EPA United States Environmental Protection Agency

In Vitro Methods for Pharmacokinetics: What Works and What Doesn't for Extrapolation to *In Vivo* Exposures?

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U.S. Environmental Protection Agency

RTP Drug Metabolism Discussion Group
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EPA Office of Research and Development

- The Office of Research and Development (ORD) is the scientific research arm of EPA
  - 626 peer-reviewed journal articles in 2017 and 456 so far in 2018

- Research is conducted by ORD's three national laboratories, four national centers, and two offices organized to address:
  - Hazard, exposure, risk assessment, and risk management

- 13 facilities across the United States

- Six research programs
  - Air, Climate, and Energy; Chemical Safety for Sustainability; Human Health Risk Assessment; Homeland Security; Safe and Sustainable Water Resources; Sustainable and Healthy Communities

- Research conducted by a combination of Federal scientists; contract researchers; and postdoctoral, graduate student, and post-baccalaureate trainees

ORD Facility in
Research Triangle Park, NC

Chemical Regulation in the United States

- Park *et al.* (2012): At least 3221 chemicals present in pooled human blood samples, many appear to be exogenous albeit at low levels
  - A tapestry of laws covers the chemicals people are exposed to in the United States (Breyer, 2009)
  - Different testing requirements exist for food additives, pharmaceuticals, and pesticide active ingredients (NRC, 2007)

November 29, 2014

Chemical Regulation in the United States

- Different testing requirements exist for food additives, pharmaceuticals, and pesticide active ingredients (NRC, 2007)
  - Most industrial chemicals, ranging from industrial waste to dyes to packing materials, are covered by the Toxic Substances Control Act (TSCA) and regulated by EPA
  - TSCA was amended by the U.S. Congress in June, 2016 and new approach methodologies (NAMs) are being considered to inform prioritization of chemicals for testing and evaluation*

*"Alternative Test Methods and Strategies to Reduce Vertebrate Animal Testing," US EPA, June 2016

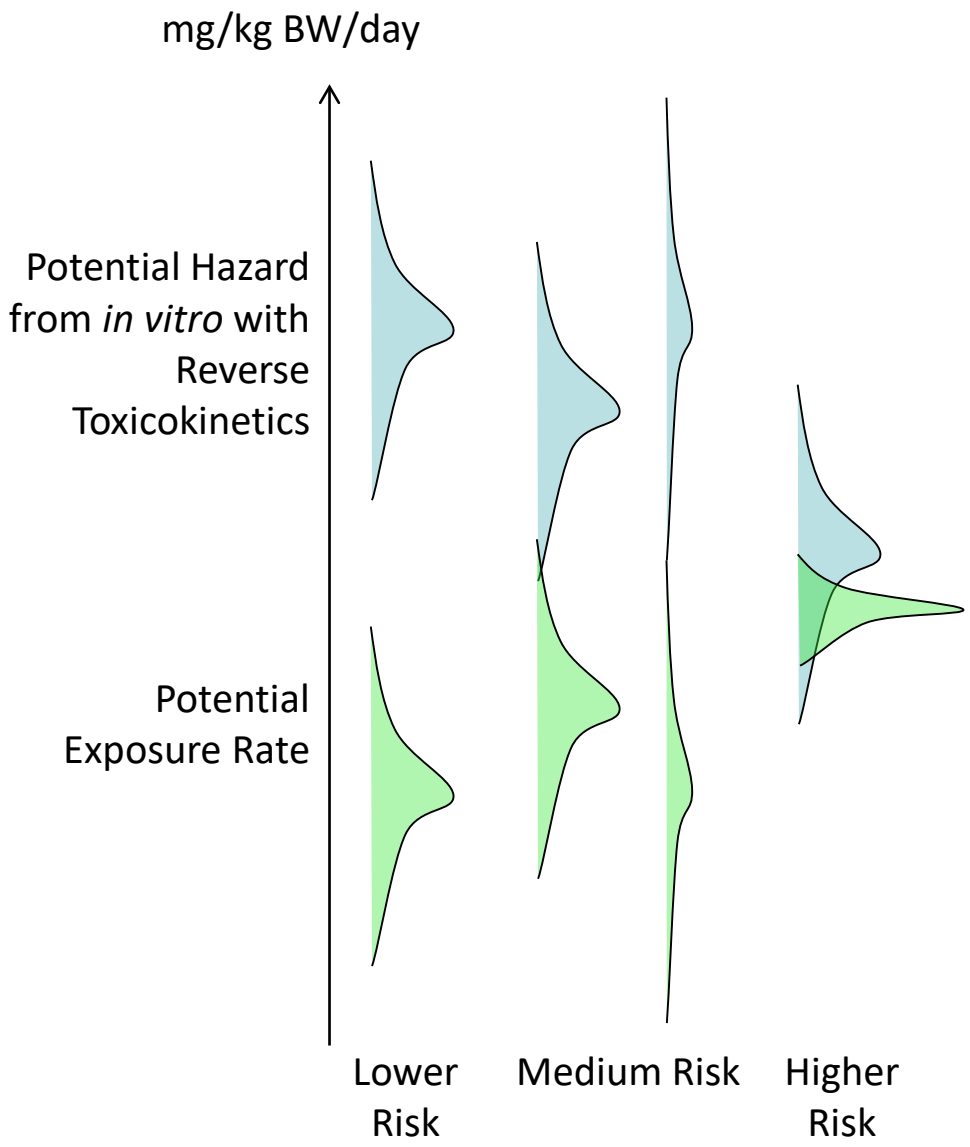
November 29, 2014

Chemical Risk = Hazard x Exposure

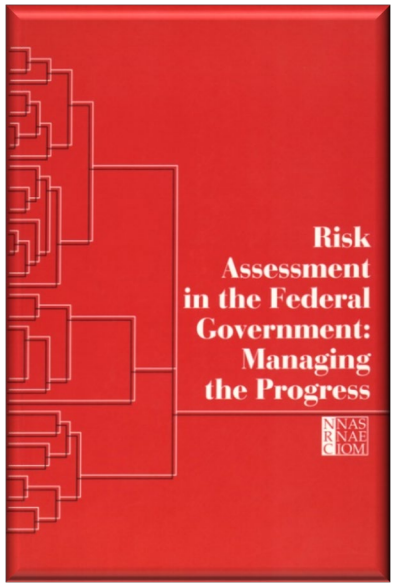
- The U.S. National Research Council (1983) identified chemical risk as a function of both inherent hazard and exposure

- To address thousands of chemicals, we need new approach methodologies (NAMs) that can inform prioritization of chemicals most worthy of additional study

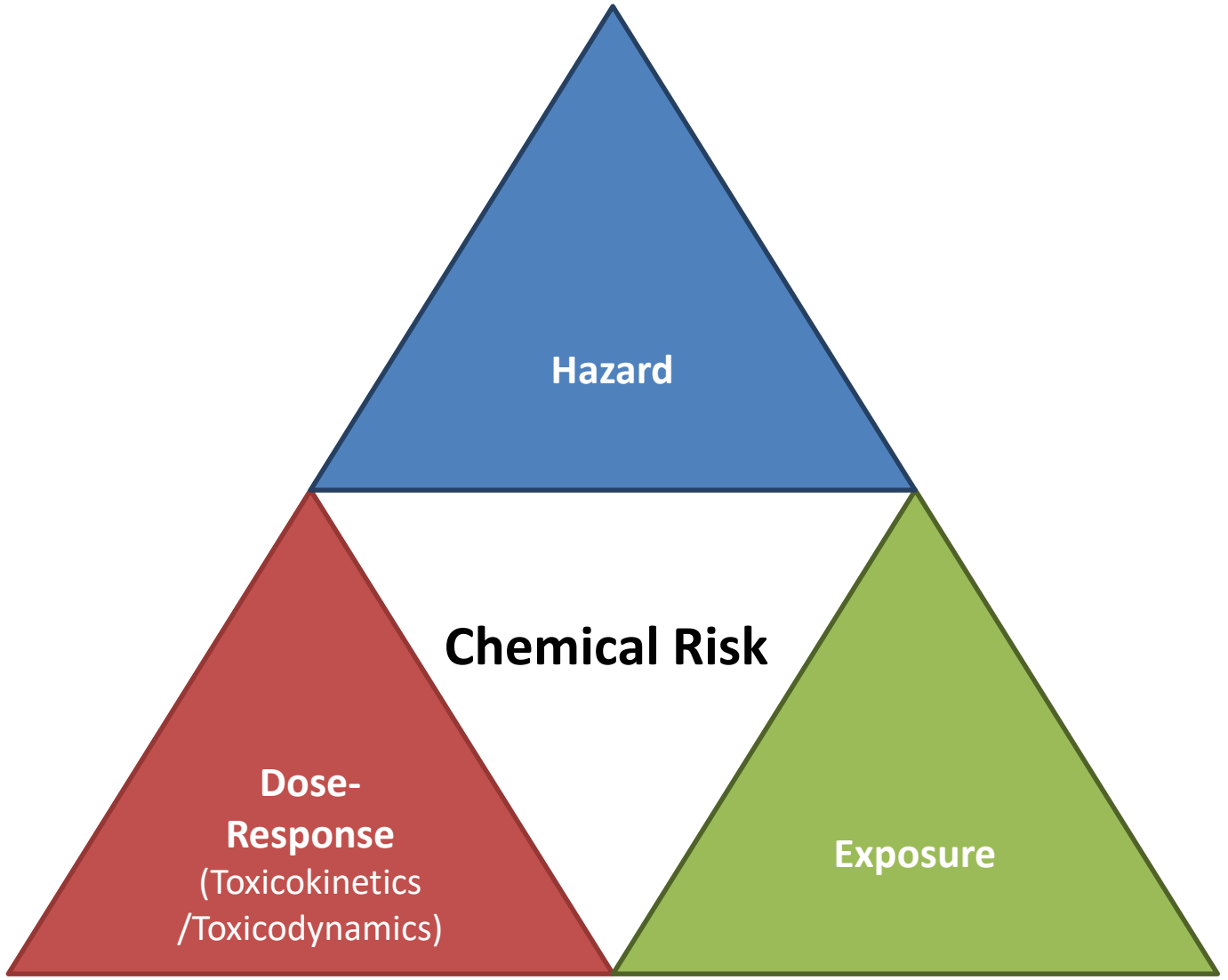
- High throughput risk prioritization needs:
  1. High throughput hazard characterization (Dix et al., 2007, Collins et al., 2008)
  2. High throughput exposure forecasts (Wambaugh et al., 2013, 2014)
  3. High throughput toxicokinetics (i.e., dose-response relationship) linking hazard and exposure (Wetmore et al., 2012, 2015)



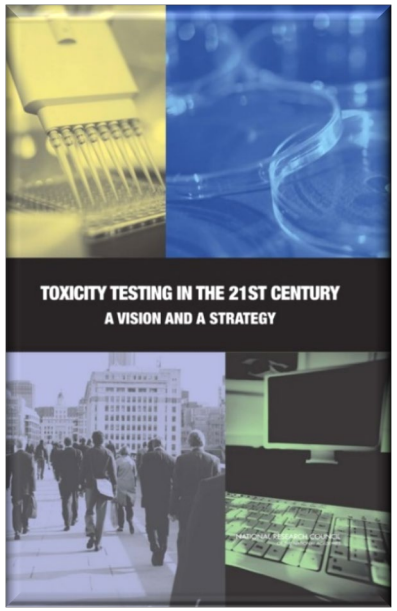
Three Components for Chemical Risk



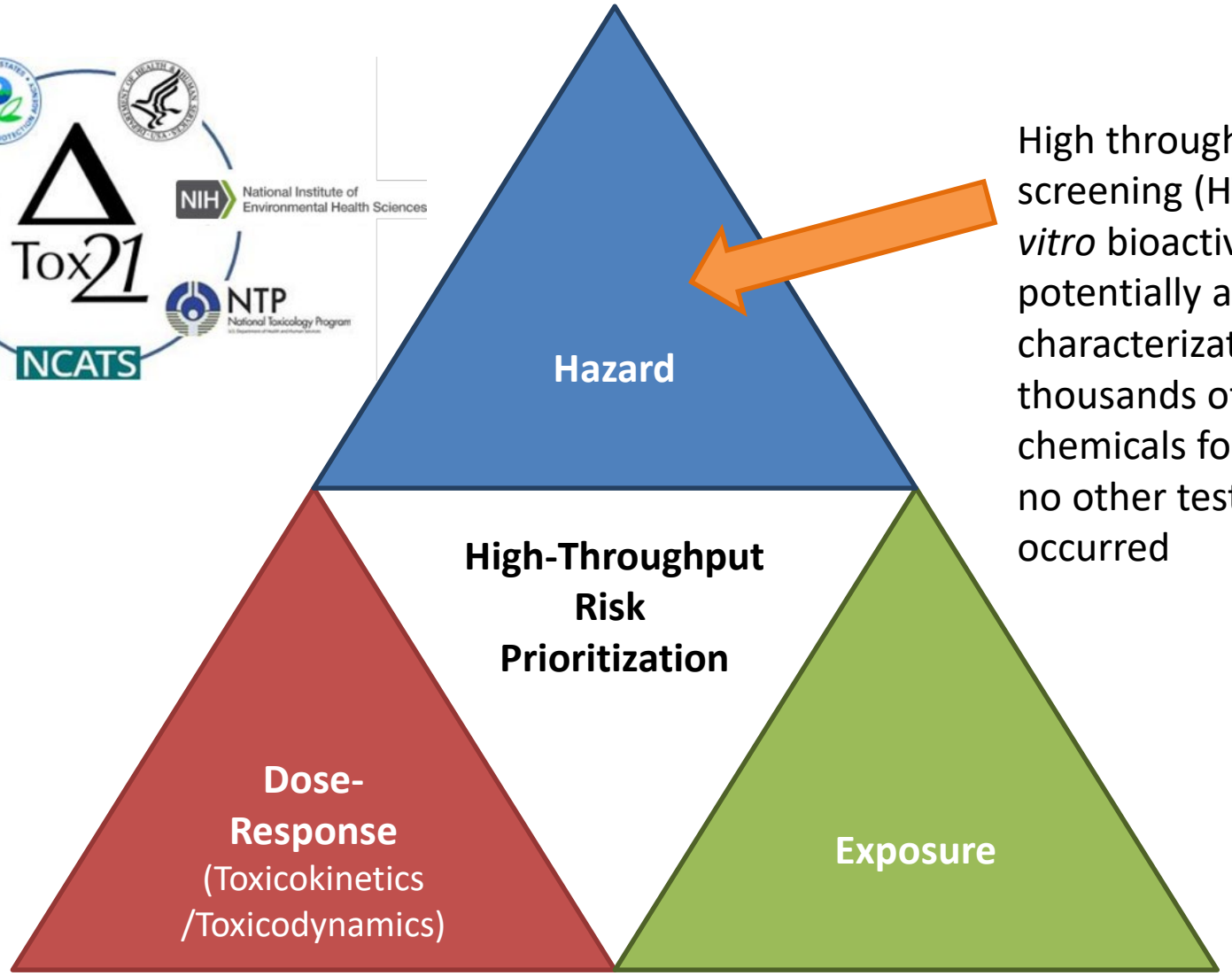
NRC (1983)



High-Throughput Risk Prioritization



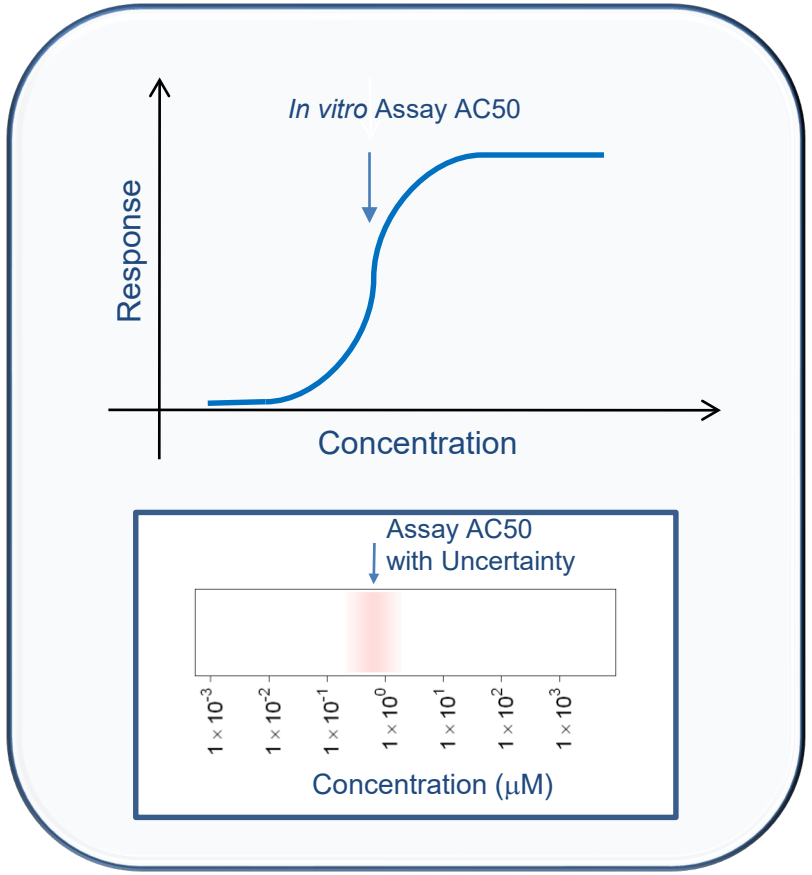
NRC (2007)



High throughput screening (HTS) for *in vitro* bioactivity potentially allows characterization of thousands of chemicals for which no other testing has occurred

High-Throughput Bioactivity Screening

- We might estimate points of departure *in vitro* using high throughput screening (HTS)
- **Tox21**: Examining >8,000 chemicals using ~50 assays intended to identify interactions with biological pathways (Schmidt, 2009)
- **ToxCast**: For a subset (>2000) of Tox21 chemicals ran >1100 additional assays (Kavlock *et al.*, 2012)
- Most assays conducted in dose-response format (identify 50% activity concentration – AC_{50} – and efficacy if data described by a Hill function, Filer *et al.*, 2016)
- All data are public: http://comptox.epa.gov/dashboard/



In Vitro - In Vivo Extrapolation (IVIVE)

Use of *in vitro* experimental data to predict phenomena *in vivo*

- IVIVE-PK/TK (Pharmacokinetics/Toxicokinetics):
  - Fate of molecules/chemicals in body
  - Considers absorption, distribution, metabolism, excretion (ADME)
  - Uses empirical PK and physiologically-based (PBPK) modeling

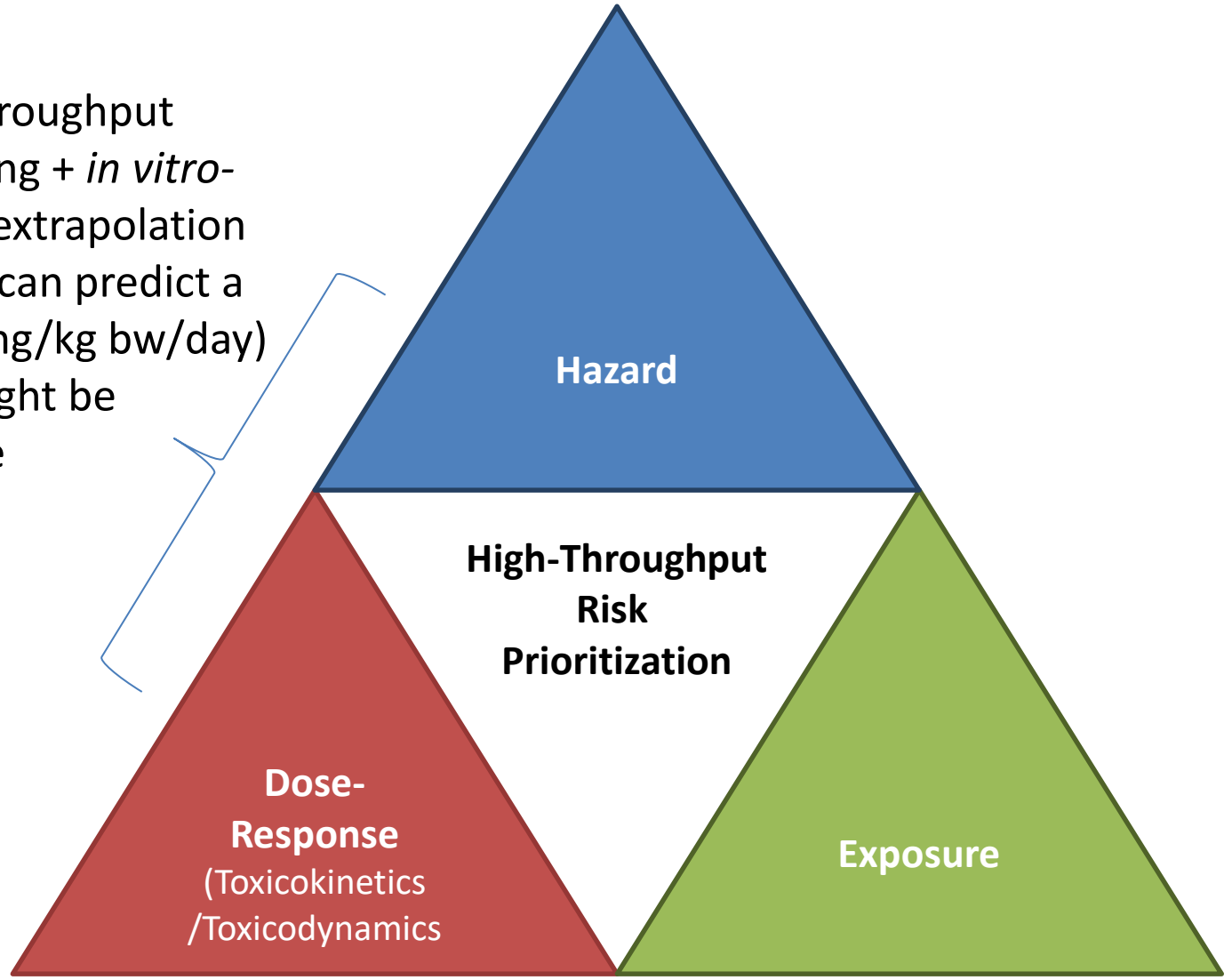
- IVIVE-PD/TD (Pharmacodynamics/Toxicodynamics):
  - Effect of molecules/chemicals at biological target *in vivo*
  - Assay design/selection important
  - Perturbation as adverse/therapeutic effect, reversible/ irreversible

- Both contribute to predict *in vivo* effects

Slide from Barbara Wetmore

New Exposure Data and Models

High throughput screening + *in vitro-in vivo* extrapolation (IVIVE) can predict a dose (mg/kg bw/day) that might be adverse



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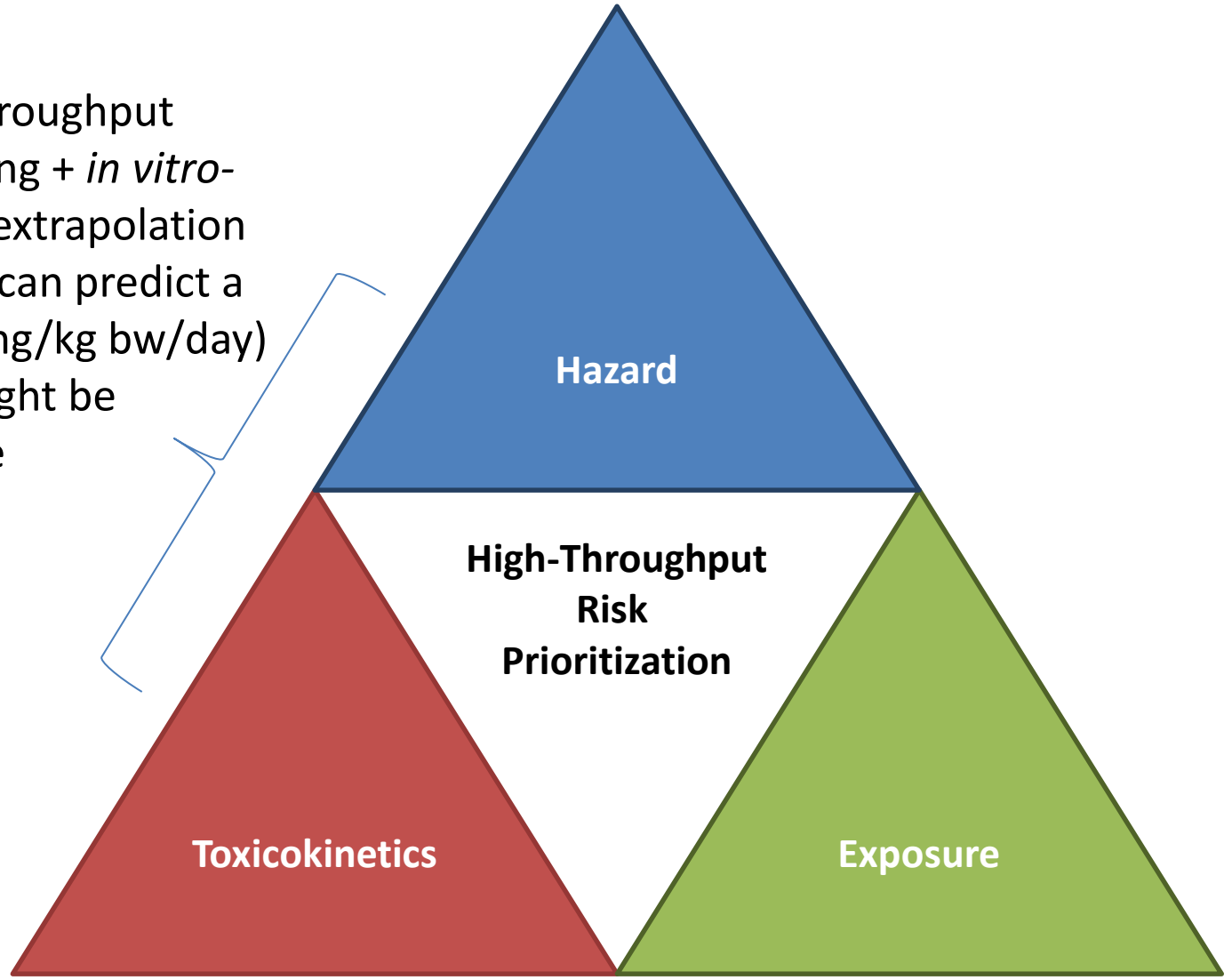

