Building Fit-for-purpose Pharmacokinetic Models

John Wambaugh
National Center for Computational Toxicology
Office of Research and Development
U.S. Environmental Protection agency
wambaugh.john@epa.gov

In Vitro to _In Vivo_ Extrapolation for High Throughput Prioritization and Decision Making Webinar Series

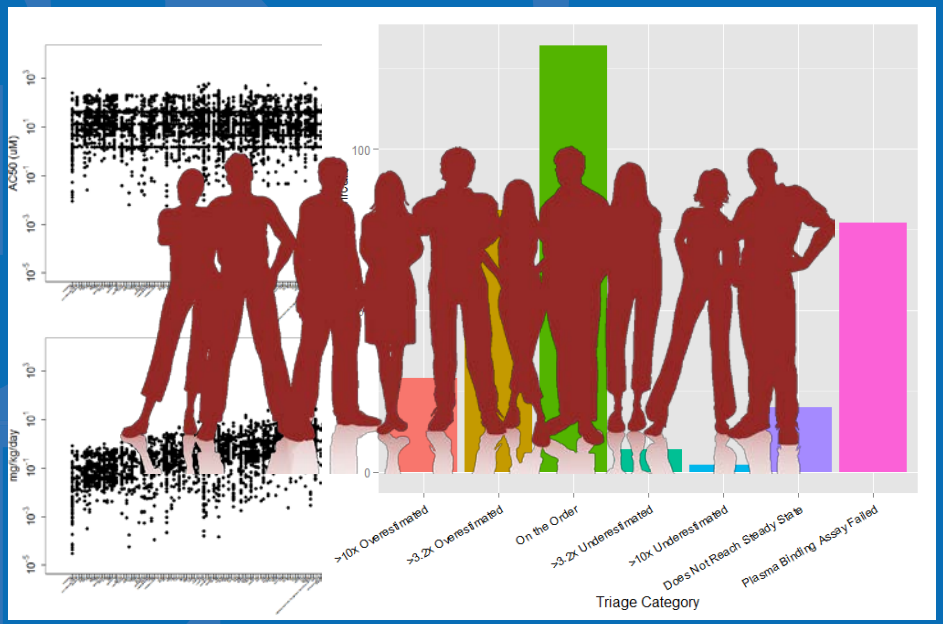


Figure includes image from Thinkstock

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

ORCID: 0000-0002-4024-534X

Introduction

- Toxicokinetics (TK) provide a bridge between hazard (e.g., what tissue concentration causes an effect?) and exposure (e.g., what dose do we get exposed to?)

- Traditional TK methods are resource intensive

- Relatively high throughput TK (HTTK) methods have been used by the pharmaceutical industry to prospectively evaluate success of planned clinical trials (Jamei, *et al.*, 2009; Wang, 2010)
  - A key application of HTTK has been "reverse dosimetry" (also called Reverse TK or RTK) (Tan et al., 2006)
  - RTK can approximately convert *in vitro* HTS results to daily doses needed to produce similar levels in a human for comparison to exposure data (Wetmore, *et al.,* 2012)
  - How accurate do predictions need to be?

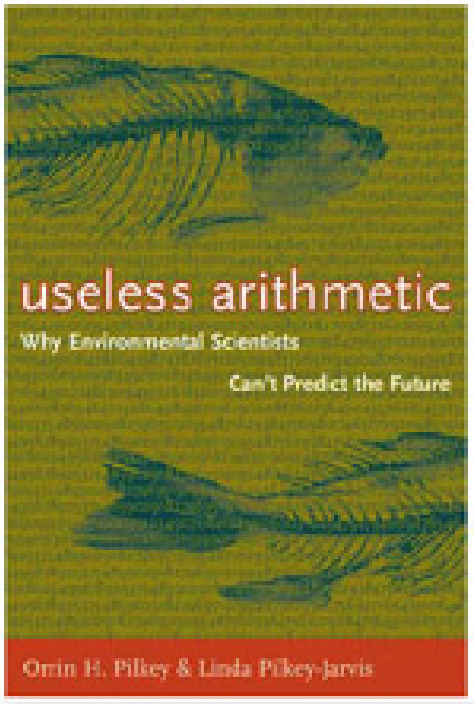
Lex Parsimoniae
"Law of Parsimony"

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

"…when you have eliminated the impossible, whatever remains, *however improbable*, must be the truth…"
Sherlock Holmes (Arthur Conan Doyle)

"PBPK? My immediate response: Junk in, junk out. The take-home is that most of the models [are] only as good as your understanding of the complexity of the system."
Louis Guillette, Medical University of South Carolina

"As far as the laws of mathematics refer to reality, they are not certain; and as far as they are certain, they do not refer to reality." Albert Einstein

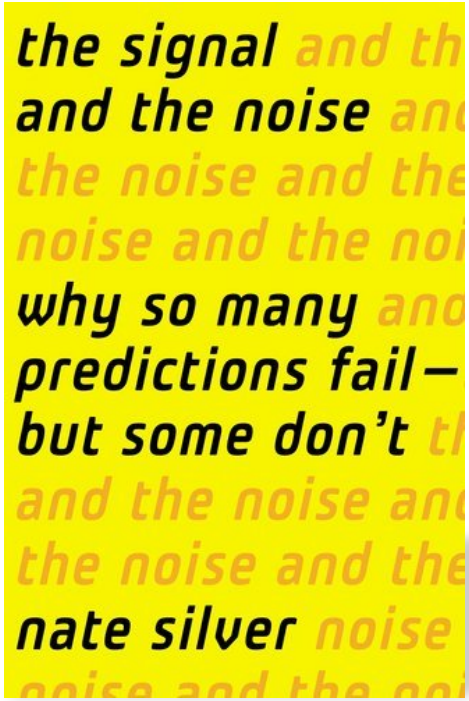


Orrin Pilkey &
Olinda Pilkey-Jarvis
(2007)

Accuracy vs. Precision

"Models can offer a means of avoiding the conclusions derived from actual experiments." Kristin Shrader-Frechette, University of Notre Dame

"Essentially, all models are wrong, but some are useful." George Box, University of Wisconsin



Nate Silver (2012)

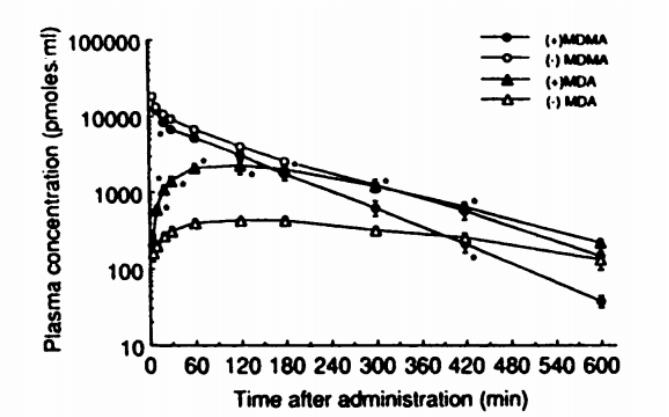
1. Think probabilistically: Evaluate model performance systematically across as many chemicals (and chemistries) as possible

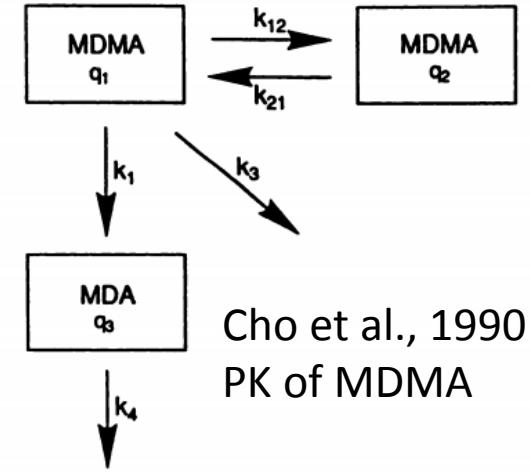
2. Forecasts change: Today's forecast reflects the best available data today but we must accept that new data and new models will cause predictions to be revised

3. Look for consensus: Evaluate as many models and predictors/predictions as possible

Complexity should fit 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, University of Wisconsin





Cho et al., 1990
PK of MDMA

Jones et al., 2012
PK of Statins

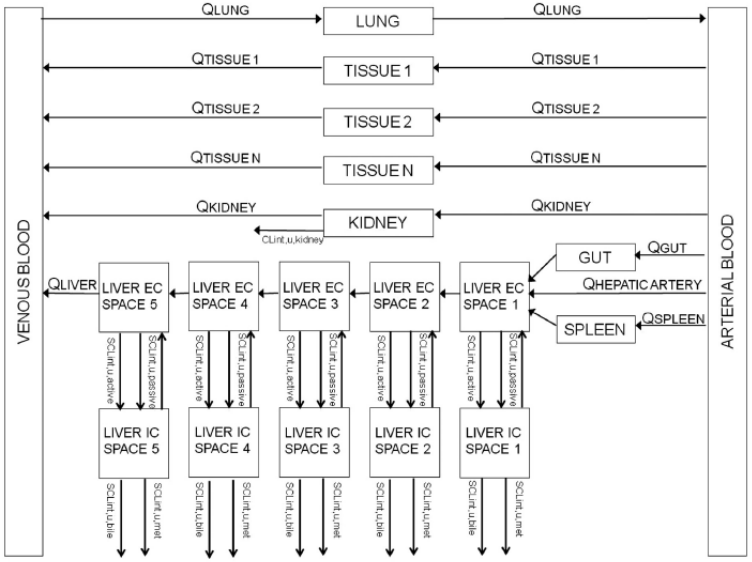
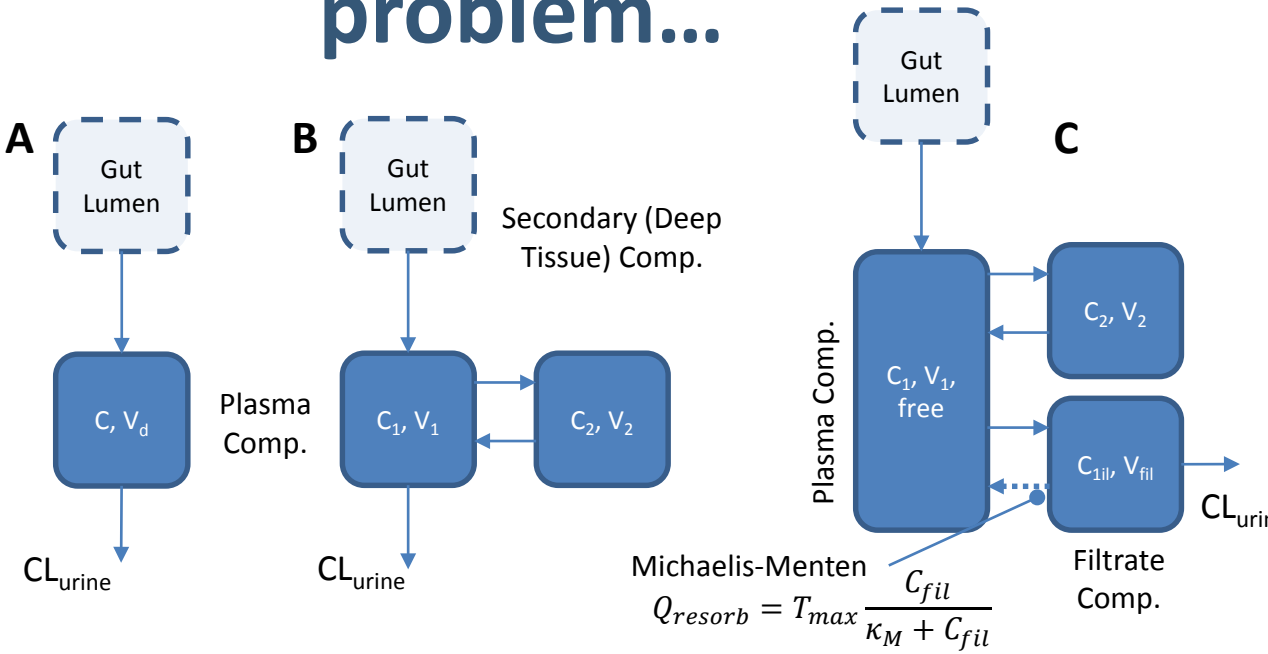
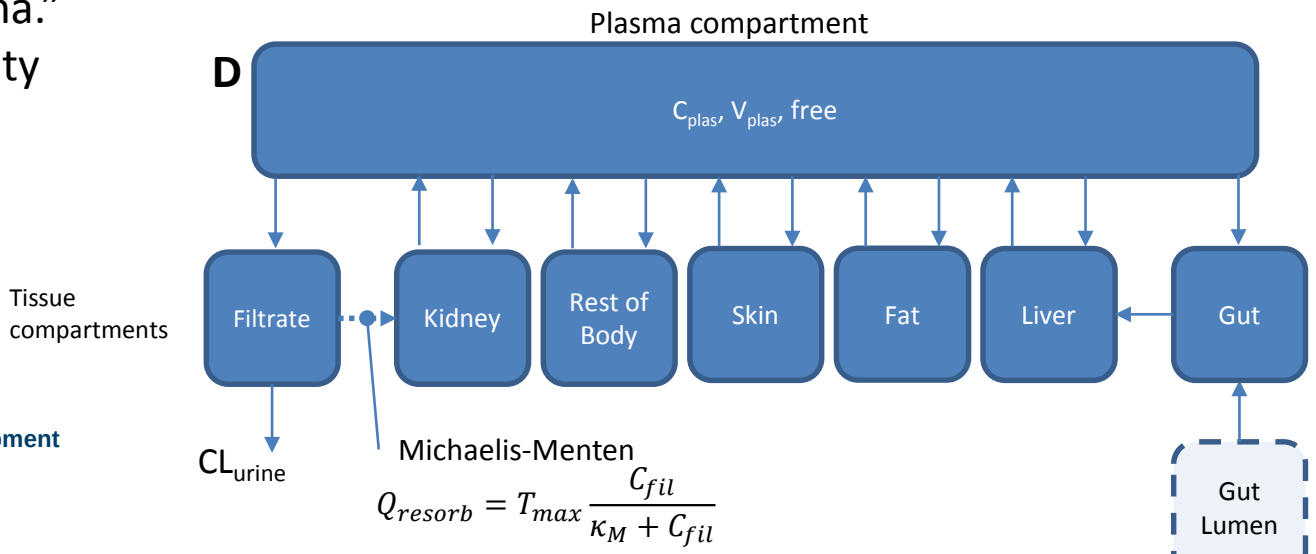


FIG. 2. Schematic diagram of the in vivo PBPK model. EC, extracellular; IC, intracellular.

