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United States Environmental Protection Agency

High-Throughput Toxicokinetics (HTTK)

John Wambaugh
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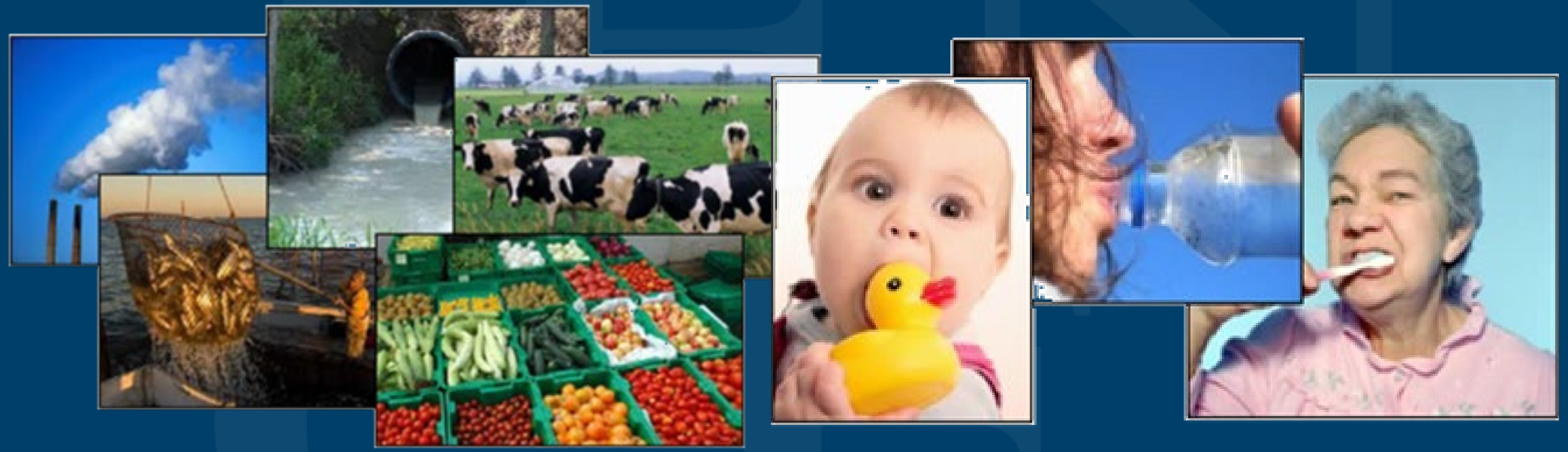

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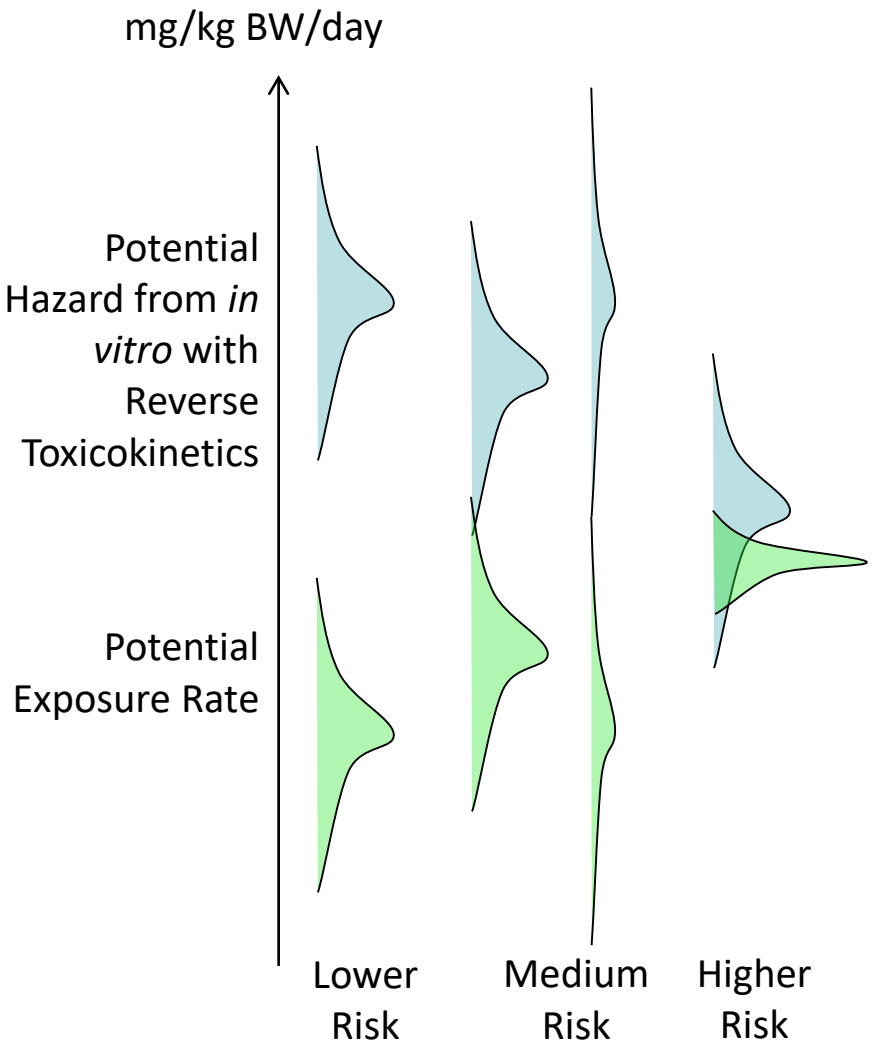
September 20, 2018

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)

- **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)

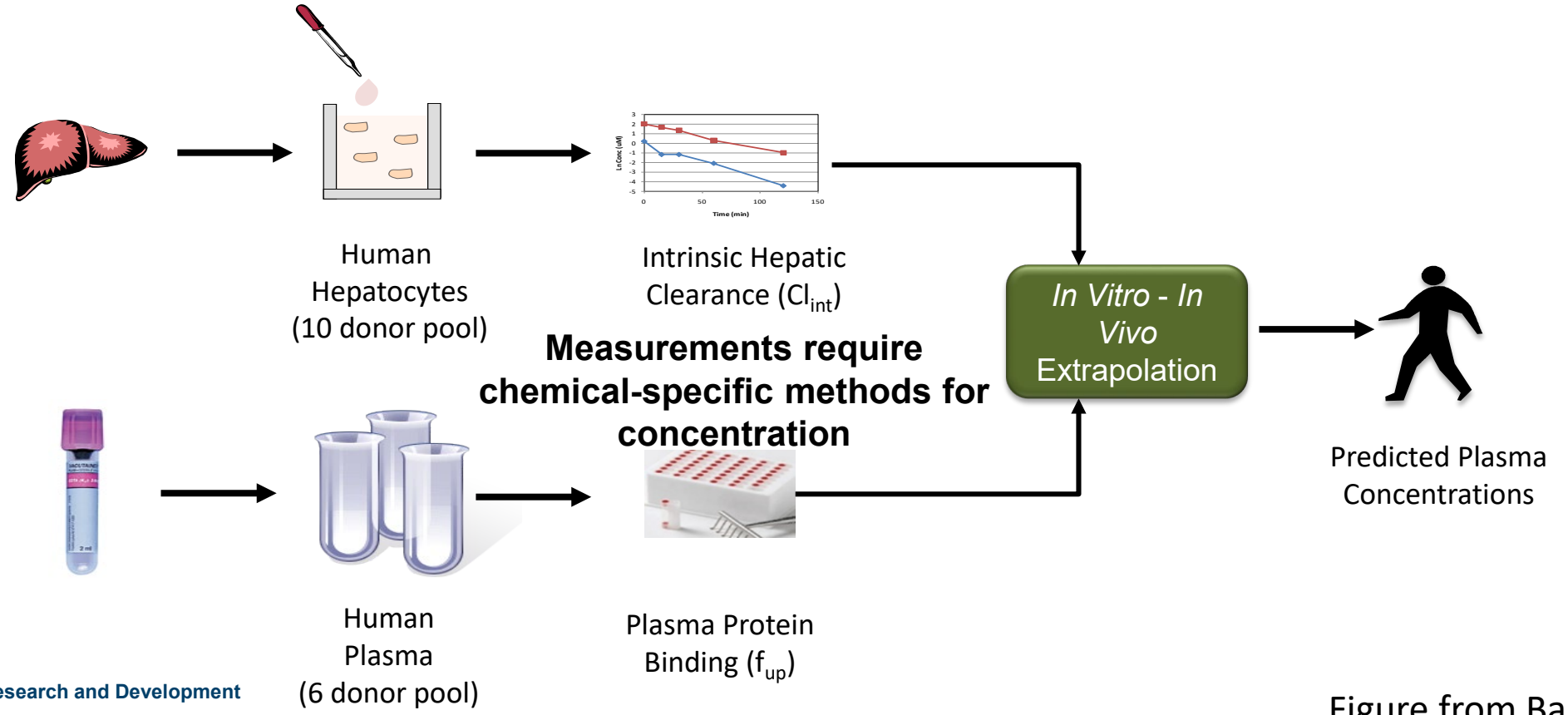


Figure from Barbara Wetmore

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

- **Most chemicals do not have TK data** – we use *in vitro* HTTK methods adapted from pharma to fill gaps

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Environmental chemicals:

Rotroff et al. (2010) 35 chemicals
Wetmore et al. (2012) +204 chemicals
Tonnelier et al. (2012) +20 chemicals
Wetmore et al. (2015) +163 chemicals
Wambaugh et al. (in prep.) + ~400 chemicals

