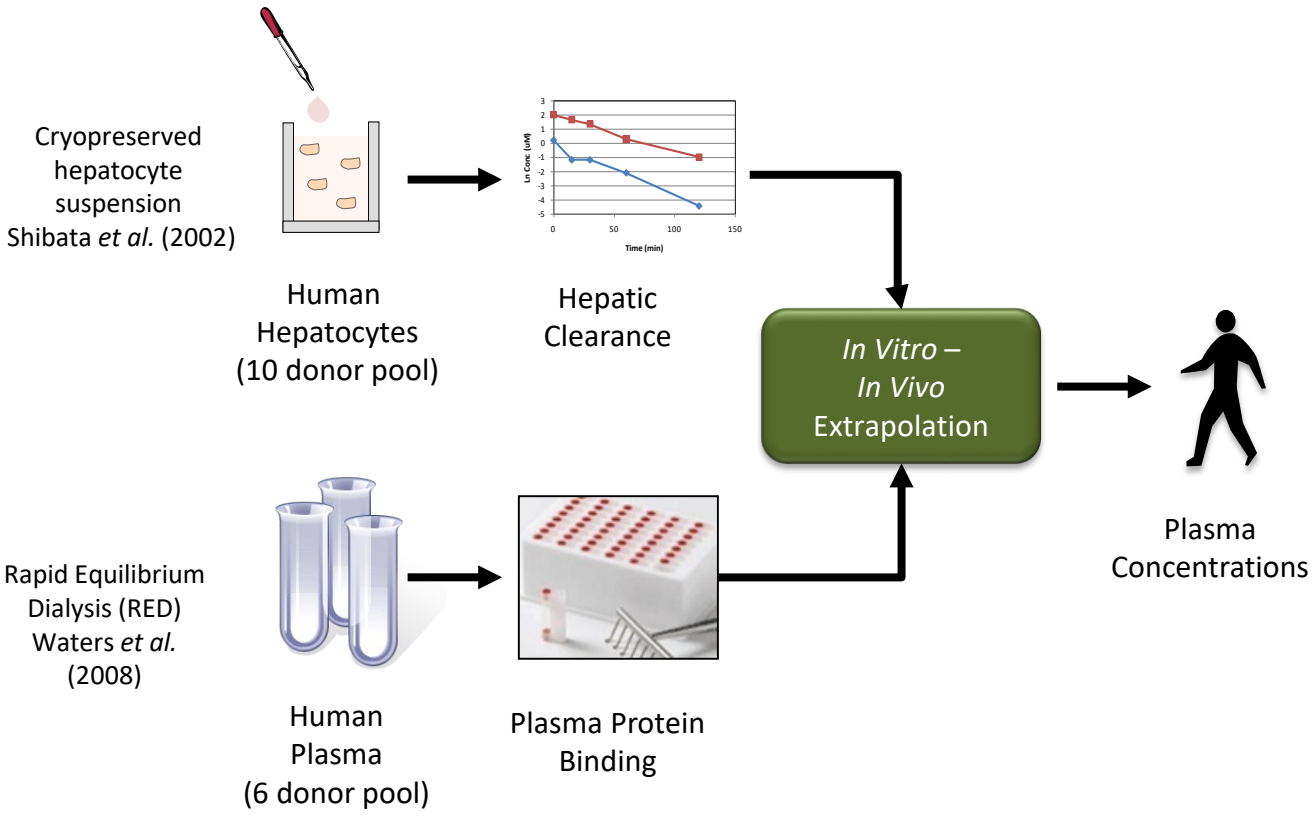

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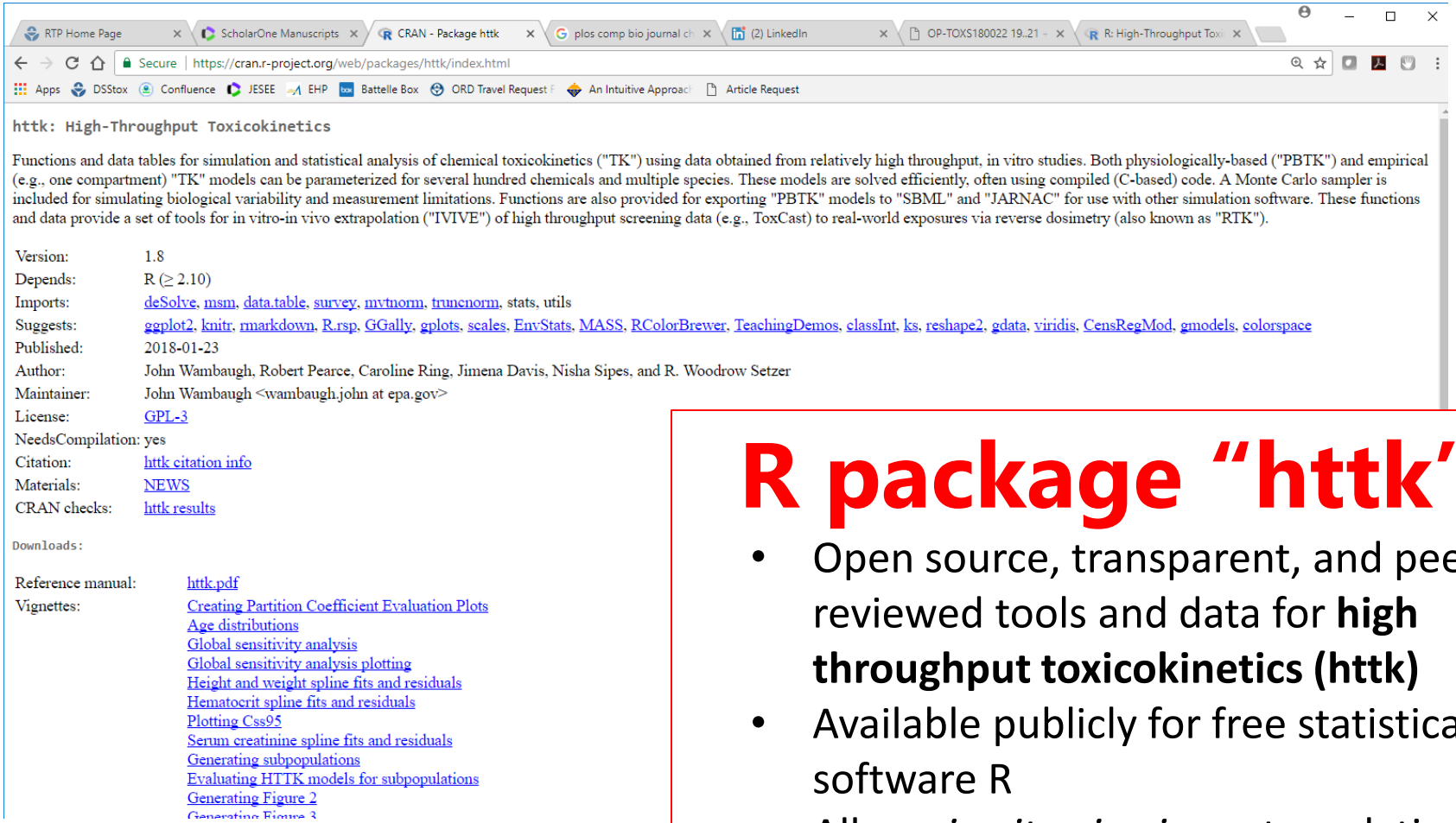
Environmental chemicals:

Rotroff et al. (2010) 35 chemicals
Wetmore et al. (2012) +204 chemicals
Wetmore et al. (2015) +163 chemicals
Wambaugh et al. (in prep.) + ~400 chemicals

Open Source Tools and Data for HTTK

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



RTP Home Page | ScholarOne Manuscripts | CRAN - Package httk | plos comp bio journal ch | (2) LinkedIn | OP-TOXS180022 19..21 | R: High-Throughput Toxi

Secure | https://cran.r-project.org/web/packages/httk/index.html

Apps | DSStox | Confluence | JESEE | EHP | Battelle Box | ORD Travel Request | An Intuitive Approach | Article Request

httk: High-Throughput Toxicokinetics

Functions and data tables for simulation and statistical analysis of chemical toxicokinetics ("TK") using data obtained from relatively high throughput, in vitro studies. Both physiologically-based ("PBTK") and empirical (e.g., one compartment) "TK" models can be parameterized for several hundred chemicals and multiple species. These models are solved efficiently, often using compiled (C-based) code. A Monte Carlo sampler is included for simulating biological variability and measurement limitations. Functions are also provided for exporting "PBTK" models to "SBML" and "JARNAC" for use with other simulation software. These functions and data provide a set of tools for in vitro-in vivo extrapolation ("IVIVE") of high throughput screening data (e.g., ToxCast) to real-world exposures via reverse dosimetry (also known as "RTK").