Wetmore et al. (2012, 2015)

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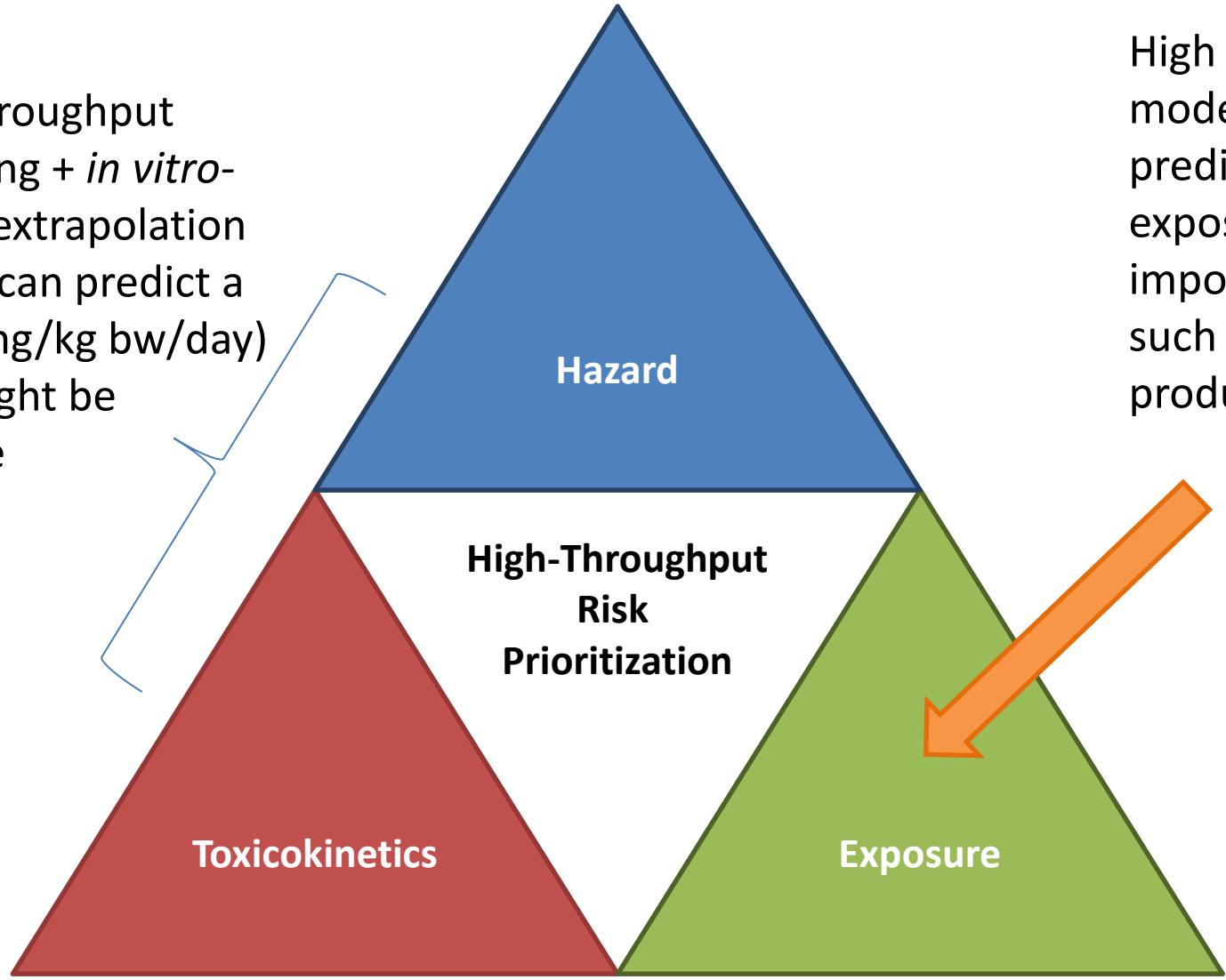
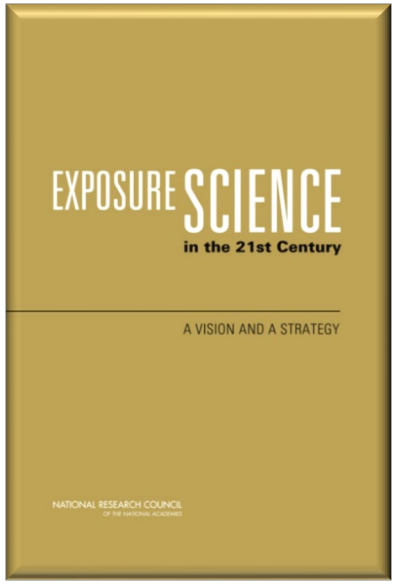


High throughput models exist to make predictions of exposure via specific, important pathways such as residential product use and diet

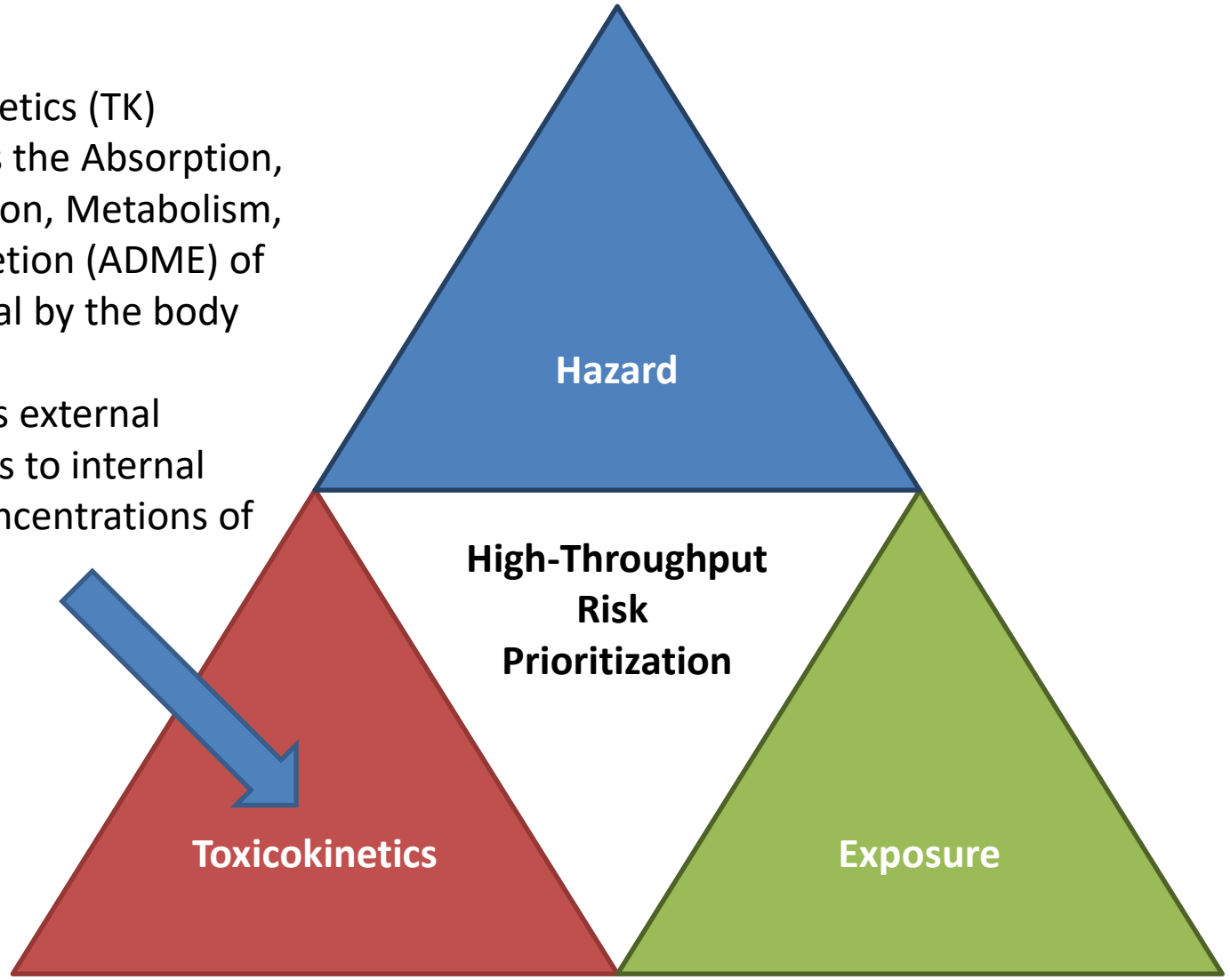


NRC (2012)

High Throughput Toxicokinetics (HTTK)

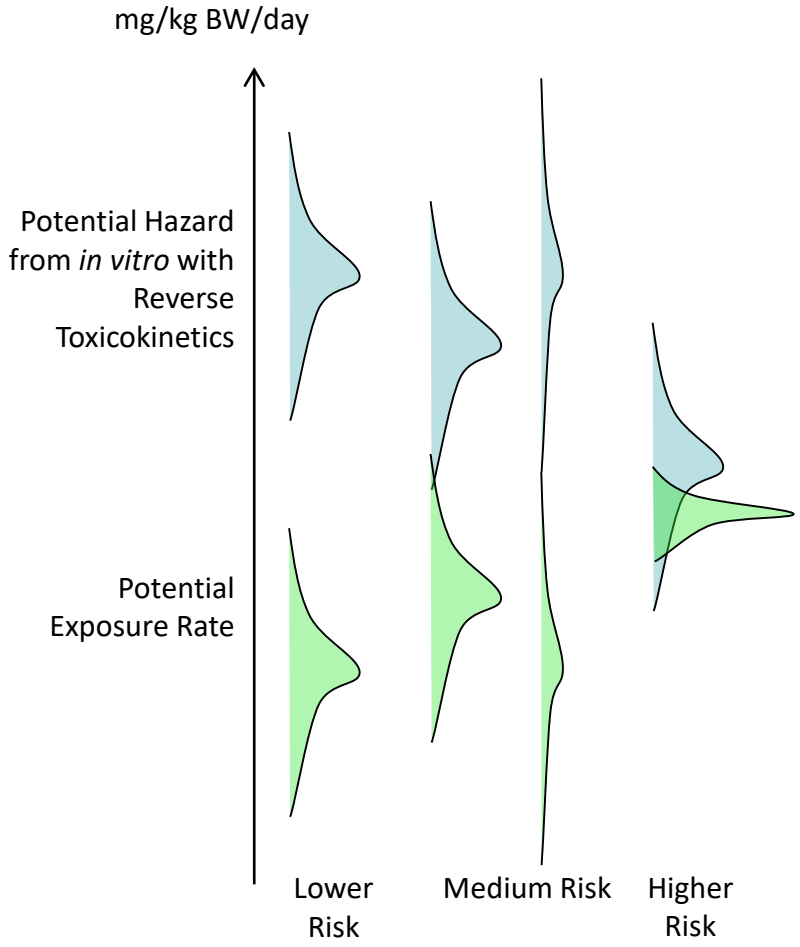
Toxicokinetics (TK) describes the Absorption, Distribution, Metabolism, and Excretion (ADME) of a chemical by the body

TK relates external exposures to internal tissue concentrations of chemical



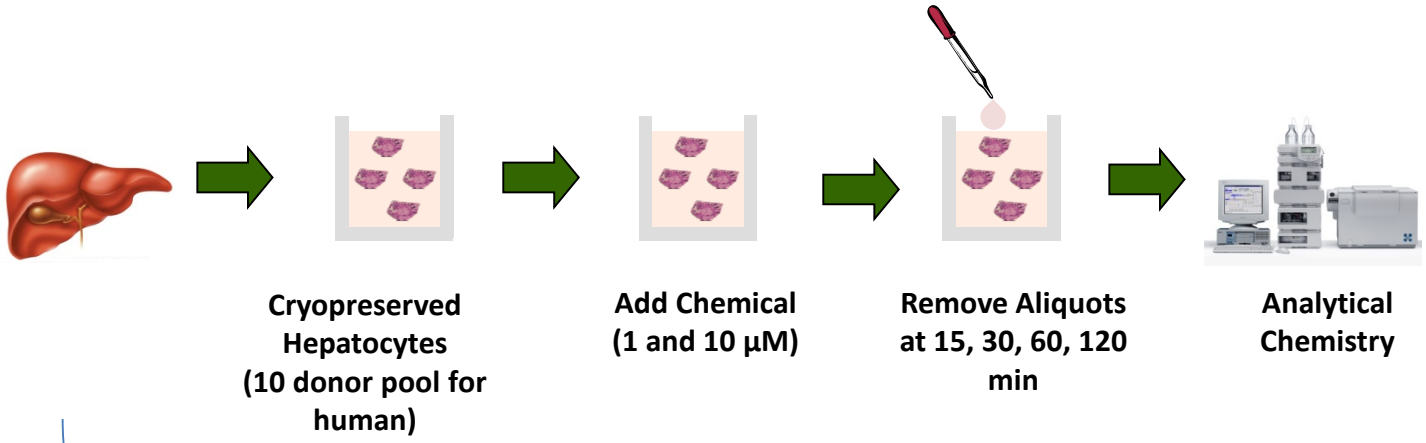
High Throughput Toxicokinetics (HTTK)

- **Most chemicals do not have TK data**
- 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)

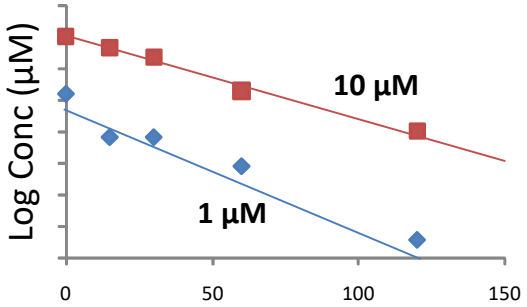


In Vitro Data for HTTK

Cryopreserved hepatocyte suspension Shibata *et al.* (2002)



The rate of disappearance of parent compound (slope of line) is the **hepatic clearance** (µL/min/10^6 hepatocytes)



We perform the assay at 1 and 10 µM to check for saturation of metabolizing enzymes.

- **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)

In Vitro Data for HTTK

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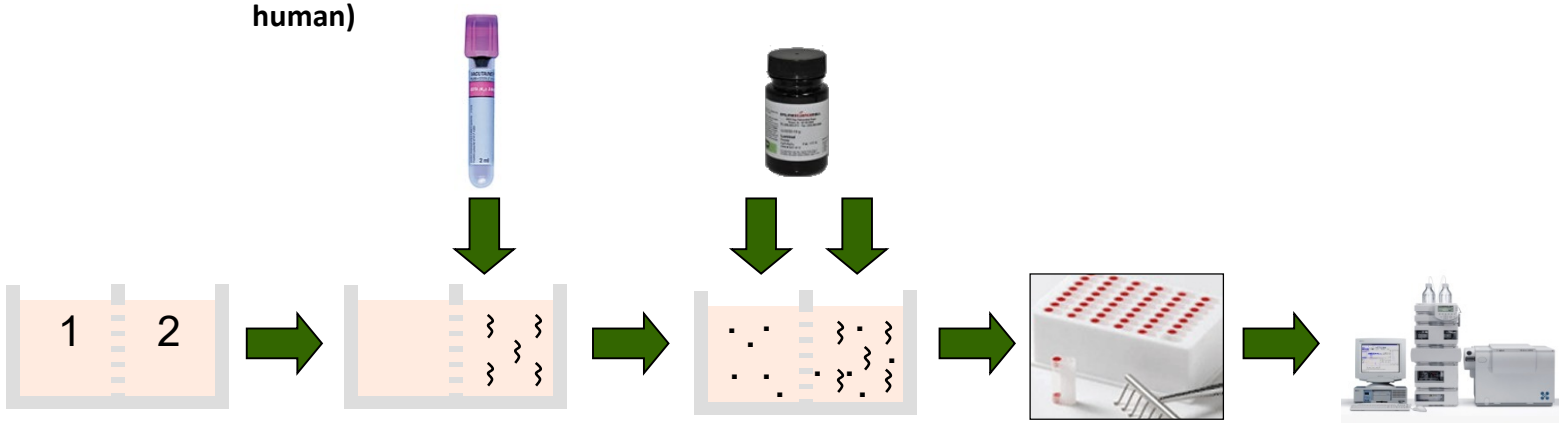
Rapid Equilibrium Dialysis (RED)
Waters *et al.* (2008)



$$F_{ub,p} = \frac{C_{well1}}{C_{well2}}$$

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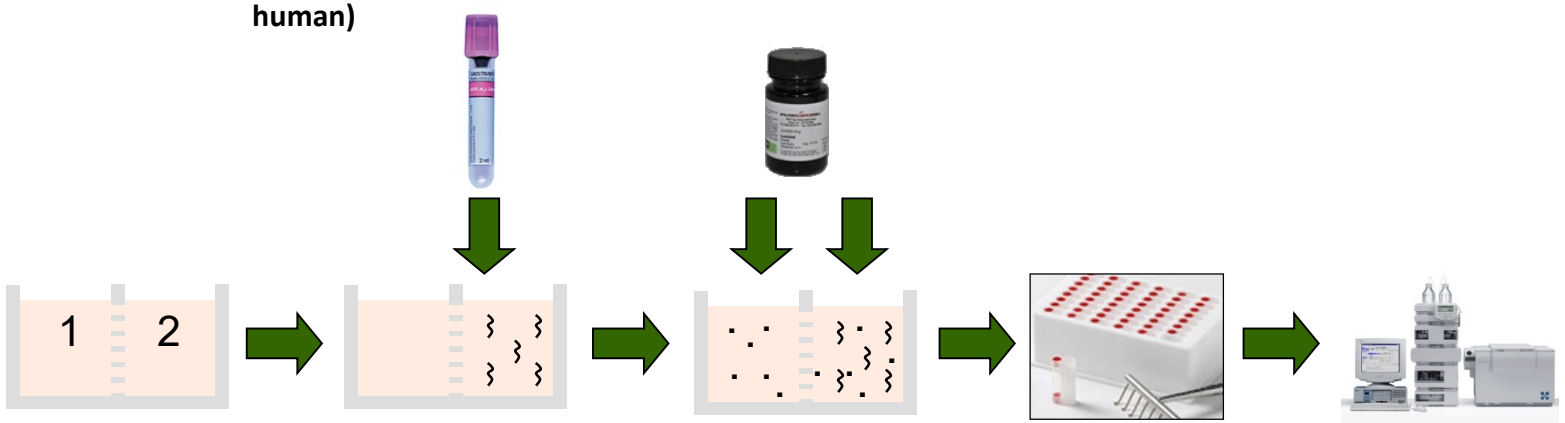
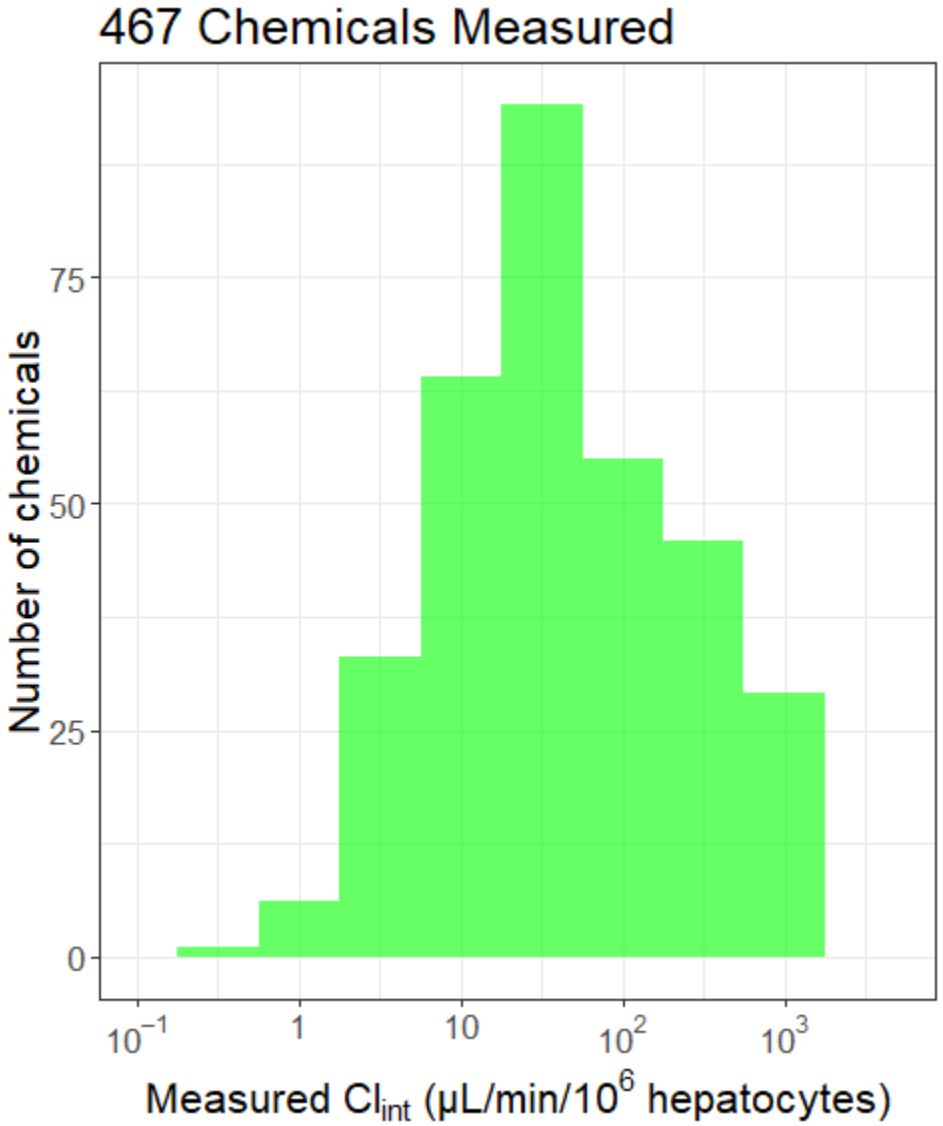


$$F_{ub,p} = \frac{C_{well1}}{C_{well2}}$$

- **Most chemicals do not have TK data** – we use *in vitro* HTTK methods adapted from pharma to fill gaps
- 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.) **+389** chemicals

Hepatic Clearance for ToxCast Chemicals

Cryopreserved hepatocyte suspension
Shibata *et al.* (2002)



ToxCast covers a range of chemistries including:

- Pesticides
- Industrial chemicals
  - Plasticizers
  - Phthalates
  - Per- and poly-fluorinated alkyl substances
- Consumer product chemicals
  - Parabens
  - Flame retardants
- Pharmaceuticals
- Reference toxicants