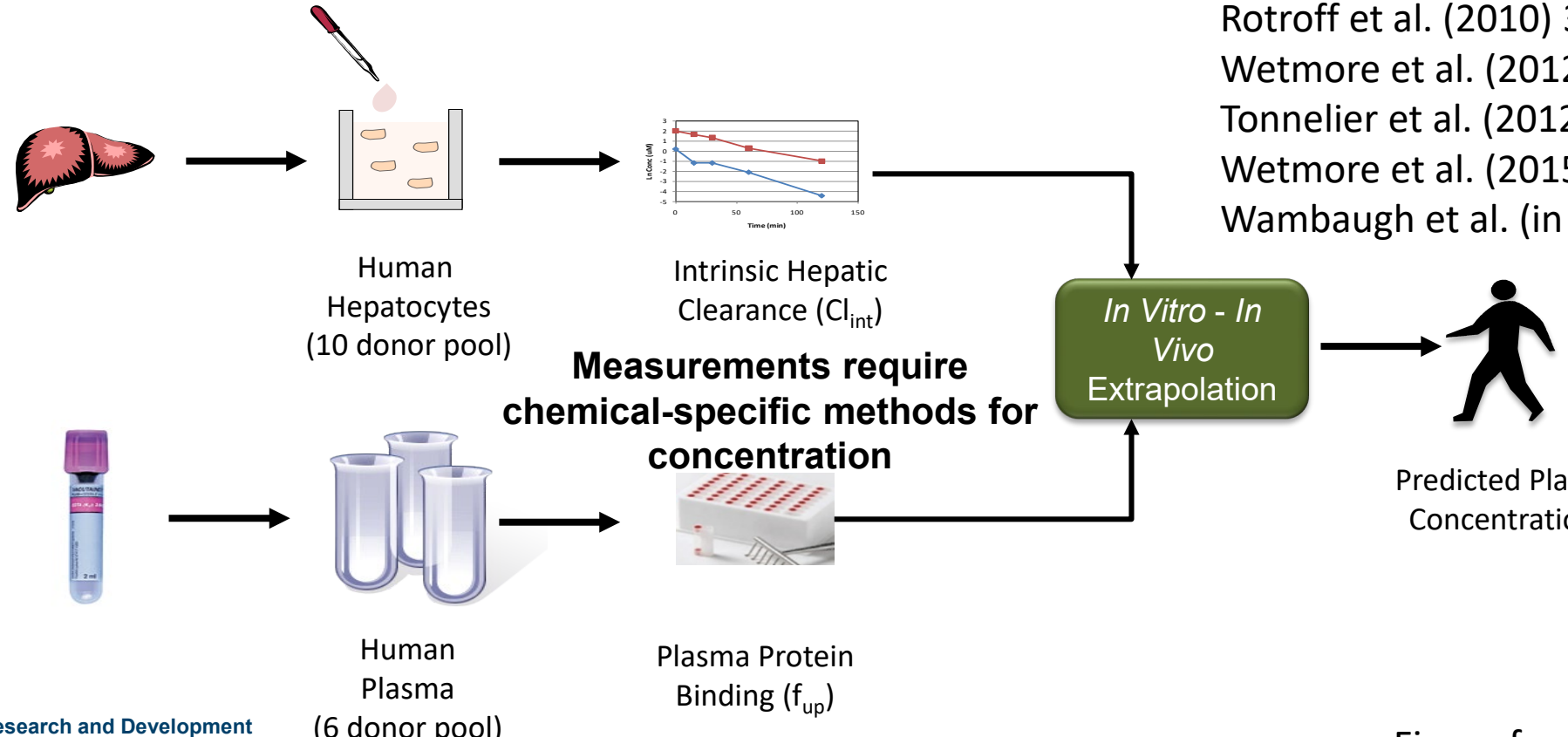


Figure from Barbara Wetmore

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

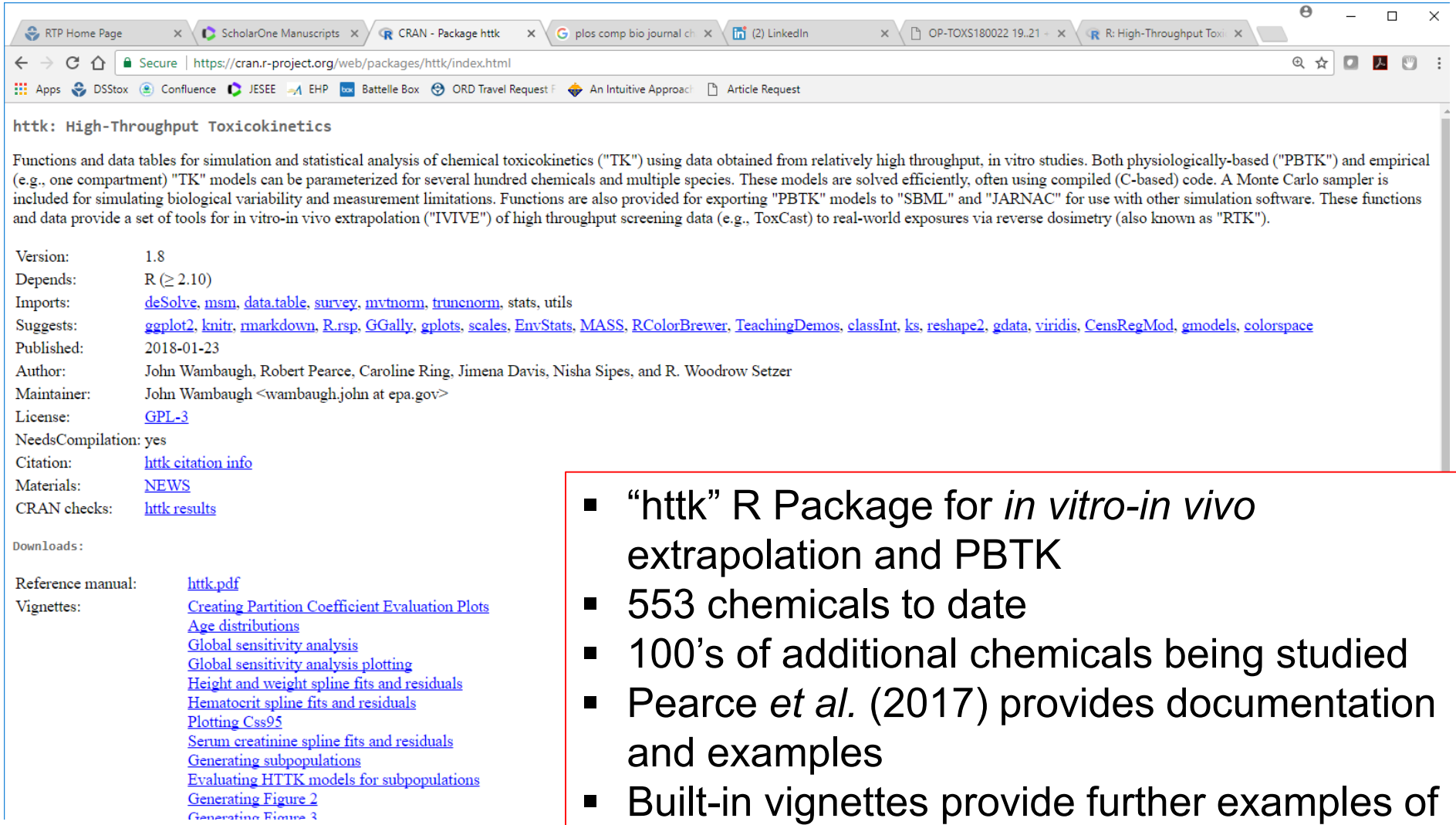
Open Source Tools and Data for HTTK

United States Environmental Protection Agency

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

Can access this from the R GUI:

"Packages" then "Install Packages"



RTP Home Page | ScholarOne Manuscripts | CRAN - Package httk | plos comp bio journal | (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

- "httk" R Package for *in vitro-in vivo* extrapolation and PBTK
- 553 chemicals to date
- 100's of additional chemicals being studied
- Pearce *et al.* (2017) provides documentation and examples
- Built-in vignettes provide further examples of how to use many functions

Doing Statistical Analysis with HTTK

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

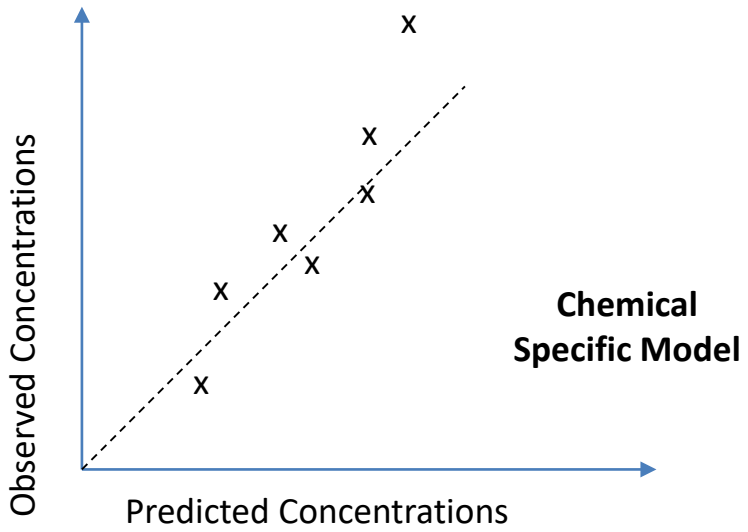
- In drug development, HTTK methods estimate therapeutic doses for clinical studies – predicted concentrations are typically on the order of values measured in clinical trials (Wang, 2010)
  - For most compounds in the environment there will be no clinical trials