Version:	1.8
Depends:	R (≥ 2.10)
Imports:	deSolve, msm, data.table, survey, mvtnorm, truncnorm, stats, utils
Suggests:	ggplot2, knitr, rmarkdown, R.rsp, GGally, gplots, scales, EnvStats, MASS, RColorBrewer, TeachingDemos, classInt, ks, reshape2, gdata, viridis, CensRegMod, gmodels, colorspace
Published:	2018-01-23
Author:	John Wambaugh, Robert Pearce, Caroline Ring, Jimena Davis, Nisha Sipes, and R. Woodrow Setzer
Maintainer:	John Wambaugh <wambaugh.john at epa.gov>
License:	GPL-3
NeedsCompilation:	yes
Citation:	httk citation info
Materials:	NEWS
CRAN checks:	httk results

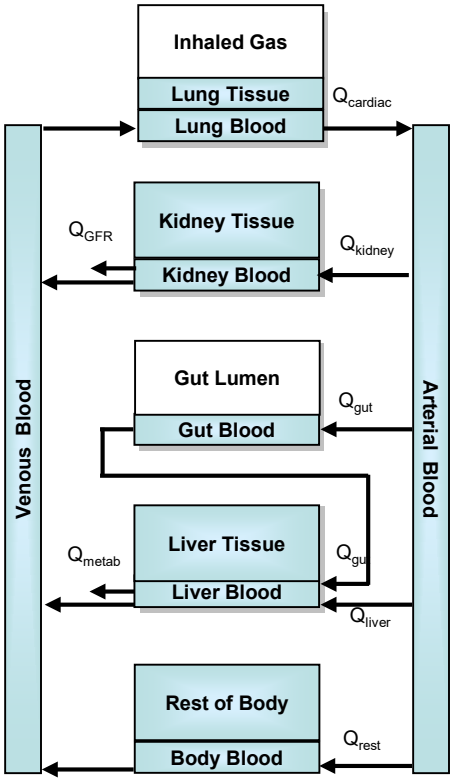
Downloads:

Reference manual:	httk.pdf
Vignettes:	Creating Partition Coefficient Evaluation Plots
	Age distributions
	Global sensitivity analysis
	Global sensitivity analysis plotting
	Height and weight spline fits and residuals
	Hematocrit spline fits and residuals
	Plotting Css95
	Serum creatinine spline fits and residuals
	Generating subpopulations
	Evaluating HTTK models for subpopulations
	Generating Figure 2
	Generating Figure 3

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)

A General Physiologically-based Toxicokinetic (PBTK) Model



- "httk" includes a generic PBTK model
- Some tissues (e.g. arterial blood) are simple compartments, while others (e.g. kidney) are compound compartments consisting of separate blood and tissue sections with constant partitioning (i.e., tissue specific partition coefficients)
- Exposures are absorbed from reservoirs (gut lumen)
- Some specific tissues (lung, kidney, gut, and liver) are modeled explicitly, others (e.g. fat, brain, bones) are lumped into the "Rest of Body" compartment.
- The only ways chemicals "leave" the body are through metabolism (change into a metabolite) in the liver or excretion by glomerular filtration into the proximal tubules of the kidney (which filter into the lumen of the kidney).

High-Throughput Toxicokinetics (HTTK) for *In Vitro-In Vivo* Extrapolation (IVIVE)

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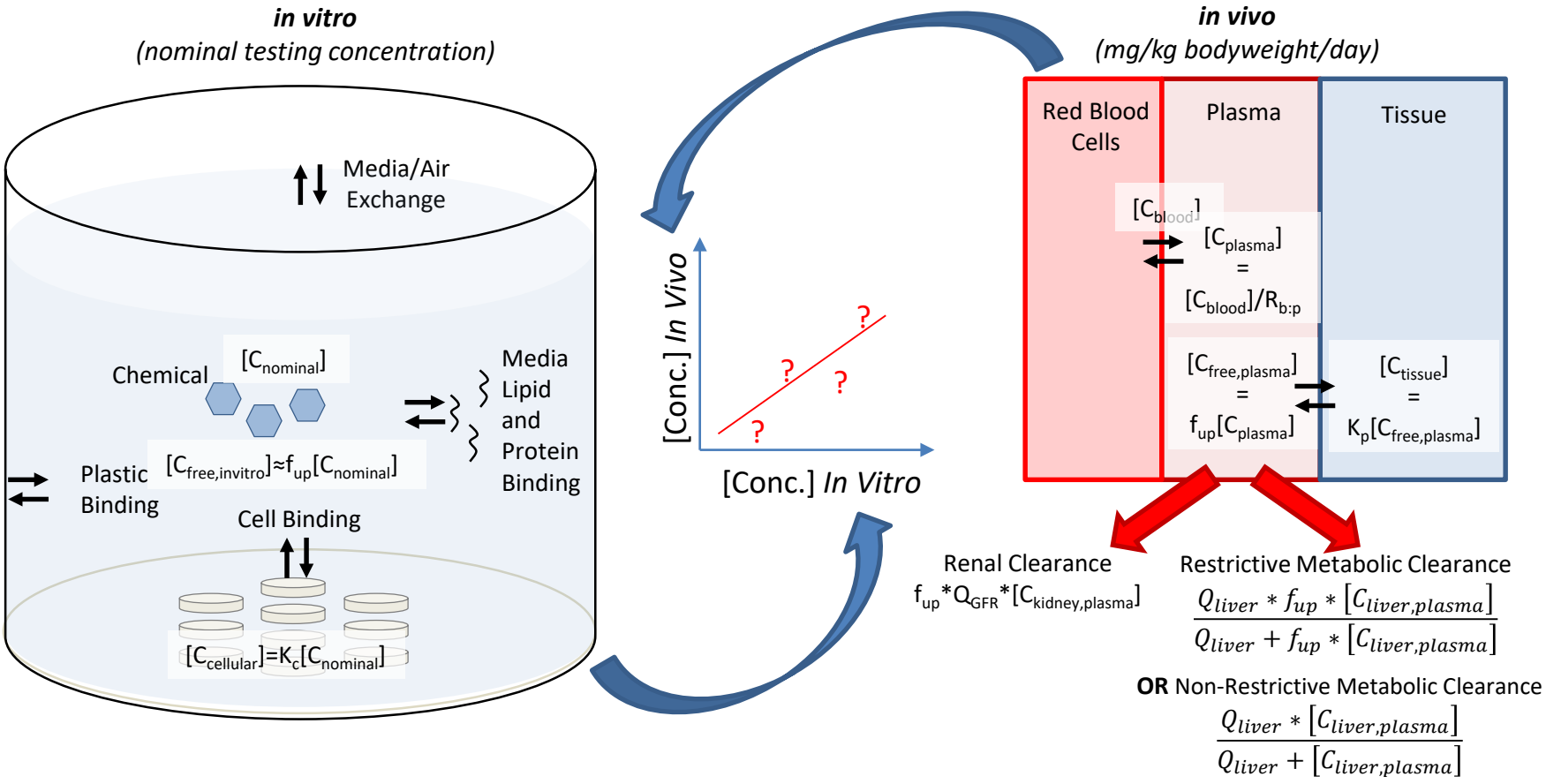

$$\frac{Q_{liver} * f_{up} * [C_{liver,plasma}]}{Q_{liver} + f_{up} * [C_{liver,plasma}]}$$

$$\frac{Q_{liver} * [C_{liver,plasma}]}{Q_{liver} + [C_{liver,plasma}]}$$

Selecting the appropriate *in vitro* and *in vivo* concentrations for extrapolation

Honda et al, in prep.