Complexity should fit the problem…

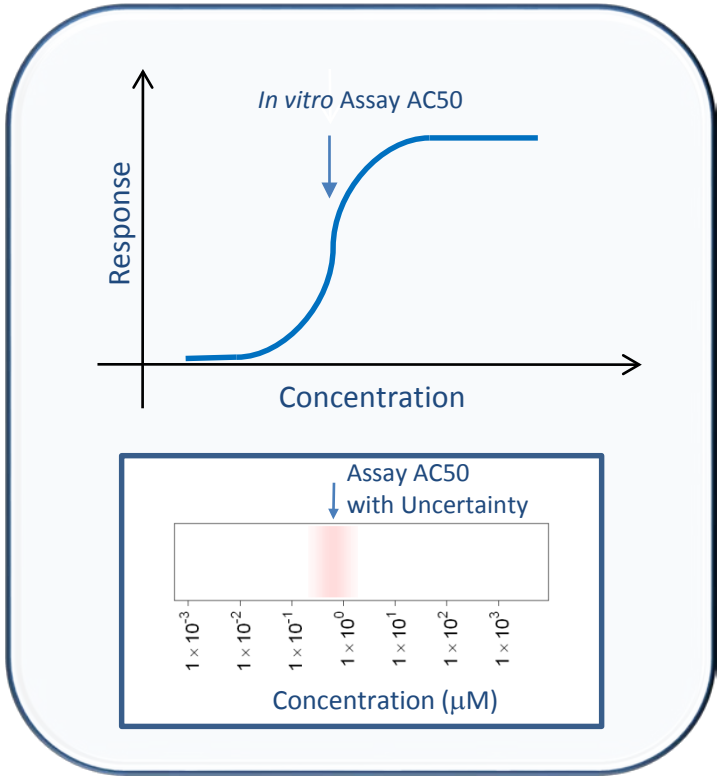
"Since all models are wrong the scientist cannot obtain a 'correct' one by excessive elaboration. On the contrary[,] following William of Occam[, they] should seek an economical description of natural phenomena." George Box, University of Wisconsin





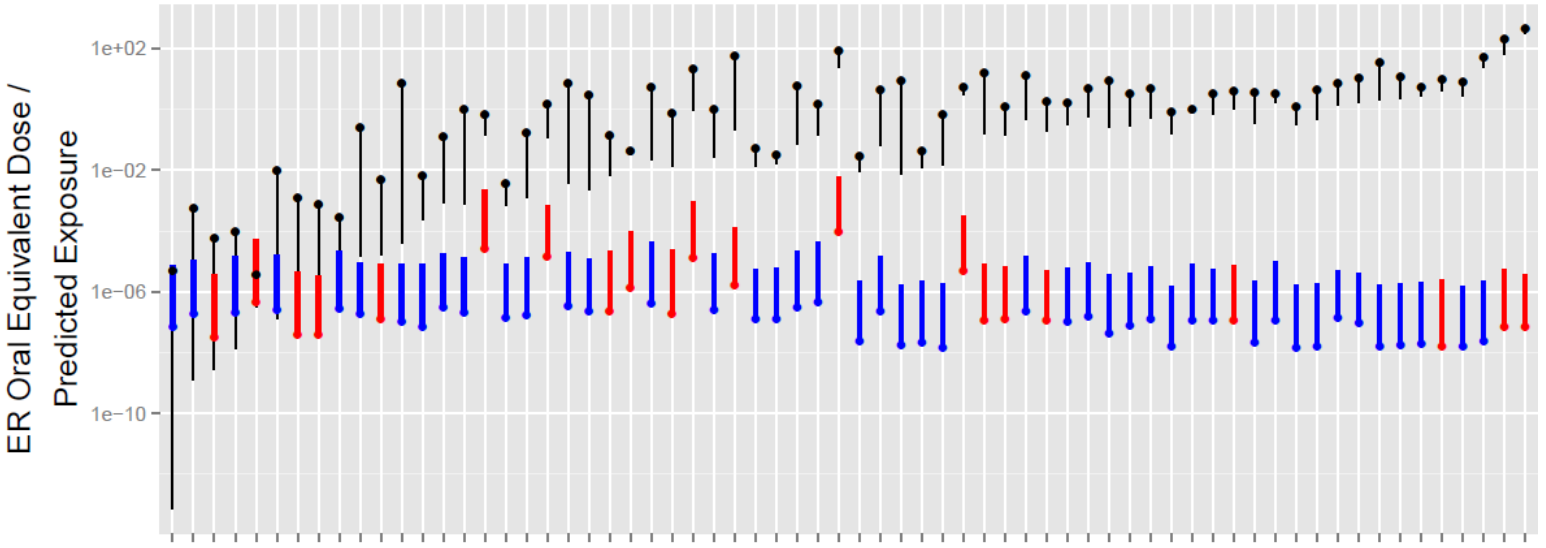
High-Throughput Bioactivity

- **Tox21**: Examining >10,000 chemicals using ~50 assays intended to identify interactions with biological pathways (Schmidt, 2009)
- **ToxCast**: For a subset (>1000) of Tox21 chemicals ran >800 additional assay endpoints (Judson et al., 2010)
- Most assays conducted in dose-response format (identify 50% activity concentration – AC50 – and efficacy if data described by a Hill function)
- All data is public: http://actor.epa.gov/dashboard2



Pharmacokinetics Allows Context for High Throughput Screening

Endocrine disruption AOP *(Judson et al., in prep.)*



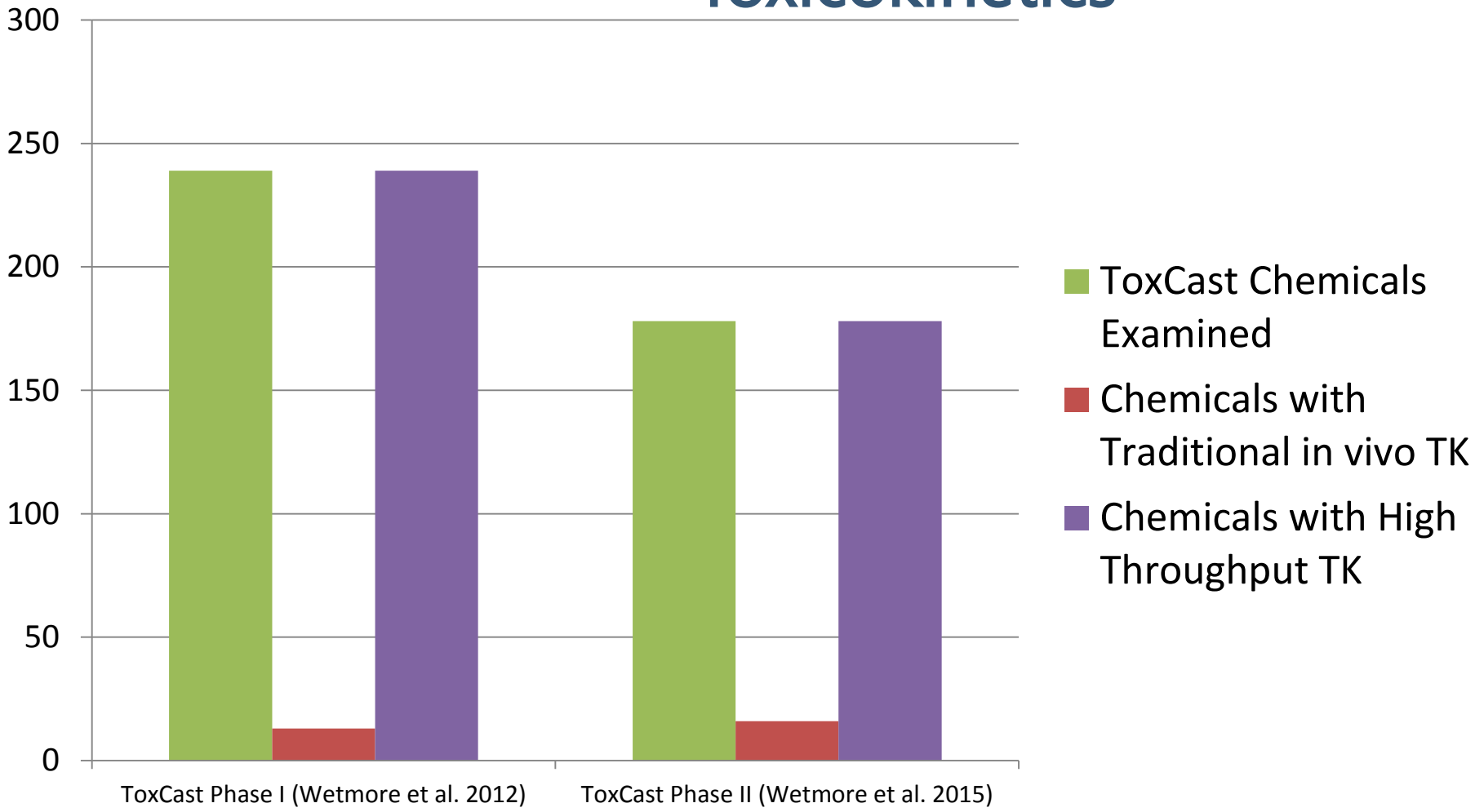
ToxCast Bioactivity Converted to mg/kg/day with HTTK (Wetmore et al., 2012)

ExpoCast Exposure Predictions (Wambaugh *et al.*, 2014)

December, 2014 Panel:
"Scientific Issues Associated with Integrated Endocrine Bioactivity and Exposure-Based Prioritization and Screening"

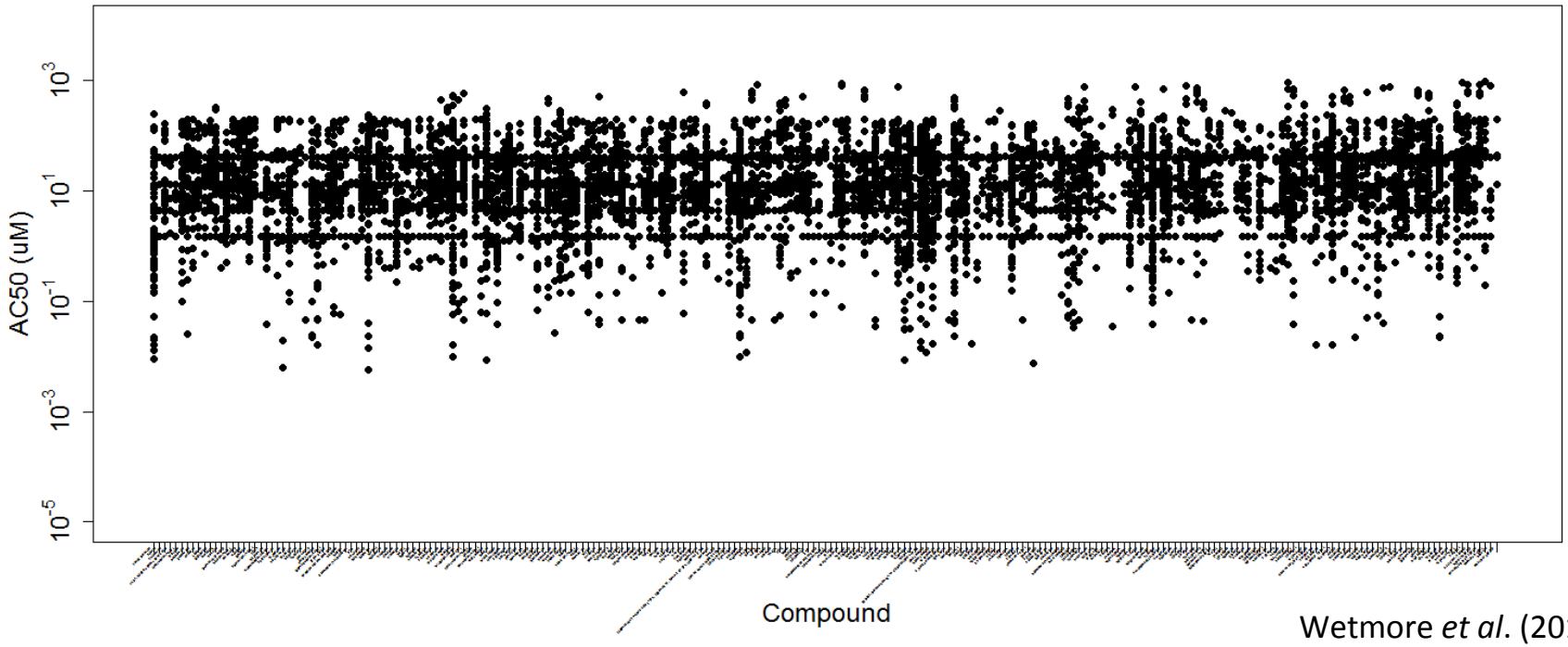
DOCKET NUMBER: EPA–HQ–OPP–2014–0614

The Need for *In Vitro* Toxicokinetics



Studies like Wetmore et al. (2012),addressed the need for TK data using *in vitro* methods

ToxCast *in vitro* Bioactive Concentrations



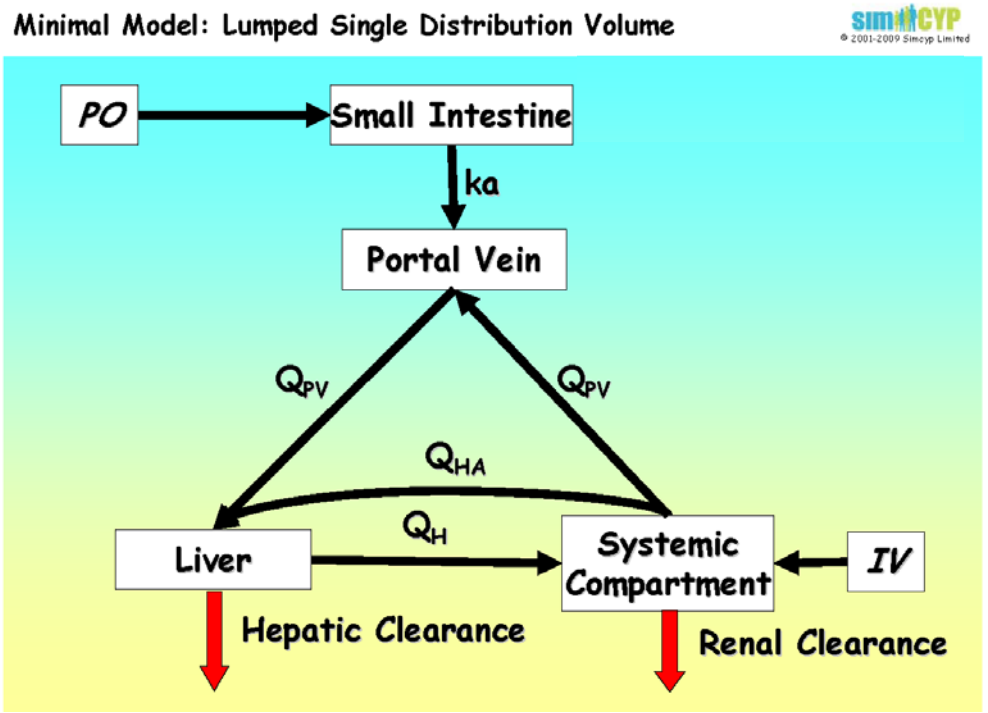
Wetmore *et al*. (2012)

- One point for each chemical-*in vitro* assay combination with a systematic (Hill function) concentration response curve
- How can we use toxicokinetics to convert these to human doses?