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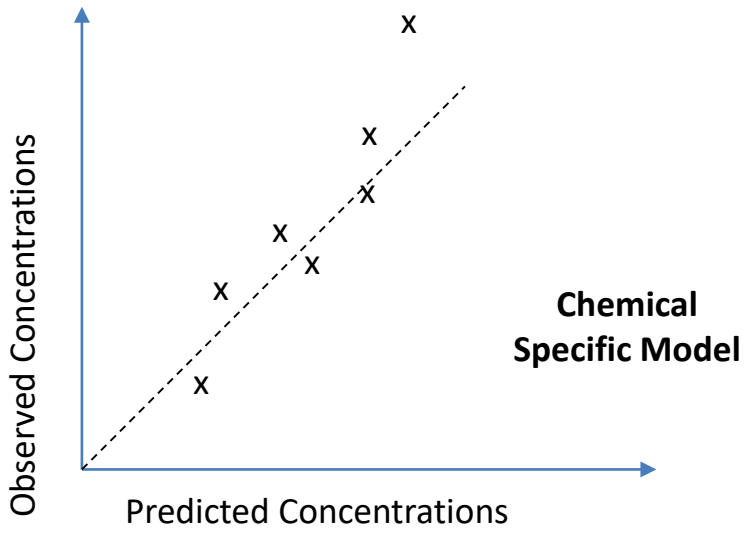
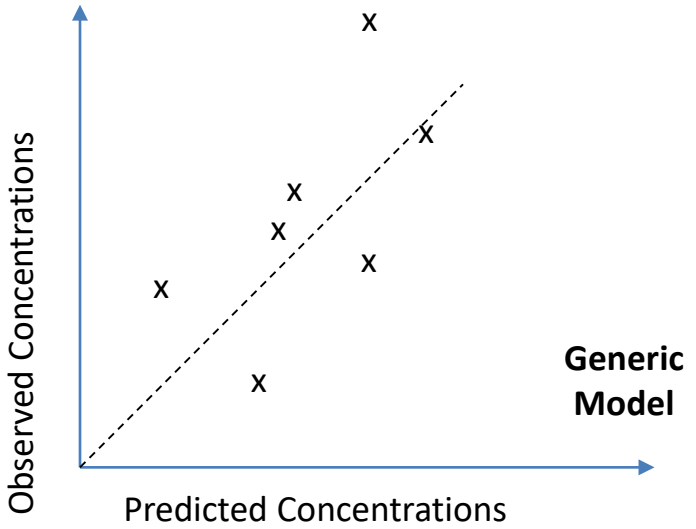
- We can parameterize a **generic TK model**, and evaluate that model for as many chemicals as we do have data
  - We do expect larger uncertainty, but also greater confidence in model implementation
  - Estimate bias and uncertainty, and try to correlate with chemical-specific properties
  - Can again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





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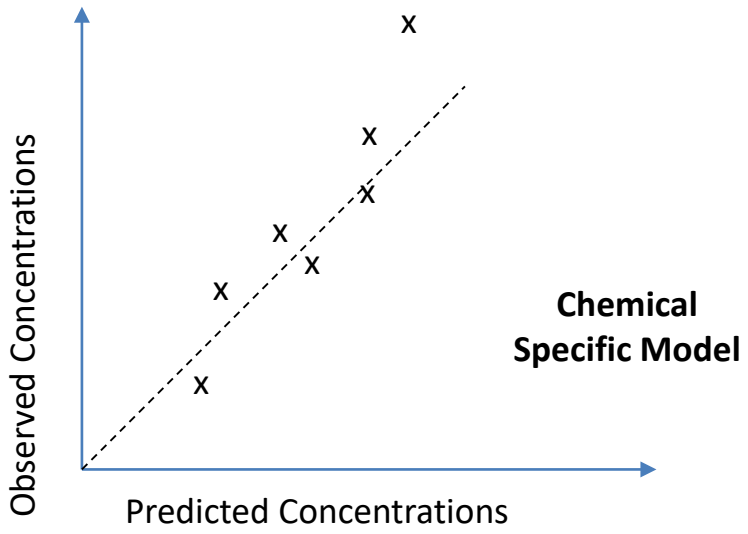

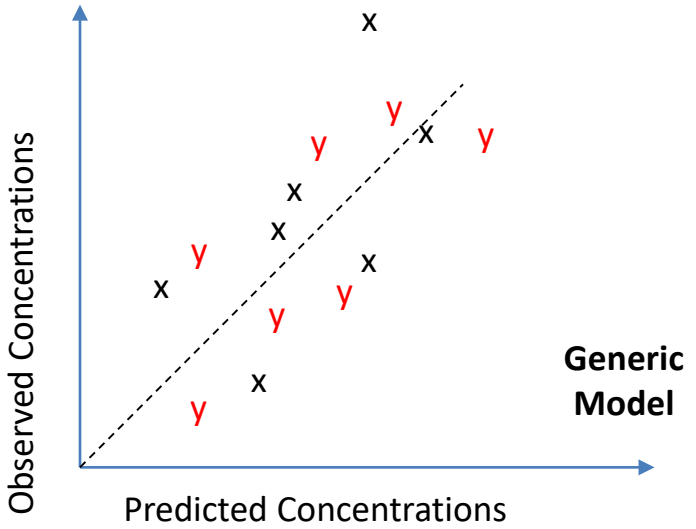

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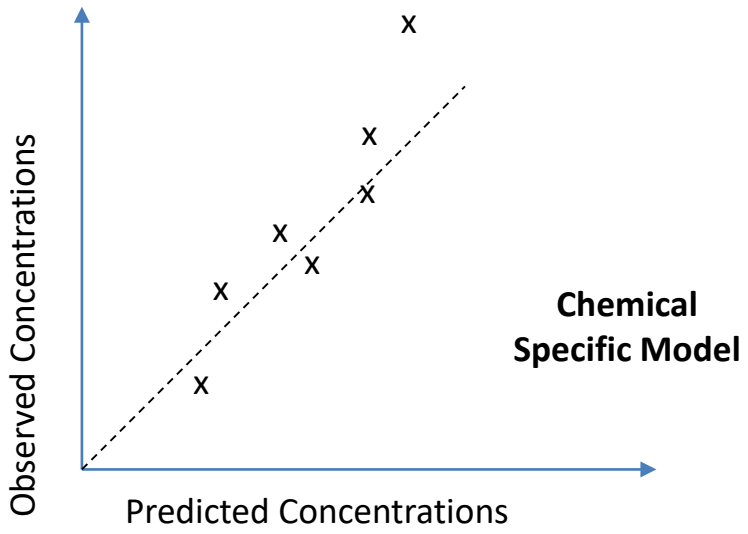
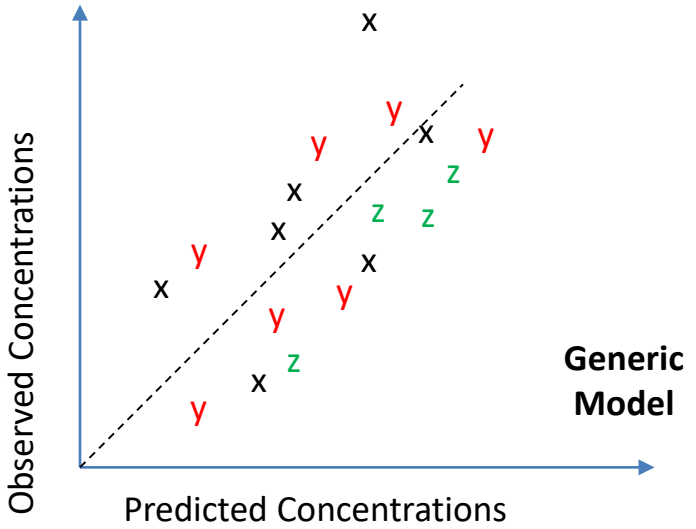
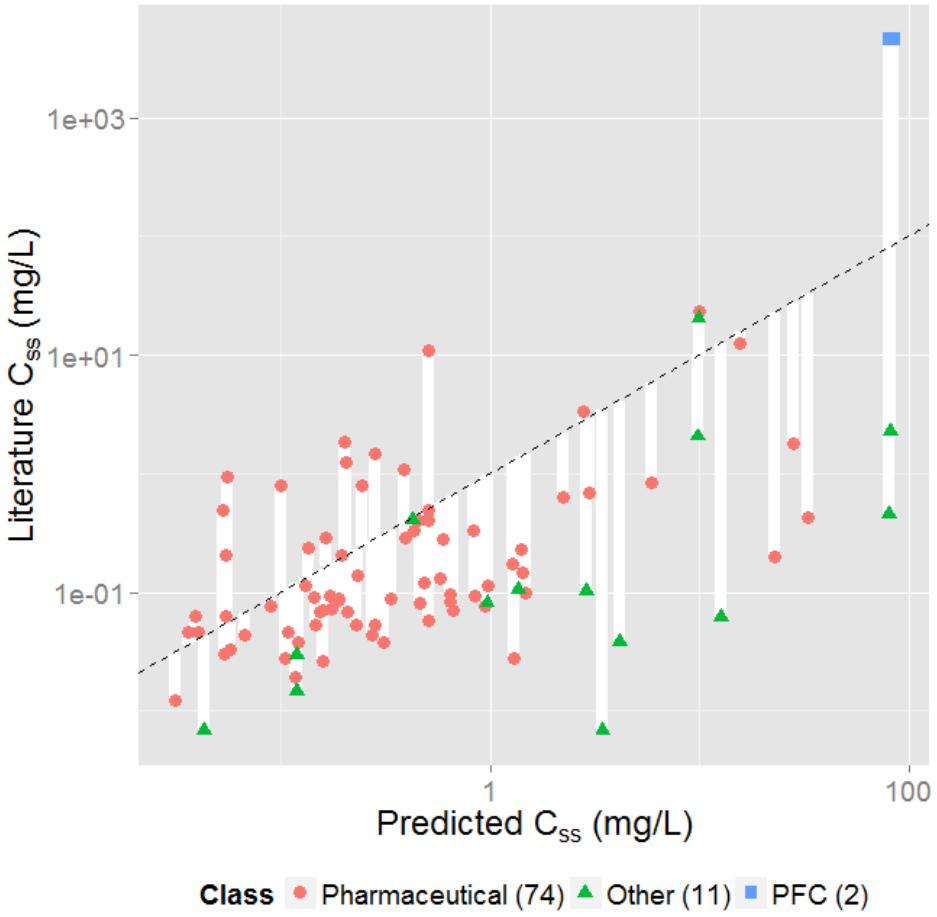
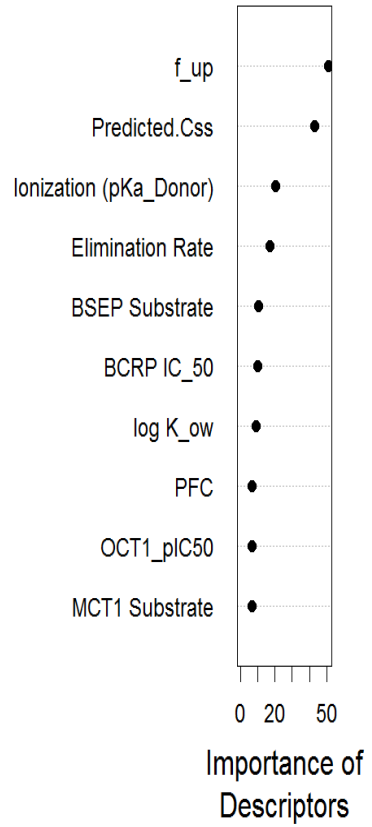