Optimizing HTTK-based IVIVE

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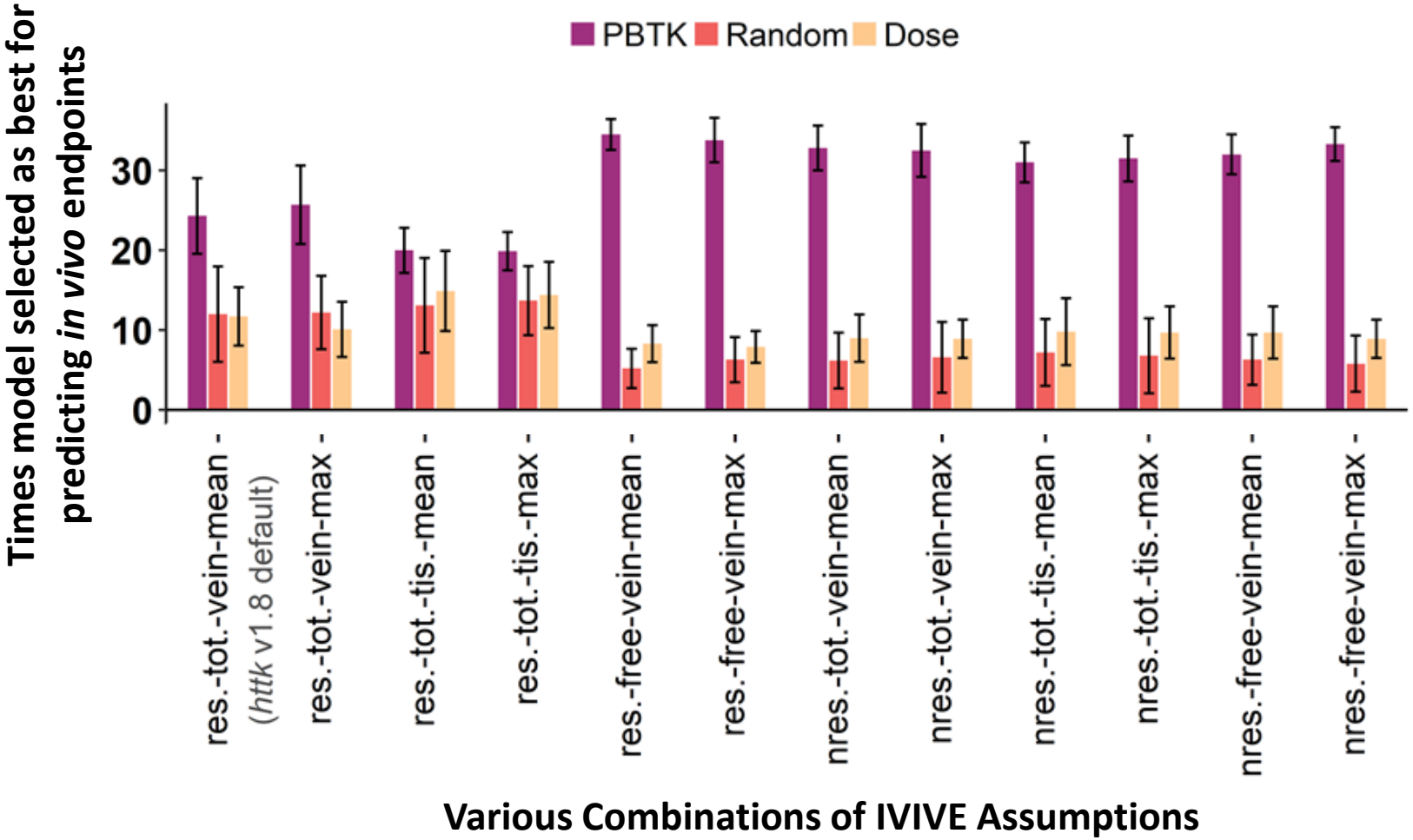

Honda et al, in prep.

Selecting Candidates for Prioritization

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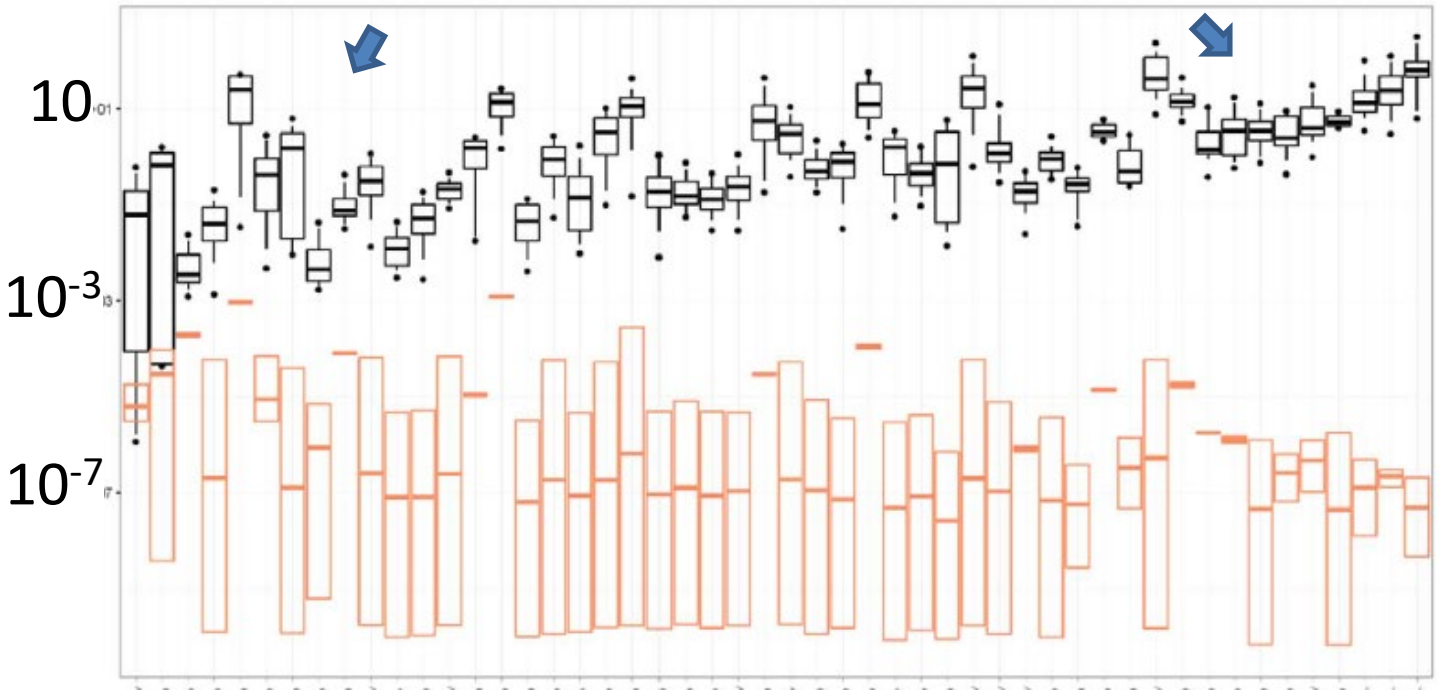
High Throughput Screening + HTTK can estimate doses needed to cause bioactivity (Wetmore, et al., 2012, 2015)



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

National Health and Nutrition Examination Survey (NHANES) is an ongoing survey that covers ~10,000 people every two years

Most NHANES chemicals do not have traditional PK models (Strope et al., 2018)

Ring *et al*. (2017)

Why Build Another Generic PBTK Tool?

	SimCYP	ADMET Predictor / GastroPlus	MEGen	IndusChemFate	httk
Maker	SimCYP Consortium / Certara	Simulations Plus	UK Health and Safety Laboratory	Cefic LRI	US EPA
Availability	License, but inexpensive for research	License, but inexpensive for research	Free: http://xnet.hsl.gov.uk/megen	Free: http://cefic-lri.org/lri_toolbox/induschemfate/	Free: https://CRAN.R-project.org/package=httk
Open Source	No	No	**Yes**	No	**Yes**
Default PBPK Structure	**Yes**	**Yes**	No	**Yes**	**Yes**
Expandable PBPK Structure	No	No	**Yes**	No	No
Population Variability	**Yes**	No	No	No	**Yes**
Batch Mode	**Yes**	**Yes**	No	No	**Yes**
Graphical User Interface	**Yes**	**Yes**	**Yes**	Excel	No
Physiological Data	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Chemical-Specific Data Library	Many Clinical Drugs	No	No	15 Environmental Compounds	543 Pharmaceutical and ToxCast Compounds
Ionizable Compounds	**Yes**	**Yes**	Potentially	No	**Yes**
Export Function	No	No	Matlab and AcslX	No	SBML and Jarnac
R Integration	No	No	No	No	**Yes**
Easy Reverse Dosimetry	**Yes**	**Yes**	No	No	**Yes**
Future Proof XML	No	No	**Yes**	No	No

We want to do a statistical analysis (using R) for as many chemicals as possible

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Doing Statistical Analysis with HTTK

- If we are to use HTTK, we need confidence in predictive ability

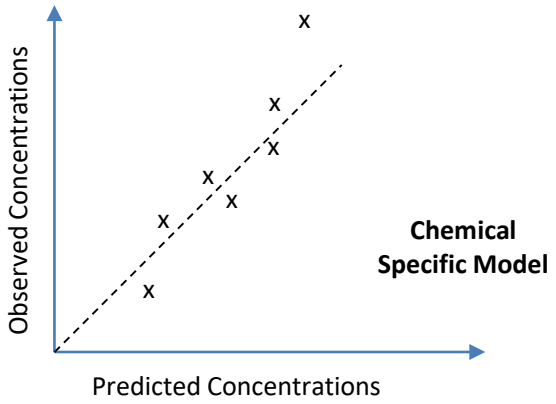
- In drug development, HTTK methods estimate therapeutic doses for clinical studies – predicted concentrations are typically on the order of values measured in clinical trials (Wang, 2010)
  - For most compounds in the environment there will be no clinical trials