High Throughput Toxicokinetics (HTTK)

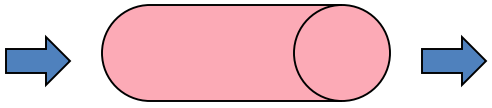
Jamei *et al*. (2009)

- *In vitro* plasma protein binding and metabolic clearance assays allow approximate hepatic and renal clearances to be calculated
- At steady state this allows conversion from concentration to administered dose
- 100% bioavailability assumed



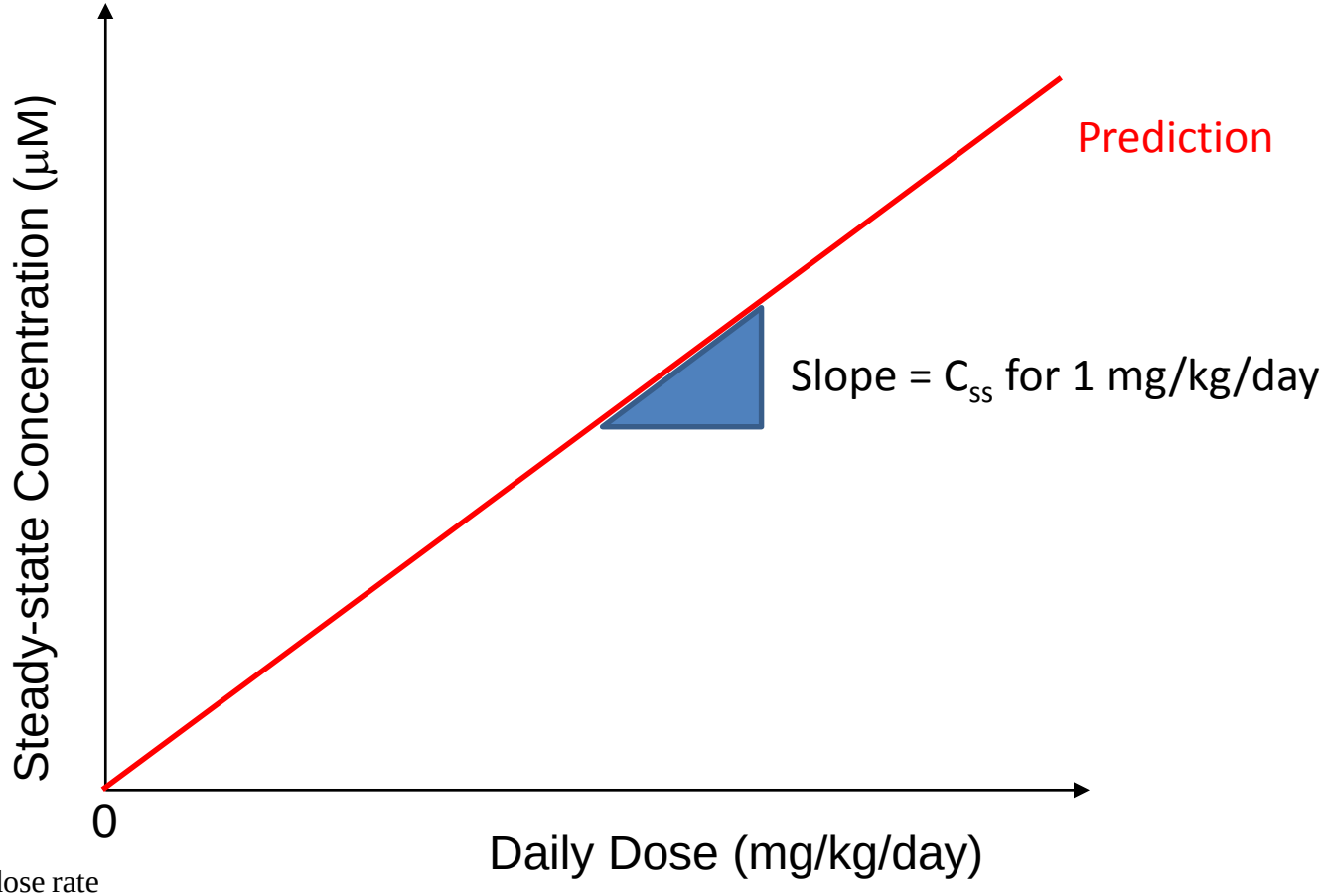
$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * \text{F}_{ub}) + \left(\text{Q}_l * \text{F}_{ub} * \frac{Cl_{int}}{\text{Q}_l + \text{F}_{ub} * Cl_{int}}\right)}$$

Oral dose in (mg/kg/day)



Sum of hepatic and renal clearance (mg/kg/day)

Steady-State is Linear with Dose

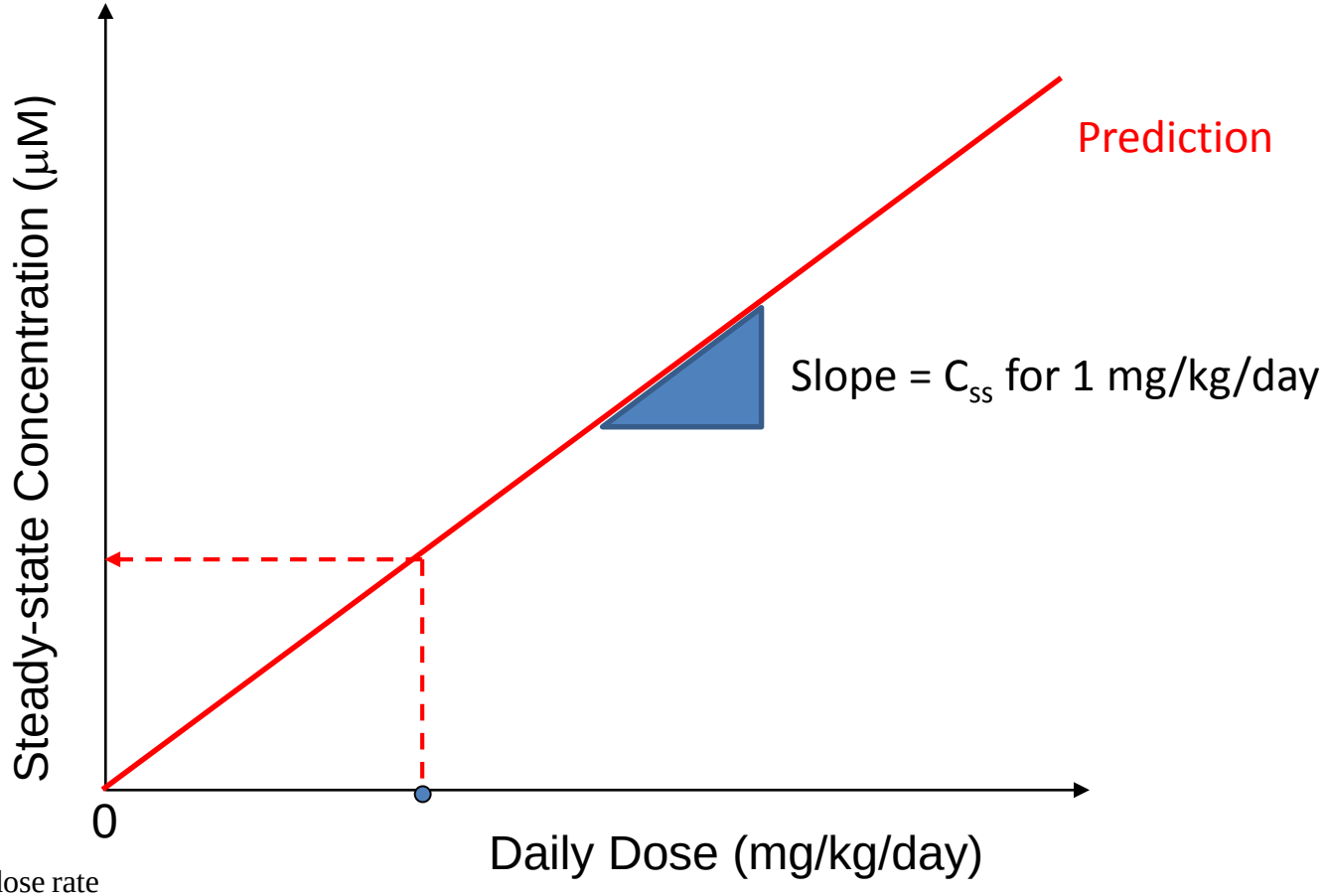


$$C_{ss} = \frac{\text{oral dose rate}}{(GFR * F_{ub}) + \left(Q_l * F_{ub} * \frac{Cl_{int}}{Q_l + F_{ub} * Cl_{int}}\right)}$$

- Can calculate predicted steady-state concentration (C_{ss}) for a 1 mg/kg/day dose and multiply to get concentrations for other doses

Wetmore *et al*. (2012)

Steady-State is Linear with Dose

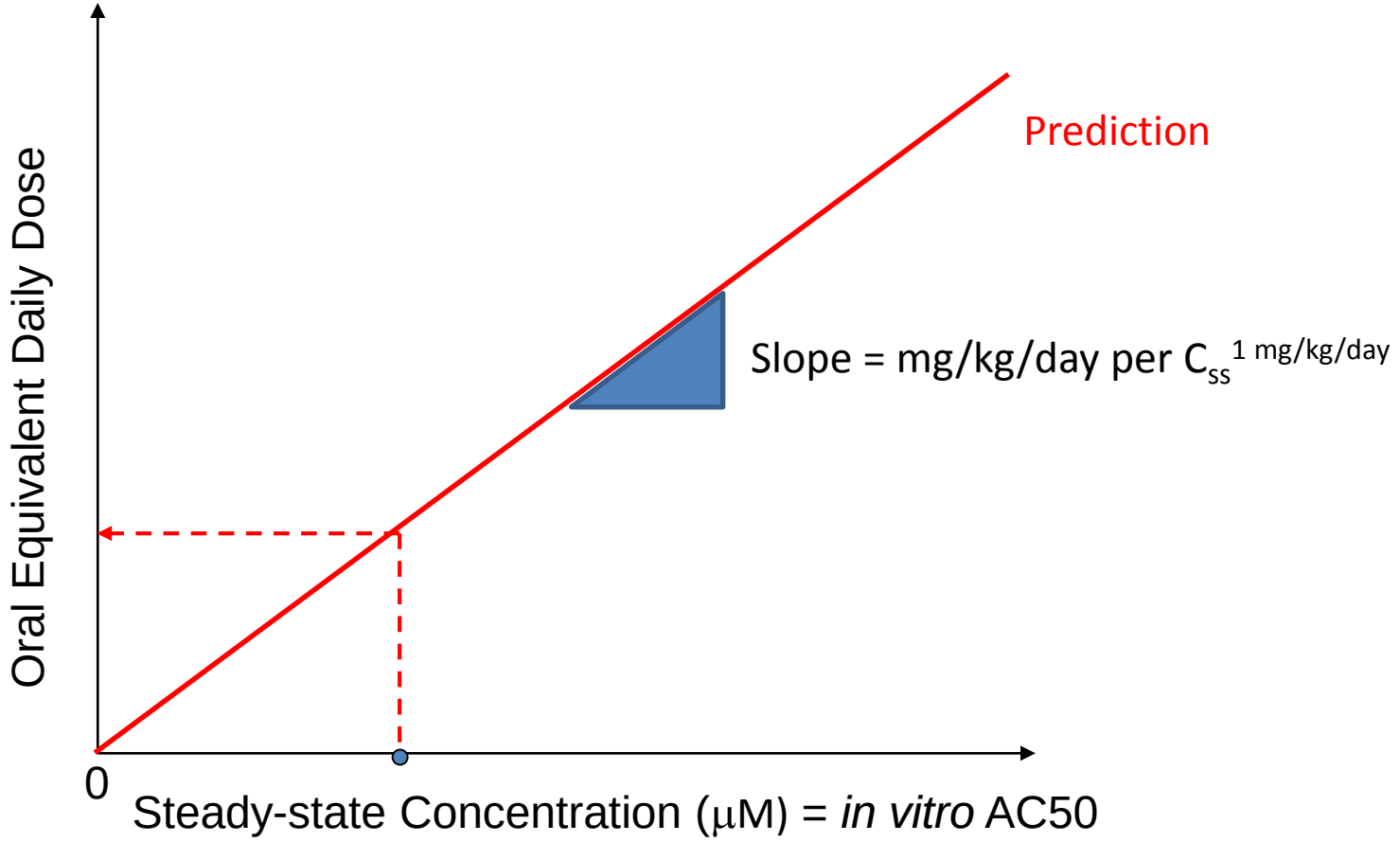


$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * \text{F}_{ub}) + \left(\text{Q}_l * \text{F}_{ub} * \frac{Cl_{int}}{\text{Q}_l + \text{F}_{ub} * Cl_{int}} \right)}$$

- Can calculate predicted steady-state concentration (C_{ss}) for a 1 mg/kg/day dose and multiply to get concentrations for other doses

Wetmore *et al*. (2012)

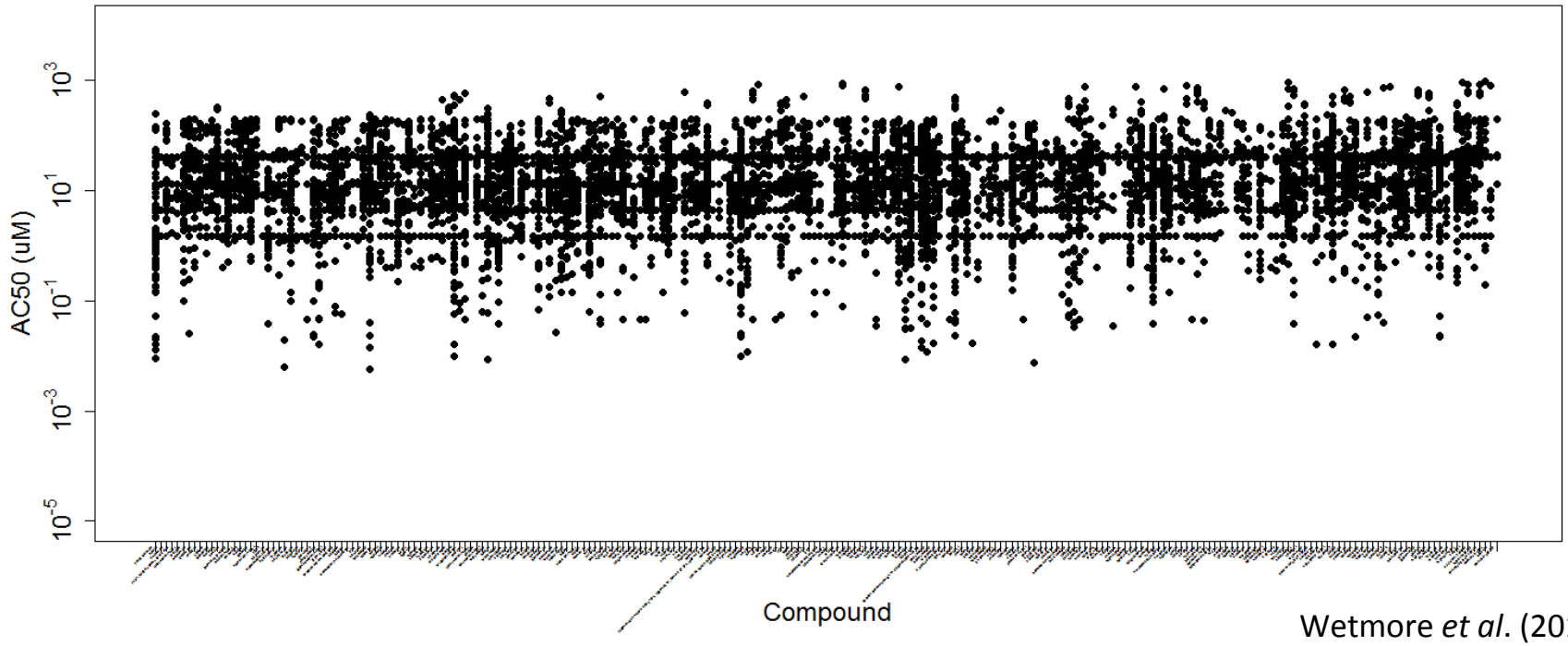
HTTK Allows Steady-State *In Vitro-In Vivo* Extrapolation (IVIVE)



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

Wetmore *et al*. (2012)