Wambaugh et al., *in preparation*

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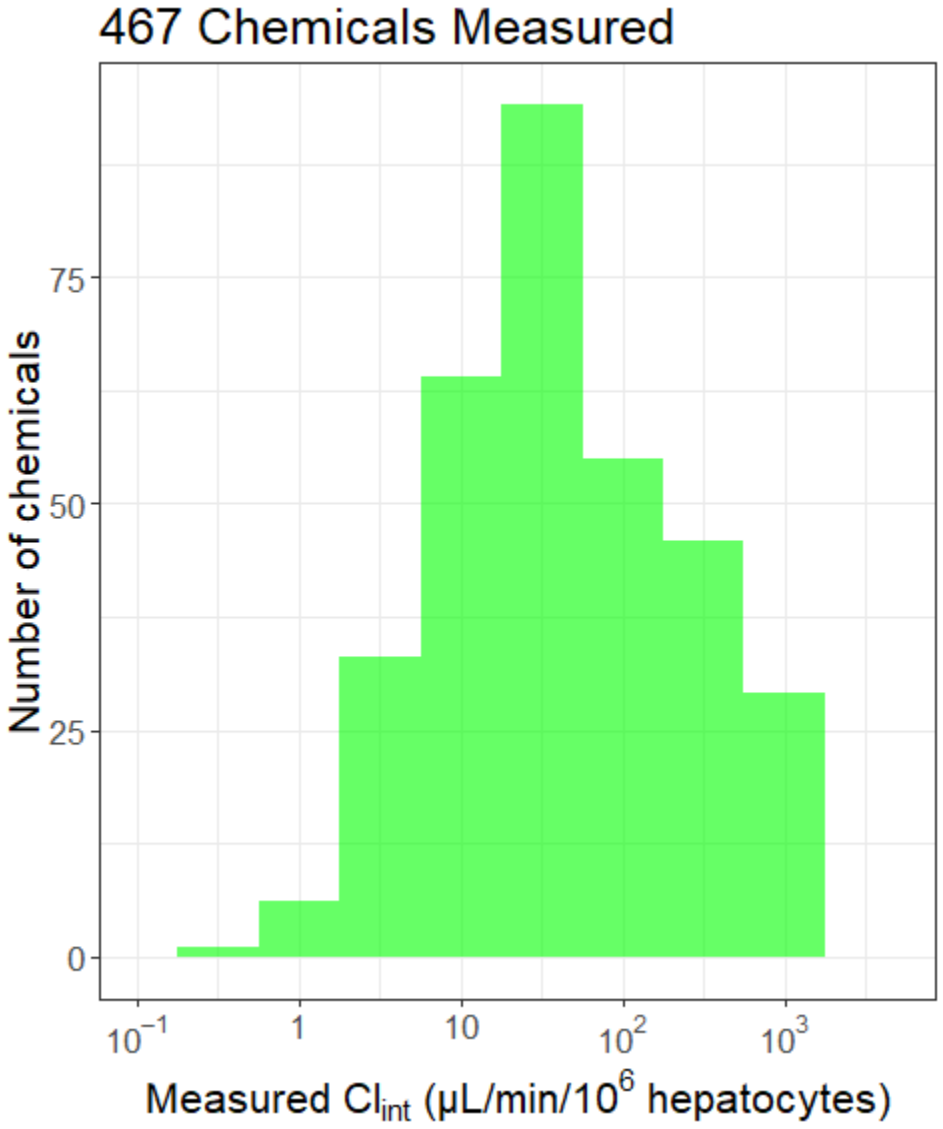
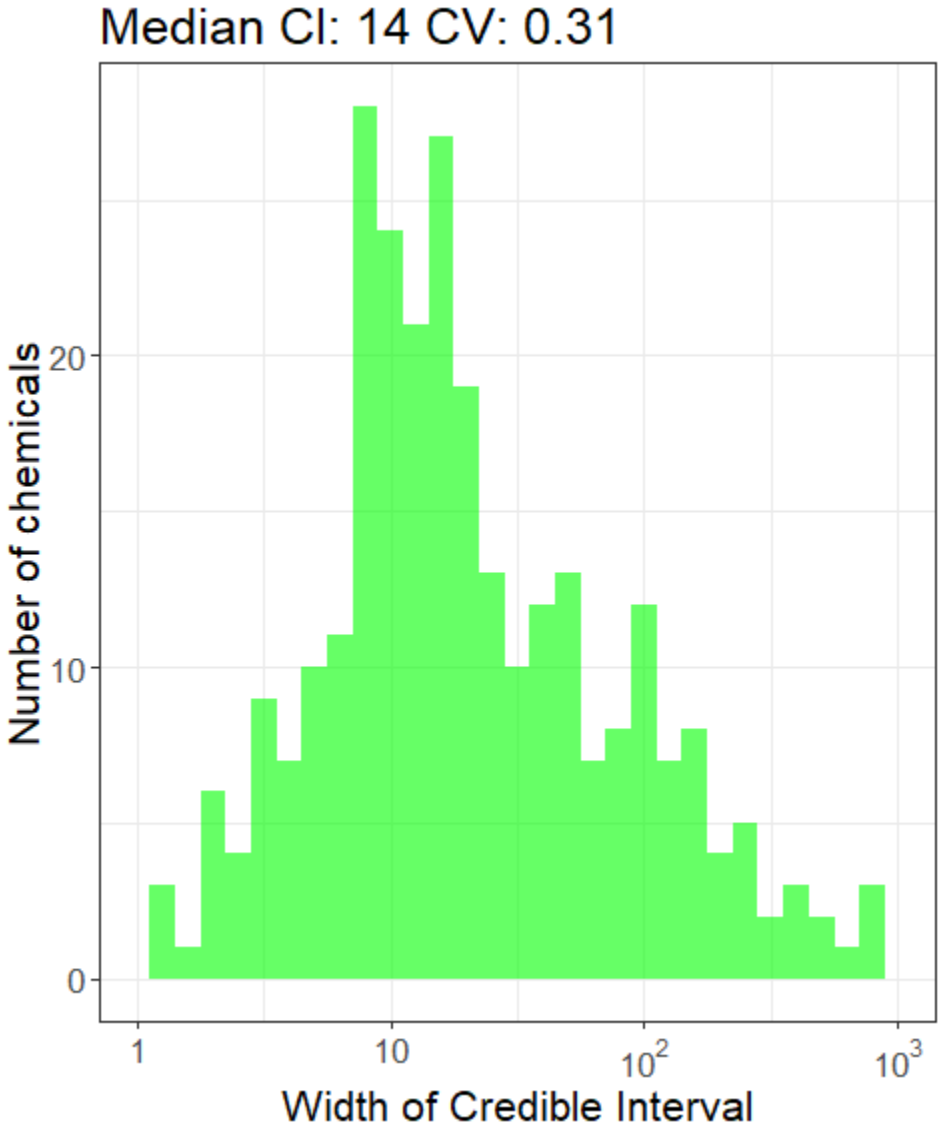
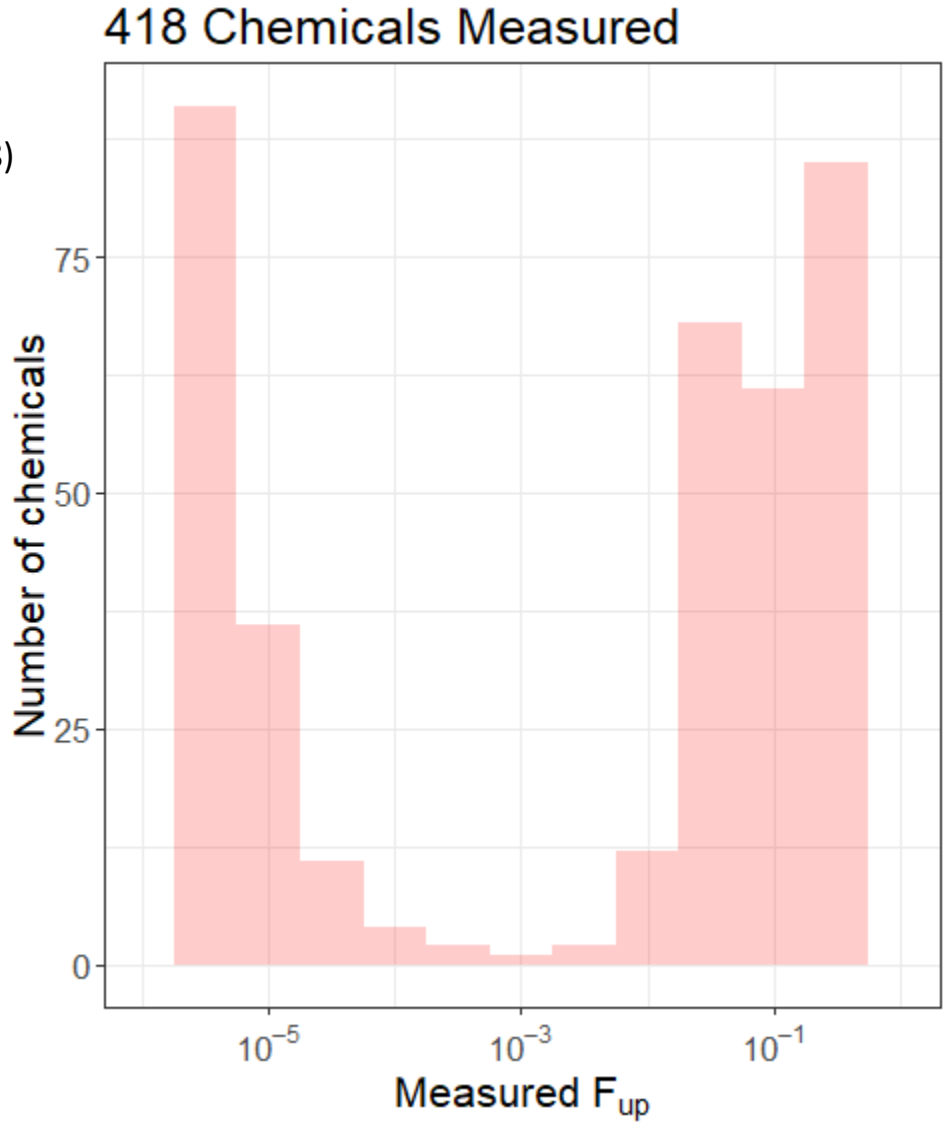




Wambaugh et al., *in preparation*

Fraction Unbound in Plasma (f_{up}) for ToxCast Chemicals

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



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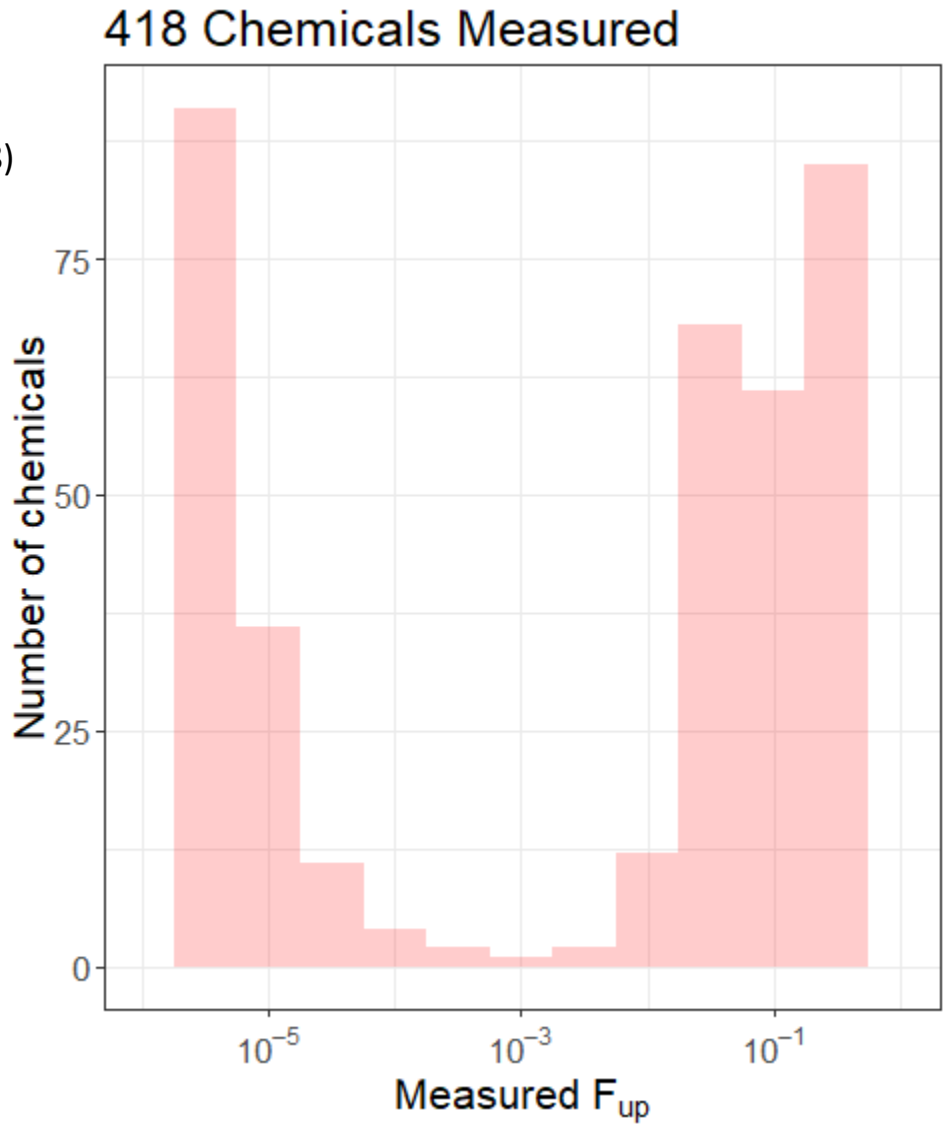

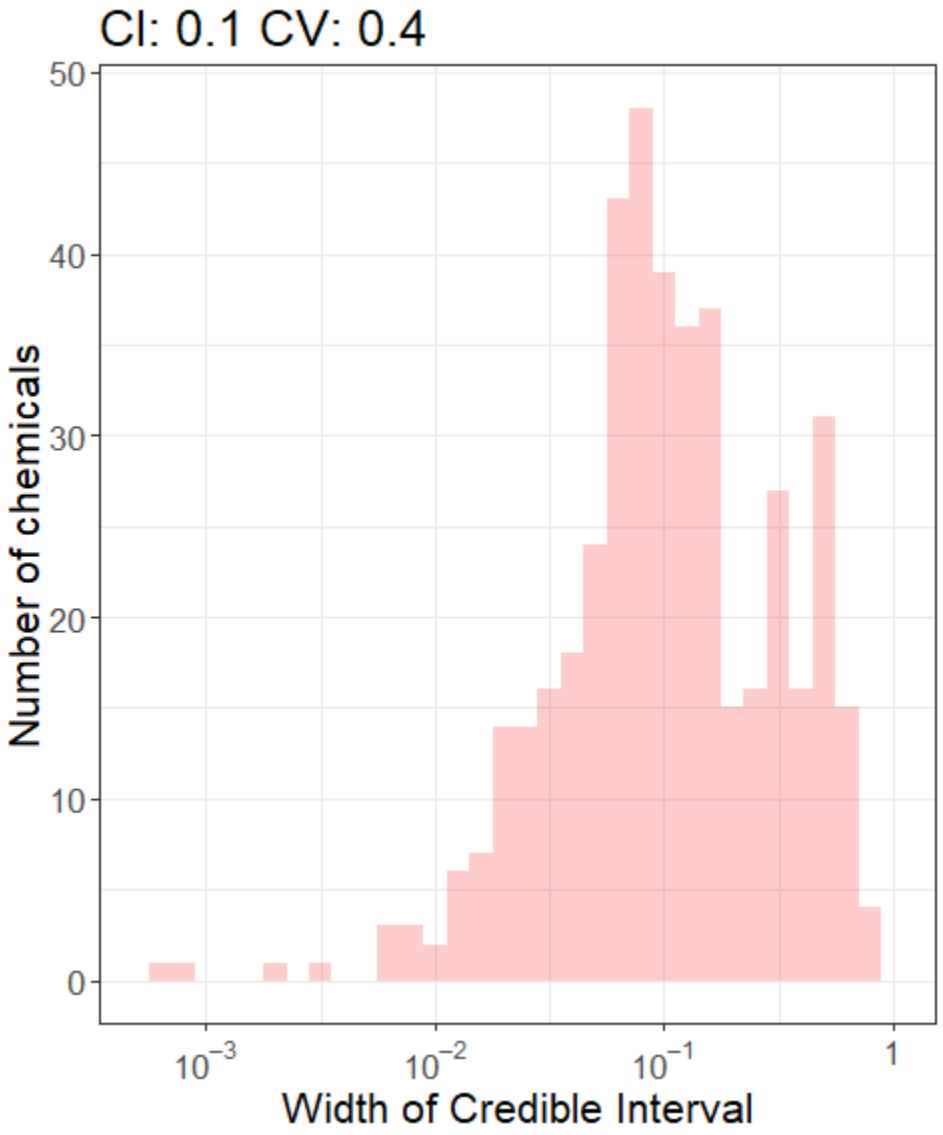

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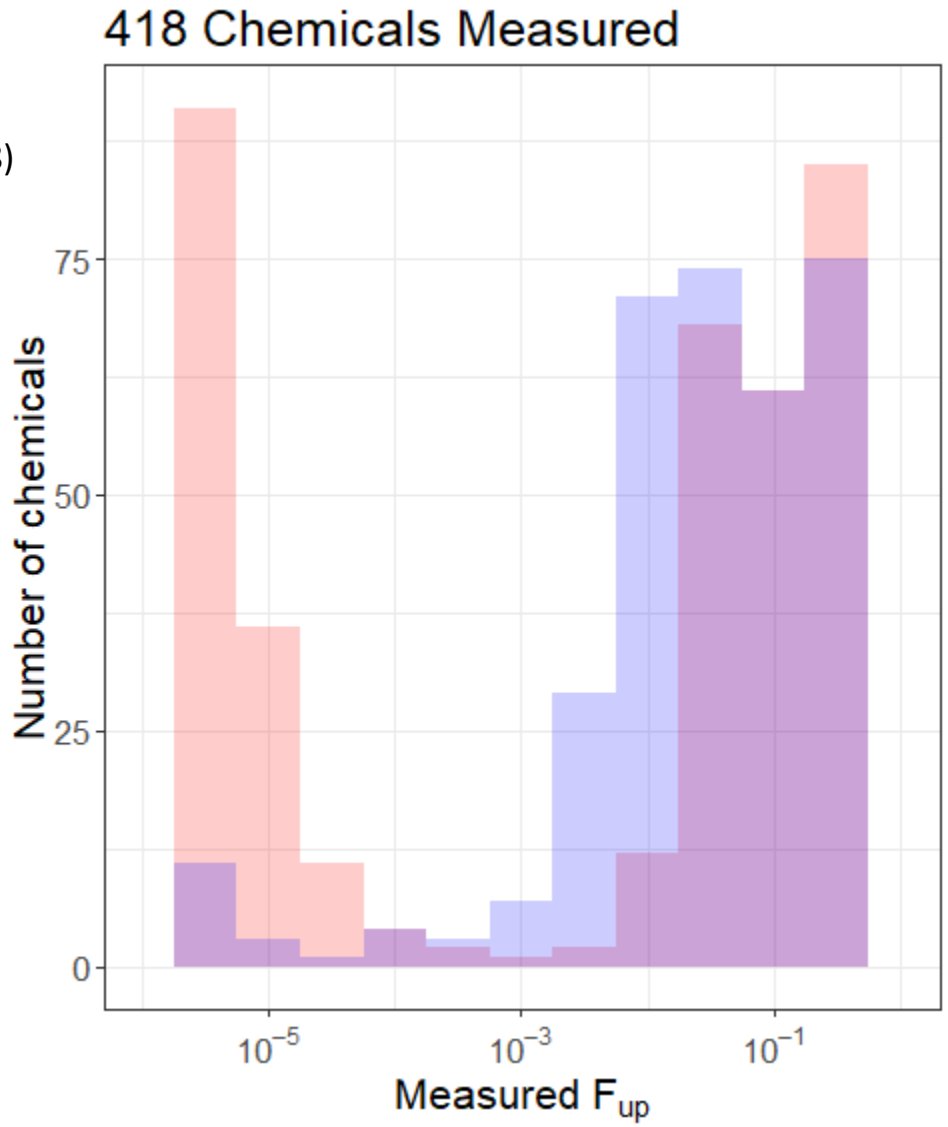
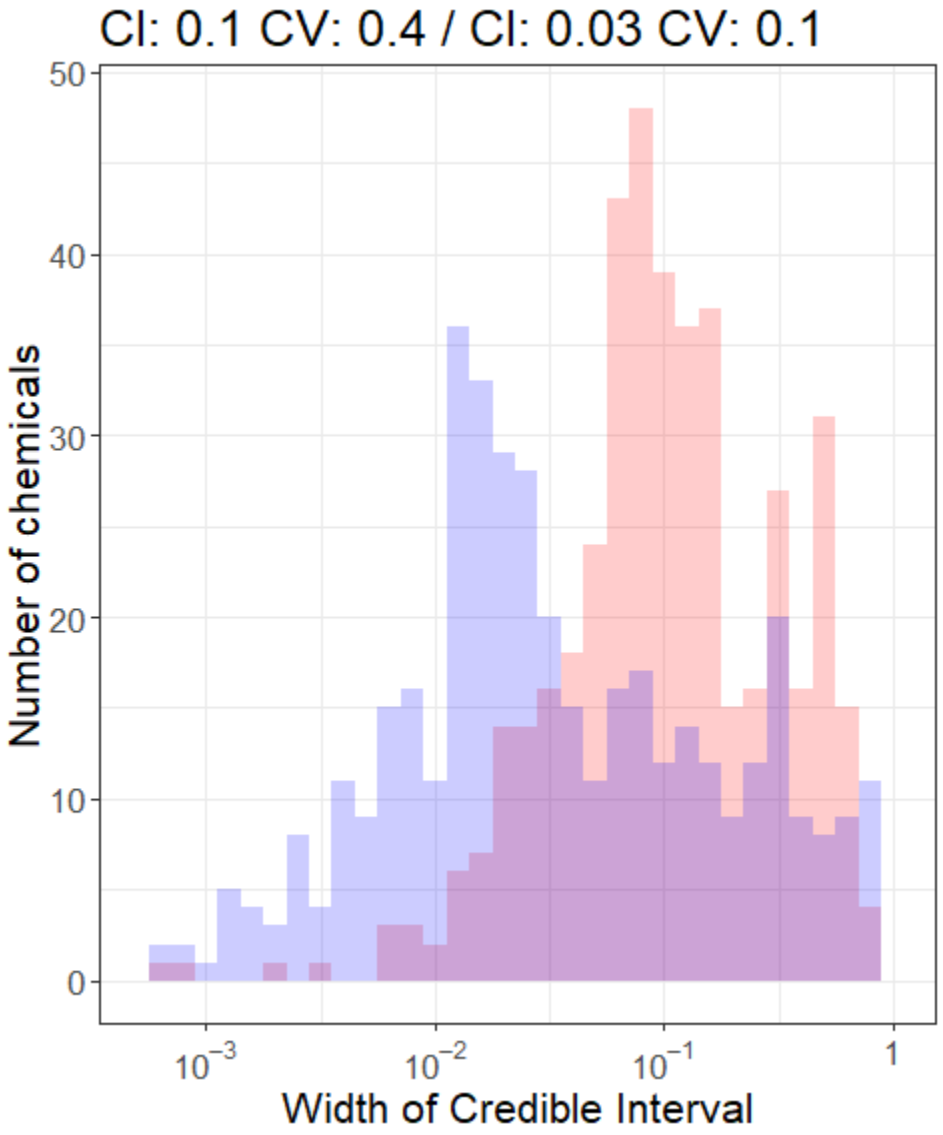
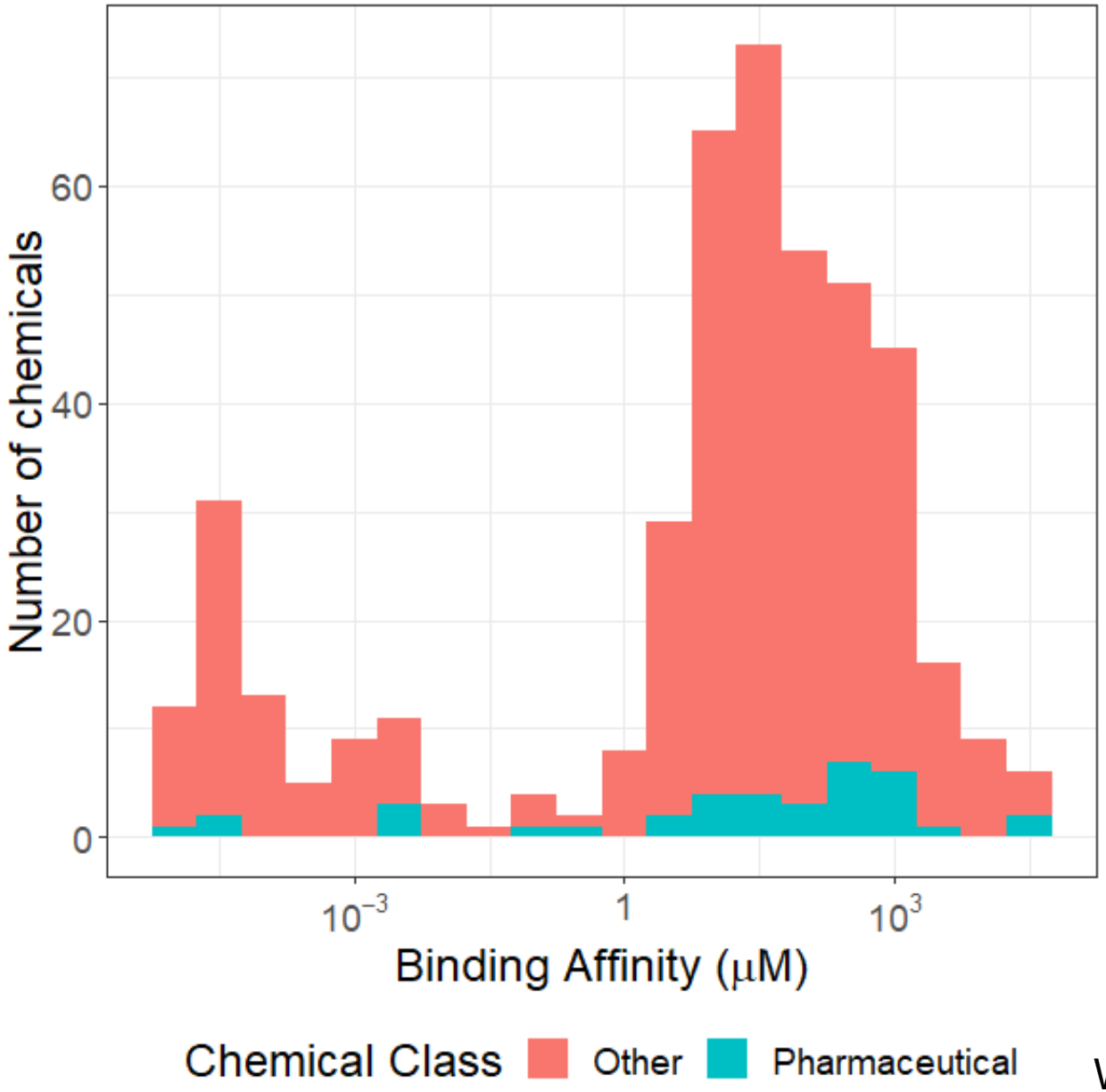