Using *in vivo* Data to Evaluate RTK



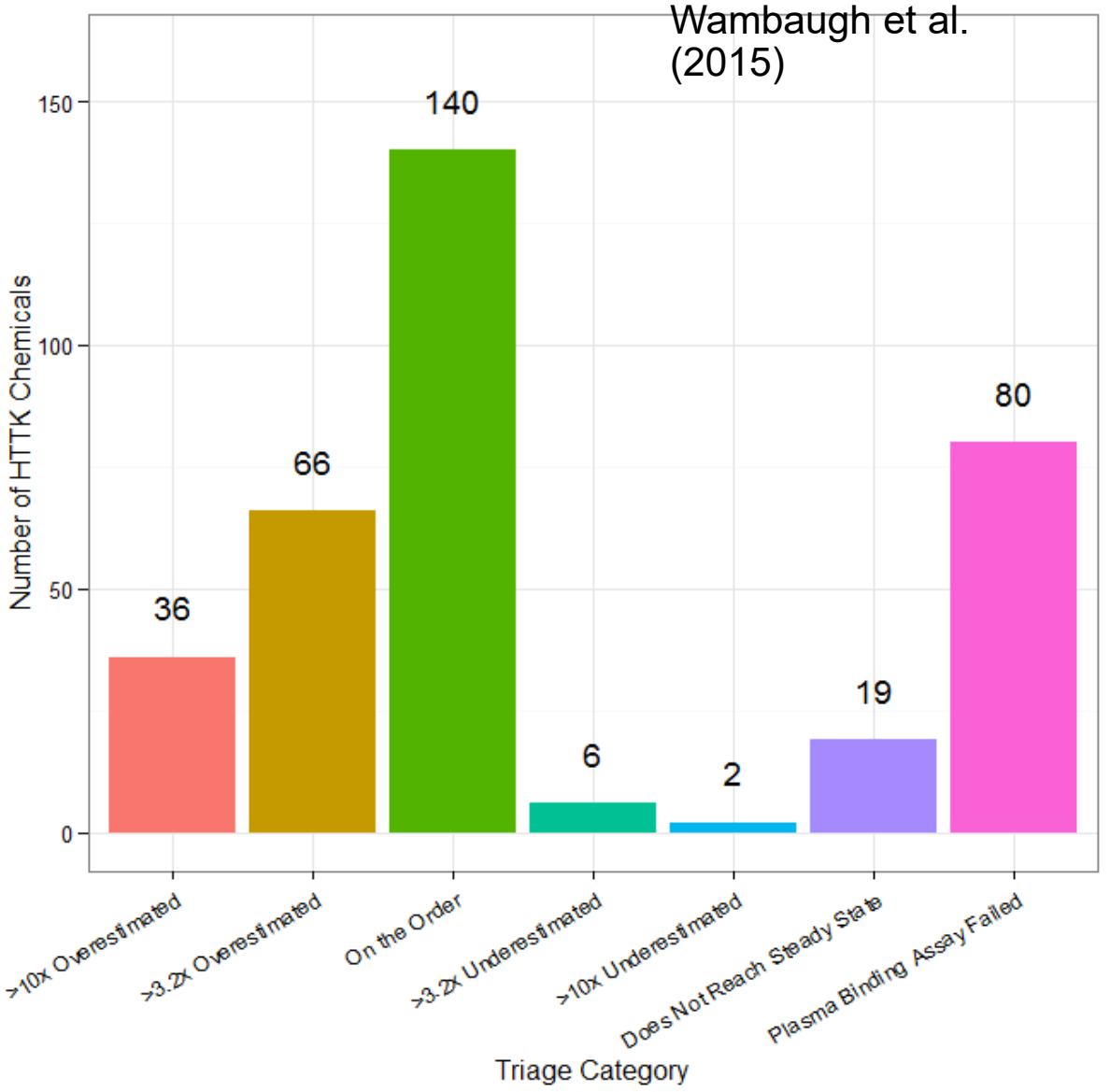
- When we compare the C_{ss} predicted from *in vitro* HTTK with *in vivo* C_{ss} values determined from the literature we find limited correlation (R^2 ~0.34)
- The dashed line indicates the identity (perfect predictor) line:
  - Over-predict for 65
  - Under-predict for 22
- The white lines indicate the discrepancy between measured and predicted values (the residual)



Wambaugh et al. (2015)

Toxicokinetic Triage

- Through comparison to *in vivo* data, a cross-validated (random forest) predictor of success or failure of HTTK has been constructed
- Add categories for chemicals that do not reach steady-state or for which plasma binding assay fails
- All chemicals can be placed into one of seven confidence categories
- Plurality of chemicals end up in the “on the order” bin (within a factor of 3.2x) which is consistent with Wang (2010)



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."

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

TOXICOLOGICAL SCIENCES, 2018, 1–18

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Research Article

Evaluating *In Vitro-In Vivo* Extrapolation of Toxicokinetics

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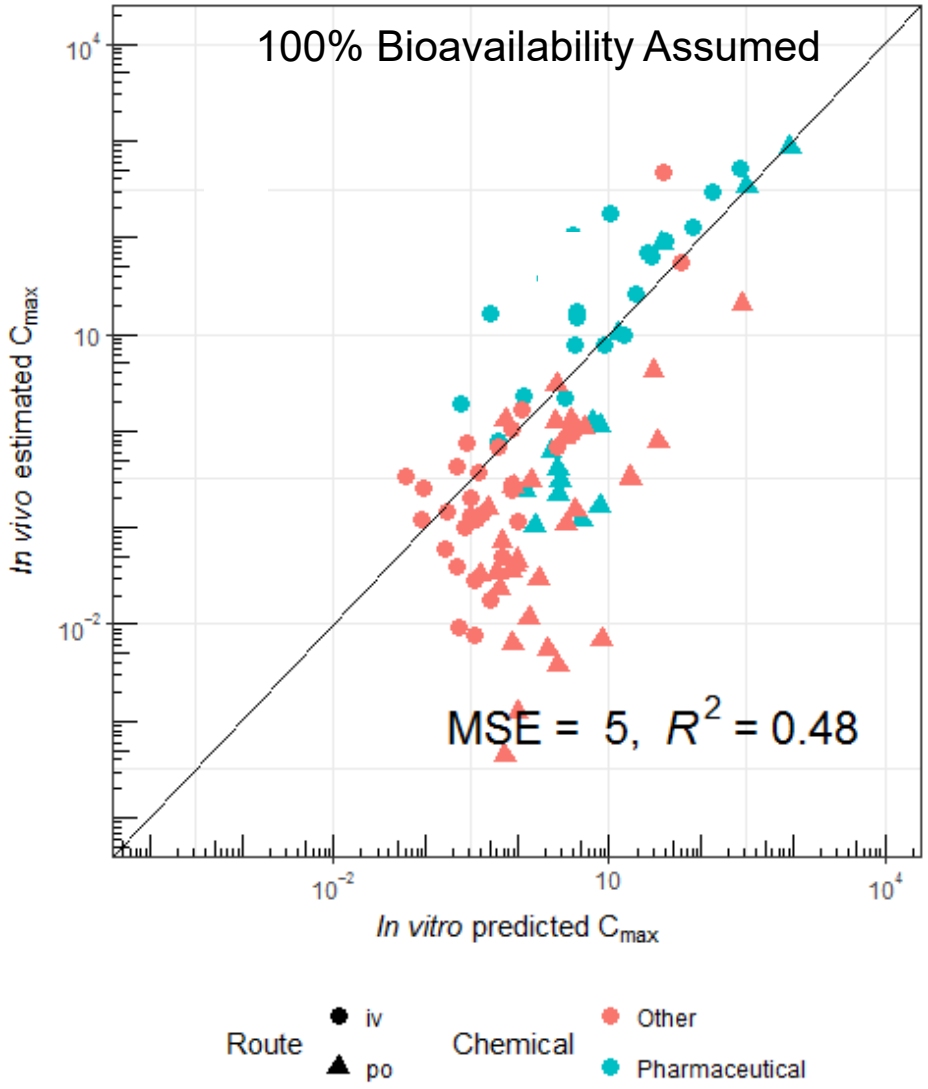
[1]To whom correspondence should be addressed. Fax: (919) 541-1194. E-mail: wambaugh.john@epa.gov.