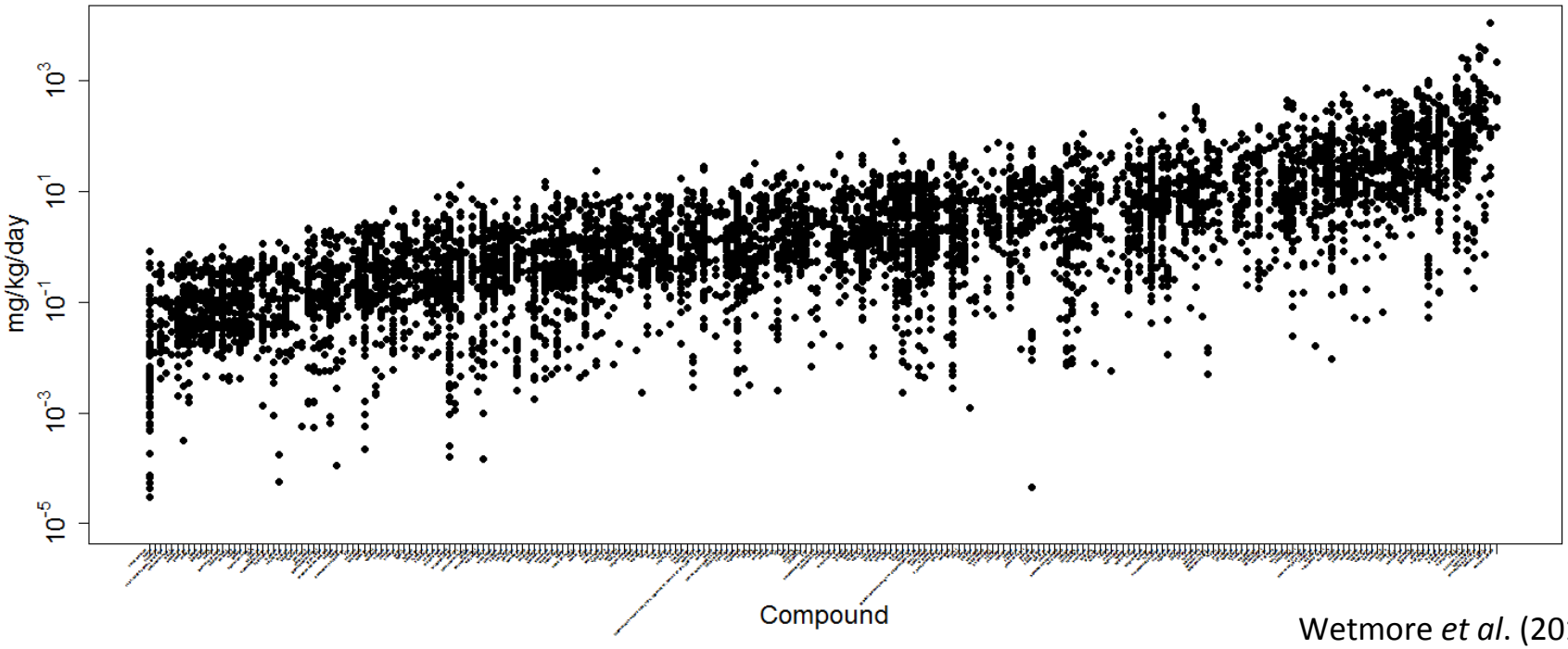
ToxCast *in vitro* Bioactive Concentrations



Wetmore *et al*. (2012)

- It appears harder to prioritize on bioactive *in vitro* concentration without *in vivo* context

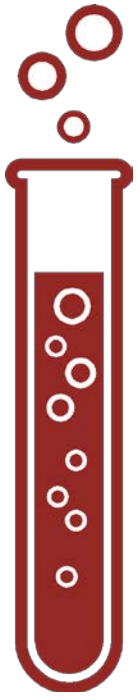
HTTK Oral Equivalents



Wetmore *et al*. (2012)

- Translation from *in vitro* to steady-state oral equivalent doses allow greater discrimination between effective chemical potencies

Reverse Dosimetry with HTTK



High Throughput *In Vitro* Bioactive Concentration

HTTK *in vitro* data

Simulated Human *In Vivo* Doses

Monte Carlo Simulation of Biological Variability

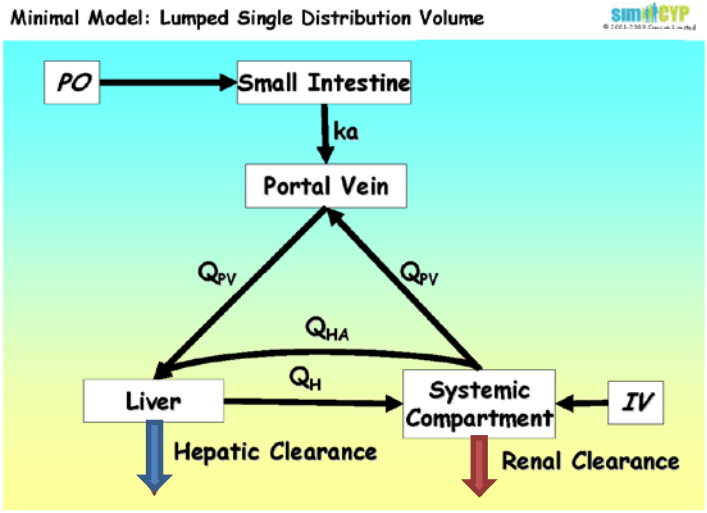
Combination of higher exposure and sensitivities

Populations that are More Sensitive

Images from Thinkstock

Variability in this Steady-State TK Model

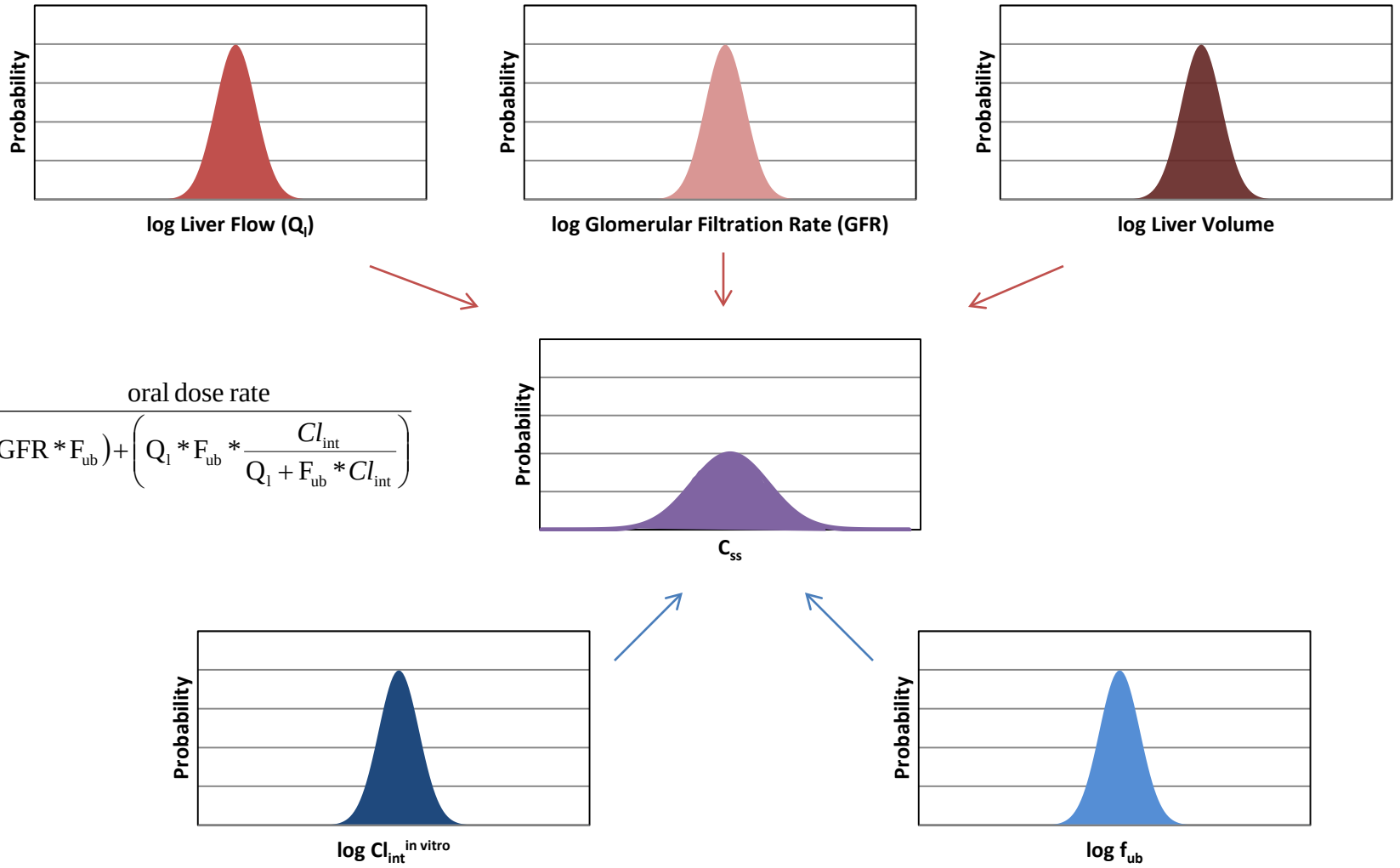
Jamei *et al*. (2009)



$$C_{ss} = \frac{\text{oral dose rate}}{\underbrace{(GFR * F_{ub})}_{\text{(Passive) Renal Clearance}} + \underbrace{\left(Q_l * F_{ub} * \frac{Cl_{int}}{Q_l + F_{ub} * Cl_{int}}\right)}_{\text{Hepatic Clearance (Metabolism)}}}$$

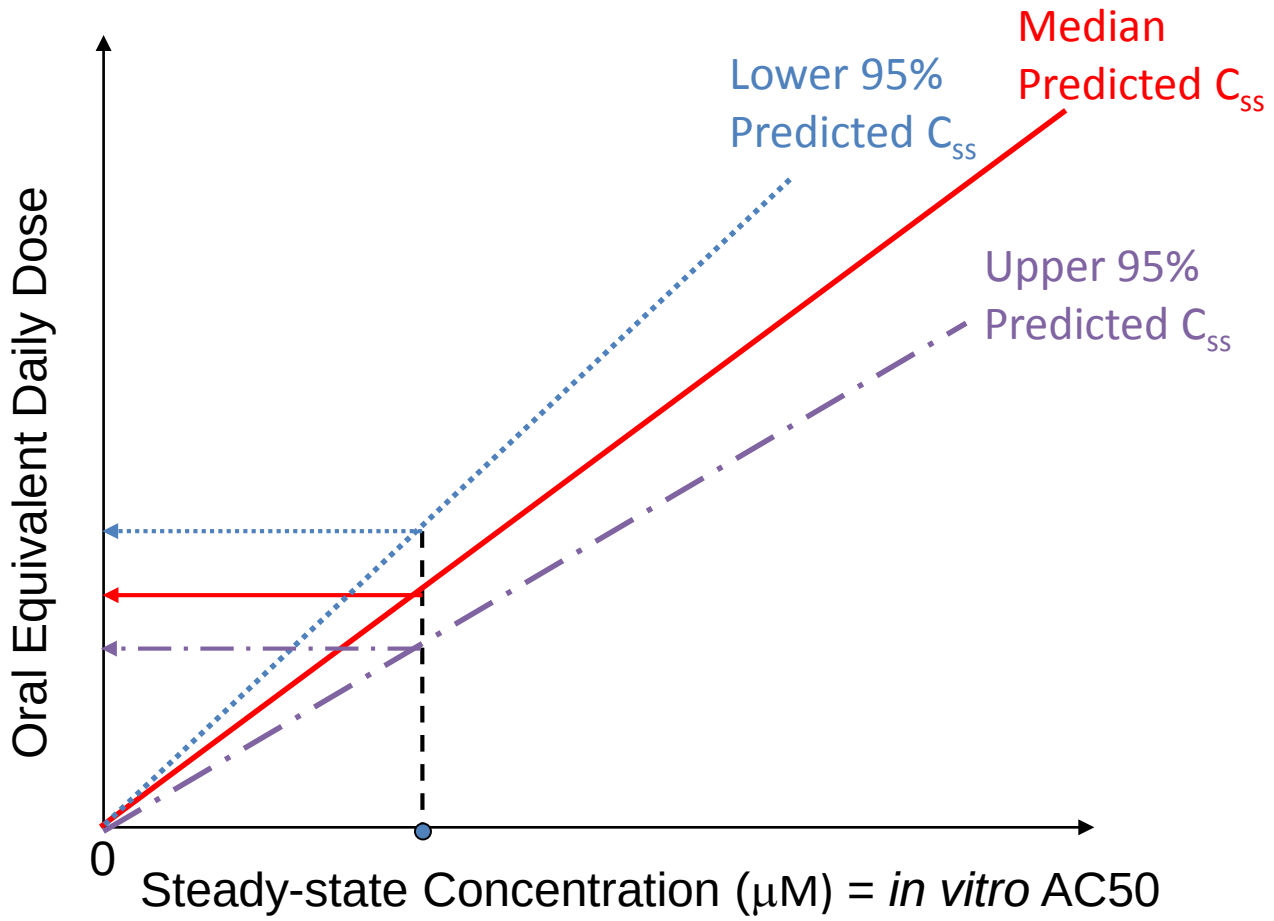
- *In vitro* clearance (µL/min/10^6 hepatocytes) is scaled to a whole organ clearance using the density of hepatocytes per gram of liver and the volume of the liver (which varies between individuals)
- Glomerular filtration rate (GFR) and blood flow to the liver (Q_l) both vary from individual to individual
- Further assume that measured HTTK parameters have 30% coefficient of variation

Monte Carlo (MC) Approach to Variability: SimCYP (Pharma) Approach



Wetmore *et al*. (2012)

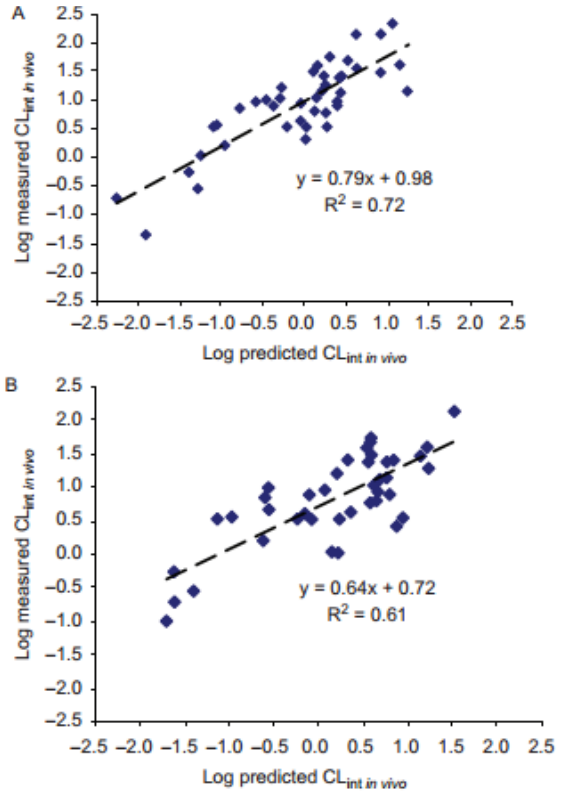
Steady-State In Vitro-In Vivo Extrapolation (IVIVE)