Original RED Assay / New Titration Assay

Wambaugh et al., *in preparation*

Plasma Protein Binding Affinity (k_a) for ToxCast Chemicals



Wambaugh et al., *in preparation*

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

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

Wilkinson and Shand (1975)

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

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

Assume that Steady-State is Linear with Dose

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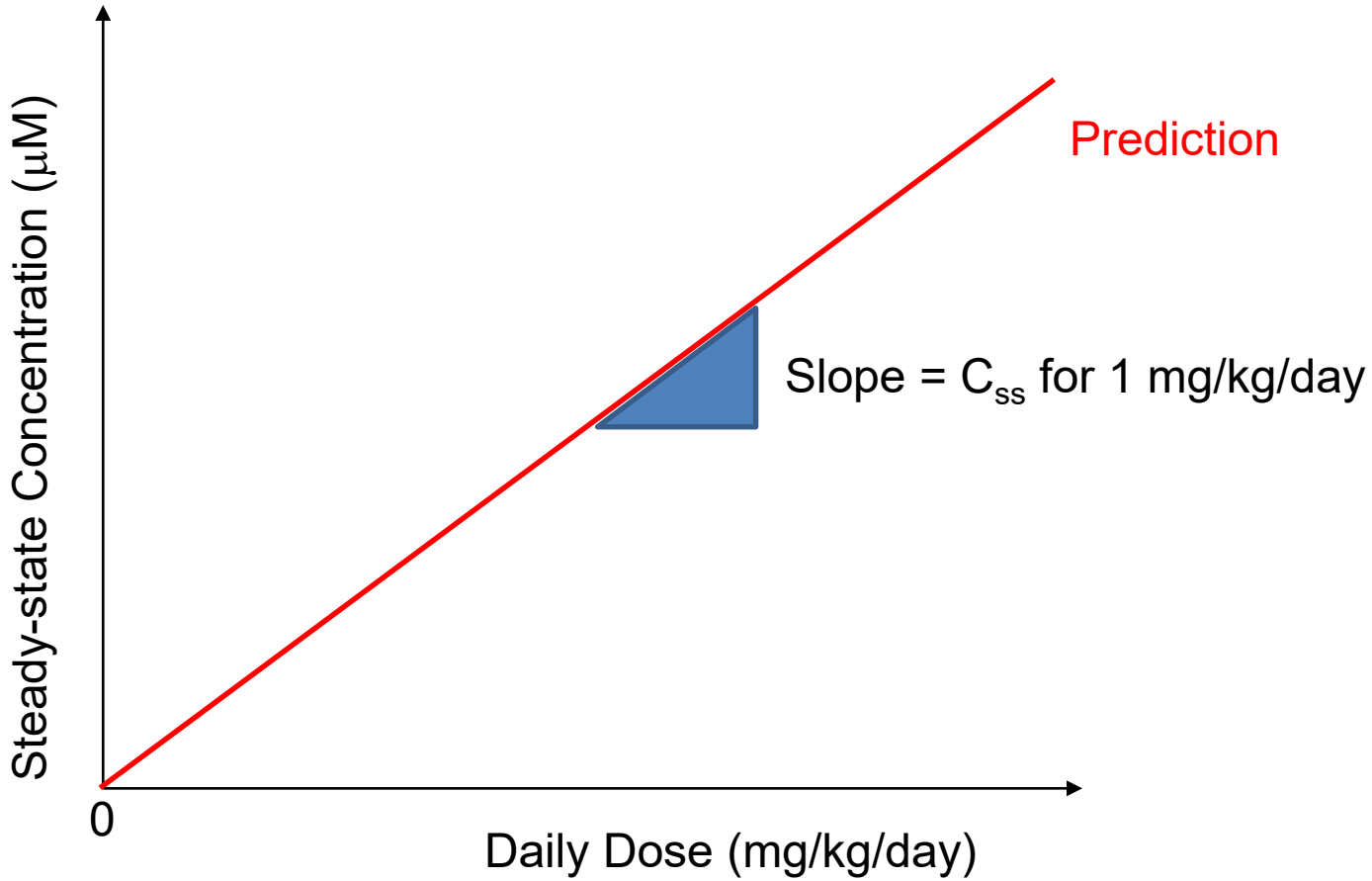

- Can calculate predicted steady-state concentration (C_{ss}) for a 1 mg/kg/day dose and multiply to get concentrations for other doses

Wetmore *et al*. (2012)

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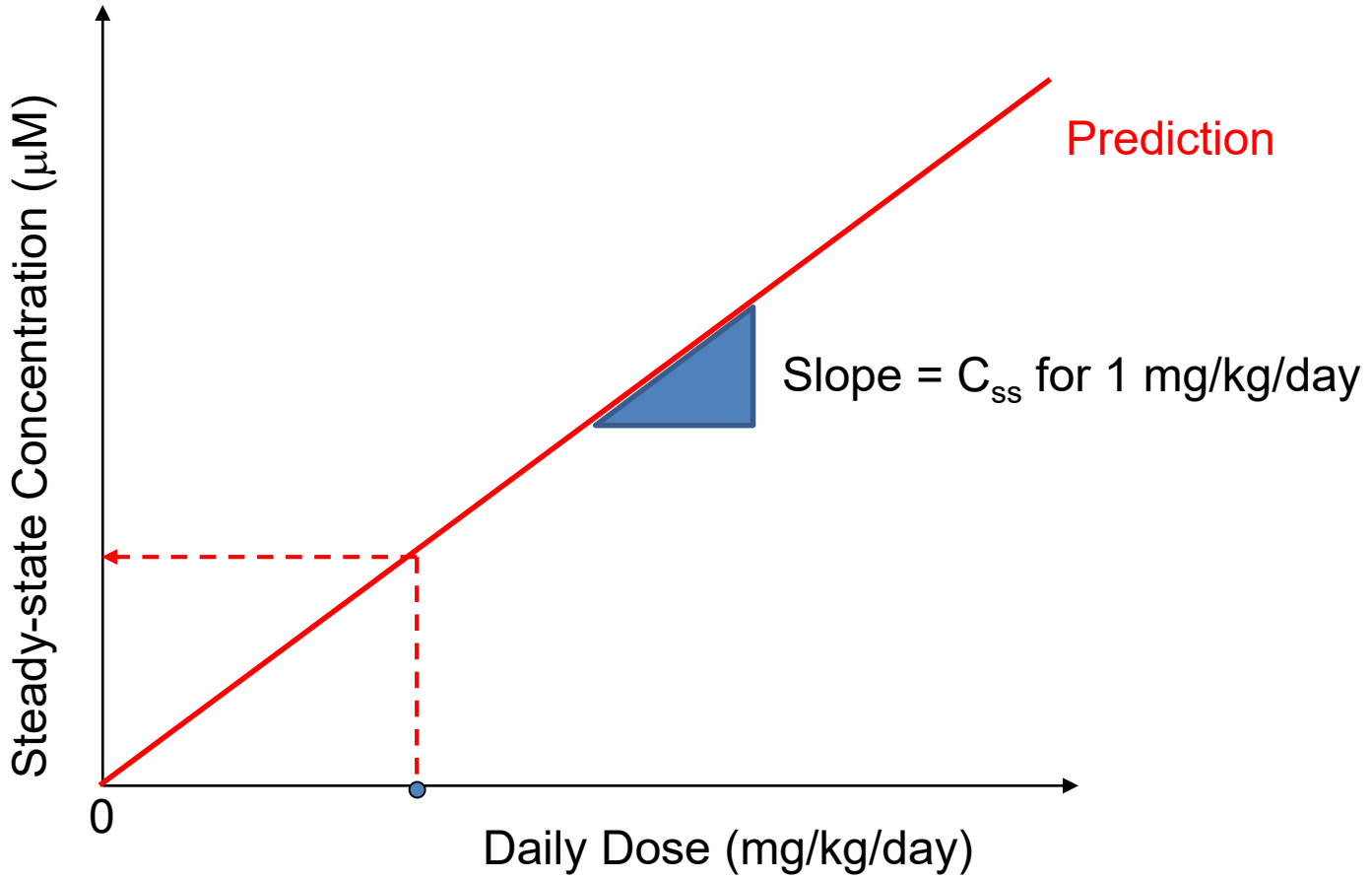

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Wetmore *et al*. (2012)

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

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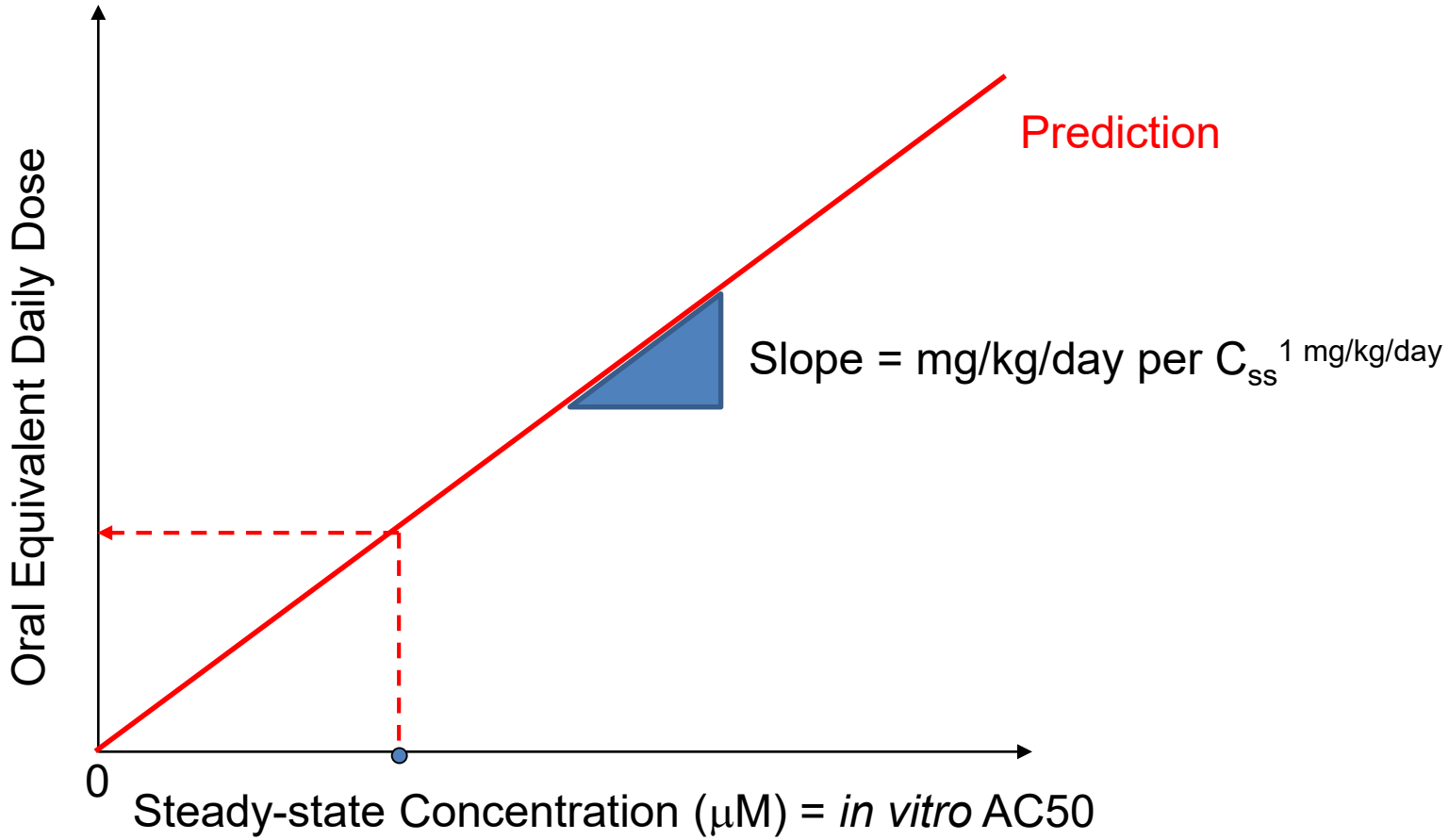

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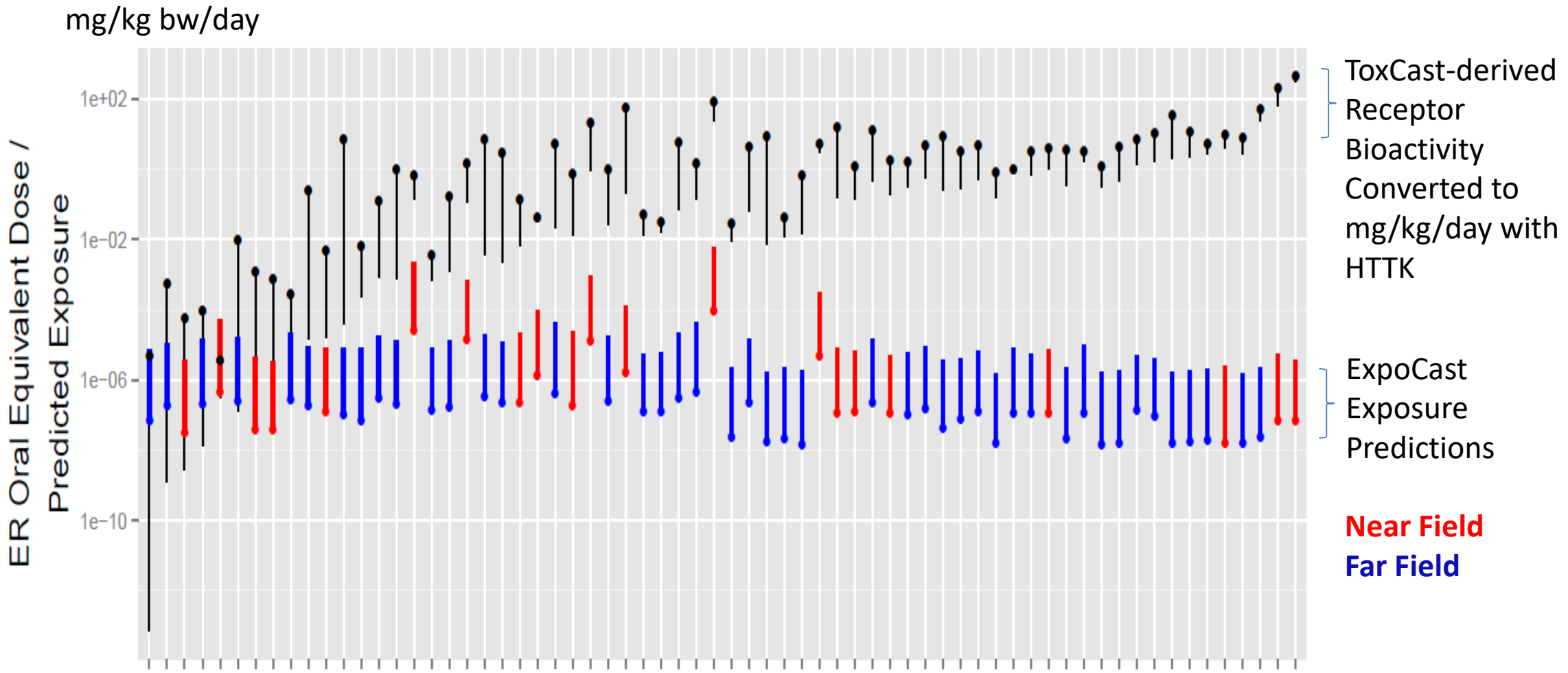
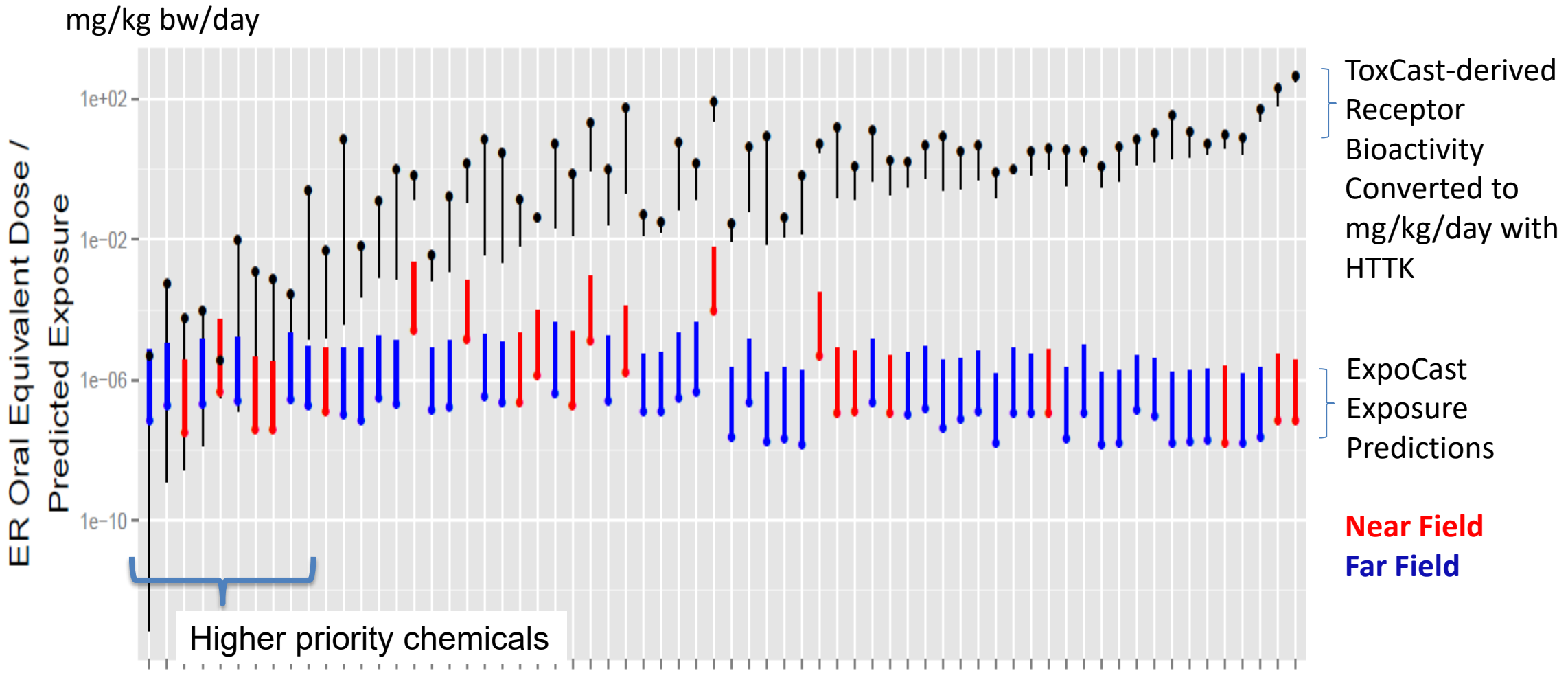
High Throughput Risk Prioritization in Practice



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

High Throughput Risk Prioritization in Practice



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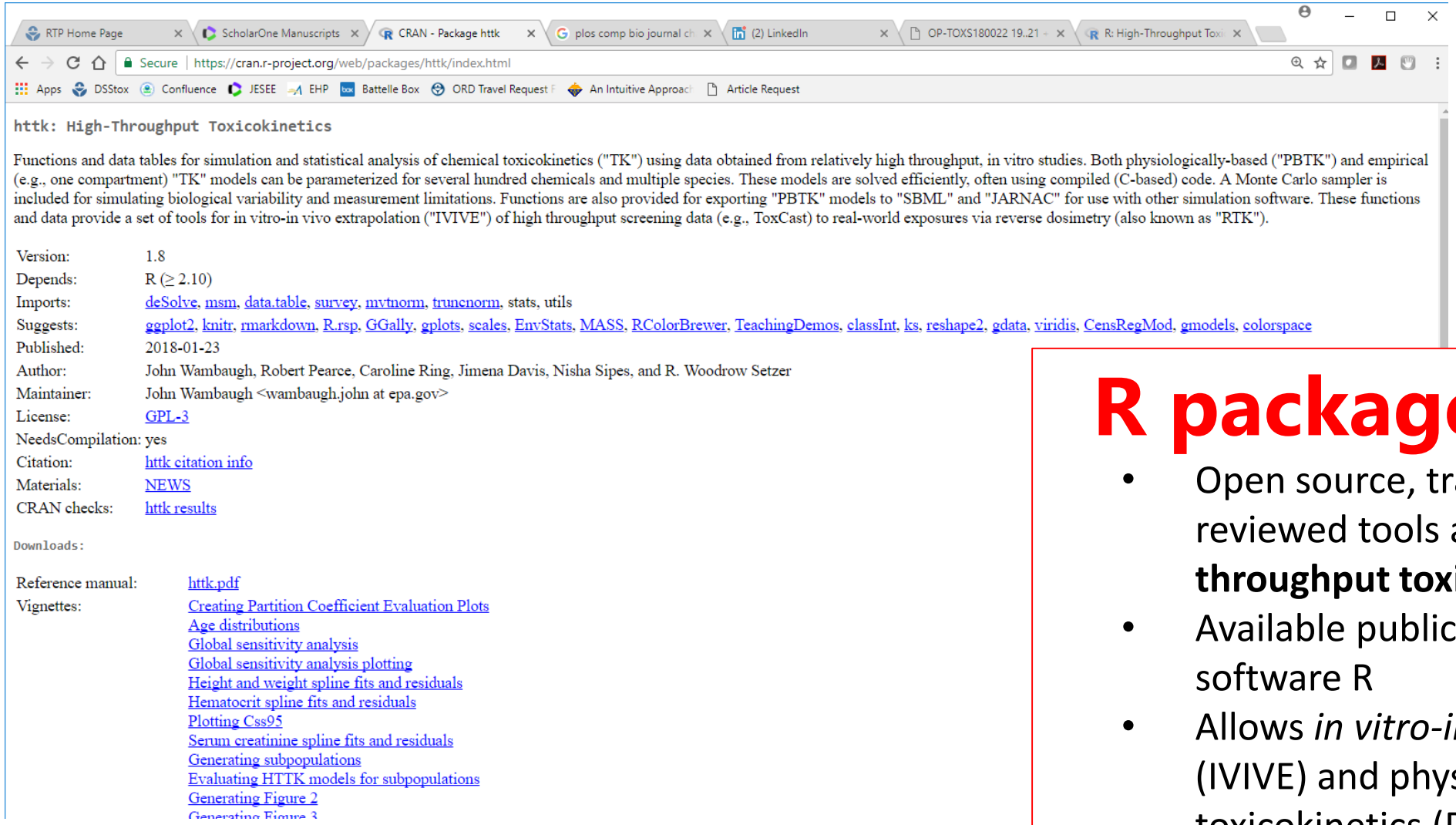
Open Source Tools and Data for HTTK

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 F | 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)

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
Reference	Jamei et al. (2009)	Lukacova et al., (2009)	Loizou et al. (2011)	Jongeneelen et al., (2013)	Pearce et al. (2017a)
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