Impact of Oral Bioavailability



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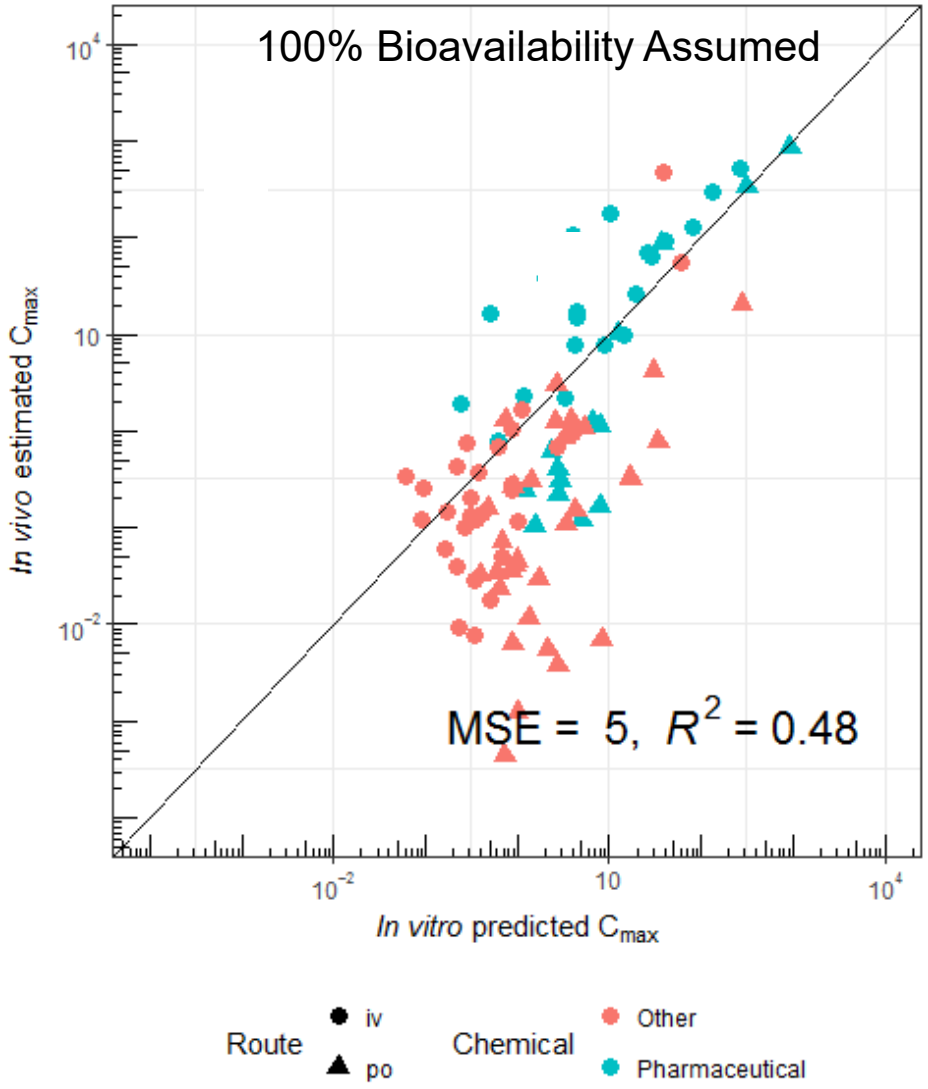

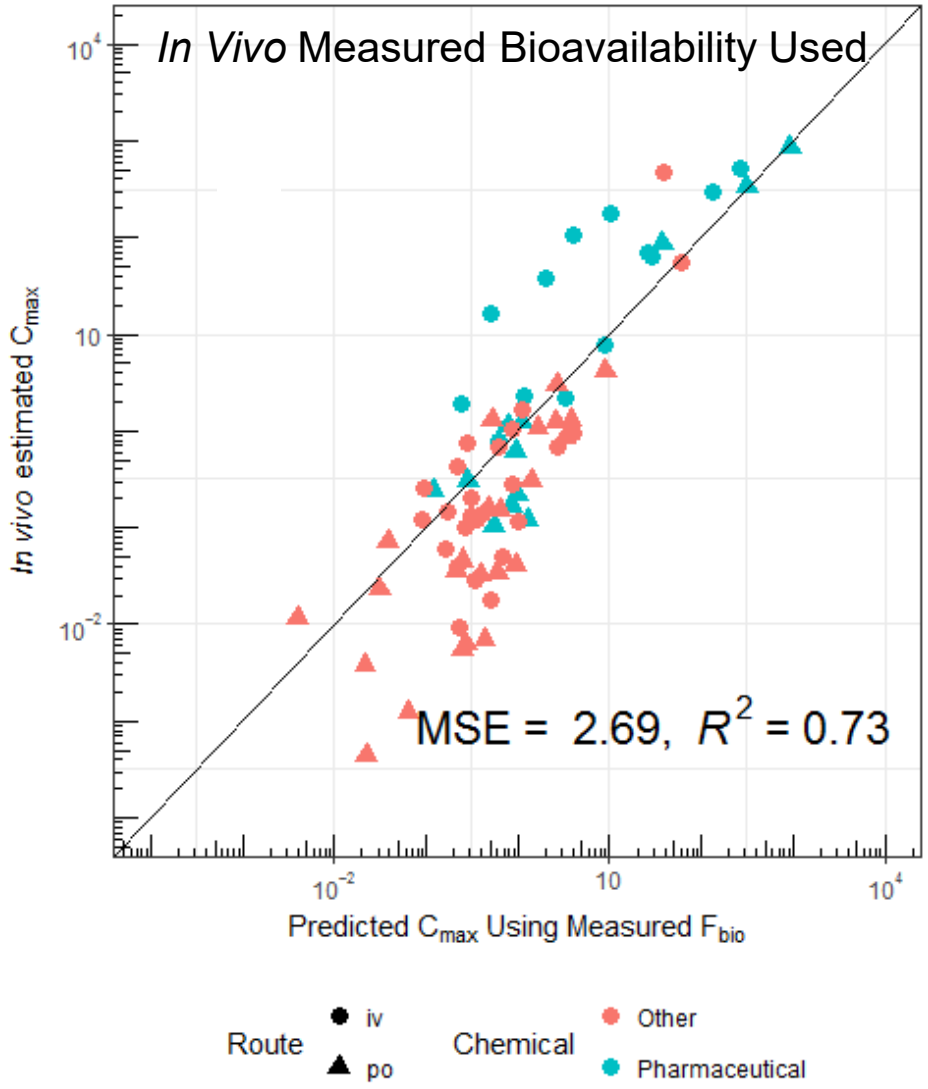

Greg Honda (NCCT) made a SOT2018 presentation on using Caco2 *in vitro* data to predict absorption for ~300 ToxCast chemicals

Key Feature of HTTK: Modern U.S. Population Simulator

Correlated Monte Carlo sampling of physiological model parameters

Sample quantities from

Sex
Race/ethnicity
Age
Height
Weight
Serum creatinine

Ring *et al*. (2017)

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(+ residual marginal variability)

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Serum creatinine

Use equations from literature
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(+ residual marginal variability)

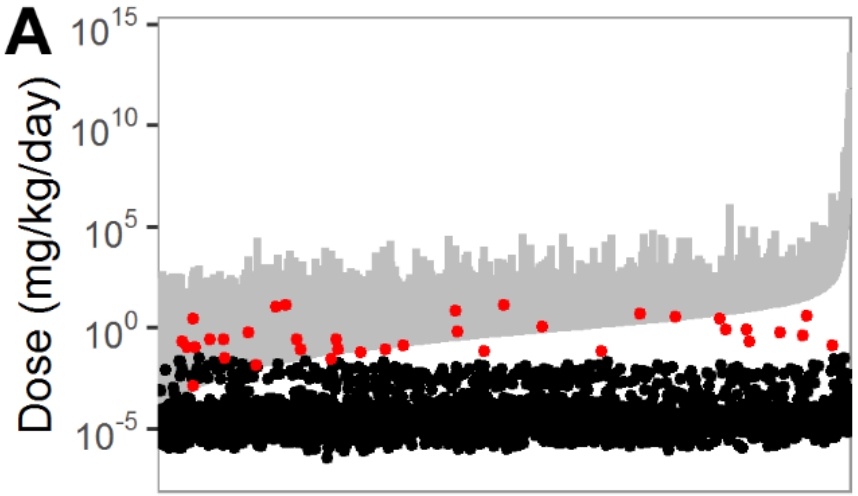
Predict physiological quantities

Tissue masses
Tissue blood flows
GFR (kidney function)
Hepatocellularity

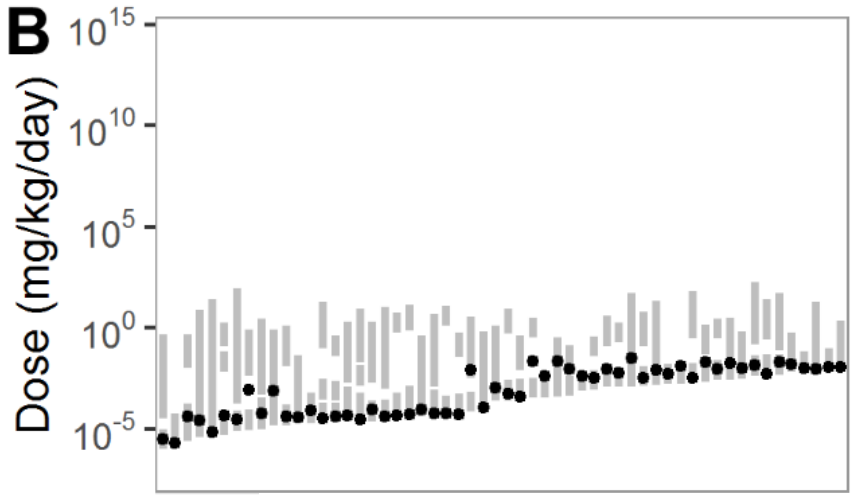
Ring *et al*. (2017)