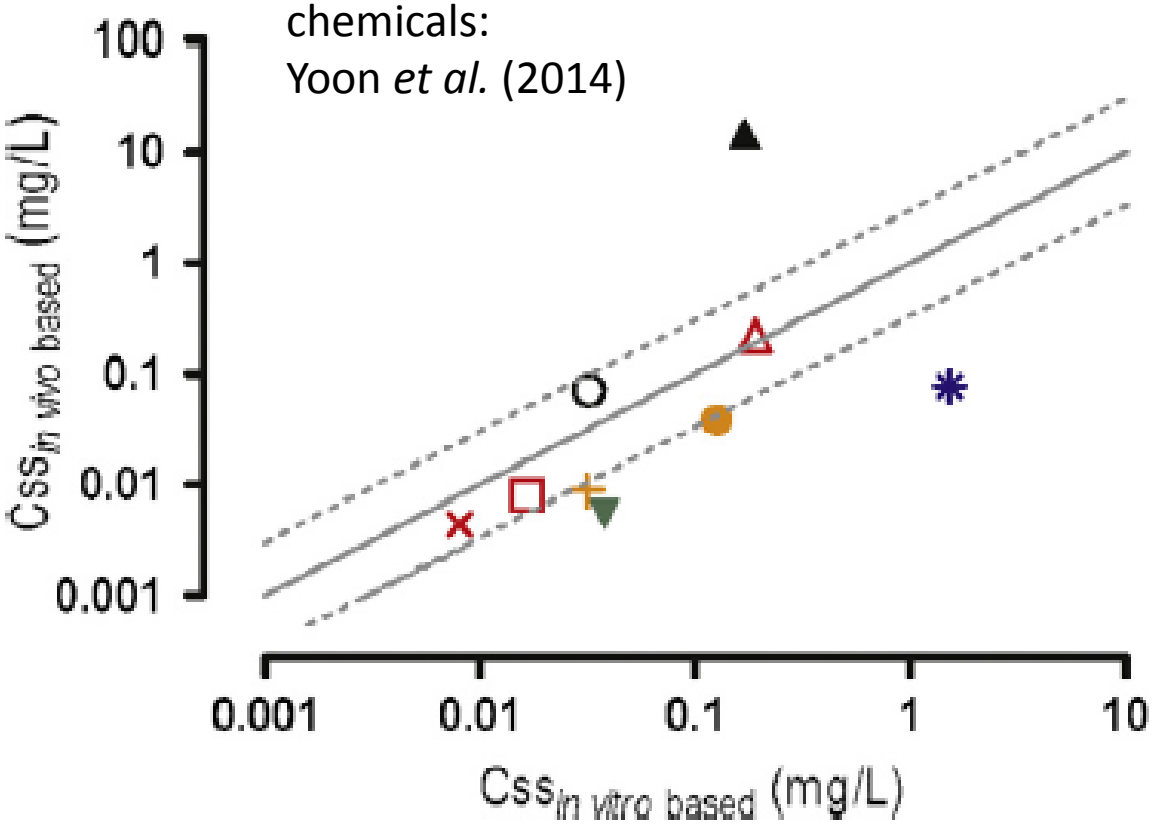
- The higher the predicted C_{ss}, the lower the oral equivalent dose, so the upper 95% predicted C_{ss} from the MC has a lower oral equivalent dose

Characterizing Accuracy of HTTK

Wang (2010): *In vitro* predictions typically within a factor of three for pharmaceuticals

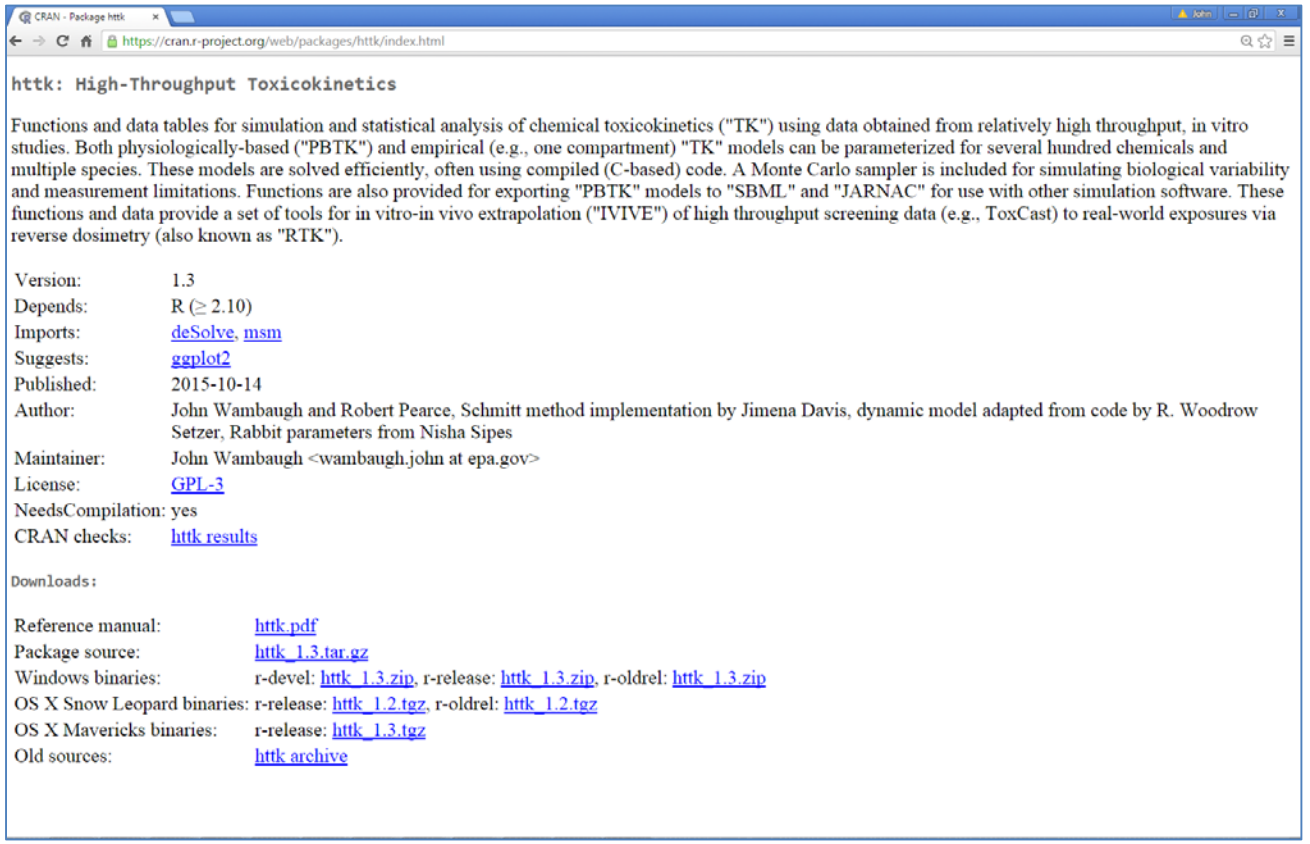


Environmental chemicals:
Yoon *et al.* (2014)



Hepatocytes and passive GFR alone tend to underestimate clearance

543 Chemicals with httk R Package



CRAN - Package httk

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

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:	1.3
Depends:	R (≥ 2.10)
Imports:	deSolve, msm
Suggests:	ggplot2
Published:	2015-10-14
Author:	John Wambaugh and Robert Pearce, Schmitt method implementation by Jimena Davis, dynamic model adapted from code by R. Woodrow Setzer, Rabbit parameters from Nisha Sipes
Maintainer:	John Wambaugh <wambaugh.john at epa.gov>
License:	GPL-3
NeedsCompilation:	yes
CRAN checks:	httk results

Downloads:

Reference manual:	httk.pdf
Package source:	httk_1.3.tar.gz
Windows binaries:	r-devel: httk_1.3.zip, r-release: httk_1.3.zip, r-oldrel: httk_1.3.zip
OS X Snow Leopard binaries:	r-release: httk_1.2.tgz, r-oldrel: httk_1.2.tgz
OS X Mavericks binaries:	r-release: httk_1.3.tgz
Old sources:	httk archive

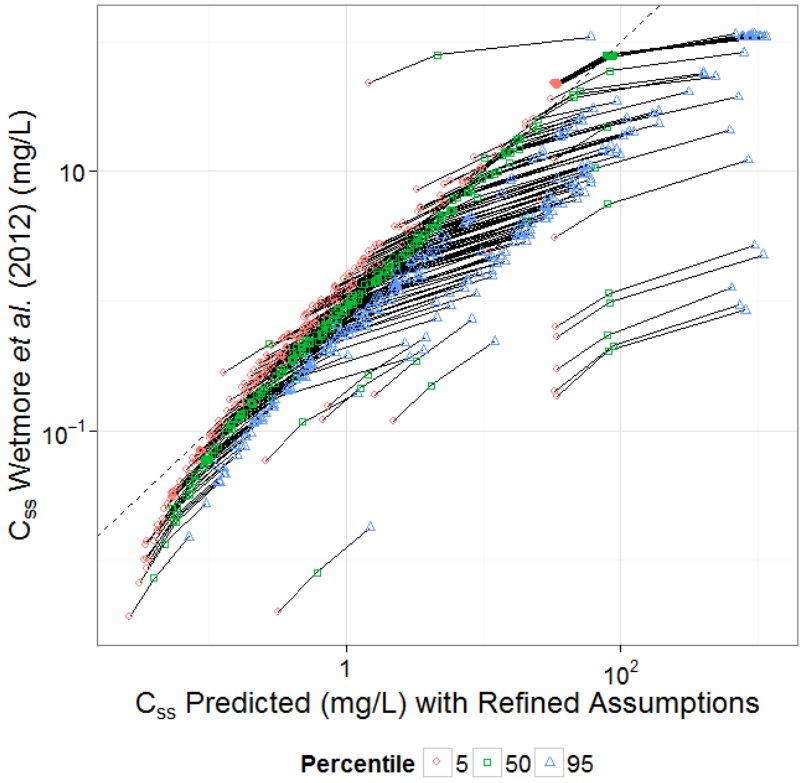
https://cran.r-project.org/web/packages/httk/

Can access this from the R GUI: “Packages” then “Install Packages”

443 with PBTK models

Lead developer Robert Peace

Comparison Between httk and SimCYP

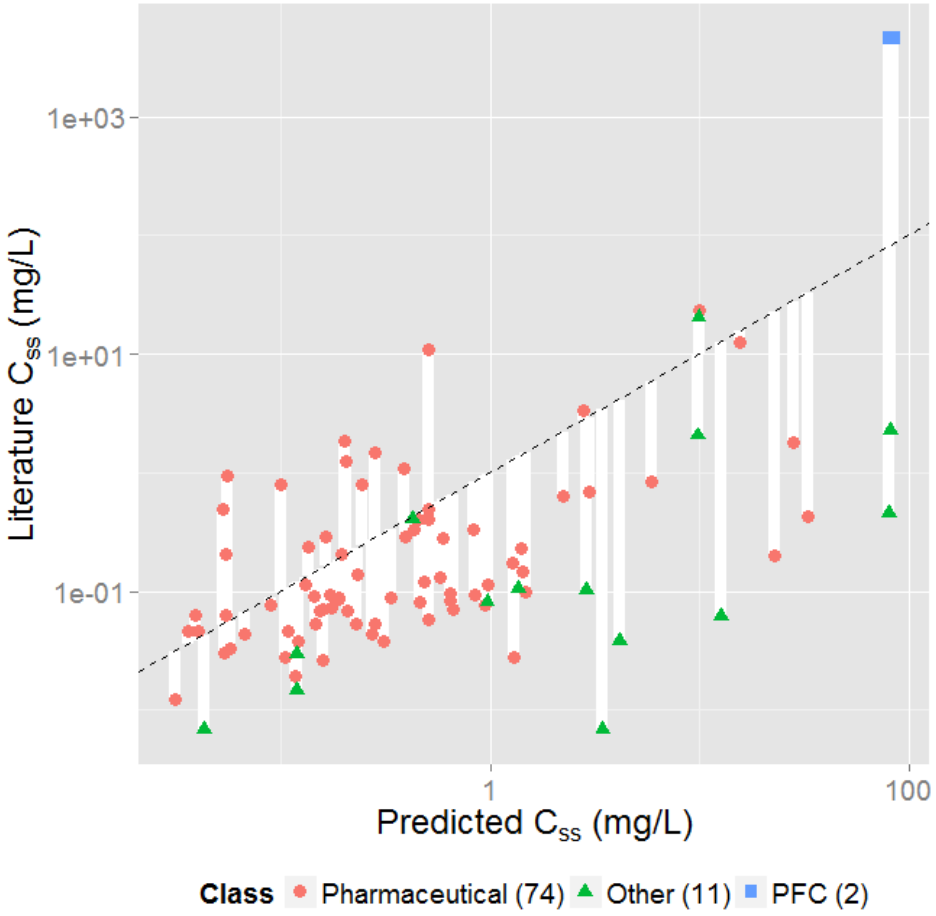


- In the Rotroff et al. (2010) and Wetmore et al. (2012, 2013, 2014, 2015) papers SimCYP was used to predict distributions of C_{ss} from *in vitro* data
  - We can reproduce the results from those publications for most chemicals using our implementation of Monte Carlo.
- Any one chemical's median and quantiles are connected by a dotted line.

The RED assay for measuring protein binding fails in some cases because the amount of free chemical is below the limit of detection. For those chemicals a default value of 0.5% free was used. We have replaced the default value with random draws from a uniform distribution from 0 to 1%.

Wambaugh et al. (2015)

Using *in vivo* Data to Evaluate RTK

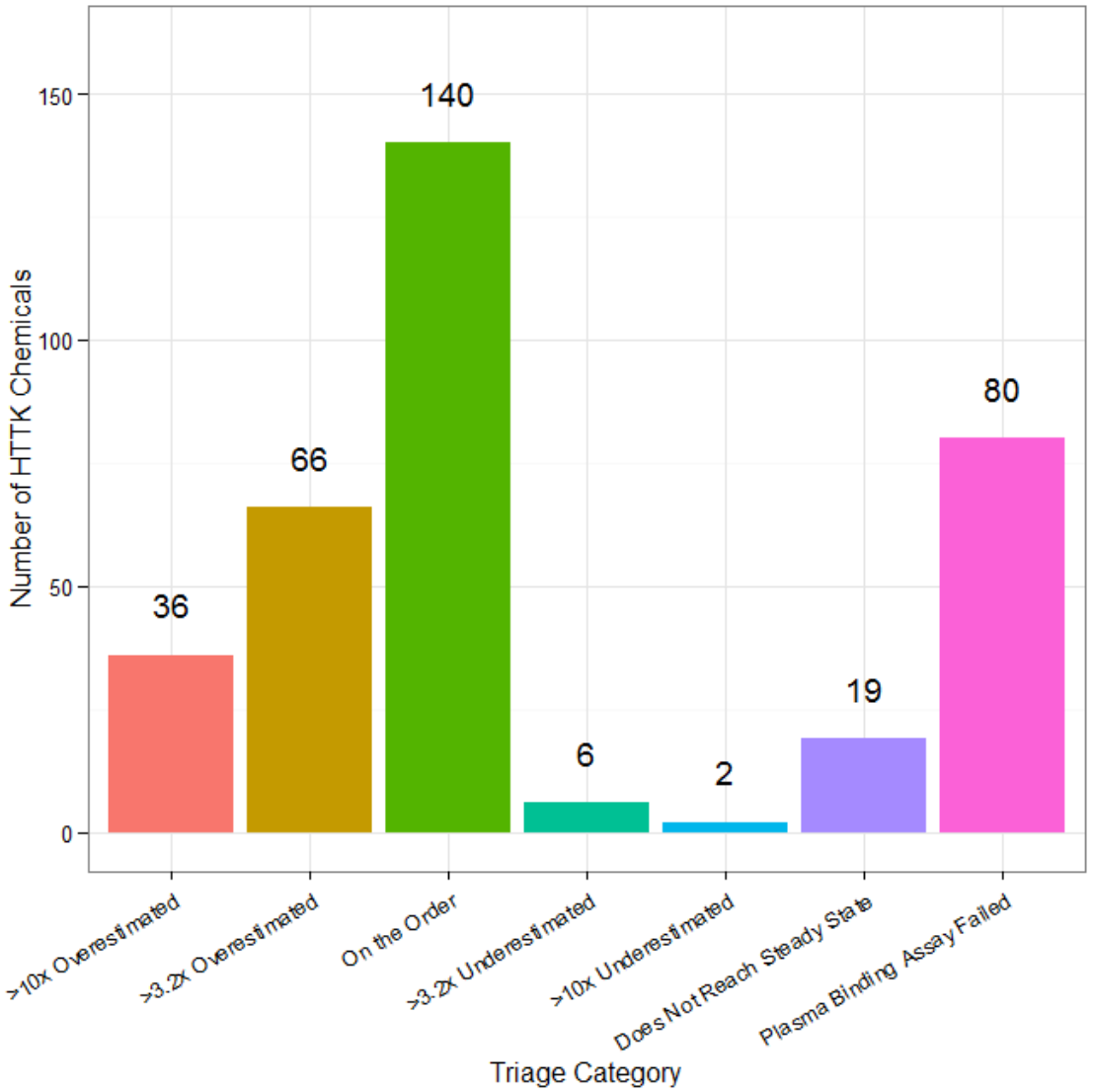


- When we compare the C_{ss} predicted from *in vitro* HTTK with *in vivo* C_{ss} values determined from the literature we find limited correlation (R^2 ~0.34)
- The dashed line indicates the identity (perfect predictor) line:
  - Over-predict for 65
  - Under-predict for 22
- The white lines indicate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