EPA United States Environmental Protection Agency

Doing Statistical Analysis with HTTK

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

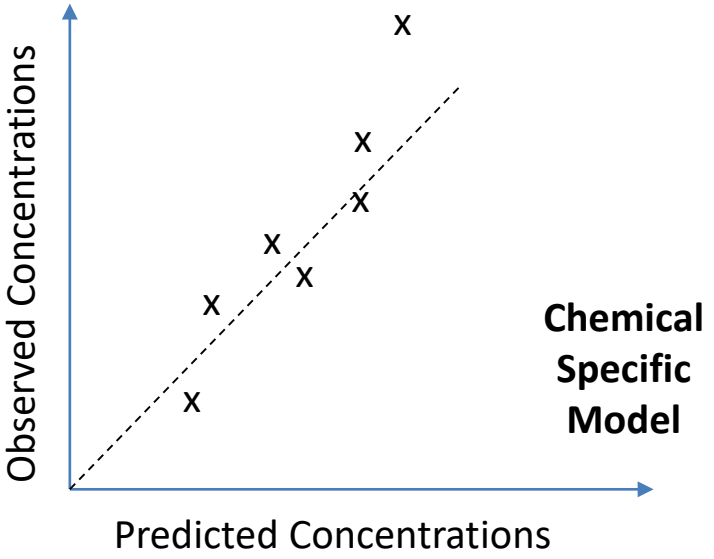
- In drug development, HTTK methods estimate therapeutic doses for clinical studies – predicted concentrations are typically on the order of values measured in clinical trials (Wang, 2010)
  - For most compounds in the environment there will be no clinical trials

- 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*., 2018)
  - 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



Cohen Hubal et al., 2018

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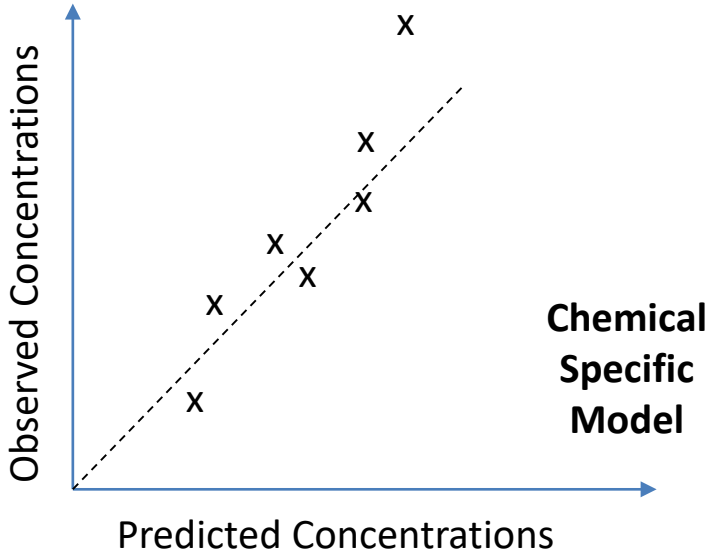
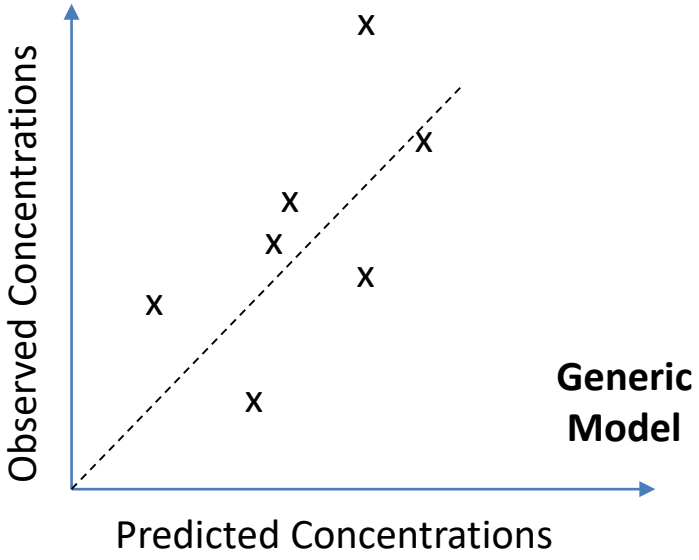
  - We do expect larger uncertainty, but also greater confidence in model implementation
  - Estimate bias and uncertainty





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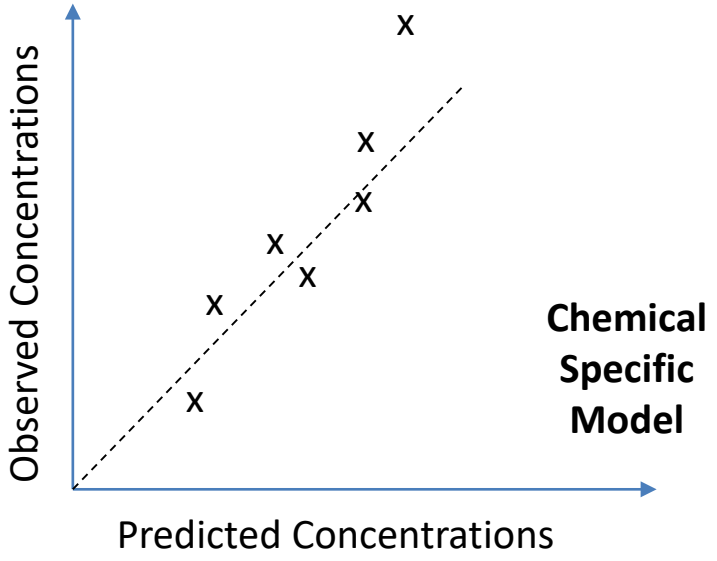

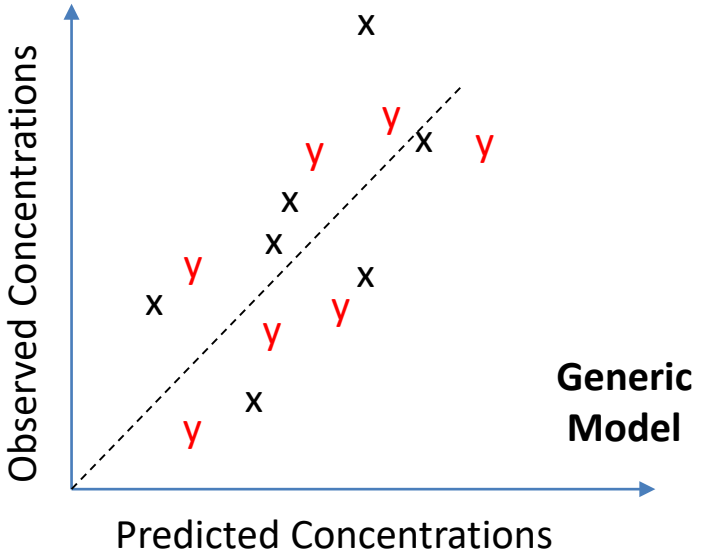

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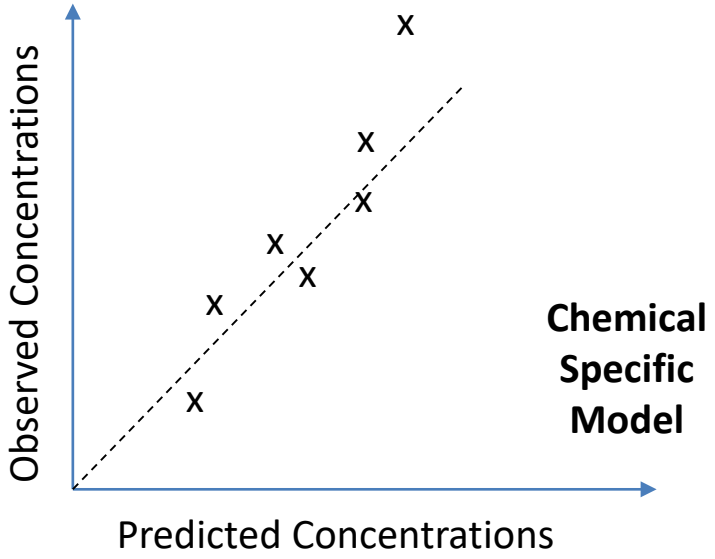
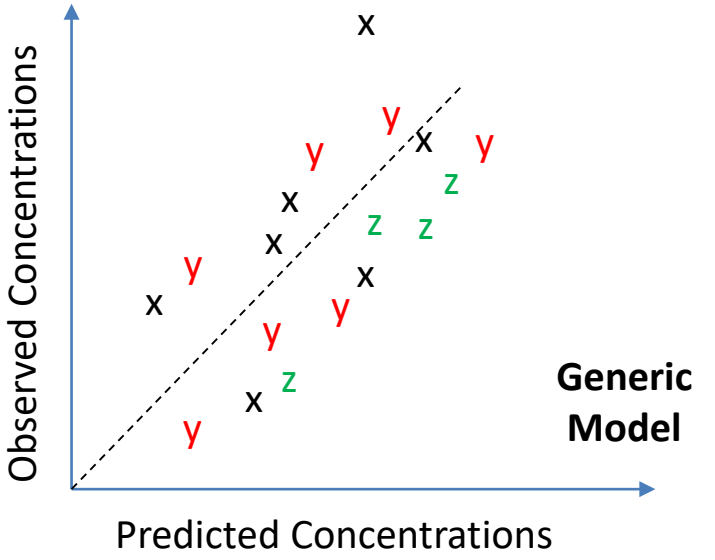




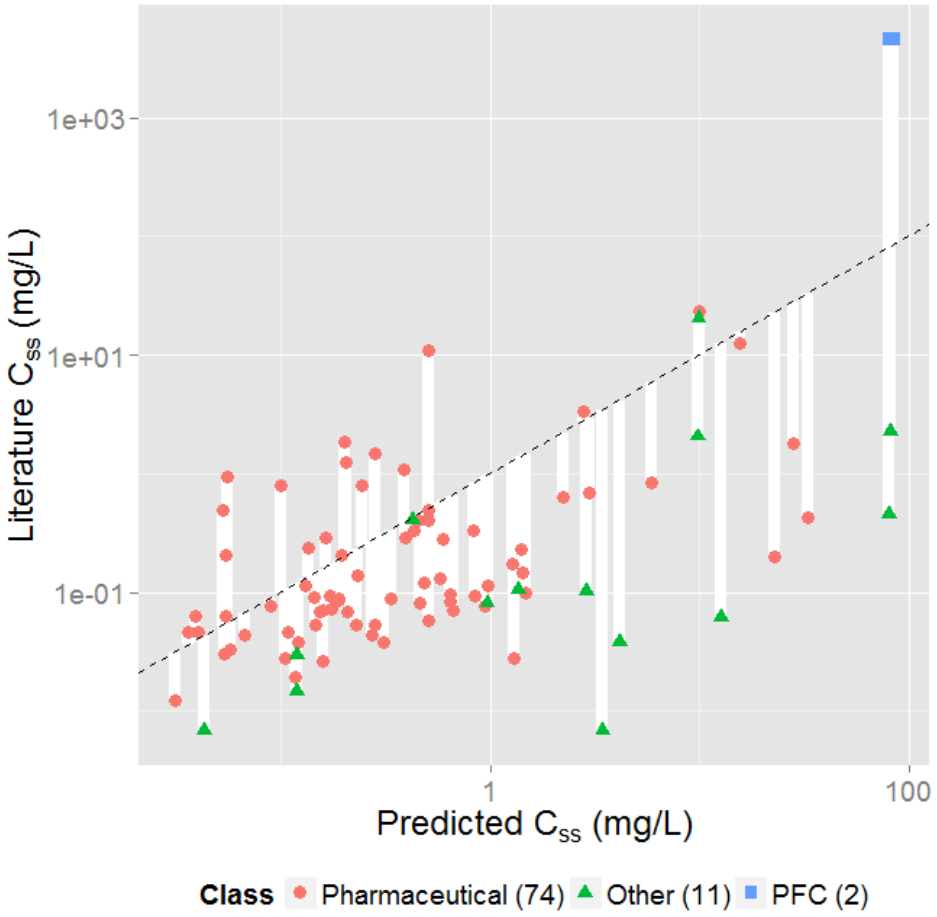
Cohen Hubal et al., 2018

Comparison Between HT-PBTK and Chemical Specific PBTK

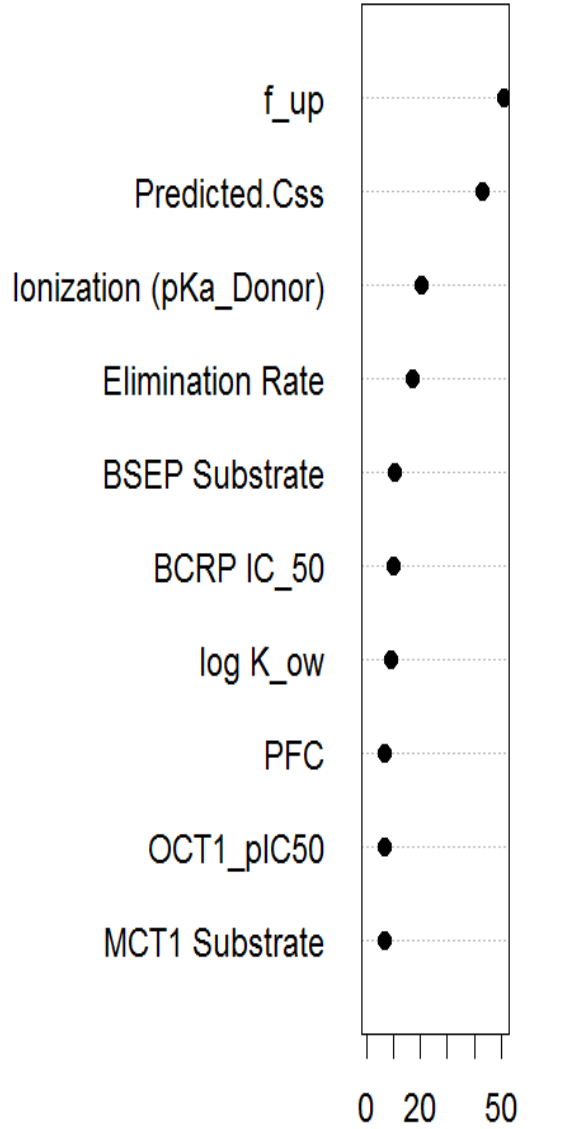
- We compared a chemical-specific human PBTK model for bisphenol A (Yang et al., 2015) to the HTTK generic PBTK model
- The fitted PBTK model from Yang et al. (2015) and the httk models yielded similar time-plasma concentration curves in the prediction of human *in vivo* data from Thayer et al. (2015)
- We assessed average-fold error (AFE) (the average quotient of the measured and predicted concentrations when the dividend is larger than the divisor)
  - The fitted model (Yang et al., 2015) performed the best, with AFE 1.4
  - However, the generic PBTK model had an AFE of 3.3
- Generally, HTTK has lower AFE than a literature model when the literature model is evaluated with an external data set

Work by Risa Sayre and Robert Pearce

Using *in vivo* Data to Evaluate RTK



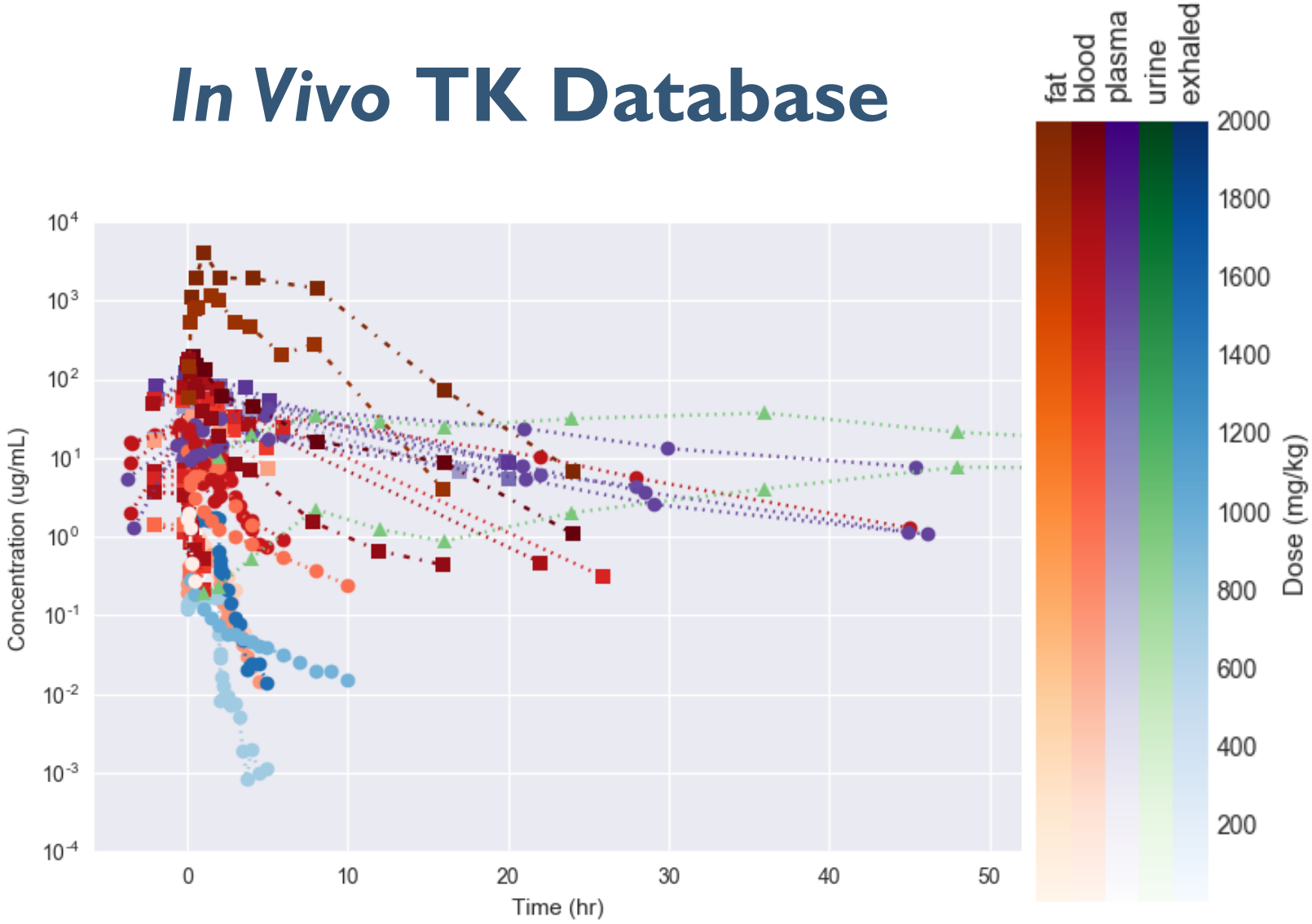
- When we compare the C_{ss} predicted from *in vitro* HTTK with *in vivo* C_{ss} values determined from the literature we find limited correlation (R^2 ~0.34)
- The dashed line indicates the identity (perfect predictor) line:
  - Over-predict for 65
  - Under-predict for 22
- The white lines indicate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



Wambaugh et al. (2015)

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

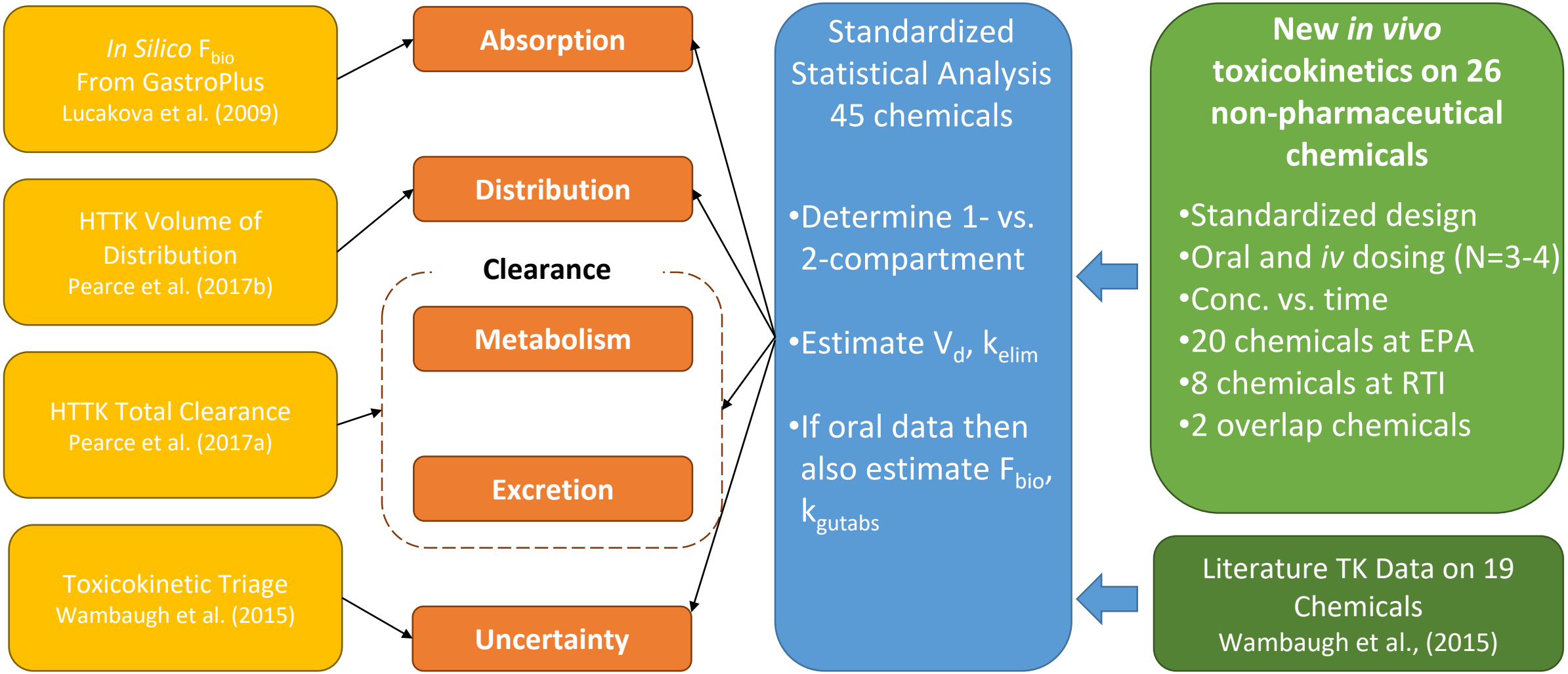


- Standardized, open source curve fitting software invivoPKfit used to calibrate models to all data: https://github.com/USEPA/CompTox-ExpoCast-invivoPKfit

Sayre et al. (in preparation)

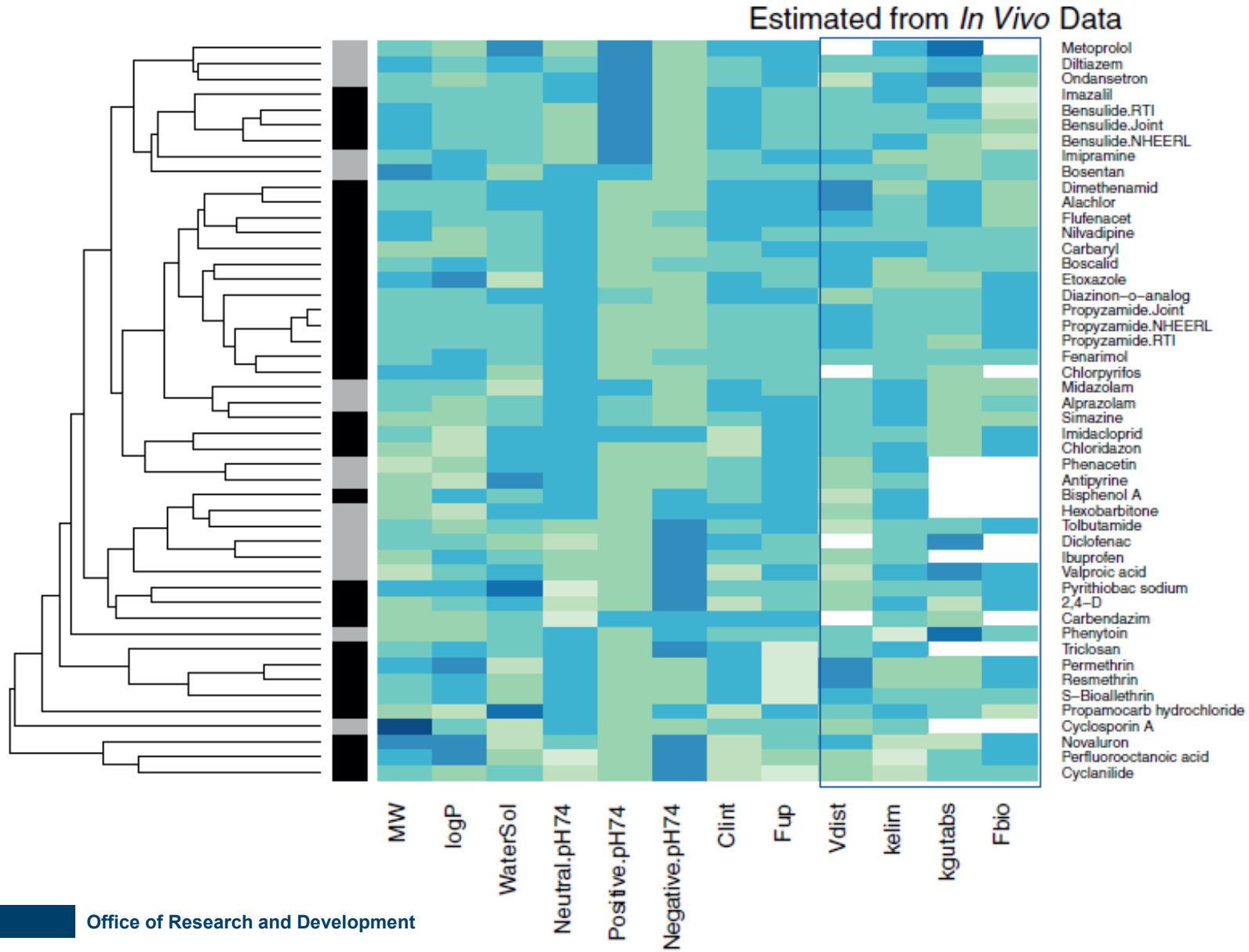
New Data for HTTK Evaluation

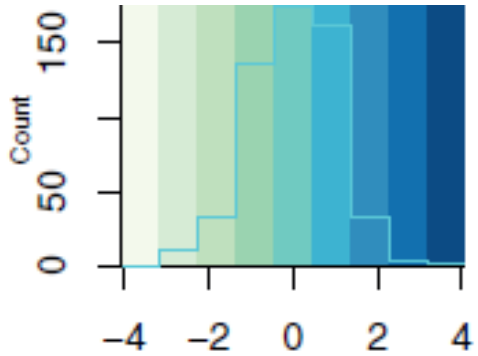
Available literature *in vivo* TK evaluation data was heavily biased toward pharmaceuticals