- 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*., submitted)
  - Any approximations, omissions, or mistakes should work to increase the estimated uncertainty when evaluated systematically across chemicals

Building Confidence in TK Models

- In order 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 don't have data

- However, we do not typically have TK data



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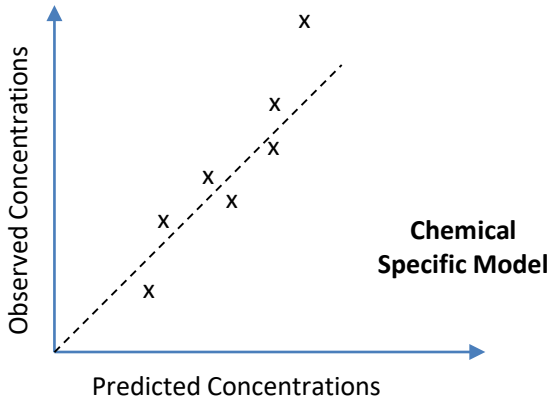
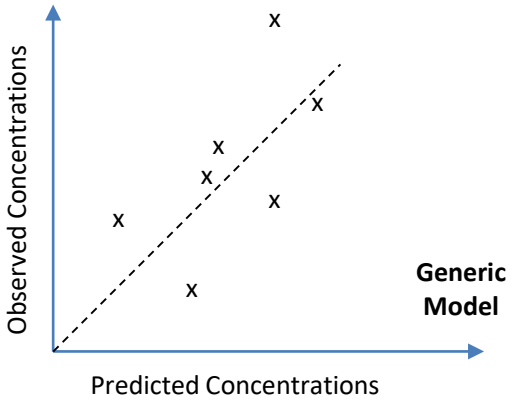
- We can parameterize a **generic TK model**, and evaluate that model for as many chemicals as we do have data
  - We do expect larger uncertainty, but also greater confidence in model implementation
  - Estimate bias and uncertainty, and try to correlate with chemical-specific properties
  - Can again consider using model to extrapolate to other situations (chemicals without *in vivo* data)





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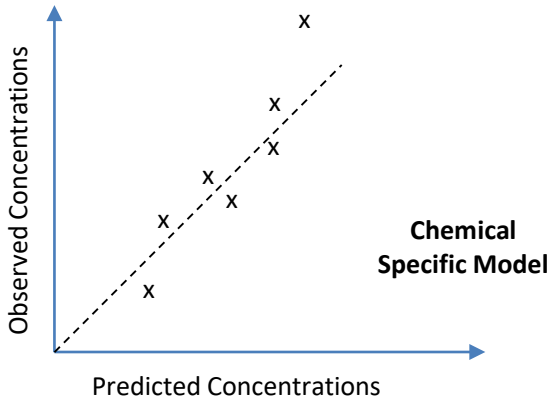

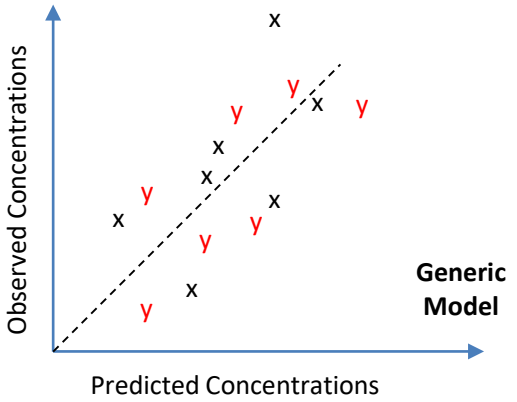

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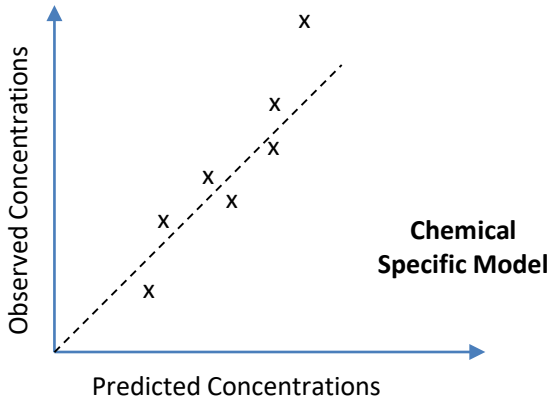
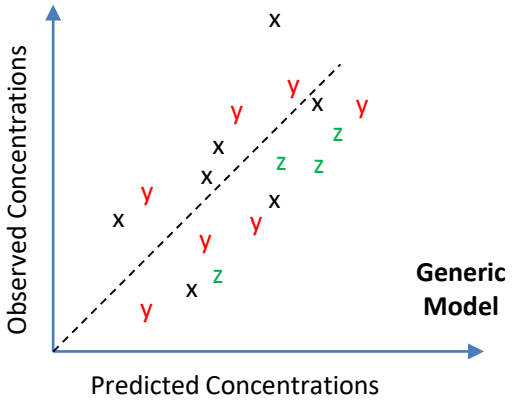
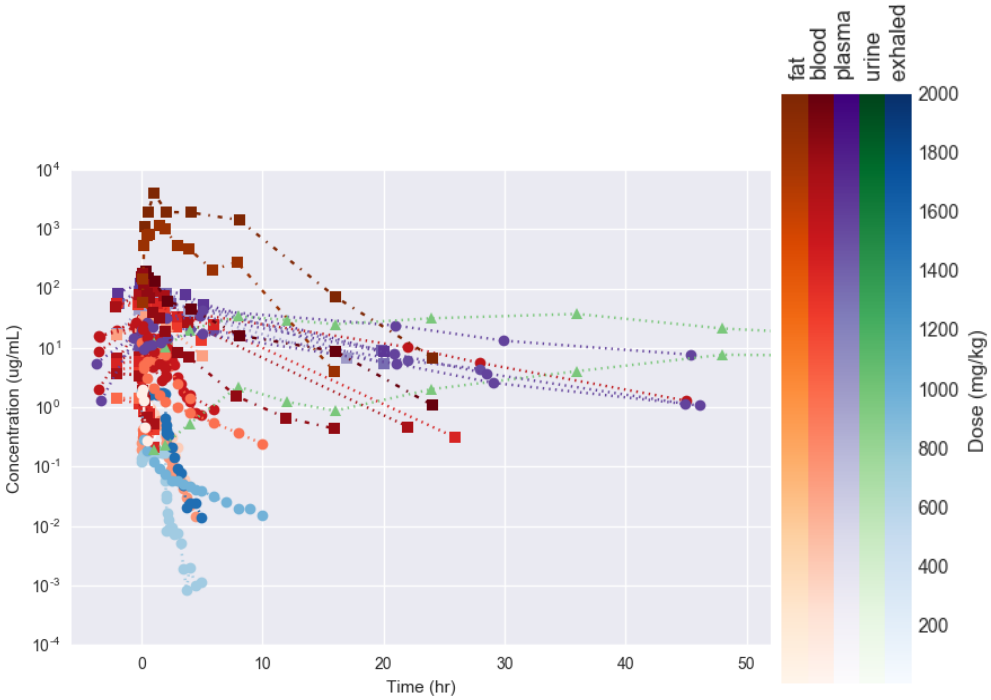




In Vivo TK Database

- EPA is developing a public database of concentration vs. time data for building, calibrating, and evaluating TK models
- Curation and development ongoing, but to date includes:
  - 198 analytes (EPA, National Toxicology Program, literature)
  - Routes: Intravenous, dermal, oral, sub-cutaneous, and inhalation exposure



- Database will be made available through web interface and through the “httk” R package
- Standardized, open source curve fitting software invivoPKfit used to calibrate models to all data:
  https://github.com/USEPA/CompTox-ExpoCast-invivoPKfit

Sayre et al., in preparation

Building Confidence in HTTK

"…the steady-state, peak, and time-integrated plasma concentrations of non-pharmaceuticals were predicted with reasonable accuracy… HTTK and IVIVE methods are adequately robust to be applied to high throughput *in vitro* toxicity screening data of environmentally-relevant chemicals for prioritizing based on human health risks."