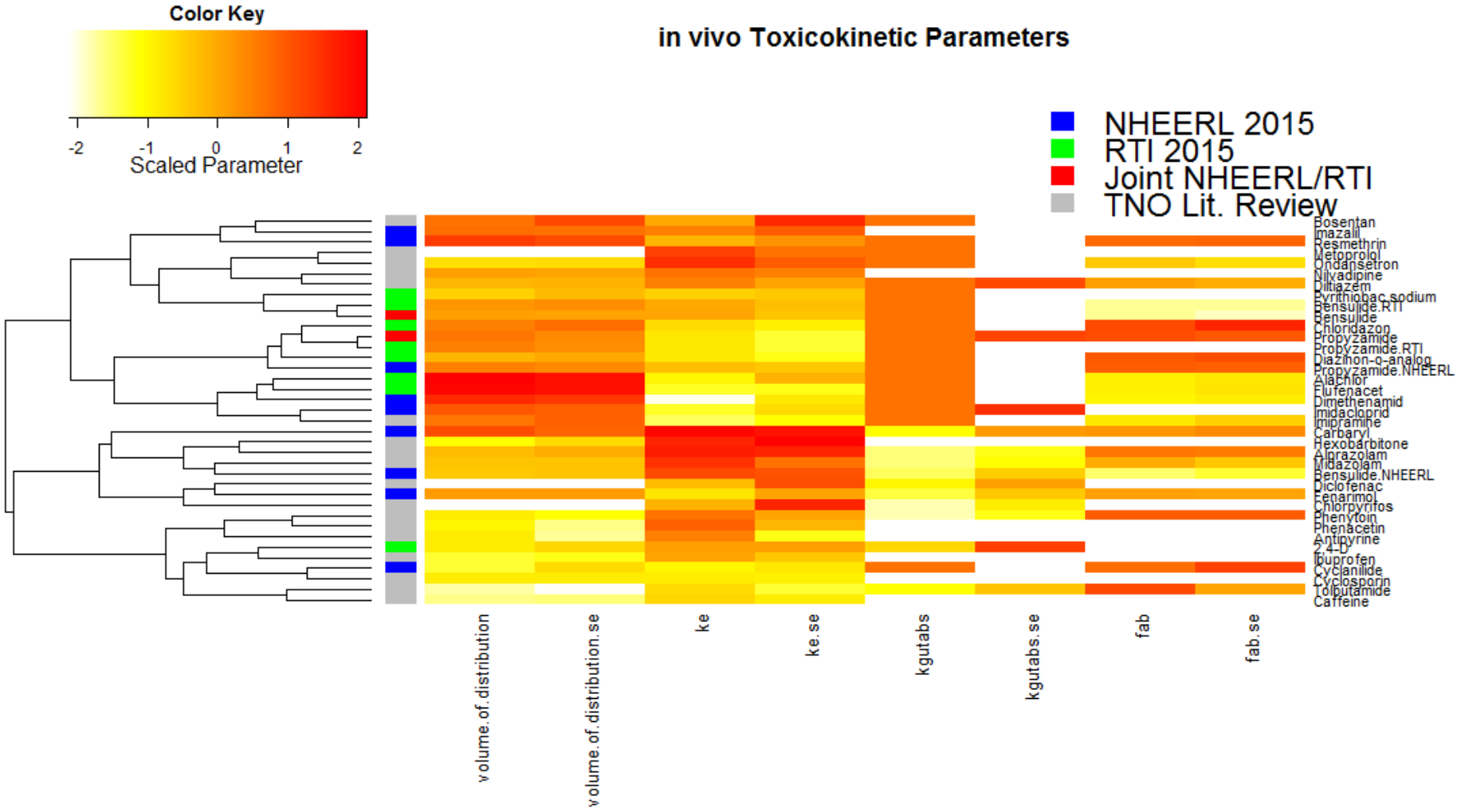


Wambaugh et al. (2015)

New *In Vivo* PK Data Set

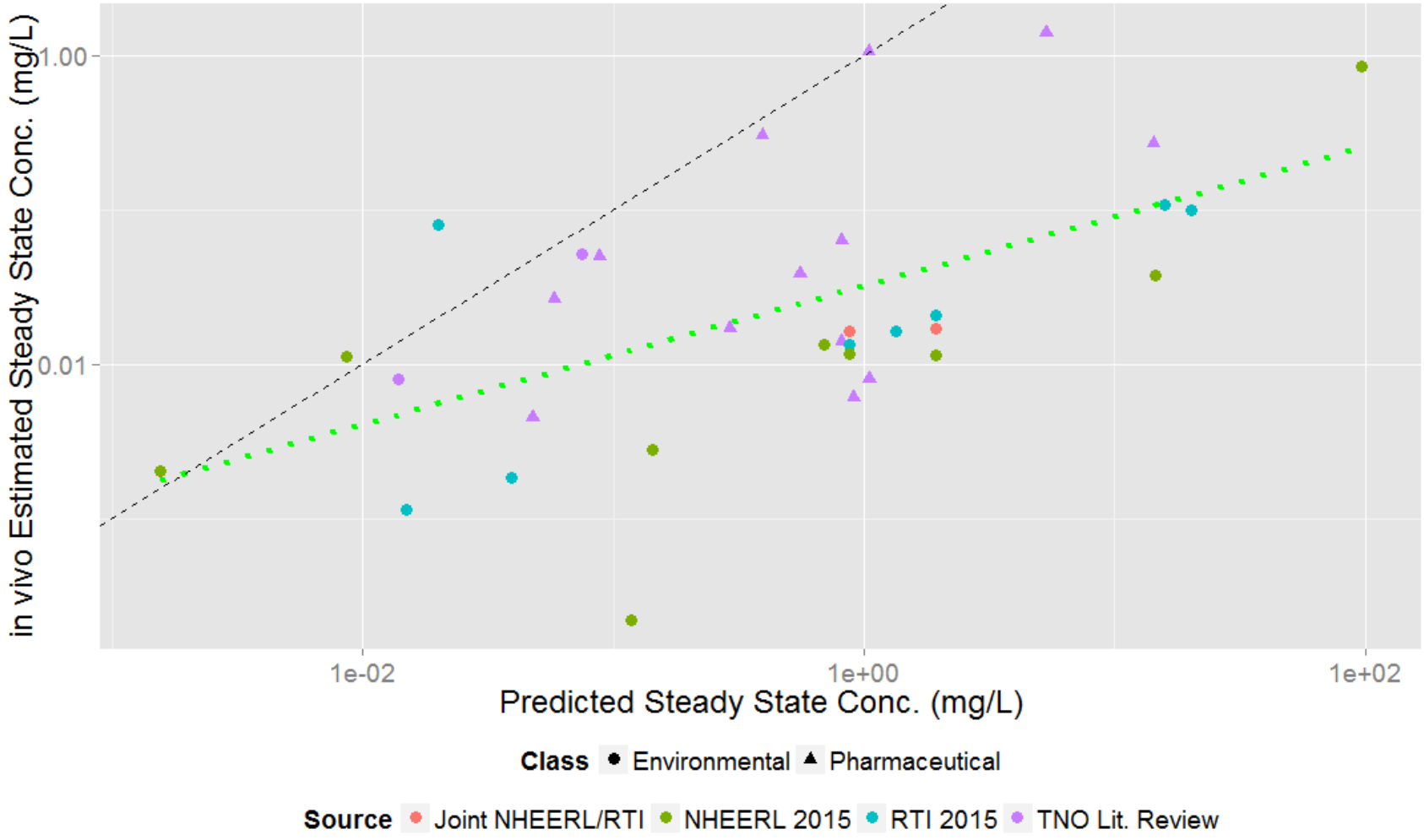
- Could the difference be related to inhomogeneous C_{ss} data?
  - Initially relying on Obach (2008) data plus data curated by TNO (Sieto Bosgra lead) from literature
- Only 13 non-pharmaceuticals examined so far
- Cross lab study:
  - 20 chemicals examined by NHEERL (Mike Hughes lead)
  - 8 chemicals examined by RTI (Tim Fennell lead)
  - 2 overlap chemicals (Bensulide and Propyzamide)

An *In Vivo* Toxicokinetic Library



Work by Mike Hughes, Caroline Ring, Tim Fennell (RTI) and many more

Evaluating Steady-state Conc. (1 mg/kg/day exposure)

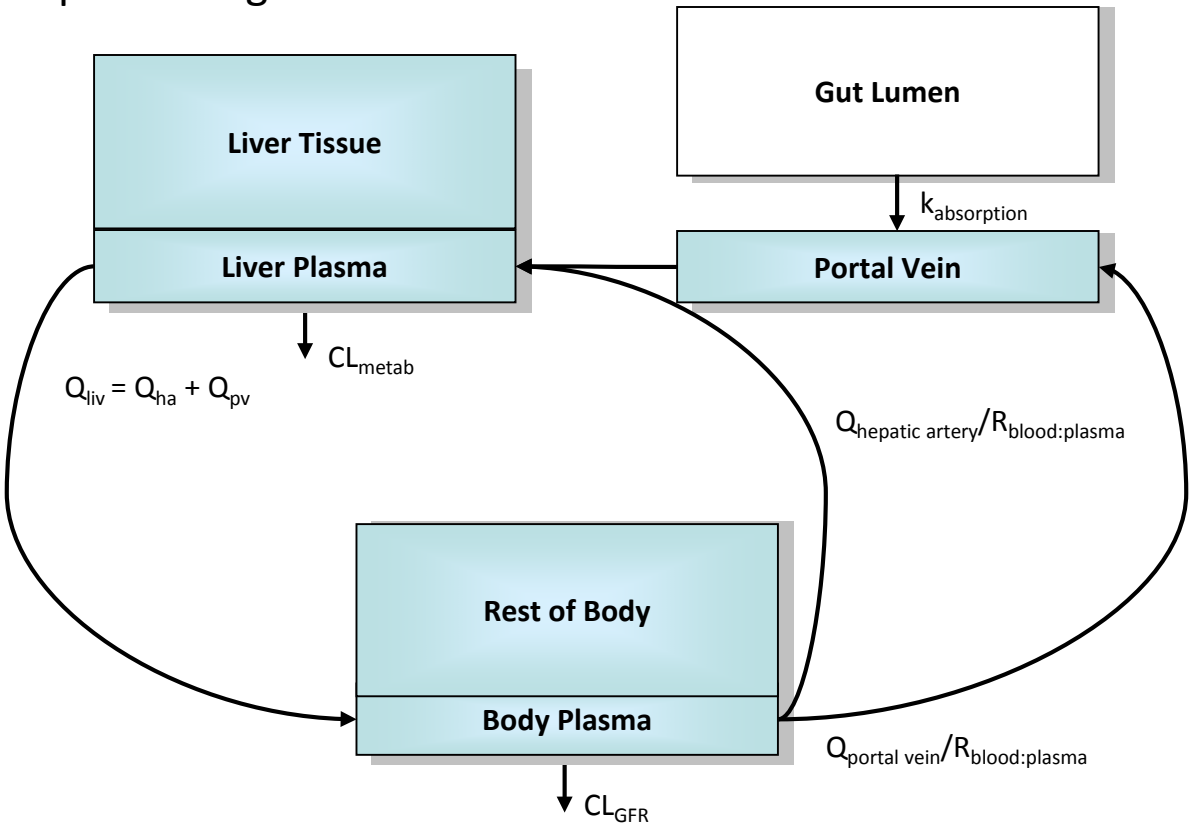


Similar to pharmaceuticals in Sohlenius-Sternbeck et al., 2010

Work by Mike Hughes, Caroline Ring, Tim Fennell (RTI) and many more

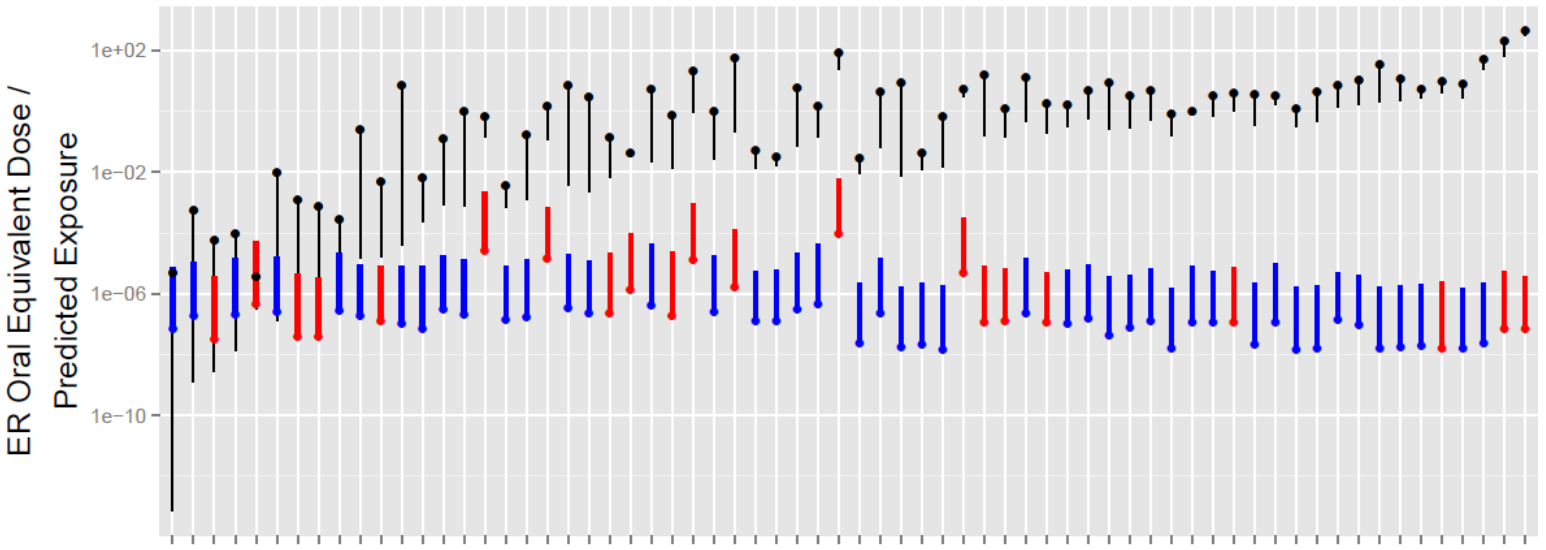
Three Compartment (SimCYP Steady-state) Model

Good enough for prioritizing chemicals…



Pharmacokinetics Allows Context for High Throughput Screening

Endocrine disruption AOP *(Judson et al., in prep.)*



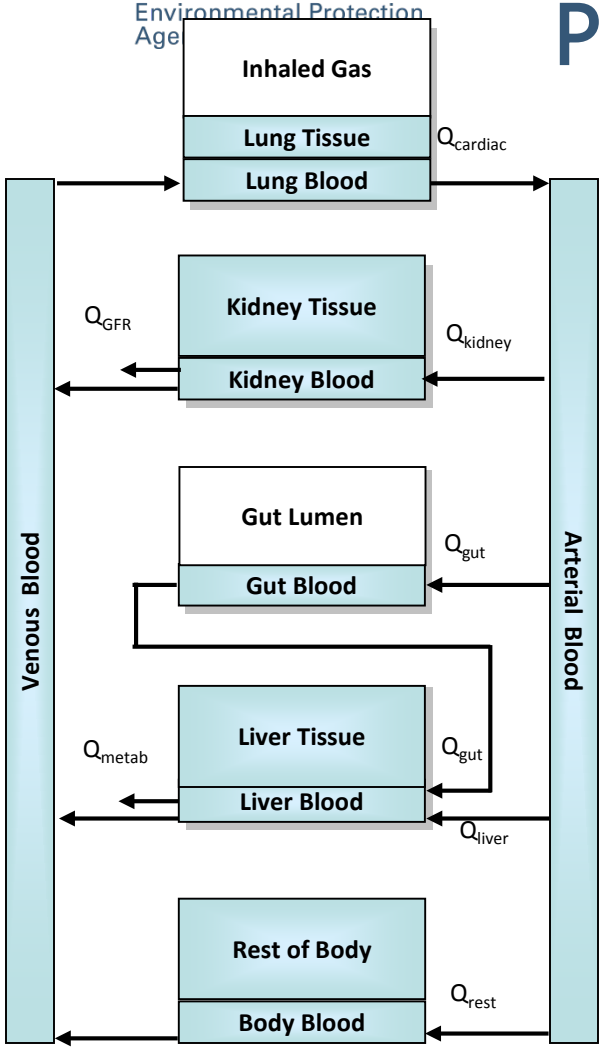
ToxCast Bioactivity Converted to mg/kg/day with HTTK (Wetmore et al., 2012)

ExpoCast Exposure Predictions (Wambaugh *et al.*, 2014)

December, 2014 Panel:
"Scientific Issues Associated with Integrated Endocrine Bioactivity and Exposure-Based Prioritization and Screening"

DOCKET NUMBER: EPA–HQ–OPP–2014–0614

A General Physiologically-based Pharmacokinetic (PBPK) 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.