Wambaugh et al. (2018)

New Data for Evaluating IVIVE





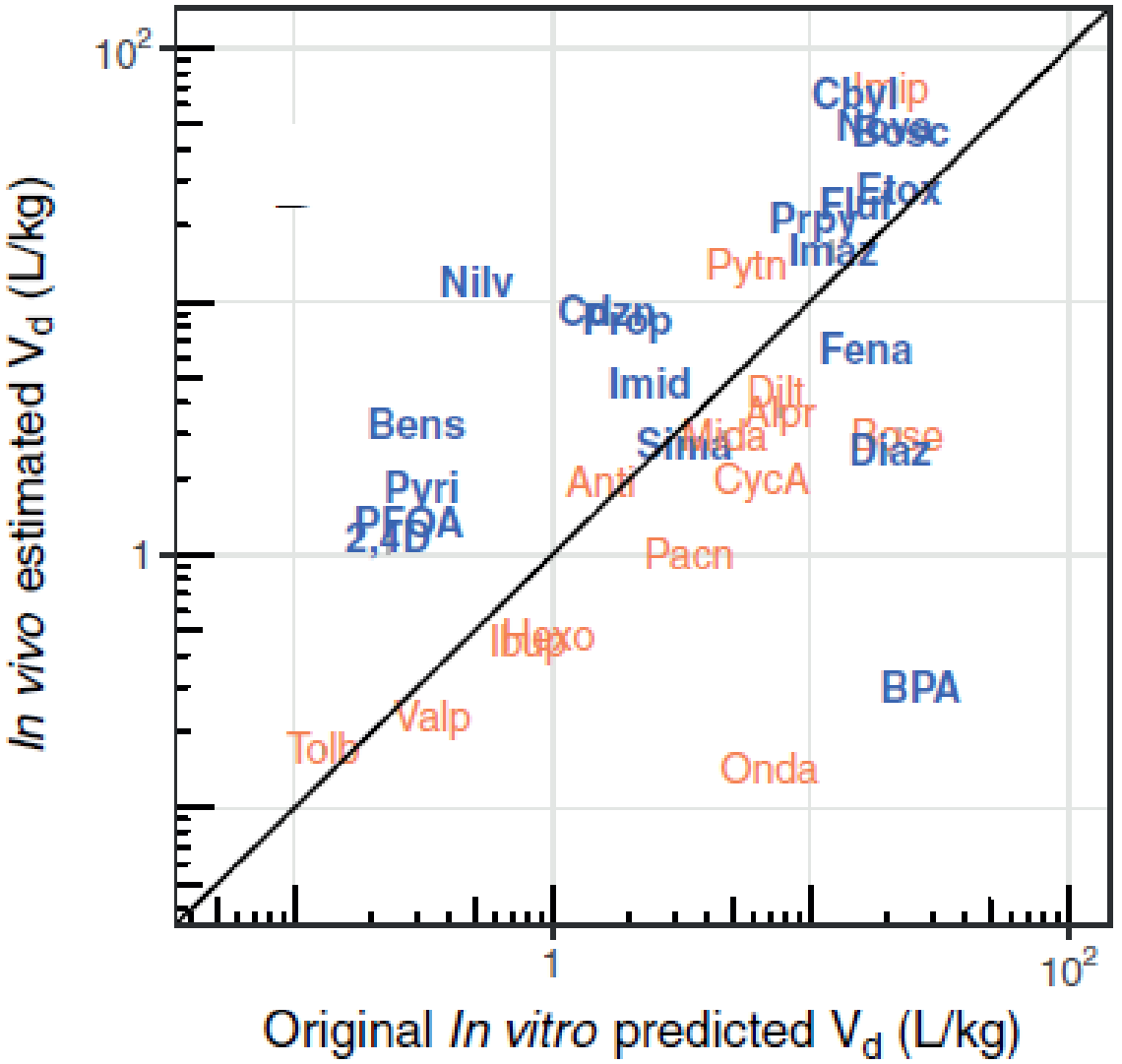
Number of Standard Deviations Above/Below Mean

- Physico-chemical properties, *in vitro* TK parameters (Wetmore et al., 2013), and TK parameters estimated from *in vivo* plasma concentration.

Wambaugh et al. (2018)

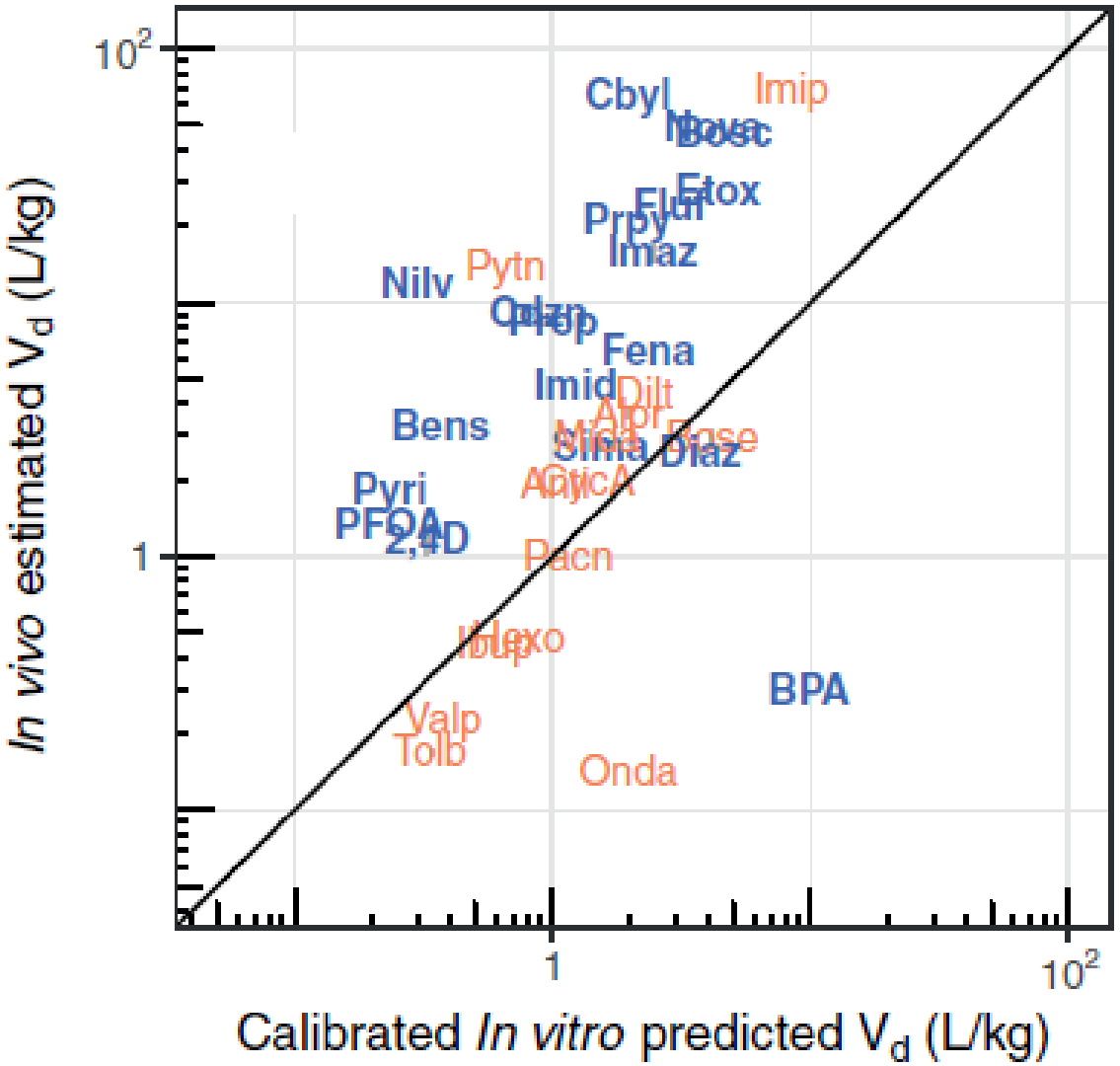
New Data for Evaluation

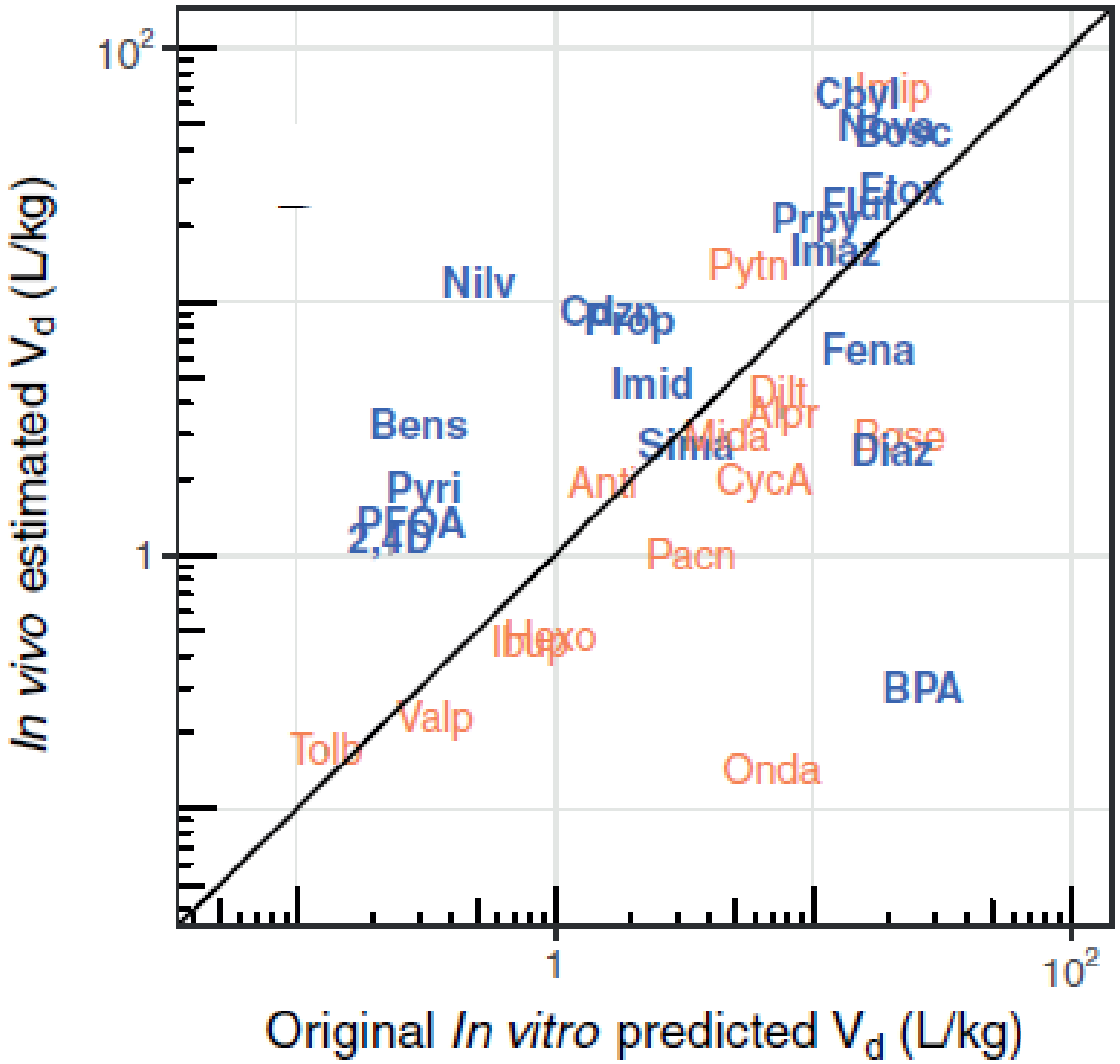
- "httk" R package predicts tissue partitioning using a hybrid of Schmitt (2008) and Peyret and Poulin (2010) algorithms
- In Pearce et al. (2017b) we calibrated these algorithms using experimentally measured partition coefficient data
- However, that data was largely for pharmaceuticals



Wambaugh et al. (2018)

New Data for Evaluation

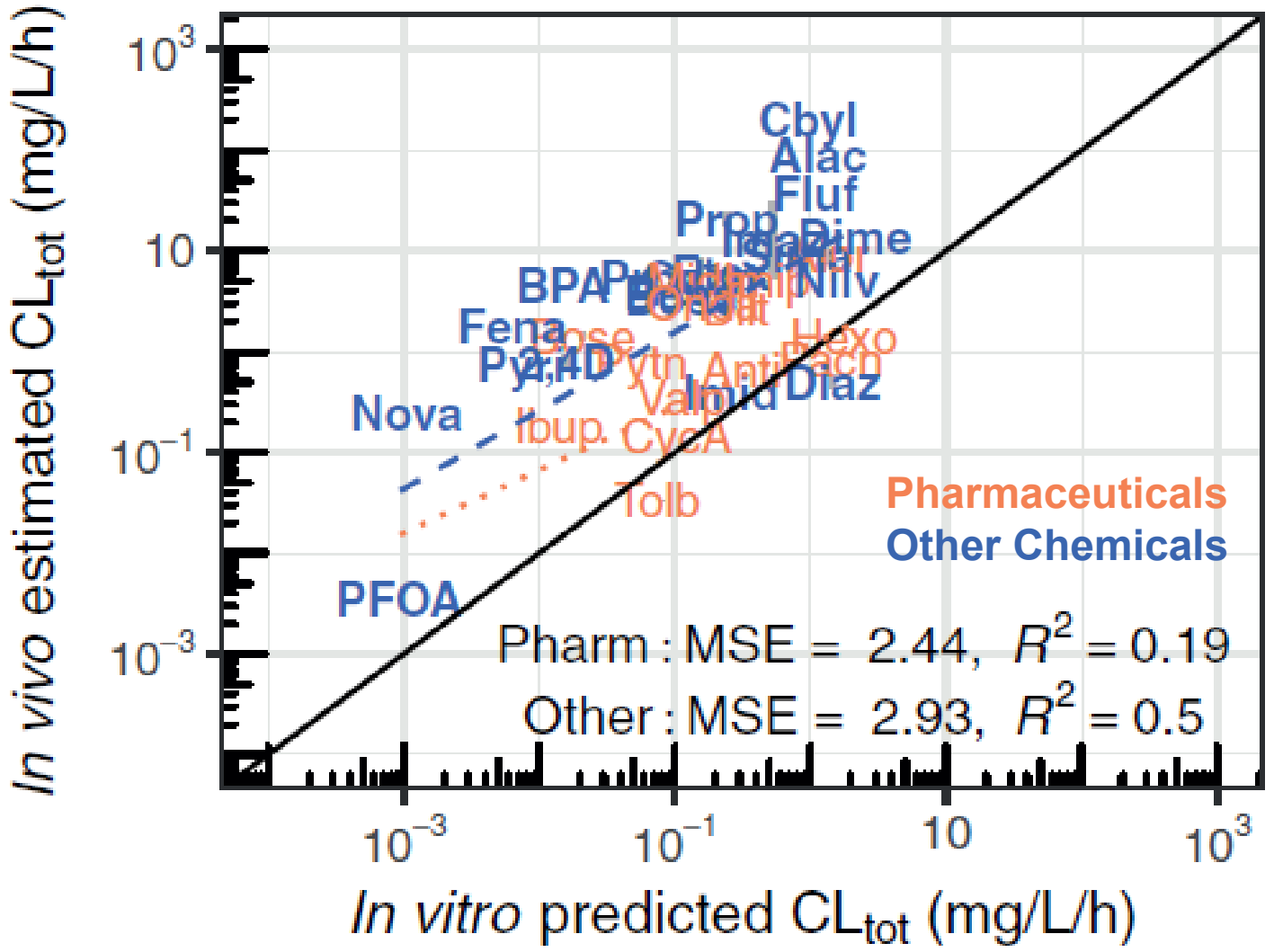




Wambaugh et al. (2018)

Observed Total Clearance

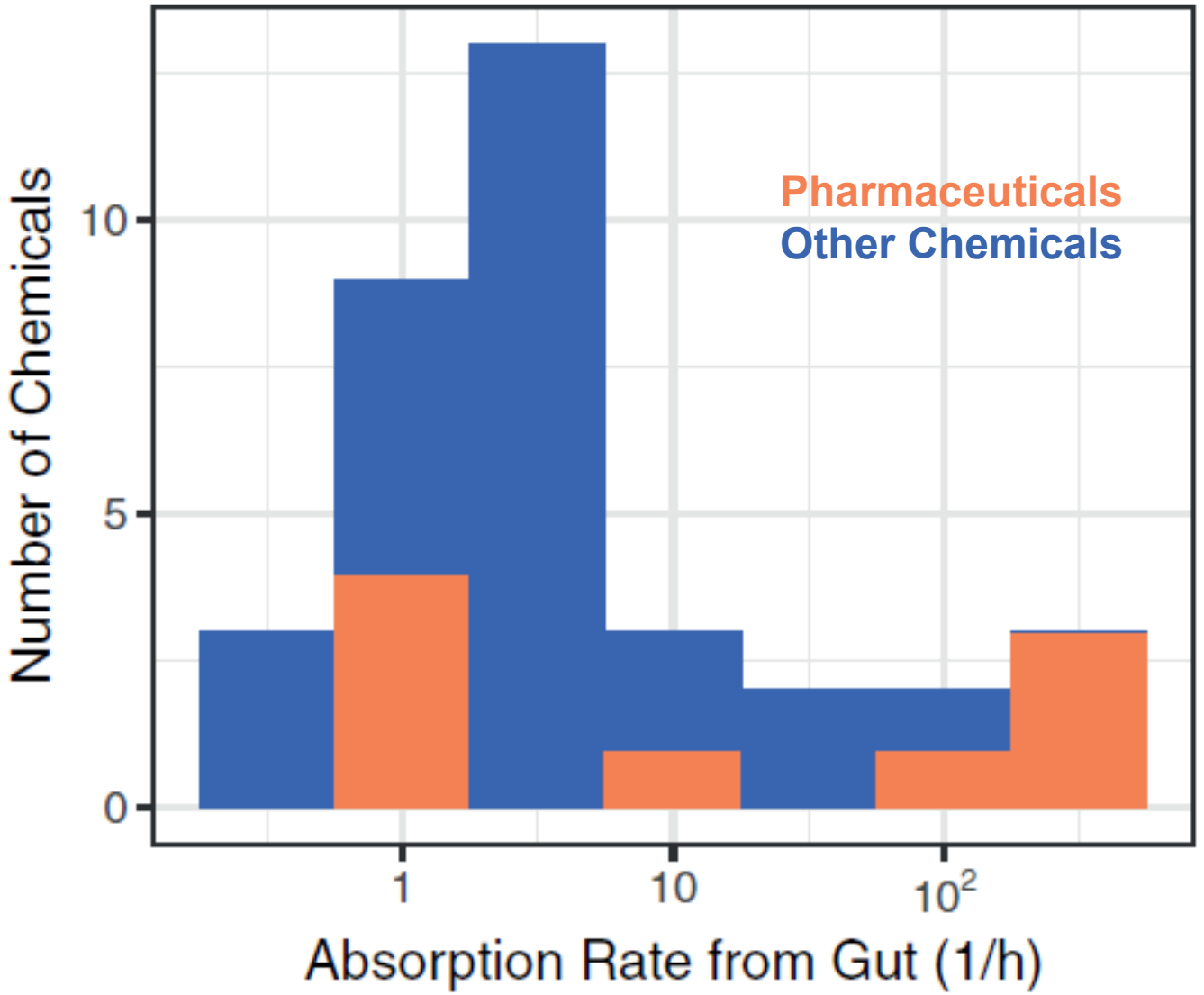
- We estimate clearance from two processes – hepatic metabolism (liver) and passive glomerular filtration (kidney)
- This appears to work better for pharmaceuticals than other chemicals
- Non-pharmaceuticals may be subject to extrahepatic metabolism and/or active transport



Wambaugh et al. (2018)

Observed Absorption Rate

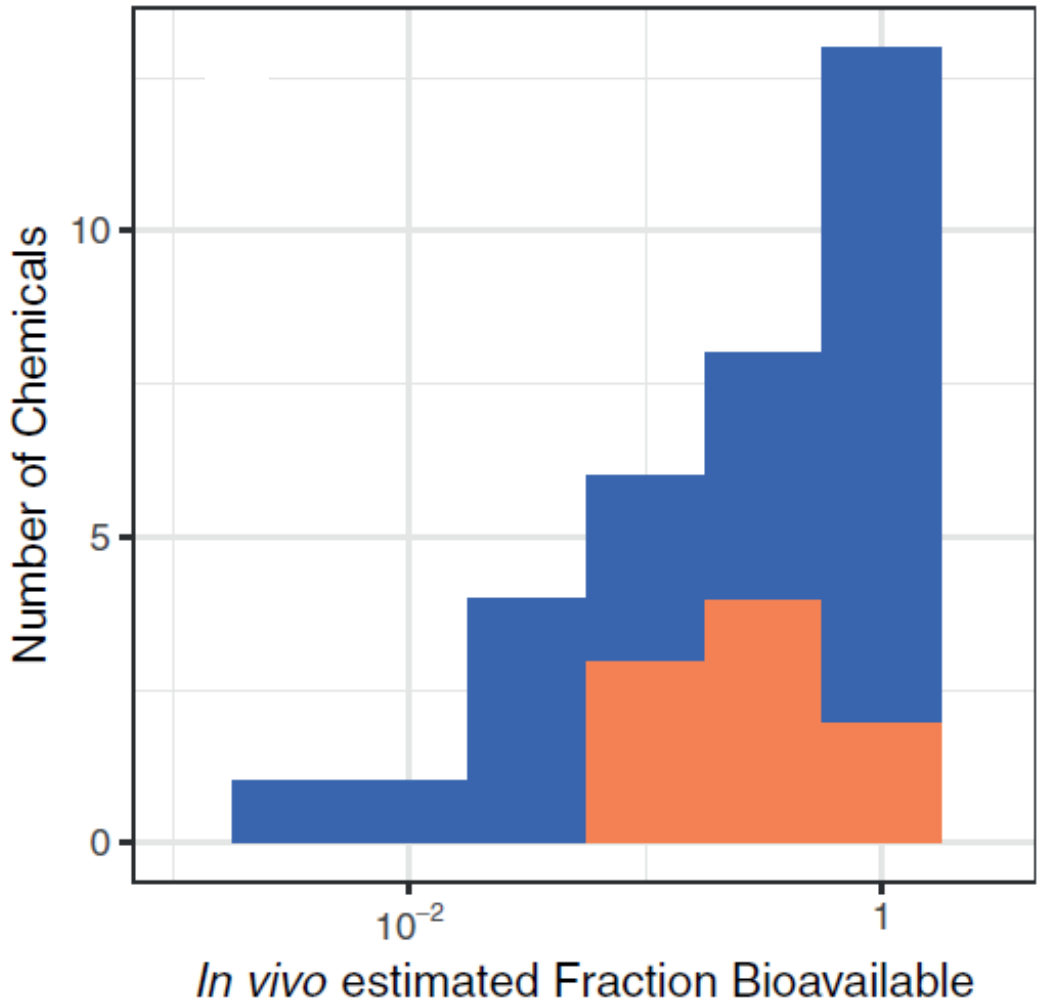
- We had previously assumed that a rate of 1/h was “Fast – most chemicals were actually absorbed somewhat faster
- We have revised the default to the median from this data set