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



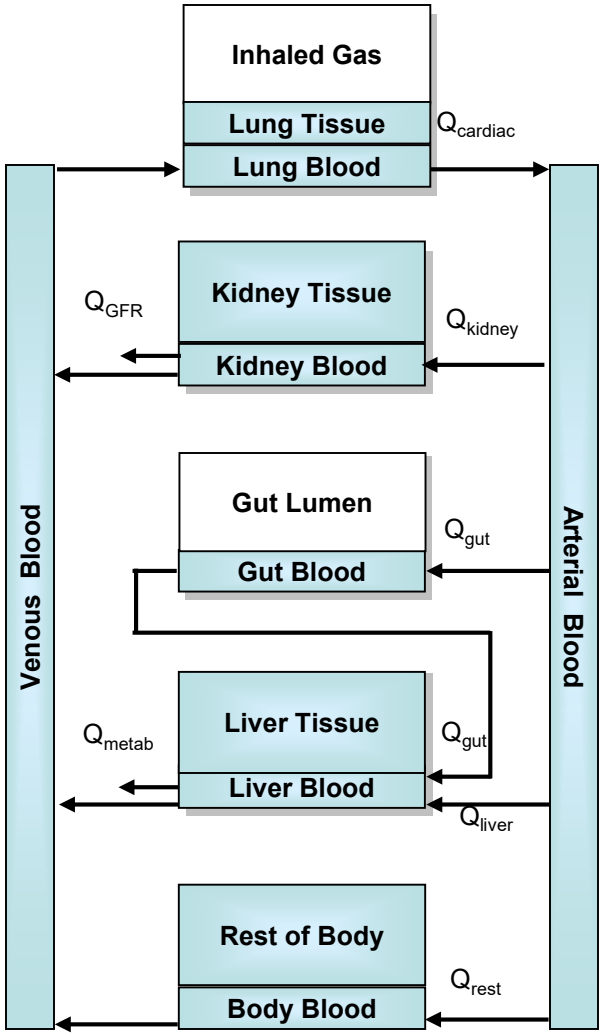
Dose range for all 3925 Tox21 compounds eliciting a 'possible'-to-'likely' human *in vivo* interaction alongside estimated daily exposure



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

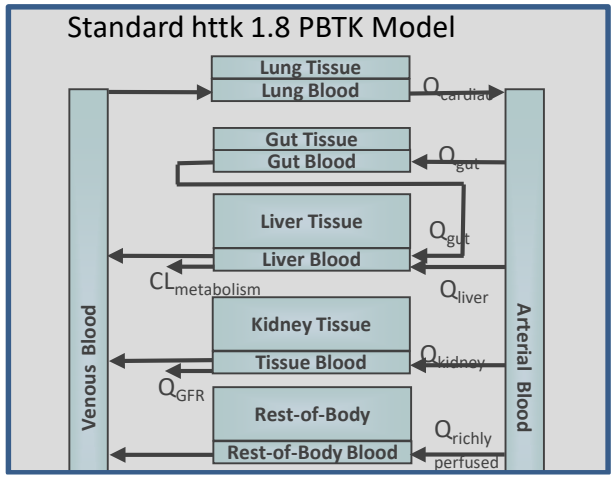
Figure from Sipes *et al.*, (2017)

A General Physiologically-based Toxicokinetic (PBTK) Model



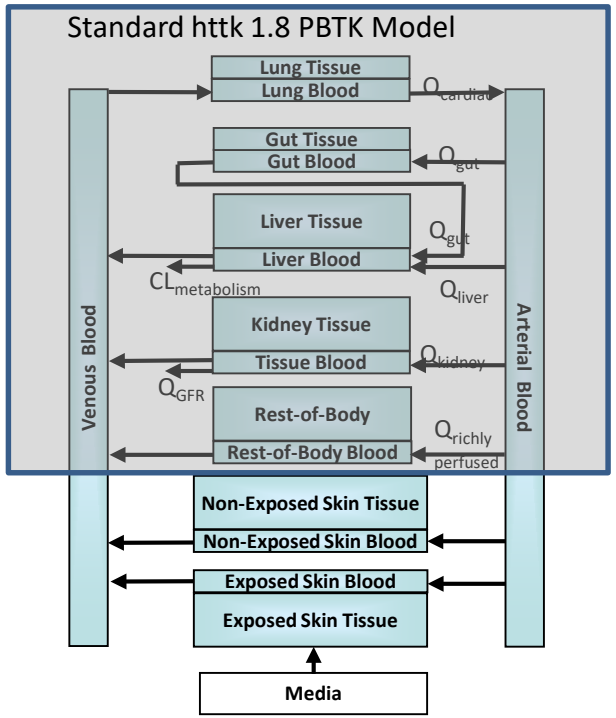
- "httk" includes a generic PBTK model
- Some tissues (e.g. arterial blood) are simple compartments, while others (e.g. kidney) are compound compartments consisting of separate blood and tissue sections with constant partitioning (i.e., tissue specific partition coefficients)
- Exposures are absorbed 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).

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

New HT-PBTK Models



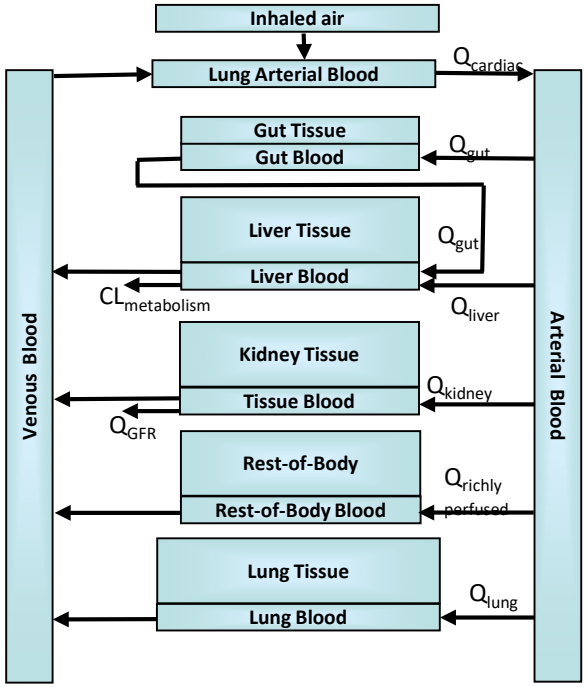
Dermal Exposure Route

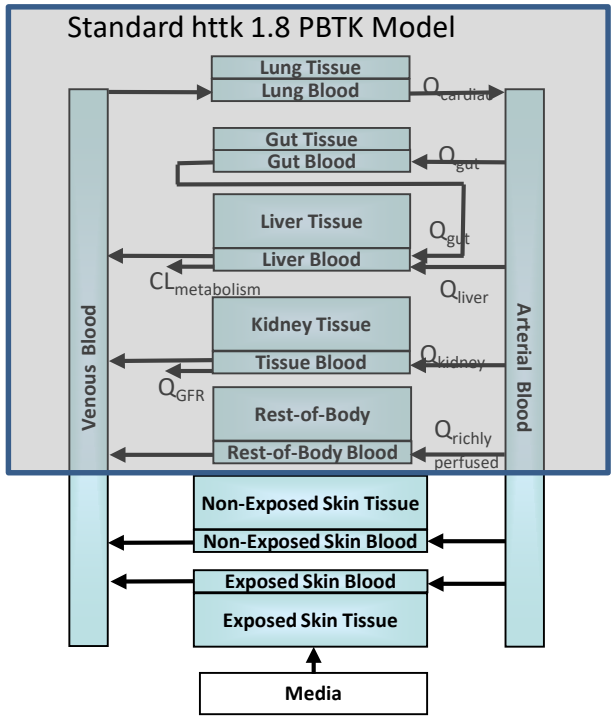
EPA, Unilever, INERIS

New HT-PBTK Models

Gas Inhalation Exposure Route

EPA, USAF





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New HT-PBTK Models

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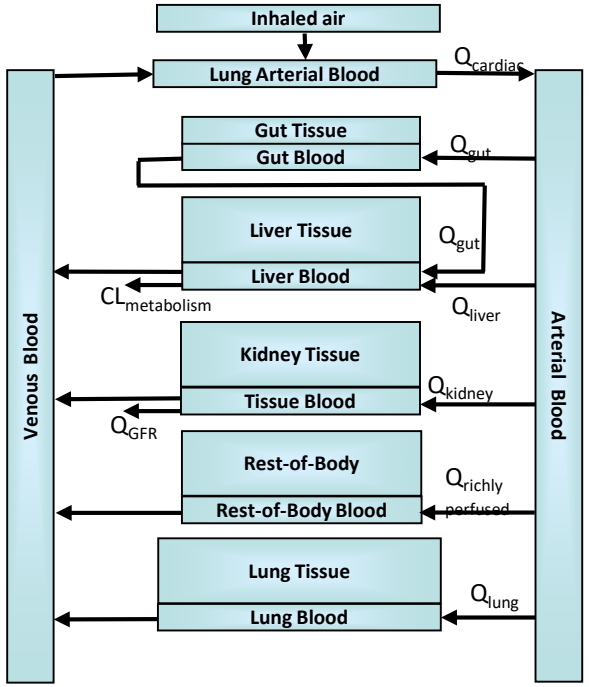
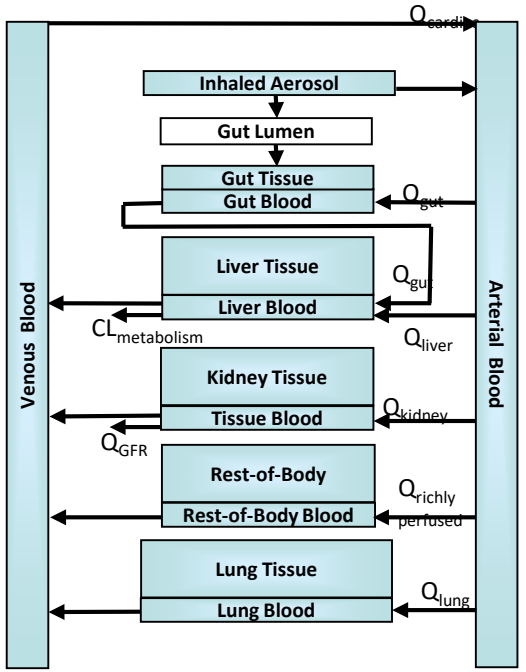
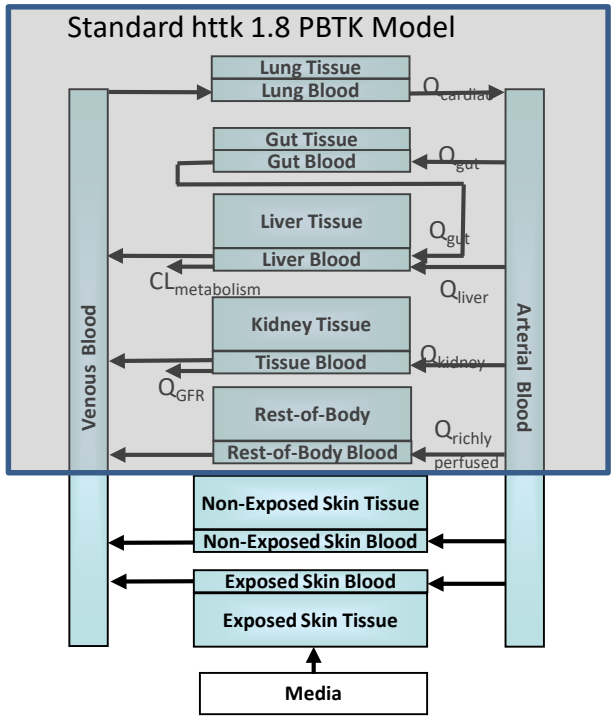
EPA, USAF