Blood flows move the chemical throughout the body. The total blood flow to all tissues equals the cardiac output.

The only ways chemicals “leaves” 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).

Physiological Data

United States Environmental Protection Agency

	Volume (L/kg)					Blood Flow (ml/min/kg)				
Tissue	**Mouse**	**Rat**	**Dog**	**Human**	**Rabbit**	**Mouse**	**Rat**	**Dog**	**Human**	**Rabbit**
Adipose	0.07	0.07	0.05	0.21	0.05	10.80	1.60	3.50	3.71	12.80
Bone	0.05	0.04	0.04	0.07	0.04	23.31	36.11	1.30	3.36	36.11
Brain	0.02	0.01	0.01	0.02	0.01	13.20	5.20	4.50	10.00	5.20
Gut	0.04	0.03	0.04	0.02	0.05	72.50	39.20	23.00	16.43	44.40
Heart	0.00	0.00	0.01	0.00	0.00	14.00	15.60	5.40	3.43	6.40
Kidneys	0.02	0.01	0.01	0.00	0.01	65.00	36.80	21.60	17.71	32.00
Liver	0.05	0.03	0.03	0.02	0.04	90.00	47.20	30.90	20.71	70.80
Lung	0.01	0.00	0.01	0.01	0.01	2.00	6.22	10.56	2.00	6.22
Muscle	0.37	0.39	0.44	0.38	0.54	45.50	30.00	25.00	10.71	62.00
Skin	0.15	0.17	0.17	0.03	0.04	20.50	23.20	10.00	4.29	23.20
Spleen	0.00	0.00	0.00	0.00	0.00	5.50	4.07	1.65	1.10	3.60
Rest	0.03	0.05	0.00	0.05	0.03	110.19	90.00	5.59	2.97	90.00

Volumes and flows from Schmitt (2008) + Nisha Sipes (Rabbit)

Other parameters from Davies and Morris (1993) + Nisha Sipes (Rabbit)

	Units	**Mouse**	**Rat**	**Dog**	**Human**	**Rabbit**
Total Body Water	ml/kg	725.00	668.00	603.60	600.00	716
Plasma Volume	ml/kg	50.00	31.20	51.50	42.86	44
Cardiac Output	ml/min/kg	400.00	296.00	120.00	80.00	212
Average BW	kg	0.02	0.25	10.00	70.00	2.5
Total Plasma Protein	g/ml	0.06	0.07	0.09	0.07	0.057
Plasma albumin	g/ml	0.03	0.03	0.03	0.04	0.0387
Plasma a-1-AGP	g/ml	0.01	0.02	0.00	0.00	0.0013
Hematocrit	fraction	0.45	0.46	0.42	0.44	0.36
Urine	ml/min/kg	0.035	0.139	0.021	0.014	0.0417
Bile	ml/min/kg	0.069	0.063	0.008	0.003	0.0833
GFR	ml/min/kg	14.0	5.2	6.1	1.8	3.12

Schmitt (2008) Tissue Composition Data

	Fraction of total volume[a]		Fraction of cell volume[b]			Fraction of total lipid			
Tissue	Cells	Interstitium	Water	Lipid	Protein	Neutral Lipid[c]	Neutral Phospholipid[c]	Acidic Phospholipid[c]	pH[d]
Adipose	0.86	0.14	0.03	0.92	0.06	1	0.0022	0.0006	7.10
Bone	0.9	0.1	0.26	0.02	0.21	0.85	0.11	0.04	7.00
Brain	1	0.004	0.79	0.11	0.08	0.39	0.48	0.13	7.10
Gut	0.9	0.096	0.78	0.07	0.15	0.69	0.26	0.05	7.00
Heart	0.86	0.14	0.7	0.11	0.19	0.48	0.43	0.09	7.10
Kidneys	0.78	0.22	0.73	0.06	0.21	0.26	0.61	0.13	7.22
Liver	0.82	0.18	0.68	0.08	0.21	0.29	0.59	0.11	7.23
Lung	0.5	0.5	0.74	0.04	0.11	0.51	0.38	0.11	6.60
Muscle	0.88	0.12	0.76	0.01	0.19	0.49	0.42	0.09	6.81
Skin	0.69	0.31	0.47	0.14	0.41	0.9	0.08	0.02	7.00
Spleen	0.79	0.21	0.75	0.02	0.23	0.3	0.54	0.15	7.00
Red blood cells	1	–	0.63	0.01	0.33	0.3	0.59	0.1	7.20

a Values taken from (Kawai et al., 1994). Original values given as fraction of total organ volume were rescaled to tissue volume by subtracting vascular volume

b Values taken from (ICRP, 1975). Original values given as fraction of total tissue mass were rescaled to cellular volume as follows: Water fraction of total tissue reduced by interstitial volume and subsequently all values normalized by cellular fraction.

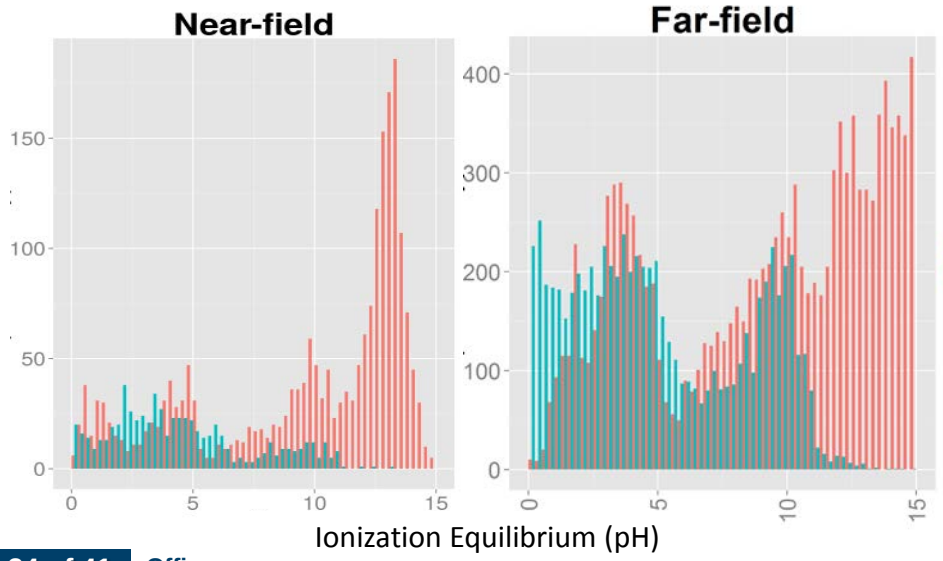
c Data taken from (Rodgers et al., 2005a).

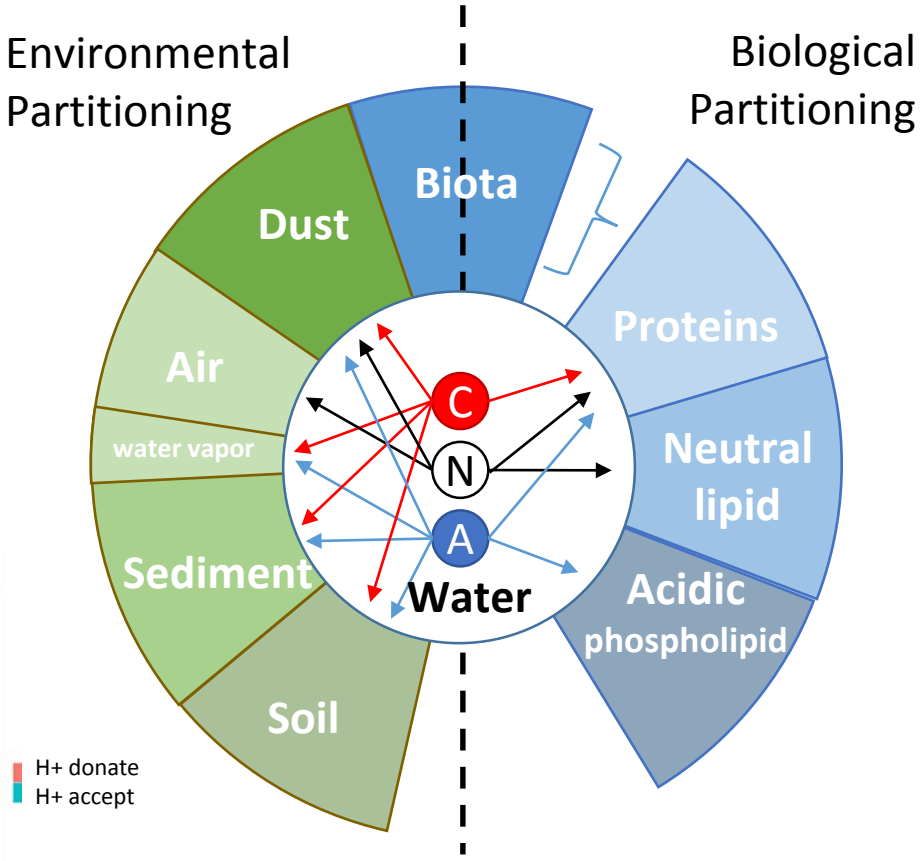
d Values taken from ([Waddell and Bates, 1969], [Malan et al., 1985], [Wood and Schaefer, 1978], [Schanker and Less, 1977], [Harrison and Walker, 1979] and [Civelek et al., 1996]). Mean values were calculated when more than one value was found for the same tissue.

e Data taken from (Gomez et al., 2002).

Prediction of Ionization

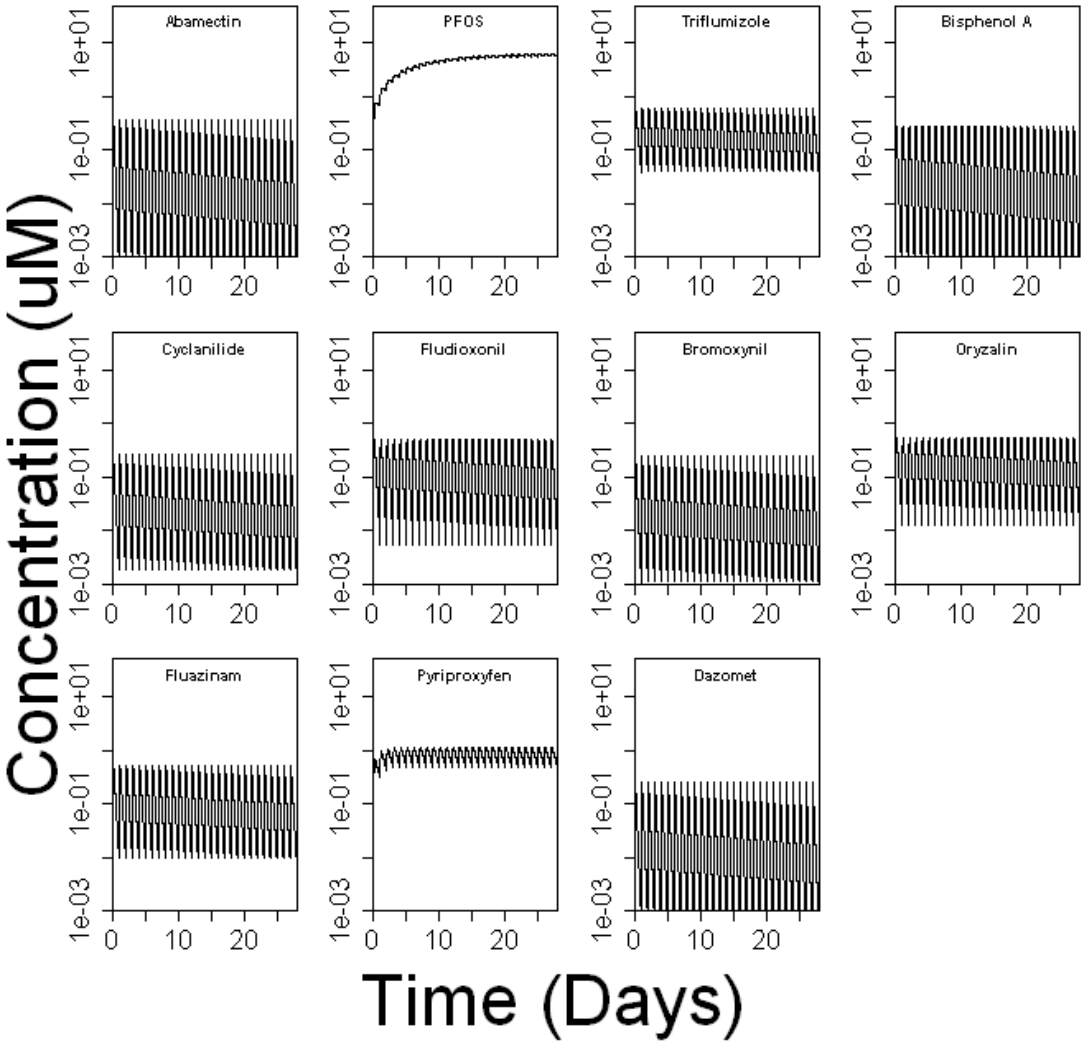
- Neutral and ionized species of the same molecule will partition differently into environmental and biological media
- Better models are needed for predicting pKa at different pH for chemicals





Project lead Cory Strope (Hamner)