Wambaugh et al. (2018)

Observed Bioavailability

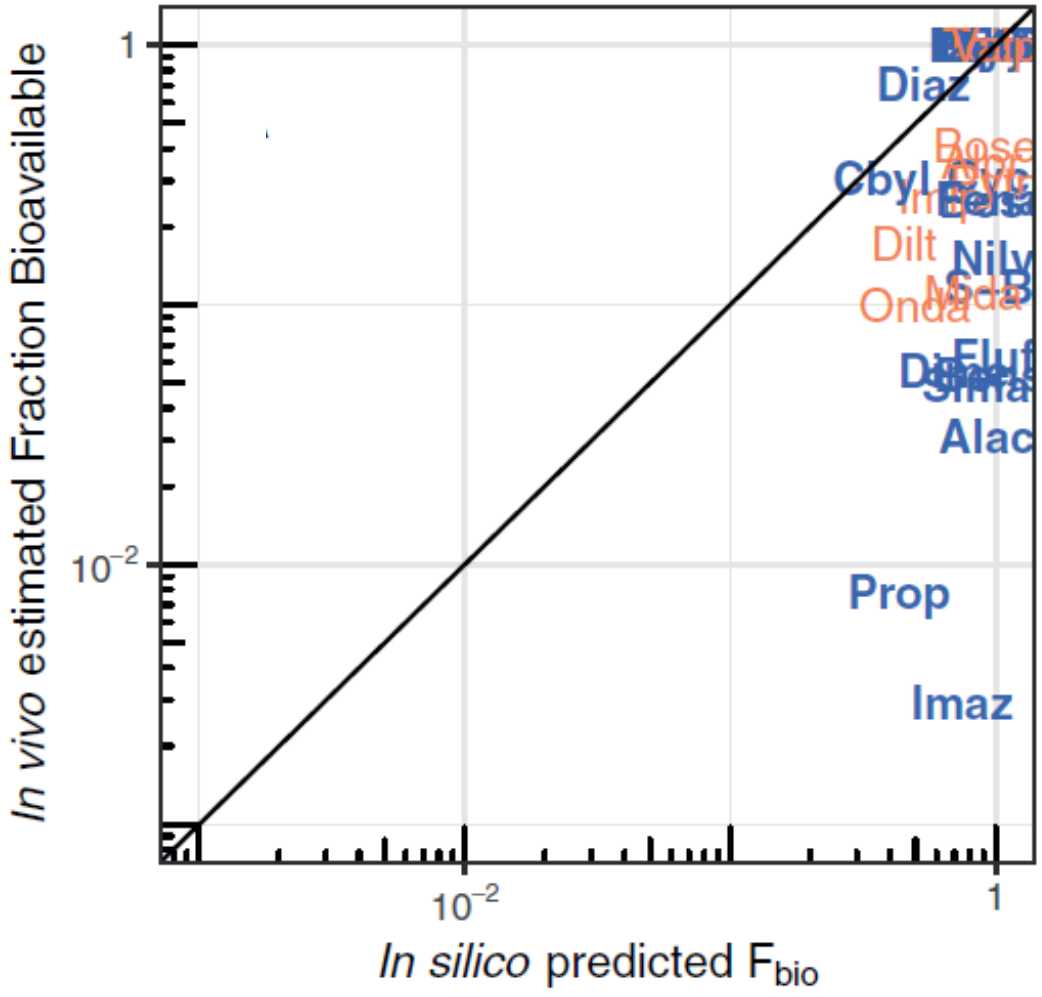
- Most chemicals were well absorbed
- We observe a greater range of bioavailabilities (fraction of oral dose that is available systemically) for non-pharmaceuticals
- Efforts to predict bioavailability were unsuccessful

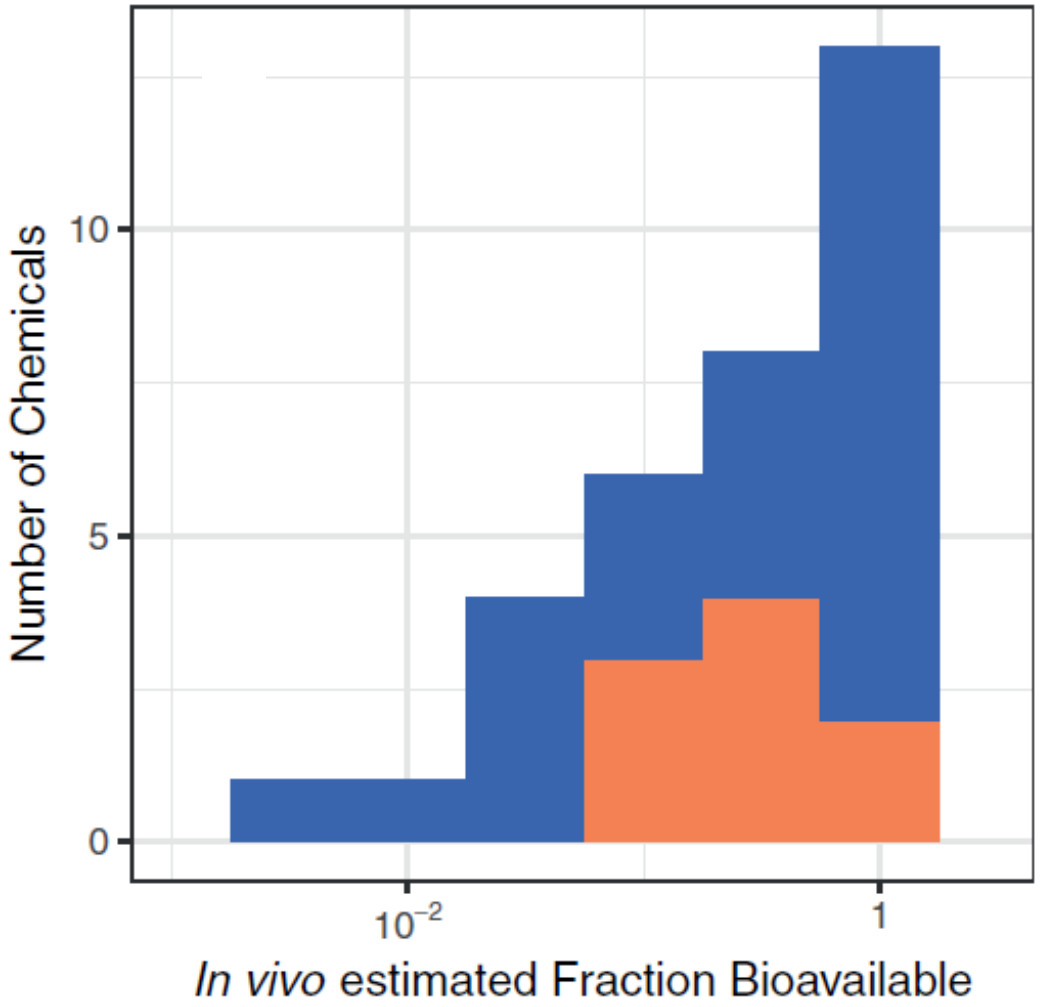


Wambaugh et al. (2018)

Observed Bioavailability

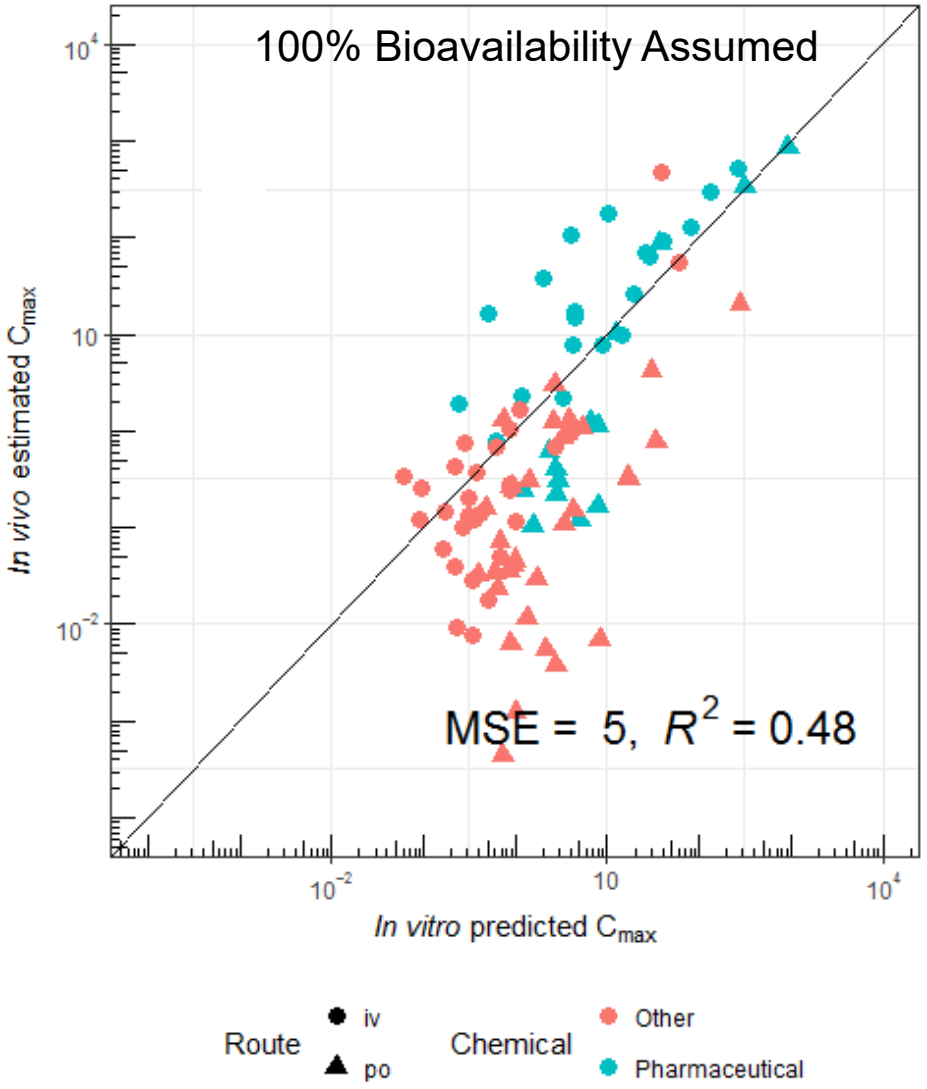
Pharmaceuticals
Other Chemicals





Wambaugh et al. (2018)

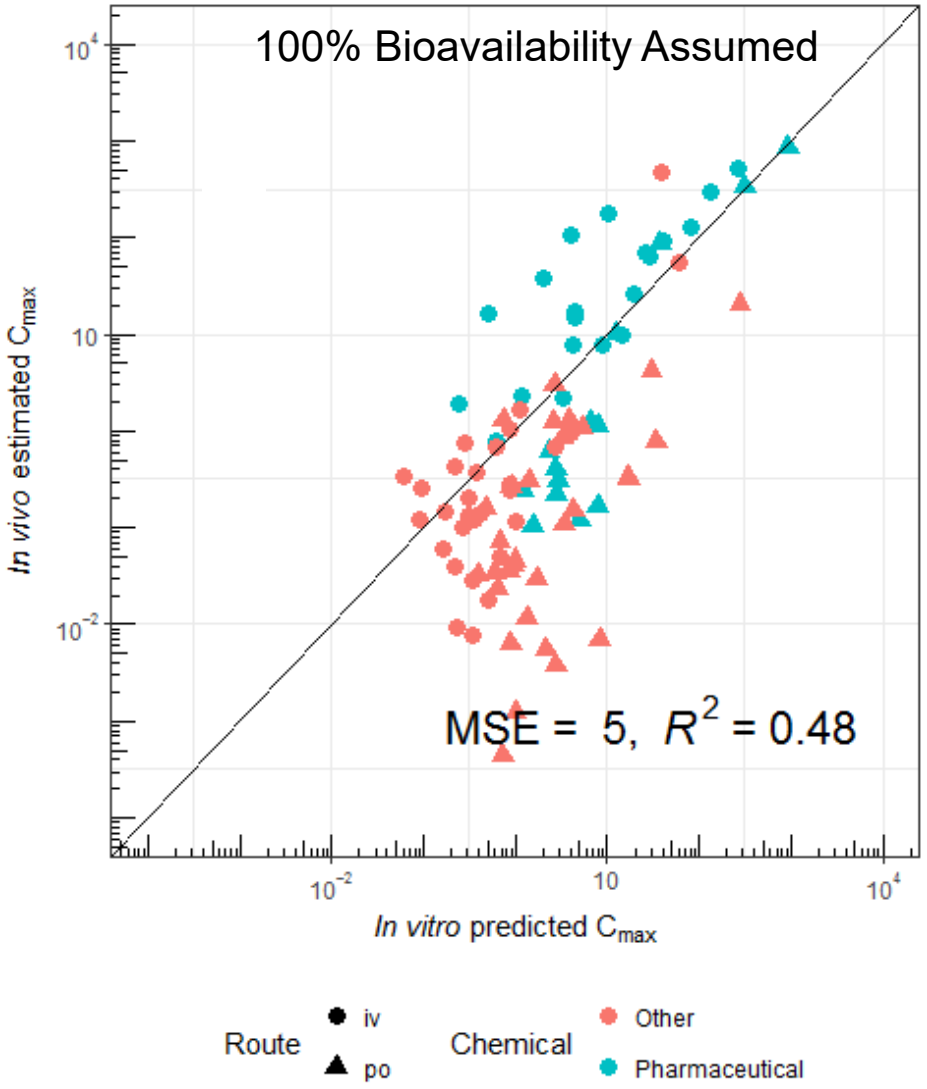
Impact of Oral Bioavailability

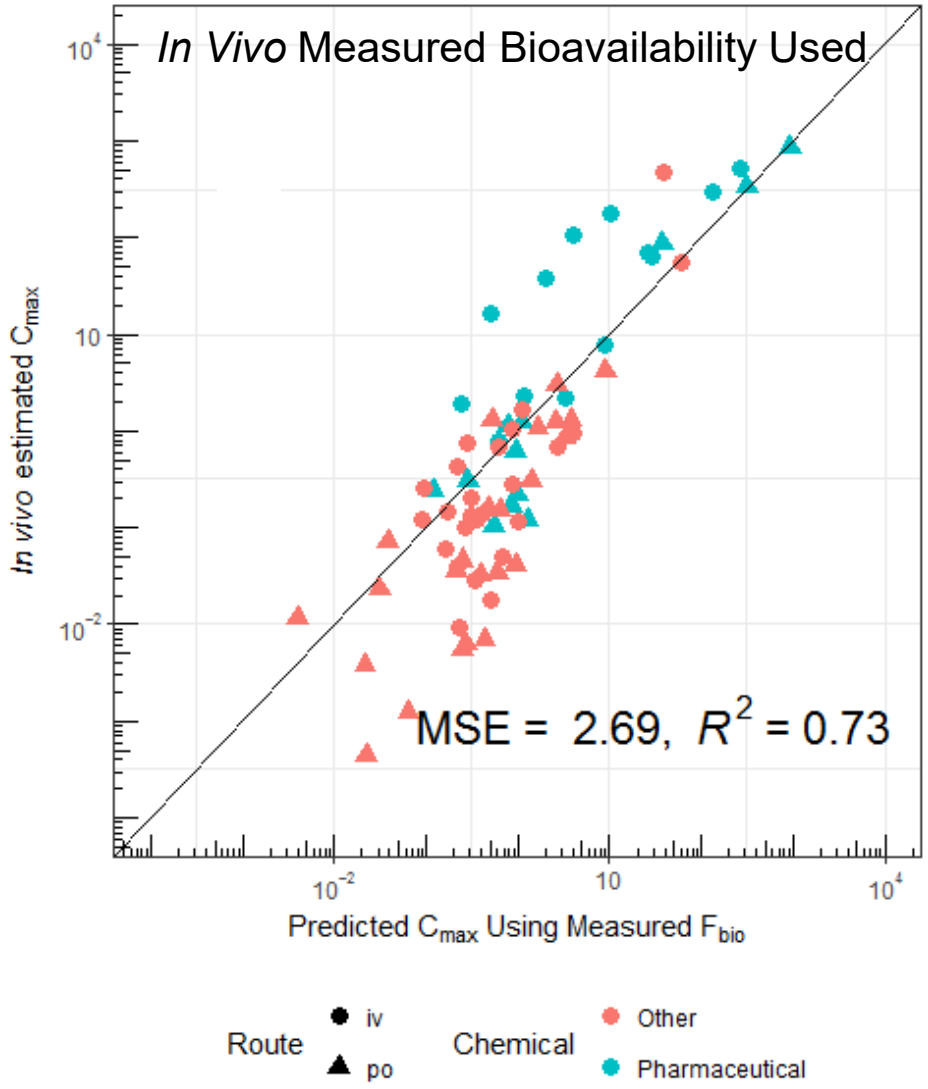


We evaluate HTTK by comparing predictions with observations for as many chemicals as possible

Wambaugh et al. (2018)

Impact of Oral Bioavailability

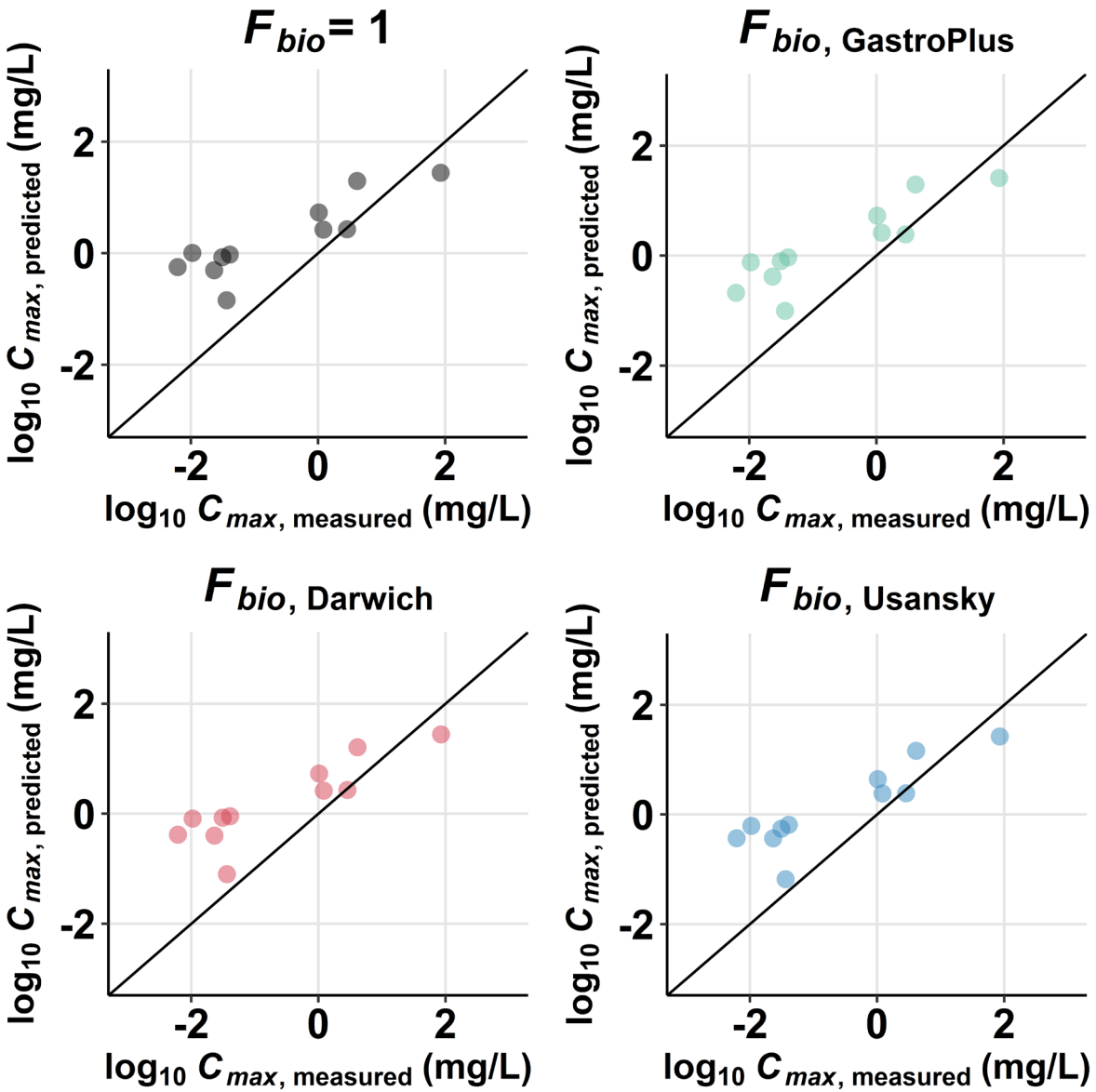




Wambaugh et al. (2018)

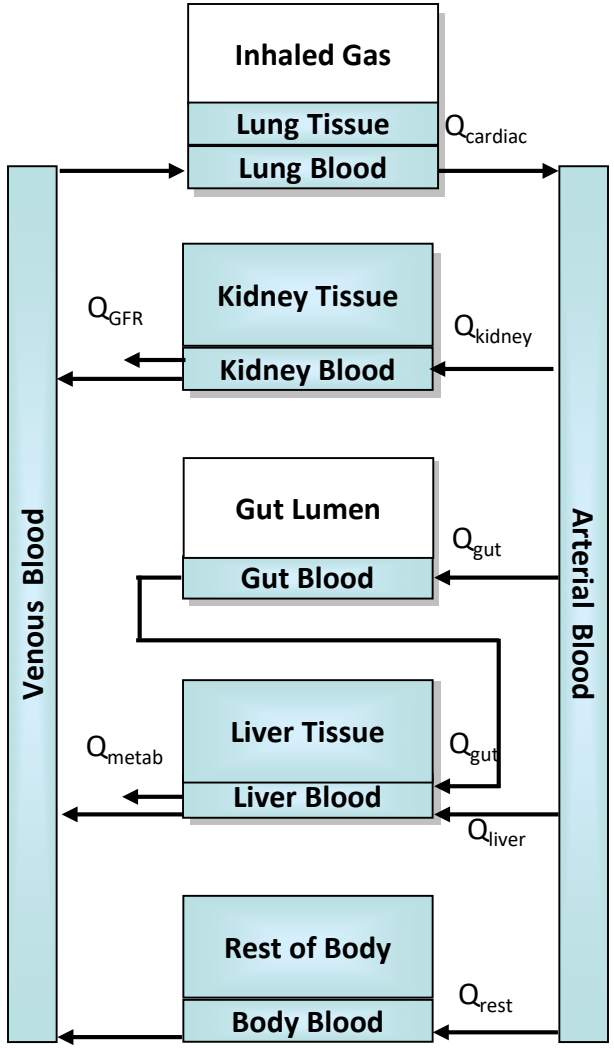
Predicting F_{bio} for Toxicokinetics

- Examining in vitro membrane permeability data (Caco2) for >300 ToxCast Chemcials
- 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



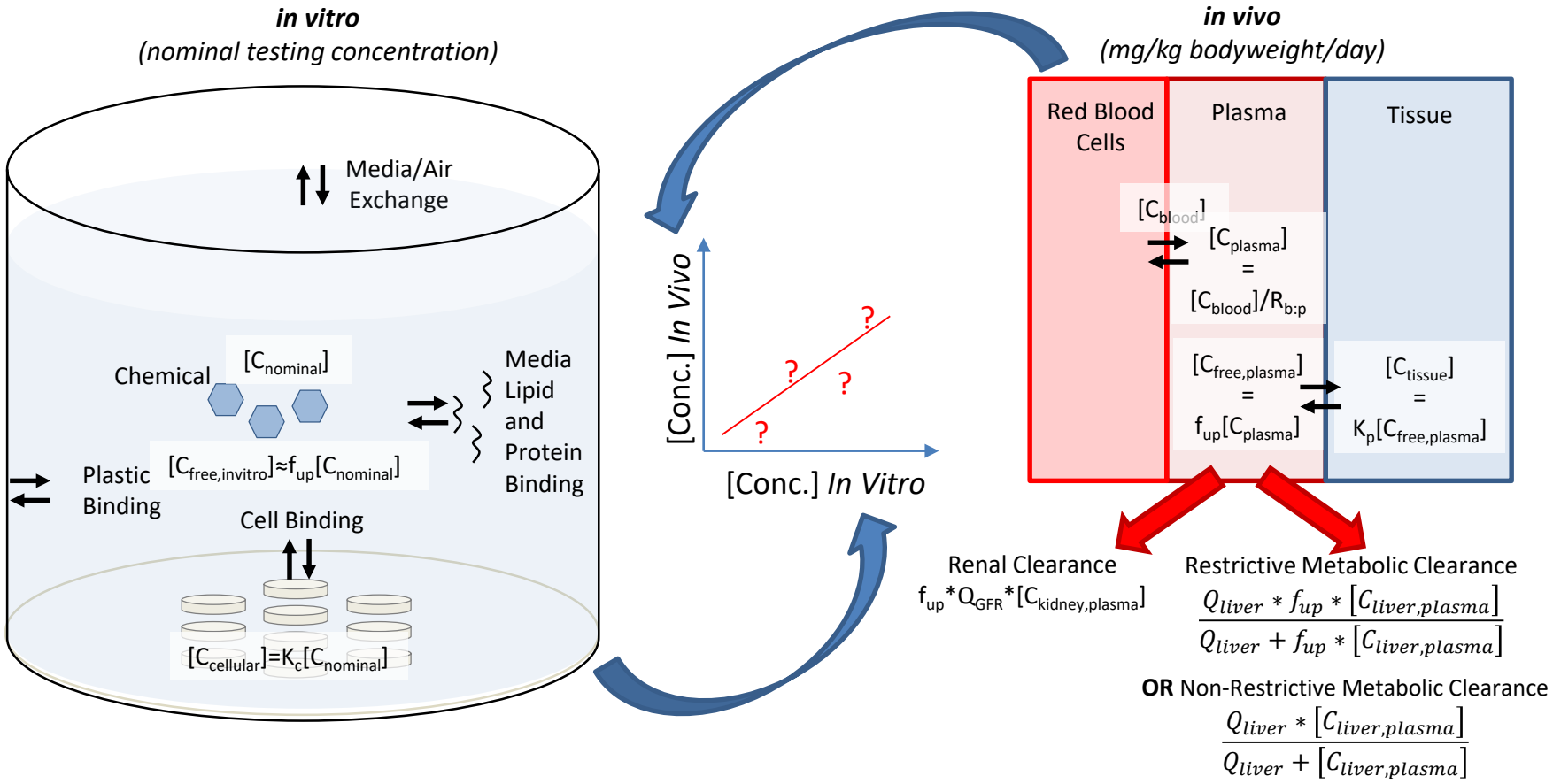
Honda et al. (in preparation)

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).