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

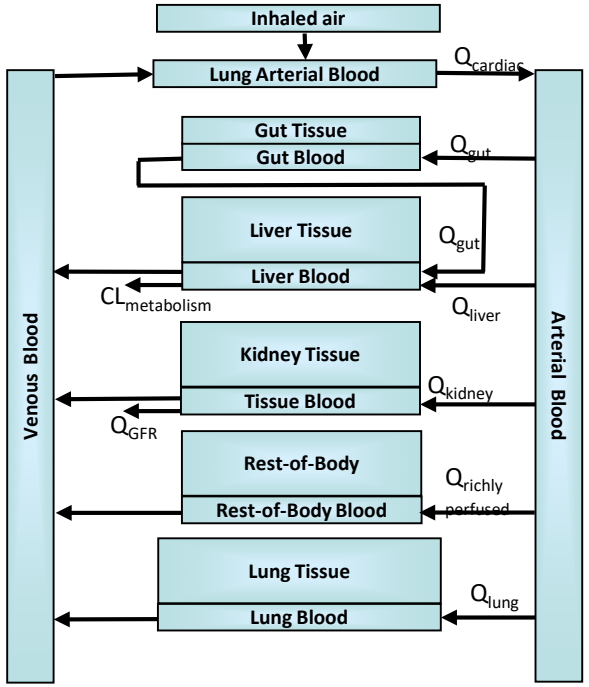




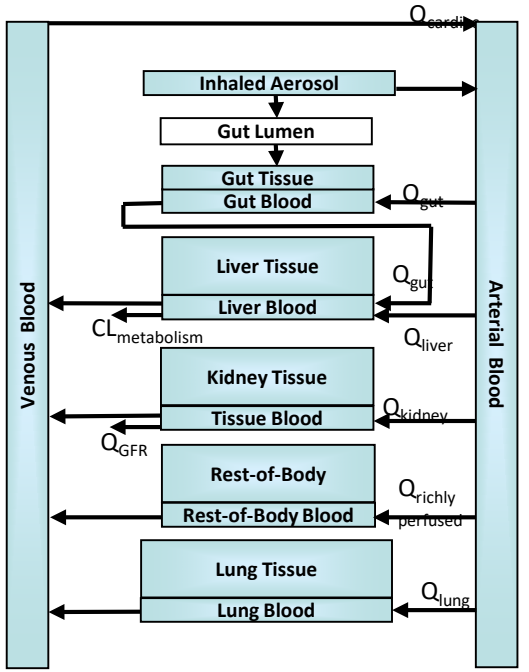
Dermal Exposure Route
EPA, Unilever, INERIS

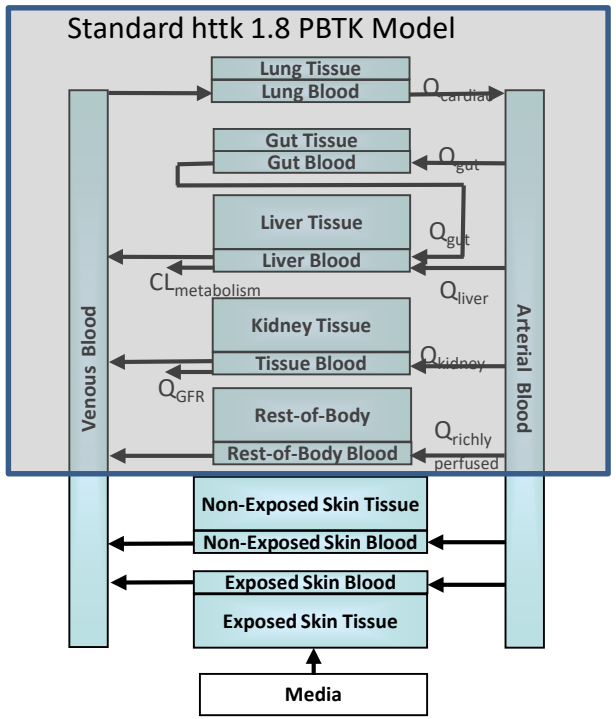
New HT-PBTK Models

Gas Inhalation Exposure Route
EPA, USAFSAM



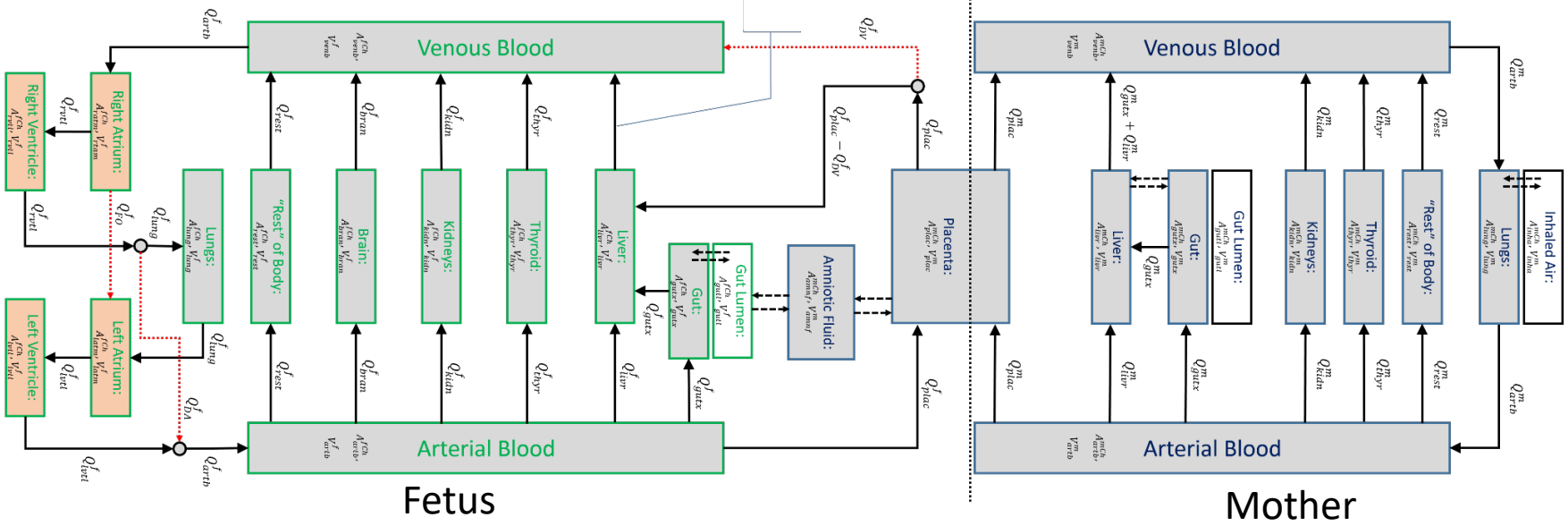
Aerosol Inhalation Exposure Route (with APEX model)
EPA, USAFSAM