Toxicological Sciences

OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org ToxSci 20 Years

TOXICOLOGICAL SCIENCES, 2018, 1–18

doi: 10.1093/toxsci/kfy020
Advance Access Publication Date: January 27, 2018
Research Article

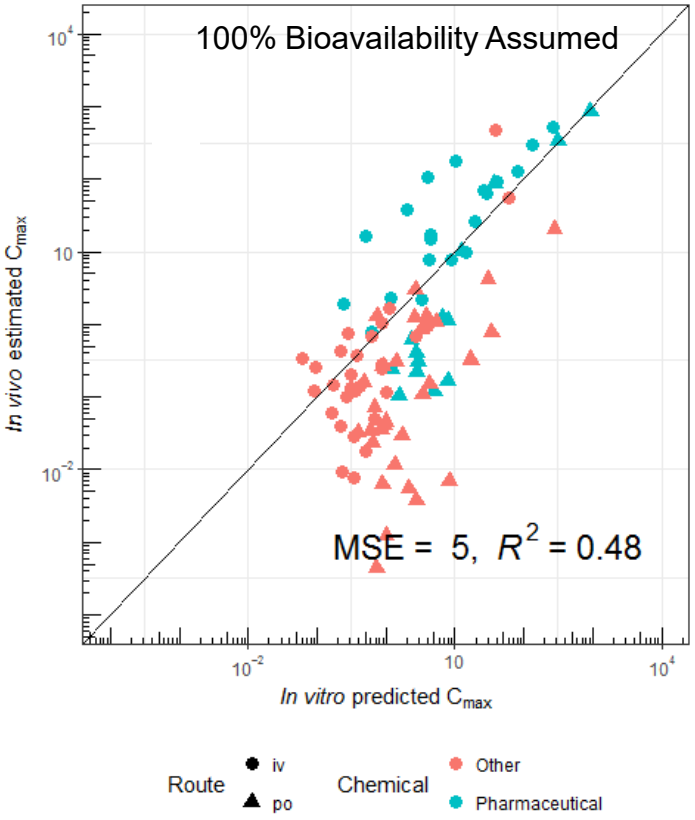
Evaluating *In Vitro-In Vivo* Extrapolation of Toxicokinetics

John F. Wambaugh,[*,1] Michael F. Hughes,[†] Caroline L. Ring,[*,‡,2] Denise K. MacMillan,[†] Jermaine Ford,[†] Timothy R. Fennell,[§] Sherry R. Black,[§] Rodney W. Snyder,[§] Nisha S. Sipes,[¶] Barbara A. Wetmore,[‖] Joost Westerhout,[‖‖] R. Woodrow Setzer,[*] Robert G. Pearce,[*,‡] Jane Ellen Simmons,[†] and Russell S. Thomas[*]

[*]National Center for Computational Toxicology; [†]National Health and Environmental Effects Research Laboratory, Office of Research and Development, United States Environmental Protection Agency, Research Triangle Park, North Carolina 27711; [‡]Oak Ridge Institute for Science and Education, Oak Ridge, Tennessee 37831; [§]RTI International, Research Triangle Park, North Carolina; [¶]National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27717; [‖]National Exposure Research Laboratory, Office of Research and Development, United States Environmental Protection Agency, Research Triangle Park, North Carolina 27711; and [‖‖]The Netherlands Organisation for Applied Scientific Research (TNO), AJ Zeist 3700, The Netherlands

[1]To whom correspondence should be addressed. Fax: (919) 541-1194. E-mail: wambaugh.john@epa.gov.

Evaluating HTTK



We evaluate HTTK by comparing predictions with observations for as many chemicals as possible

Wambaugh et al. (2018)

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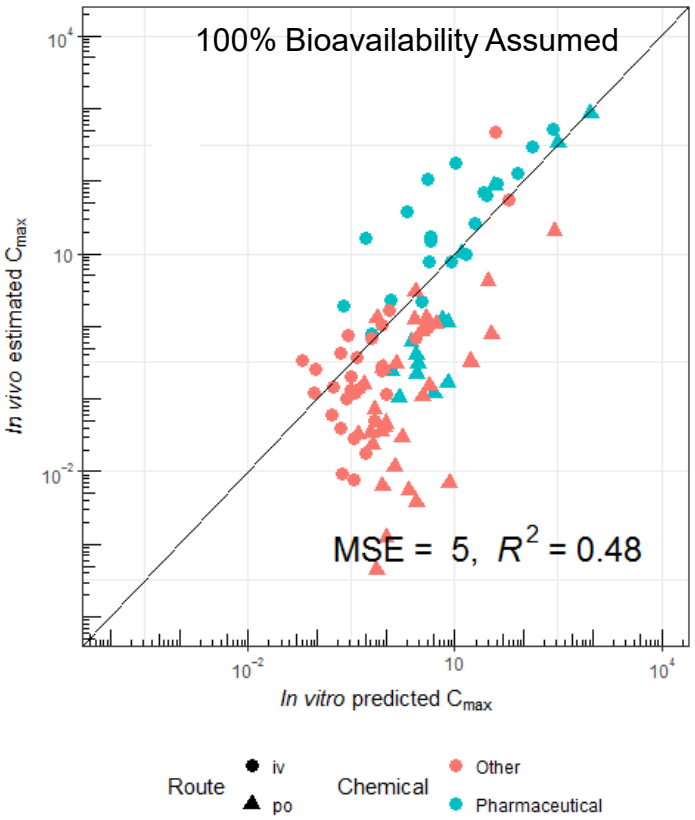
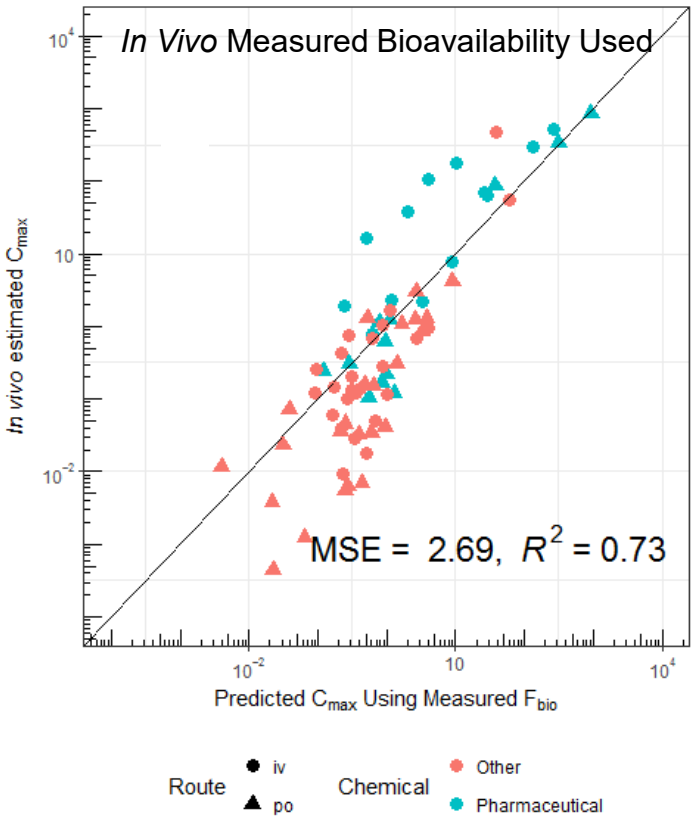
Evaluating HTTK

Impact of Oral Bioavailability Data





Wambaugh et al. (2018)

Characterizing Bioavailability *In Vitro*

Greg Honda (NCCT) made a SOT2018 presentation on using Caco2 *in vitro* data to predict absorption for ~300 ToxCast chemicals