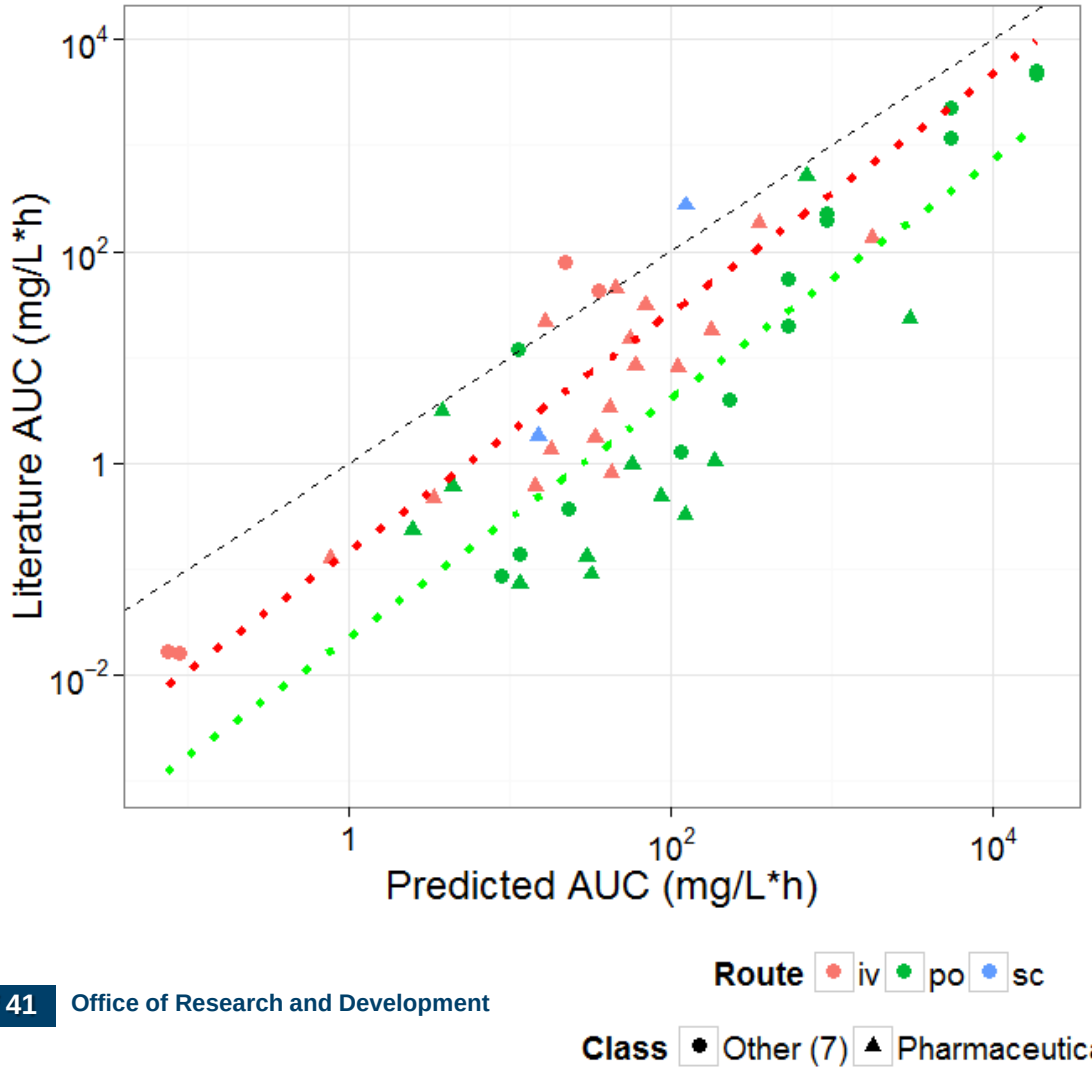
Predicted PK Metrics



Human hepatic concentration of various chemicals as a function of 28 daily doses (10 mg/kg/day)

Can predict mean and peak concentration and time integrated area under the curve (AUC) for various tissues

Evaluating HTPBPK Predictions with *In Vitro* Data

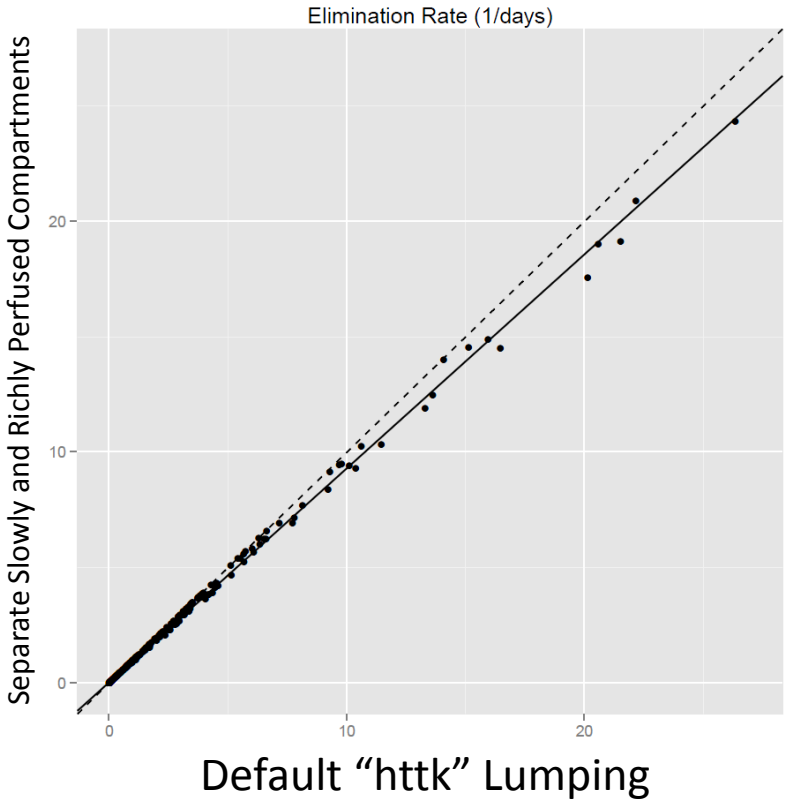


- HTPBPK predictions for the AUC (time integrated plasma concentration or Area Under the Curve)
- *in vivo* measurements from the literature for various treatments (dose and route) of rat.
- Predictions are generally conservative – *i.e.*, predicted AUC higher than measured
- Oral dose AUC ~6.4x higher than intravenous dose AUC

Wambaugh et al. (2015)

Evaluation Leads to Insight

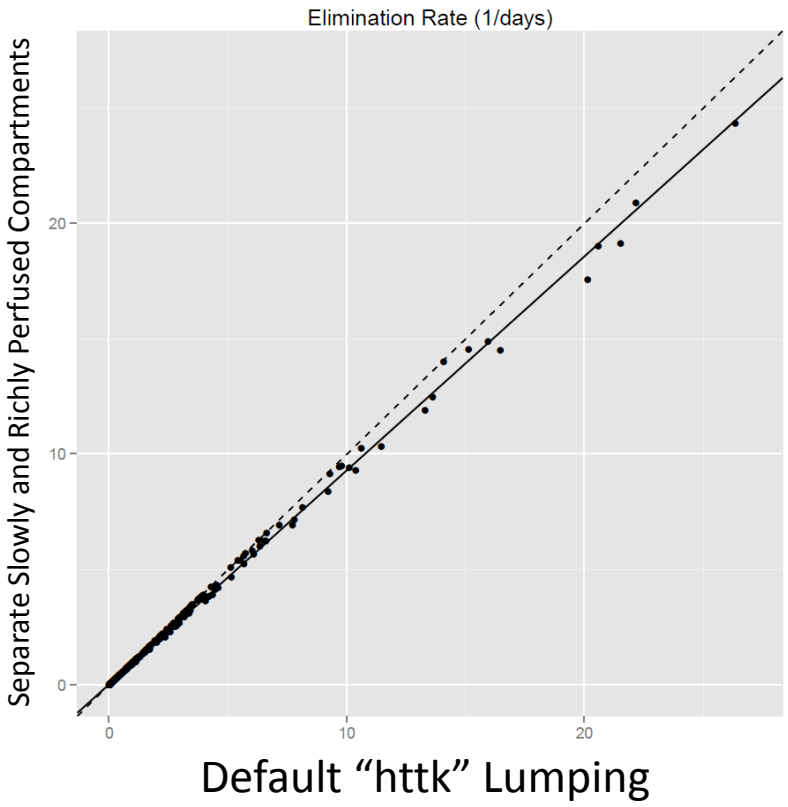
Examining the impact of lumping – default is liver, kidney, rest of body
What if we separate rest of body into richly and slowly perfused?

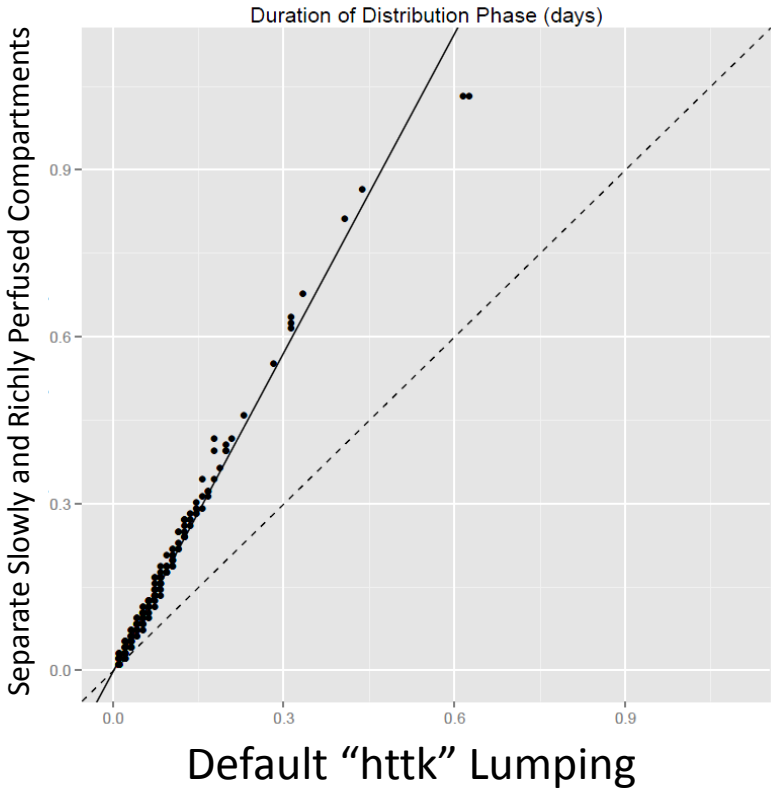


Work by Robert Pearce

Evaluation Leads to Insight

Examining the impact of lumping – default is liver, kidney, rest of body
What if we separate rest of body into richly and slowly perfused?

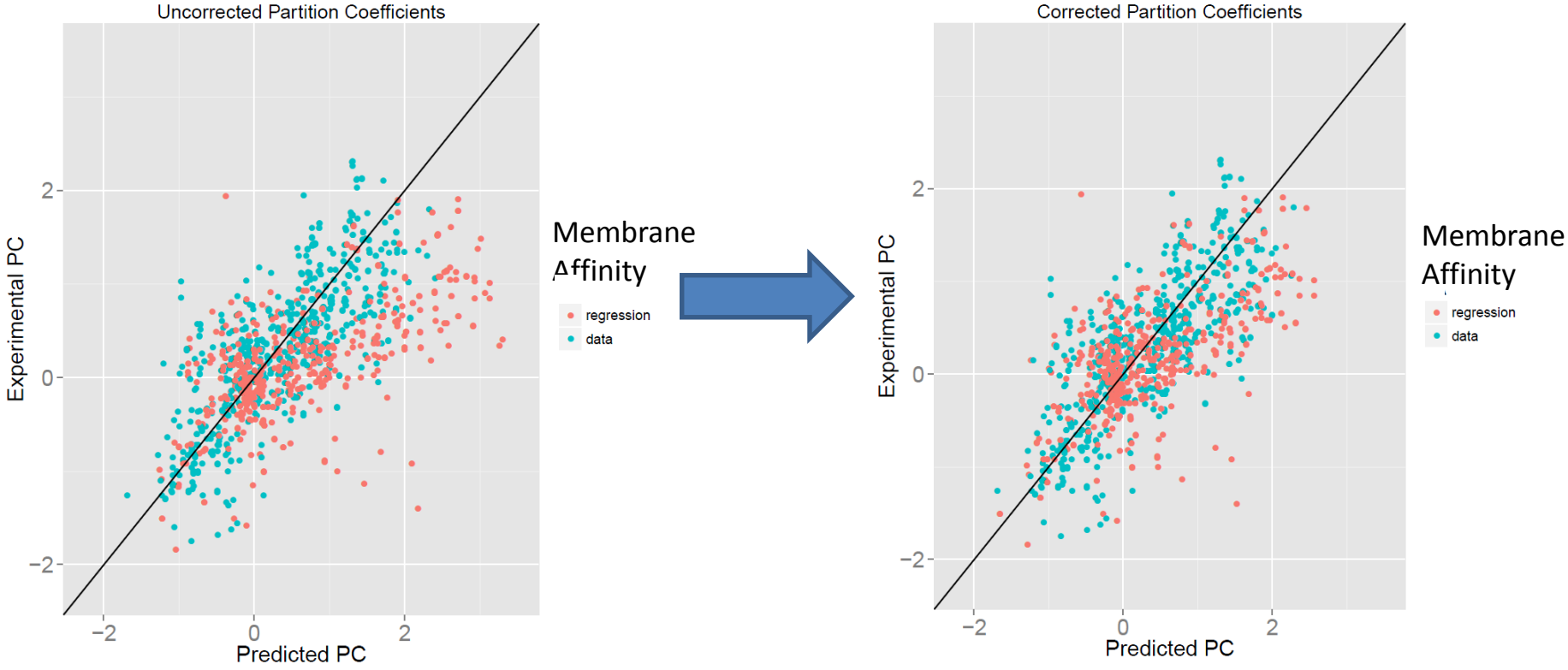




Work by Robert Pearce

Evaluation Leads to Refined Models

Ongoing refinements of tissue-specific partition coefficient predictions:
Handling high log P, better treatment of ionization (Pearce et al. manuscript)



Work by Robert Pearce

Summary

- Toxicokinetics (TK) provides a bridge between hazard and exposure by predicting tissue concentrations due to exposure
- We must keep in mind the purpose – simple models appear to allow meaningful prioritization of further research
- A primary application of HTTK is "Reverse Dosimetry" or RTK
  - Can infer daily doses that produce plasma concentrations equivalent to the bioactive concentrations,
- We can also use QSAR to build provisional PBTK models

But we must consider parsimony and domain of applicability:

- Do not build beyond the evaluation data
- Carefully determine whether, when, and why model errors are conservative
- Collect PK data from *in vivo* studies to allow larger, systematic studies

- R package "httk" freely available on CRAN allows statistical analyses

EPA United States Environmental Protection Agency

Chemical Safety for Sustainability (CSS) Rapid Exposure and Dosimetry (RED) Project

NCCT
Chris Grulke
Richard Judson
Dustin Kapruan*
Chantel Nicolas*
Robert Pearce*
James Rabinowitz
Ann Richard
Caroline Ring*
Woody Setzer
Rusty Thomas
John Wambaugh
Antony Williams

NRMRL
Yirui Liang*
Xiaoyu Liu

NHEERL
Jane Ellen Simmons
Marina Evans
Mike Hughes

*Trainees

NERL
Craig Barber
Brandy Beverly*
Derya Biryol*
Kathie Dionisio
Peter Egeghy
Kim Gaetz
Brandall Ingle*
Kristin Isaacs
Katherine Phillips*
Paul Price
Mark Strynar
Jon Sobus
Mike Tornero-Velez
Elin Ulrich
Dan Vallero

Collaborators

Arnot Research and Consulting
Jon Arnot
Battelle Memorial Institute
Anne Louise Sumner
Anne Gregg
Chemical Computing Group
Rocky Goldsmith
Hamner Institutes
Barbara Wetmore
Cory Strope
National Institute for Environmental Health Sciences (NIEHS)
Mike Devito
Steve Ferguson
Nisha Sipes
Kyla Taylor
Kristina Thayer
Netherlands Organisation for Applied Scientific Research (TNO)
Sieto Bosgra
Research Triangle Institute
Timothy Fennell
Silent Spring Institute
Robin Dodson
Southwest Research Institute
Alice Yau
Kristin Favela
University of California, Davis
Deborah Bennett
University of Michigan
Olivier Jolliet
University of North Carolina, Chapel Hill
Alex Tropsha