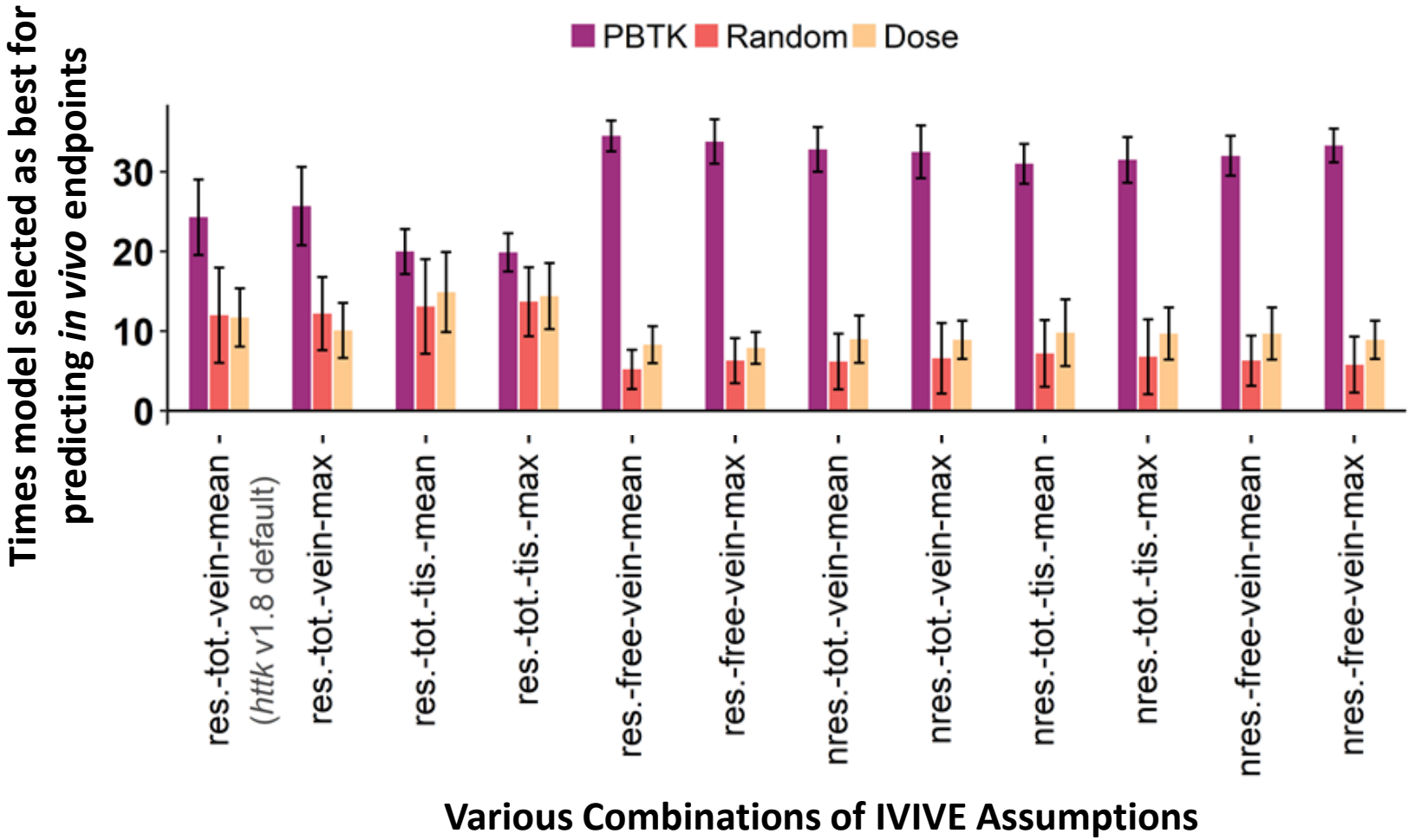
High-Throughput Toxicokinetics (HTTK) for *In Vitro-In Vivo* Extrapolation (IVIVE)



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

Honda et al, in prep.

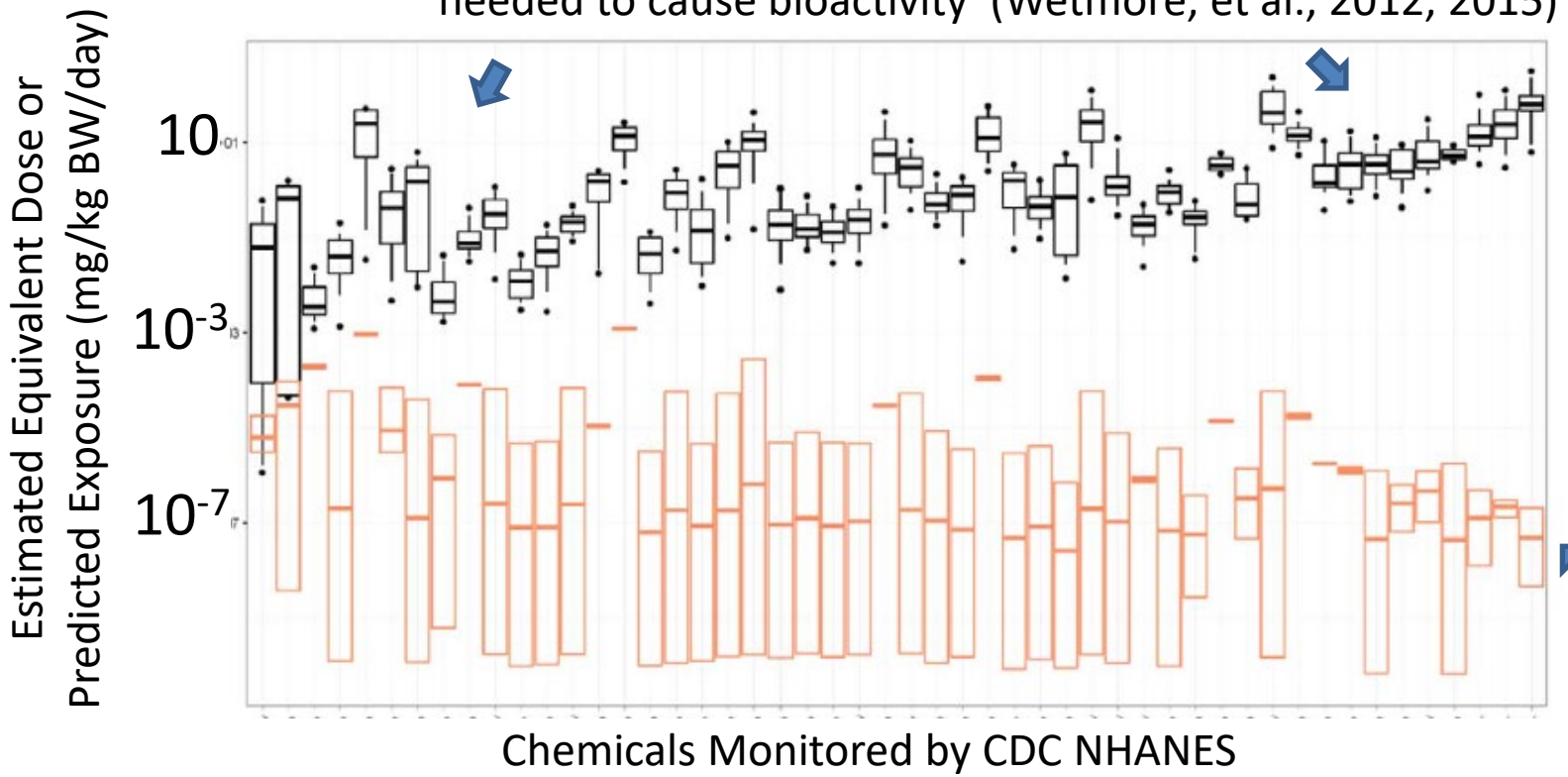
Optimizing HTTK-based IVIVE



Honda et al, in prep.

Selecting Candidates for Prioritization

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)

Population simulator for HTTK

Correlated Monte Carlo sampling of physiological model parameters built into R “httk” package (Pearce et al., 2017):

Sample NHANES biometrics for actual individuals:

- Sex
- Race/ethnicity
- Age
- Height
- Weight
- Serum creatinine

Slide from Caroline Ring (ToxStrategies)

Ring *et al.* (2017)

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

(Similar approach used in SimCYP [Jamei et al. 2009], GastroPlus, PopGen [McNally et al. 2014], P3M [Price et al. 2003], physB [Bosgra et al. 2012], etc.)

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Predict physiological quantities

Tissue masses
Tissue blood flows
GFR (kidney function)
Hepatocellularity

(Similar approach used in SimCYP [Jamei et al. 2009], GastroPlus, PopGen [McNally et al. 2014], P3M [Price et al. 2003], physB [Bosgra et al. 2012], etc.)

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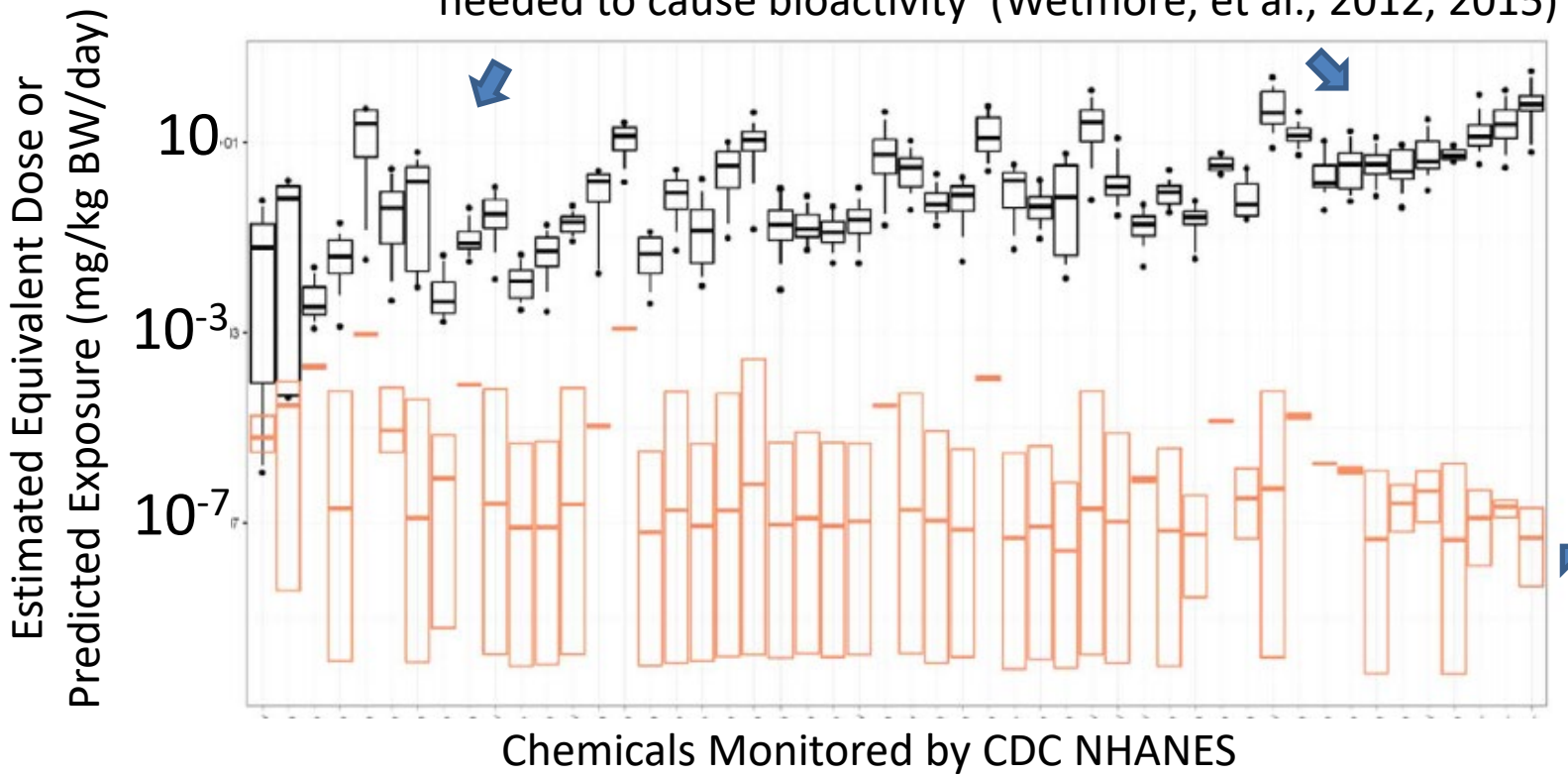

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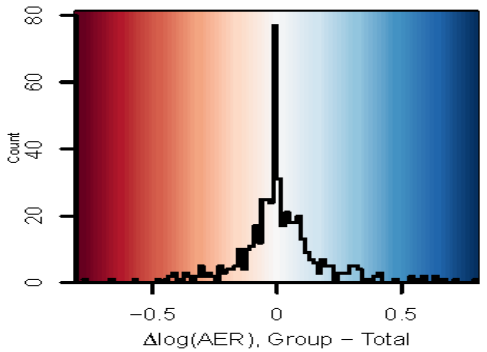
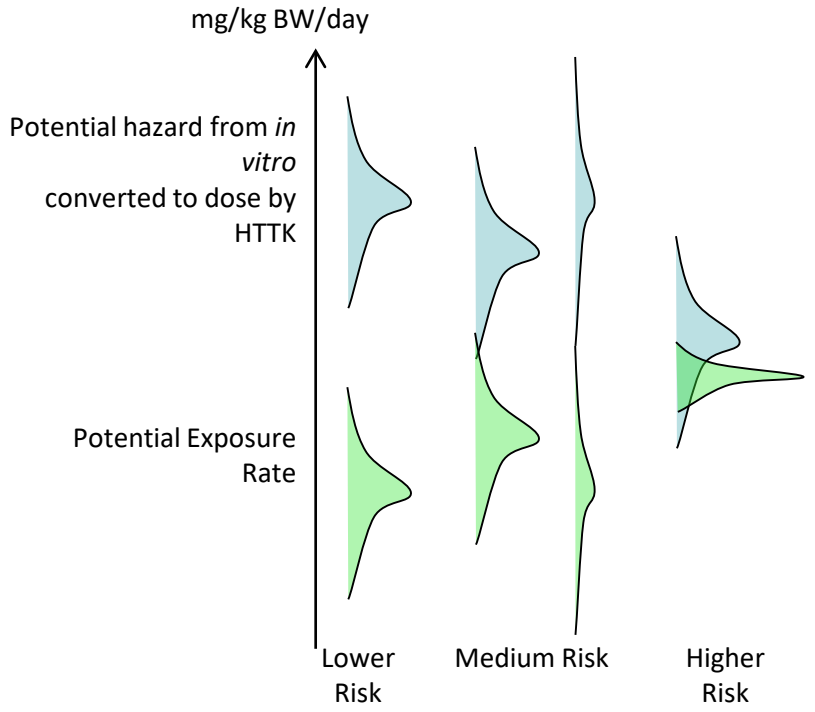
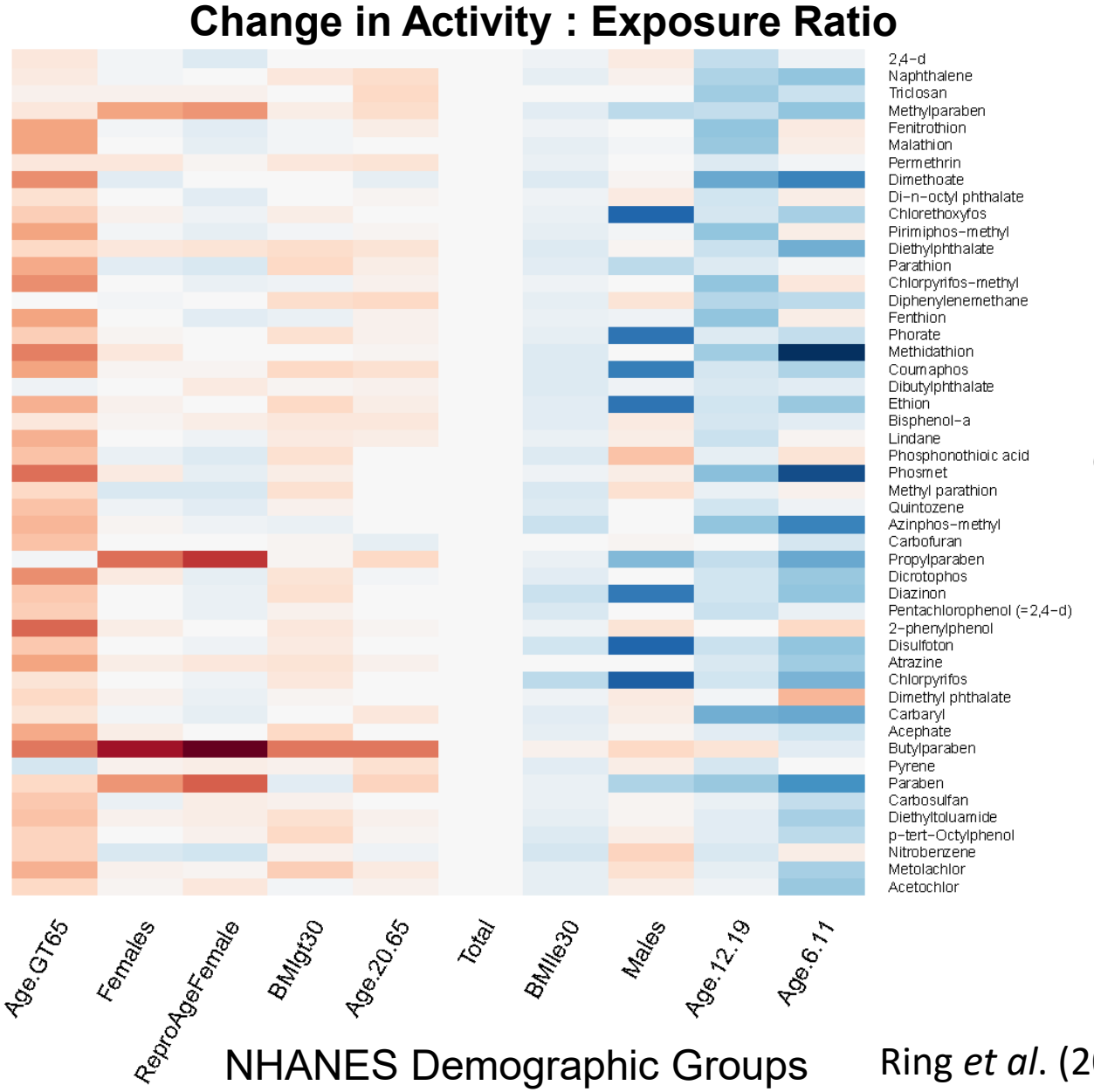
Life-stage and Demographic Specific Predictions

- We use HTTK to calculate margin between bioactivity and exposure for specific populations



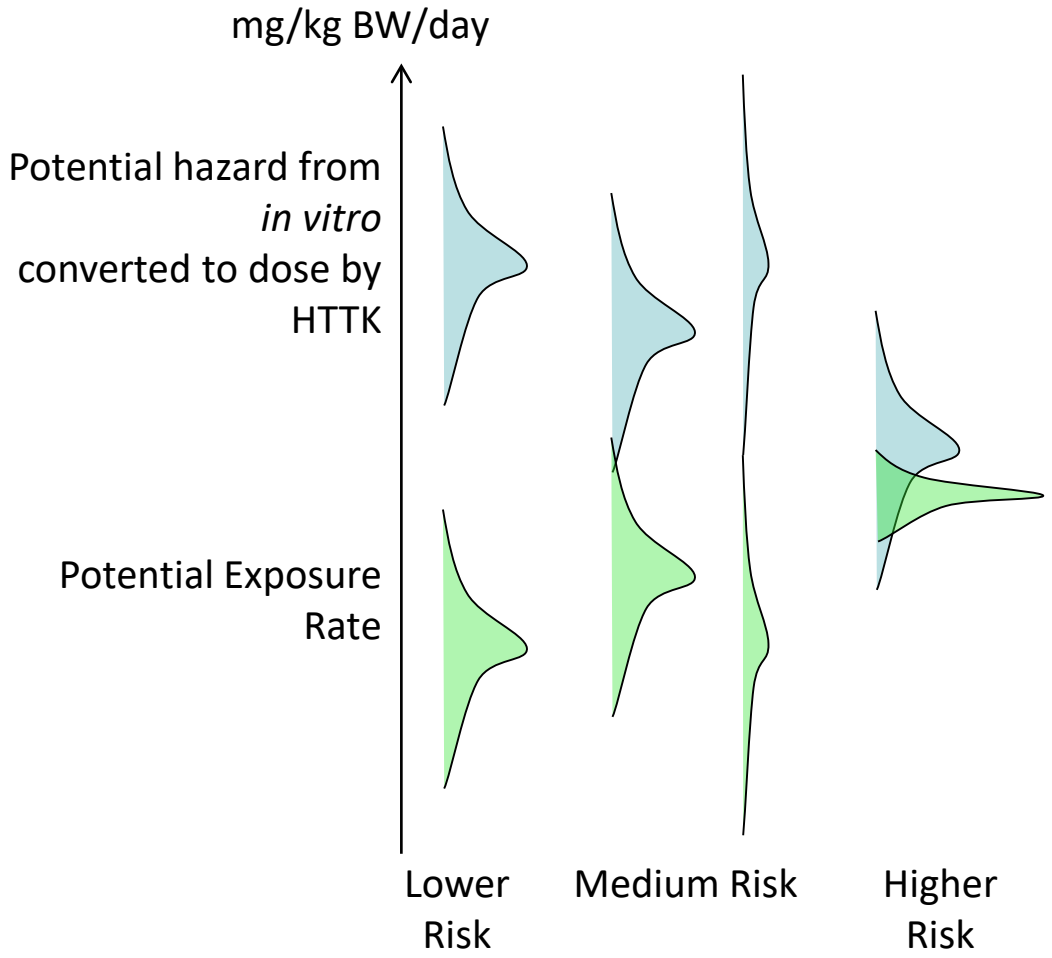




Ring *et al.* (2017)

Summary

- We would like to know more about the risk posed by thousands of chemicals in the environment – which ones should we start with?
- HTTK New approach methodologies (NAMs) are being evaluated through comparison between *in vitro* predictions and *in vivo* measurements of both plasma concentrations and doses associated with the onset of effects
- Comparison between HTTK predicted time course concentrations in plasma and *in vivo* data indicate that some properties (e.g. average and maximum concentration) can be predicted with confidence.



- NAMs for TK allow risk-based prioritization of large numbers of chemicals.

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

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