$$P_{AB} = \frac{1}{area * C_{Apical}} \frac{dA_{Basolateral}}{dt}$$

$$F_{abs} = A_{abs} / A_{gut} \approx \text{func.}\ (P_{AB})$$

$$F_{FP} \approx \frac{Q_{liv}}{Q_{liv} + f_{up} Cl / R_{b2p}}$$

$$F_{bio} = F_{abs} F_{FP}$$

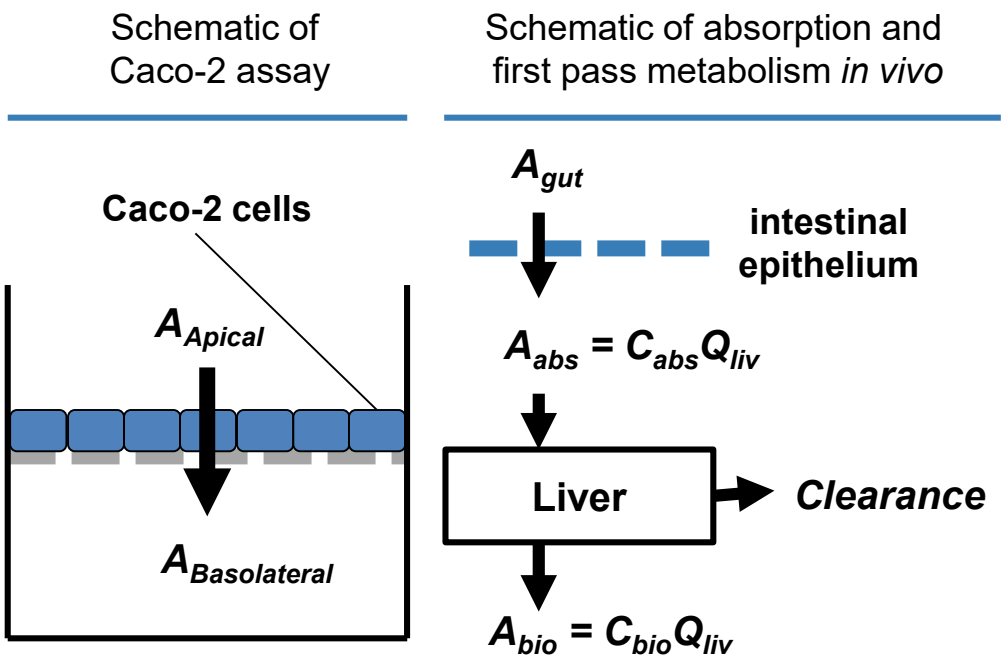


Figure from Greg Honda

Characterizing Bioavailability *In Vitro*

$$P_{AB} = \frac{1}{area * C_{Apical}} \frac{dA_{Basolateral}}{dt}$$

$$F_{abs} = A_{abs} / A_{gut} \approx \text{Darwich } (P_{AB})$$

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Darwich *et al.* 2010

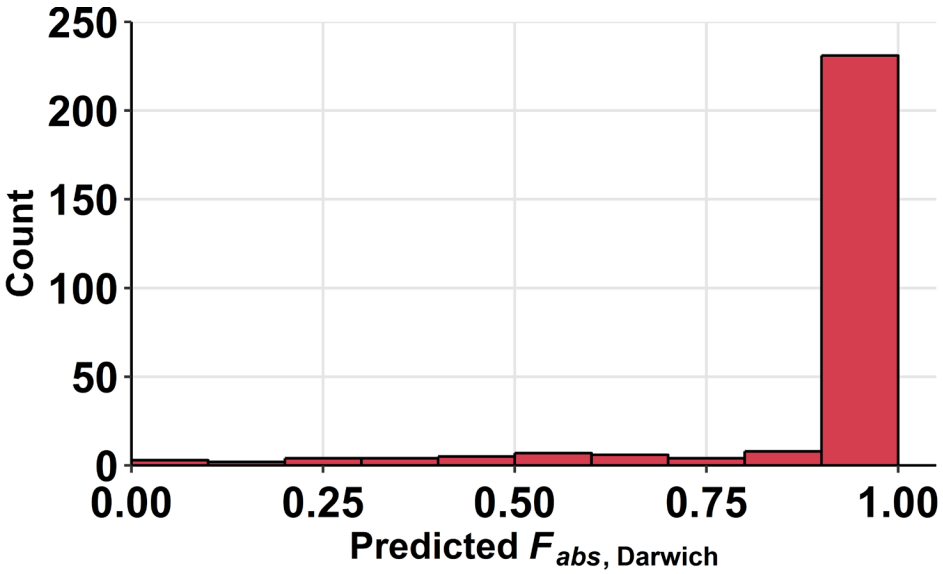

Figure from Greg Honda

Characterizing Bioavailability *In Vitro*

United States Environmental Protection Agency

Usansky and Sinko 2005

$$P_{AB} = \frac{1}{area * C_{Apical}} \frac{dA_{Basolateral}}{dt}$$

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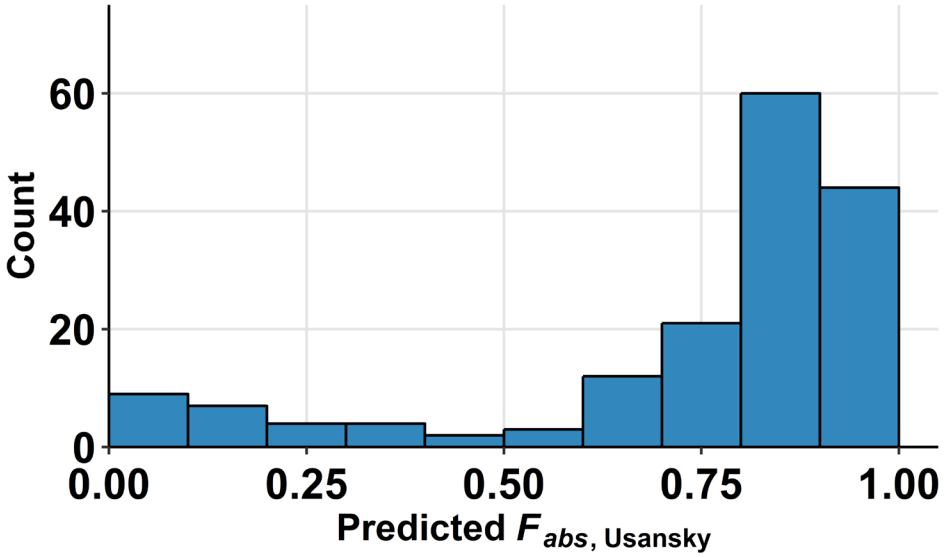


Figure from Greg Honda

Predicting F_{bio} for Toxicokinetics

- C_{max} predicted using a 1 compartment model (Wambaugh *et al.* 2018)
- Minimal difference when using estimated F_{bio} in prediction of toxicokinetics observed for this limited set of chemicals

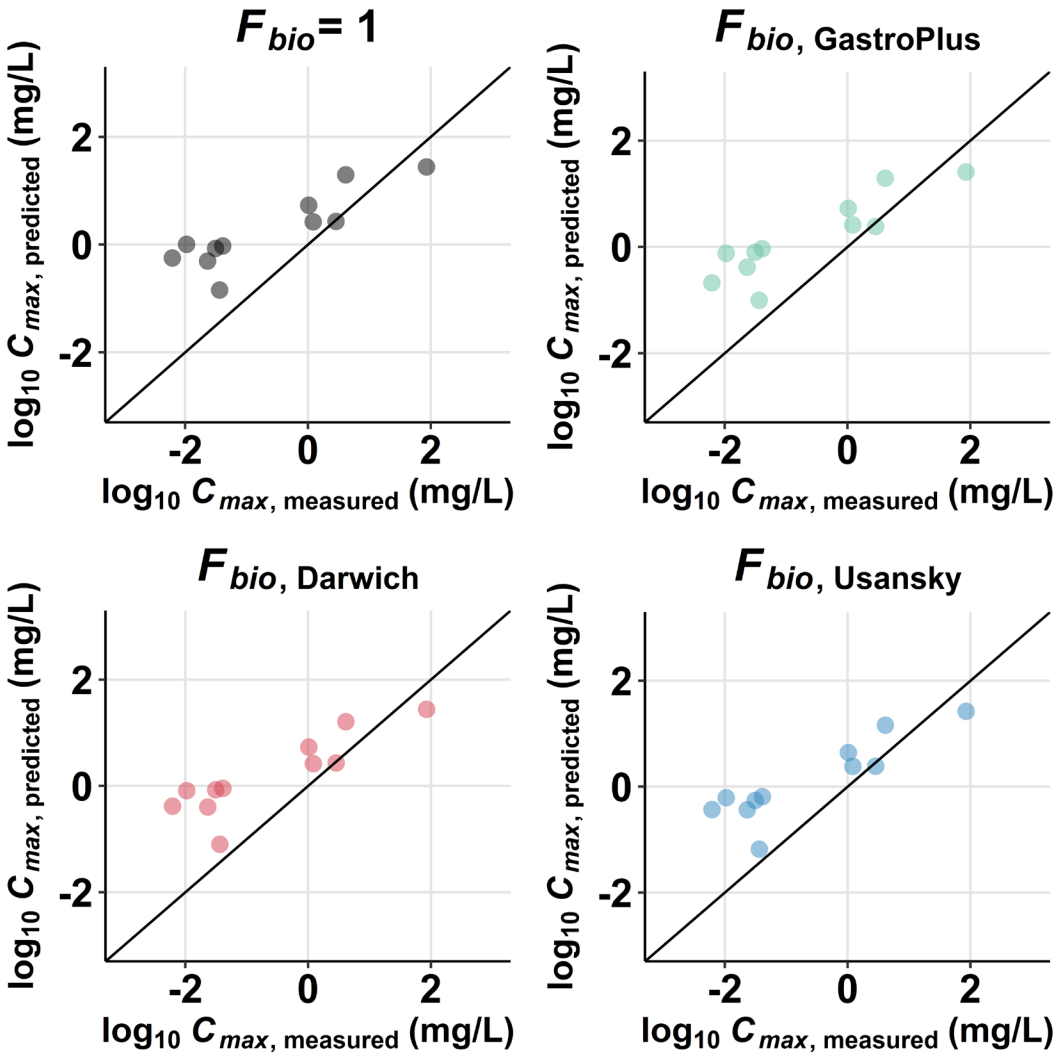
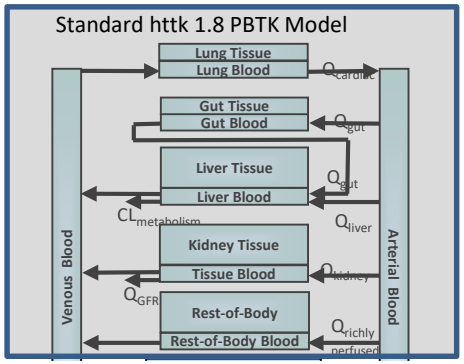