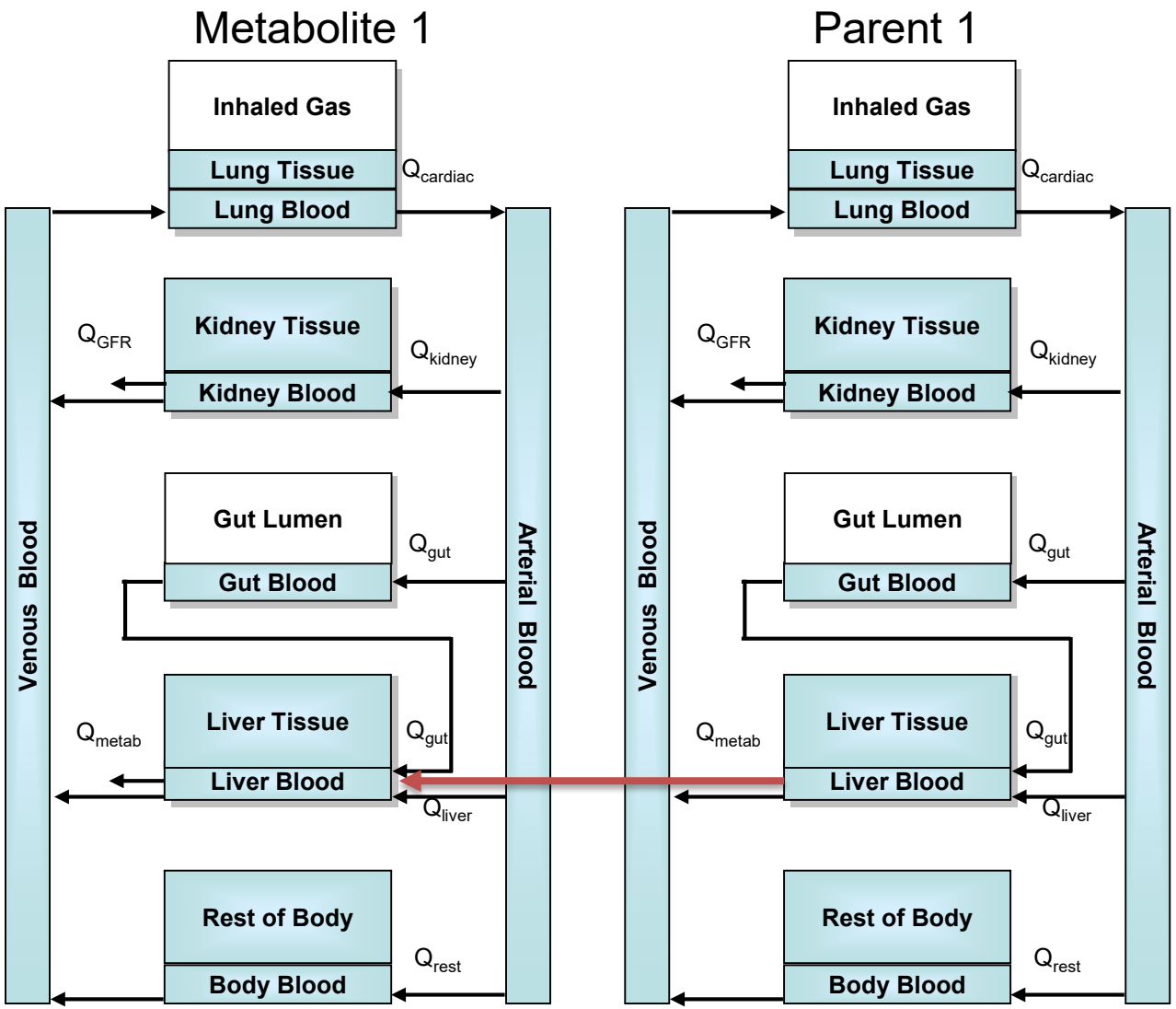


Dermal Exposure Route
EPA, Unilever, INERIS

Human Gestational Model
EPA, FDA



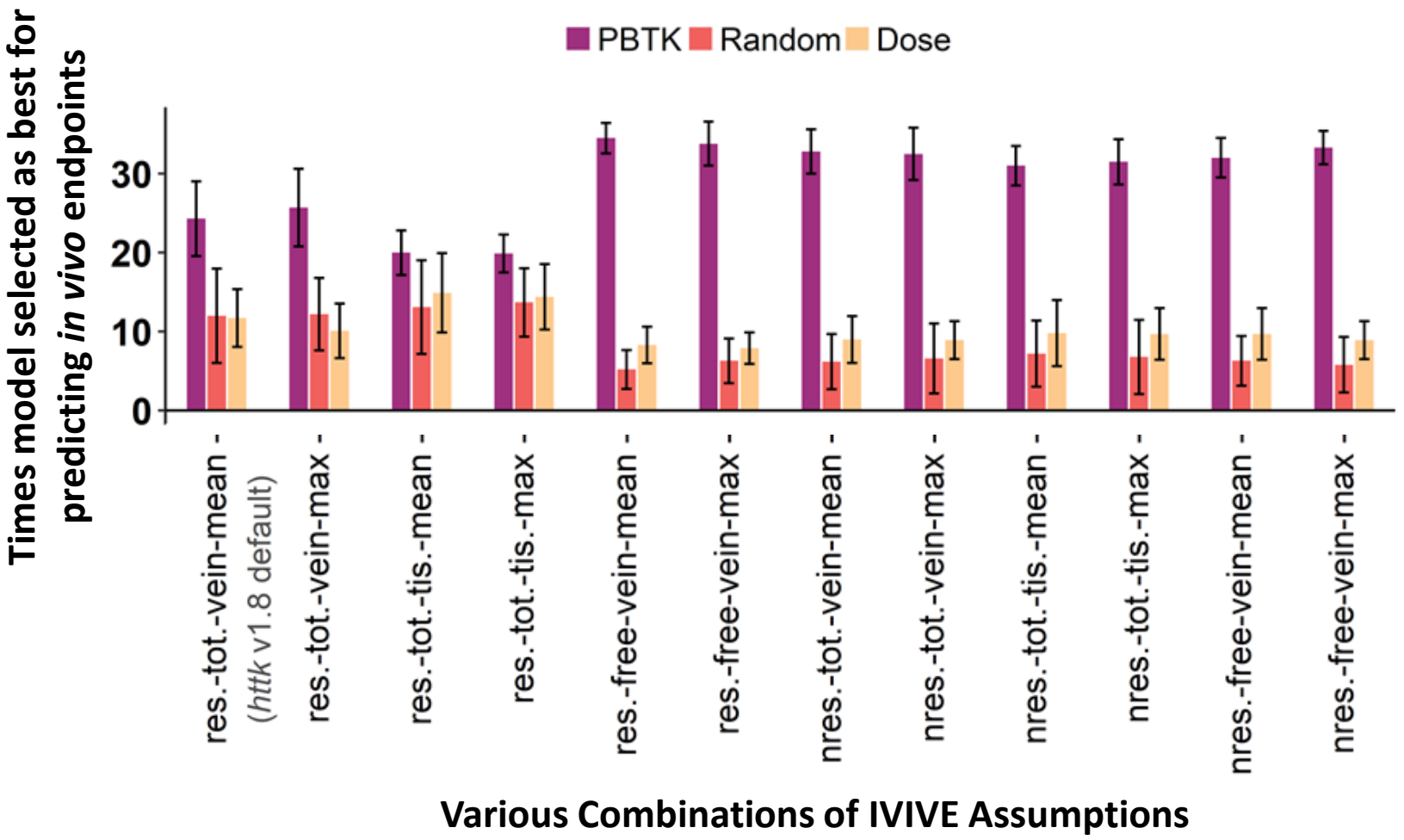
Generic Parent-Metabolite Model?



- Typically we compile the model code in advance, so the number of compartments is fixed
- However, we don't need to turn them all on
- How many compounds do we need to track?
- Perhaps eight replicate models with configurable linkages?

Generic TK enables *In Vitro-In Vivo* Extrapolation (IVIVE)

- Generic PBTK models based on HTTK seem to increase correlation between in vitro bioactivity and in vivo effects
- Histograms (at right) give number of correlated ToxCast assay and ToxRefDB *in vivo* effect pairs
- Using PBTK to predict tissue concentrations does better than using administered dose (or PBTK for random chemical)



Honda et al., in clearance

EPA United States Environmental Protection Agency

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EPA United States Environmental Protection Agency

References

- Breyer, Stephen. Breaking the vicious circle: Toward effective risk regulation. Harvard University Press, 2009
- Bosgra, Sieto, et al. "An improved model to predict physiologically based model parameters and their inter-individual variability from anthropometry." Critical reviews in toxicology 42.9 (2012): 751-767.
- Collins FS, Gray GM, Bucher JR. Transforming environmental health protection. Science. 2008;319:906–907. [PMC free article] [PubMed]
- Dix DJ, Houck KA, Martin M, Richard AM, Setzer RW, Kavlock RJ. The ToxCast program for prioritizing toxicity testing of environmental chemicals. Toxicol Sci. 2007;95:5–12
- Jamei, et al. “The Simcyp® population-based ADME simulator.” Expert opinion on drug metabolism & toxicology 2009b;5:211-223
- McNally, et al., “PopGen: a virtual human population generator.” Toxicology *2014)
- National Research Council. (1983). Risk Assessment in the Federal Government: Managing the Process Working Papers. National Academies Press.
- Park, Youngja H., et al. "High-performance metabolic profiling of plasma from seven mammalian species for simultaneous environmental chemical surveillance and bioeffect monitoring." Toxicology 295.1 (2012): 47-55.
- Pearce, Robert, et al. “httk: R Package for High-Throughput Toxicokinetics.” Journal of Statistical Software, (2017)
- Price, Paul S., et al. "Modeling interindividual variation in physiological factors used in PBPK models of humans." Critical Reviews in Toxicology 33.5 (2003): 469-503.
- Ring, Caroline L., et al. "Identifying populations sensitive to environmental chemicals by simulating toxicokinetic variability." Environment International 106 (2017): 105-118.
- Rotroff, Daniel M., et al. "Incorporating human dosimetry and exposure into high-throughput in vitro toxicity screening." Toxicological Sciences 117.2 (2010): 348-358.
- Strope, Cory L., et al. "High-throughput in-silico prediction of ionization equilibria for pharmacokinetic modeling." Science of science & technology 47.15 (2013): 8479-The Total Environment 615 (2018): 150-160.
- Wambaugh, John F., et al. "High-throughput models for exposure-based chemical prioritization in the ExpoCast project." Environmental 848.
- Wambaugh, John F., et al. "High Throughput Heuristics for Prioritizing Human Exposure to Environmental Chemicals." Environmental science & technology (2014).
- Wetmore, Barbara A., et al. "Integration of dosimetry, exposure and high-throughput screening data in chemical toxicity assessment." *Tox. Sciences* (2012)
- Wetmore, Barbara A., et al. "Incorporating population variability and susceptible subpopulations into dosimetry for high-throughput toxicity testing." Toxicological Sciences 142.1 (2014): 210-224.
- Wetmore, Barbara A., et al. "Incorporating high-throughput exposure predictions with dosimetry-adjusted in vitro bioactivity to inform chemical toxicity testing." Toxicological Sciences 148.1 (2015): 121-136.
- Waters, Nigel J., et al. "Validation of a rapid equilibrium dialysis approach for the measurement of plasma protein binding." Journal of pharmaceutical sciences 97.10 (2008): 4586-4595