Figure from Greg Honda

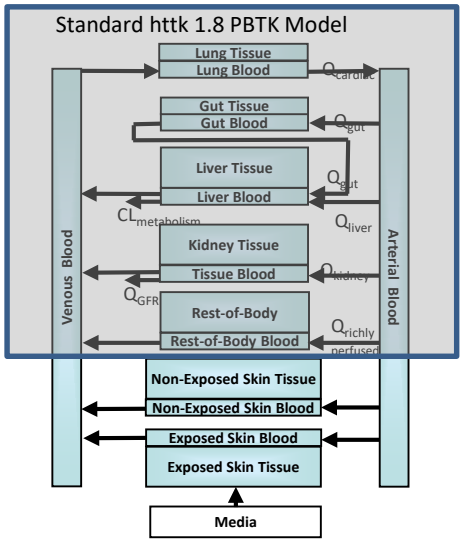
New HT-PBTK Models



- We are working to augment the basic HT-PBPTK model with new PBTK models
- Each model will be released publicly upon peer-reviewed publication
- Pre-publication models can be shared under a MTA
- We assume there will be coding errors and over-simplifications, so each publication involves curation of evaluation data from the scientific literature and through statistical analysis
- Cvt database (Sayre et al.) is critical to these efforts

New HT-PBTK Models

United States Environmental Protection Agency



Dermal Exposure Route

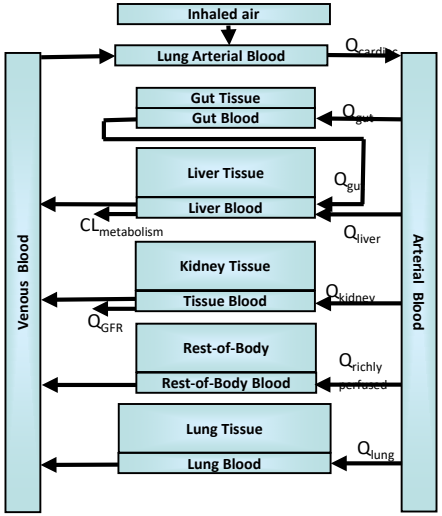
EPA, Unilever, INERIS

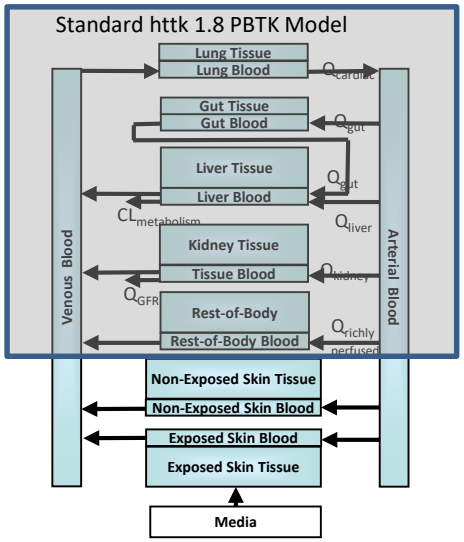
New HT-PBTK Models

United States Environmental Protection Agency

Gas Inhalation Exposure Route

EPA, USAFSAM





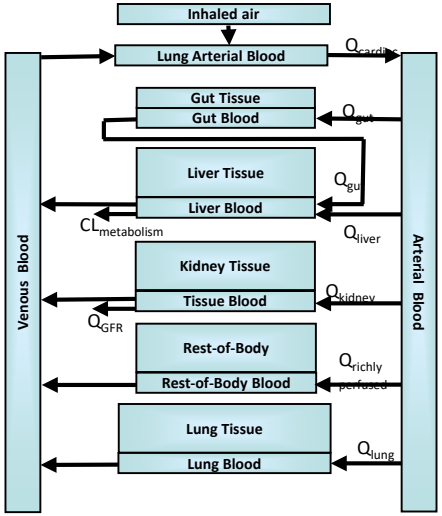
Dermal Exposure Route

EPA, Unilever, INERIS

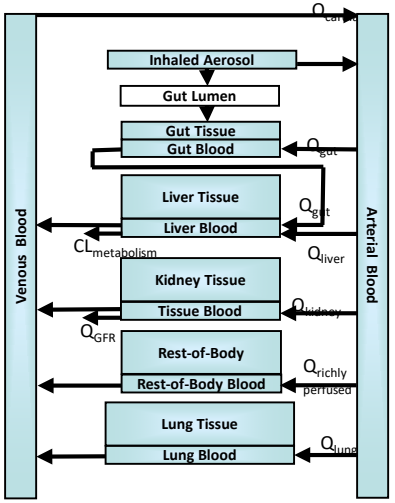
New HT-PBTK Models

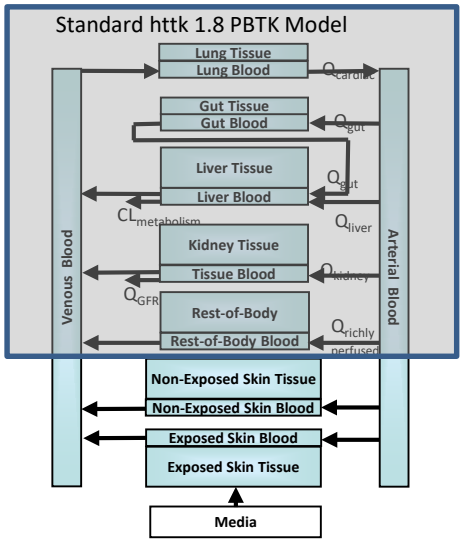
United States Environmental Protection Agency

Gas Inhalation Exposure Route
EPA, USAFSAM



Aerosol Inhalation Exposure Route (with APEX model)
EPA, USAFSAM





Dermal Exposure Route
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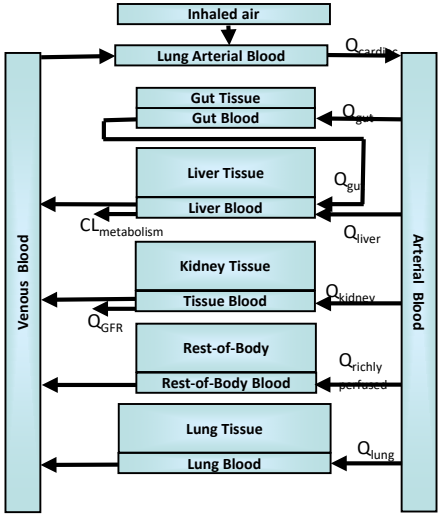

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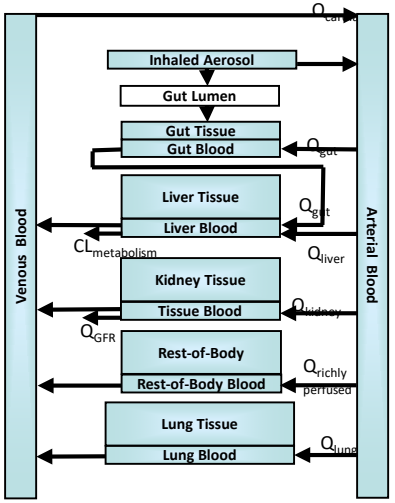
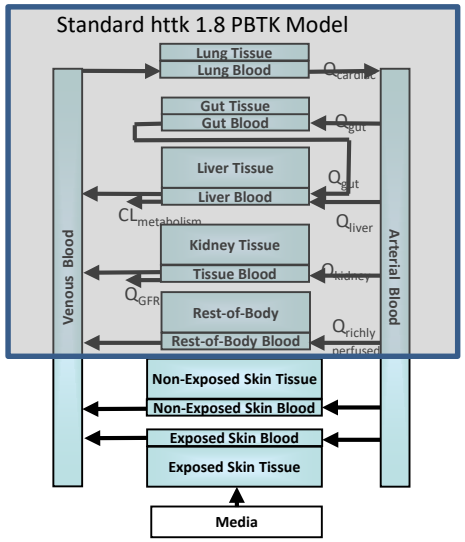


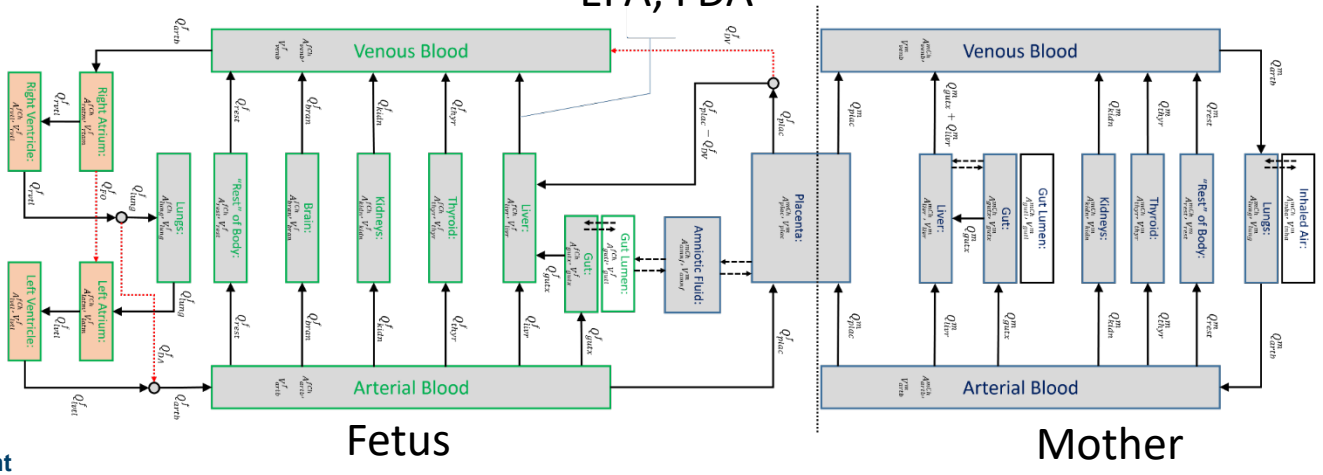


Dermal Exposure Route

EPA, Unilever, INERIS

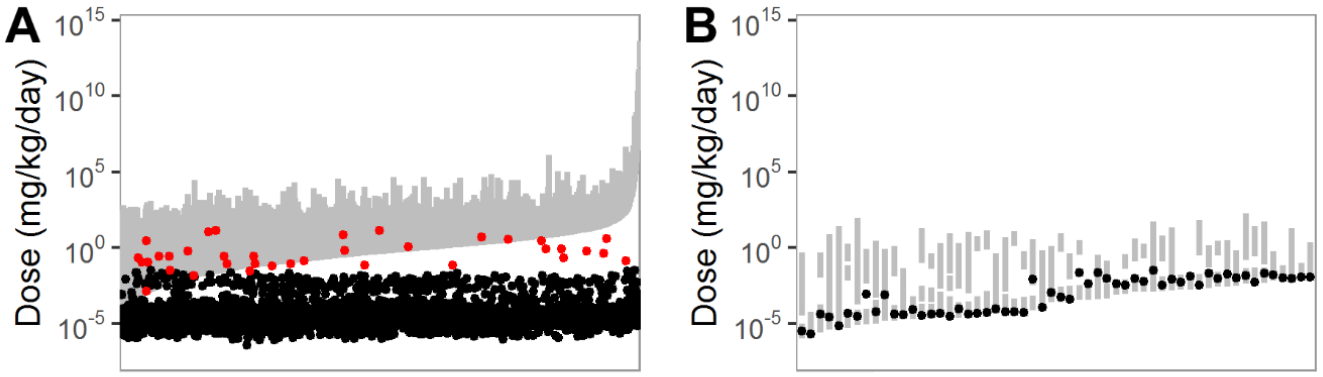
Human Gestational Model

EPA, FDA



In Silico HTTK Predictions

- Tox21 has screened >8000 chemicals – Sipes *et al.* (2017) wanted to compare *in vitro* active concentrations with HTTK predicted maximum plasma concentrations with high throughput exposure predictions from Wambaugh et al. (2014)
  - "httk" package only has ~500 chemicals
- Used Simulations Plus ADMet Predictor to predict for entire library (supplemental table) and used add_chemtable() function to add into "httk" package
- Predictions available in httk v1.8



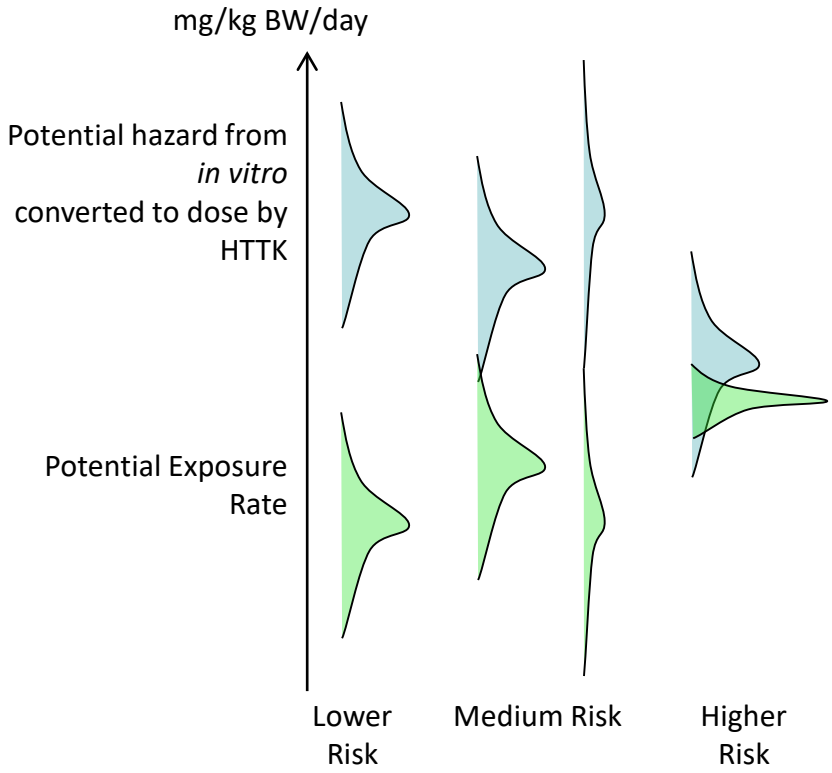
Dose range for all 3925 Tox21 compounds eliciting a 'possible'-to-'likely' human *in vivo* interaction alongside estimated daily exposure

56 compounds with potential *in vivo* biological interaction at or above estimated environmental exposures

Figure from Sipes *et al.*, (2017)

Conclusions

- We would like to know more about the risk posed by thousands of chemicals in the environment – which ones should we start with?
- In addition to toxicity, we need information on **Toxicokinetics**:
  - HTTK New approach methodologies (NAMs) are being evaluated through 1) uncertainty analysis and 2) comparison between *in vitro* predictions and *in vivo* measurements of both plasma concentrations and doses associated with the onset of effects
  - Modeling various exposure routes (e.g., inhalation of gasses and aerosols) allows extrapolation to important scenarios



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

Chemical Safety for Sustainability (CSS) Research Program

Rapid Exposure and Dosimetry (RED) Project

NCCT
Chris Grulke
Greg Honda*
Richard Judson
Matthew Linakis*
Andrew McEachran*
Ann Richard
Risa Sayre*
Woody Setzer
Rusty Thomas
John Wambaugh
Antony Williams

NRMRL
Xiaoyu Liu

NHEERL
Linda Adams
Christopher Ecklund
Marina Evans
Mike Hughes
Jane Ellen Simmons

NERL
Cody Addington*
Craig Barber
Namdi Brandon*
Peter Egeghy
Hongtai Huang*
Kristin Isaacs
Ashley Jackson*
Charles Lowe*
Dawn Mills*
Seth Newton
Katherine Phillips
Paul Price
Jeanette Reyes*
Randolph Singh*
Jon Sobus
John Streicher*
Mark Strynar
Mike Tornero-Velez
Elin Ulrich
Dan Vallero
Barbara Wetmore

Lead CSS Matrix Interfaces:
John Kenneke (NERL)
John Cowden (NCCT)

***Trainees**

Collaborators

Arnot Research and Consulting
Jon Arnot
Johnny Westgate
Institut National de l'Environnement et des Risques (INERIS)
Frederic Bois
Integrated Laboratory Systems
Kamel Mansouri
National Toxicology Program
Mike Devito
Steve Ferguson
Nisha Sipes
Ramboll
Harvey Clewell
ScitoVation
Chantel Nicolas
Silent Spring Institute
Robin Dodson
Southwest Research Institute
Alice Yau
Kristin Favela
Summit Toxicology
Lesa Aylward
Technical University of Denmark
Peter Fantke
Tox Strategies
Caroline Ring
Miyoung Yoon
Unilever
Beate Nicol
Cecilie Rendal
Ian Sorrell
United States Air Force
Heather Pangburn
University of California, Davis
Deborah Bennett
University of Michigan
Lei Huang
Olivier Jolliet
University of Texas, Arlington
Hyeong-Moo Shin