United States Environmental Protection Agency

High-Throughput PBPK: Evaluating EPA's Open-Source Data and Tools for Dosimetry and Exposure Reconstruction.

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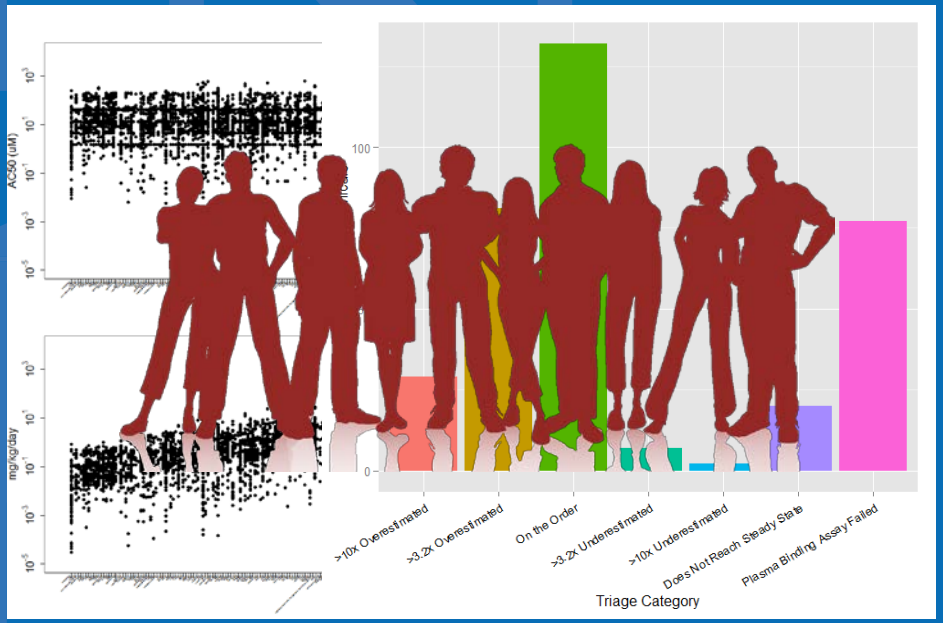

Figure includes image from Thinkstock

"Lost in Translation: Bringing the Real World to In Vitro *Data"*
Society of Toxicology Annual Meeting
Baltimore, MD

March 14, 2017

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Conflict of Interest Statement

I have no conflicts of interest to disclose

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

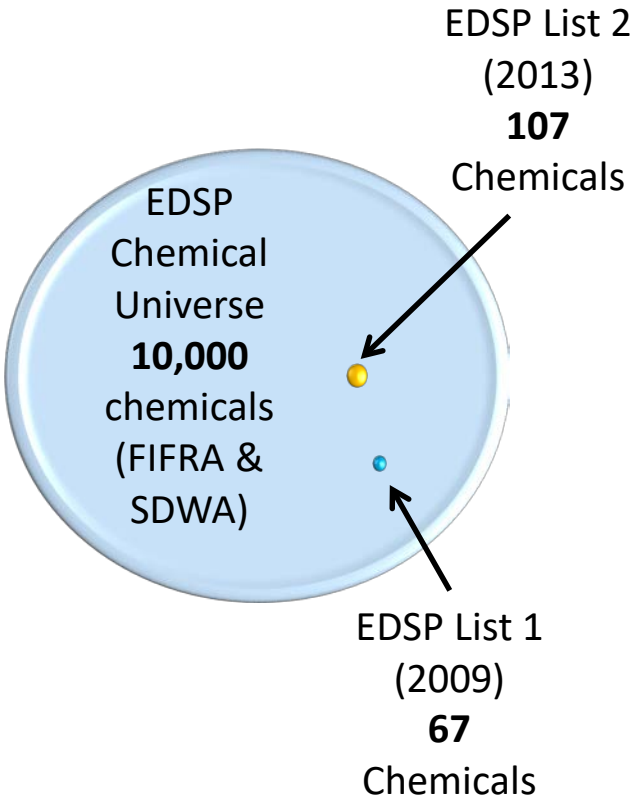
Introduction

- In order to address greater numbers of chemicals we collect *in vitro*, high throughput toxicokinetic (HTTK) data
- The goal of HTTK is to provide a human dose context for in vitro concentrations from HTS
  - This allows direct comparisons with exposure
- A key application of HTTK has been reverse dosimetry
  - Allows in vitro – in vivo extrapolation (What dose causes a bioactive concentration?)
  - Allows exposure reconstruction (What dose is consistent with a biomarker?)

Scale of the Problem

- Park *et al*. (2012): At least 3221 chemicals in humans, many appear to be exogenous

Endocrine Disruptor Screening Program (EDSP) Chemical List	Number of Compounds
Conventional Active Ingredients	838
Antimicrobial Active Ingredients	324
Biological Pesticide Active Ingredients	287
Non Food Use Inert Ingredients	2,211
Food Use Inert Ingredients	1,536
Fragrances used as Inert Ingredients	1,529
Safe Drinking Water Act Chemicals	3,616
TOTAL	**10,341**

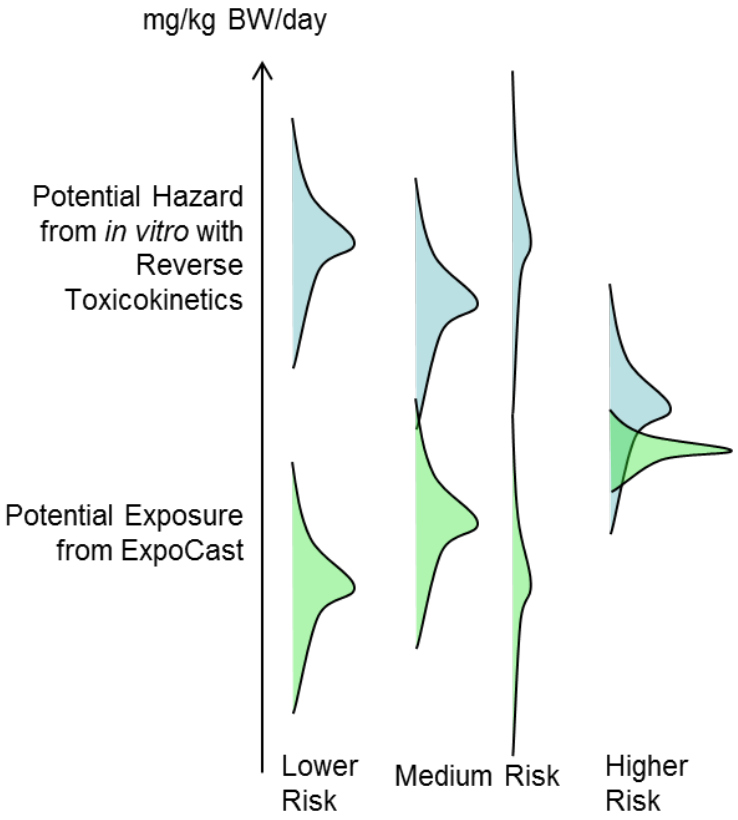


So far 67 chemicals have completed testing and an additional 107 are being tested

December, 2014 Panel: "Scientific Issues Associated with Integrated Endocrine Bioactivity and Exposure-Based Prioritization and Screening" DOCKET NUMBER: EPA–HQ–OPP–2014–0614

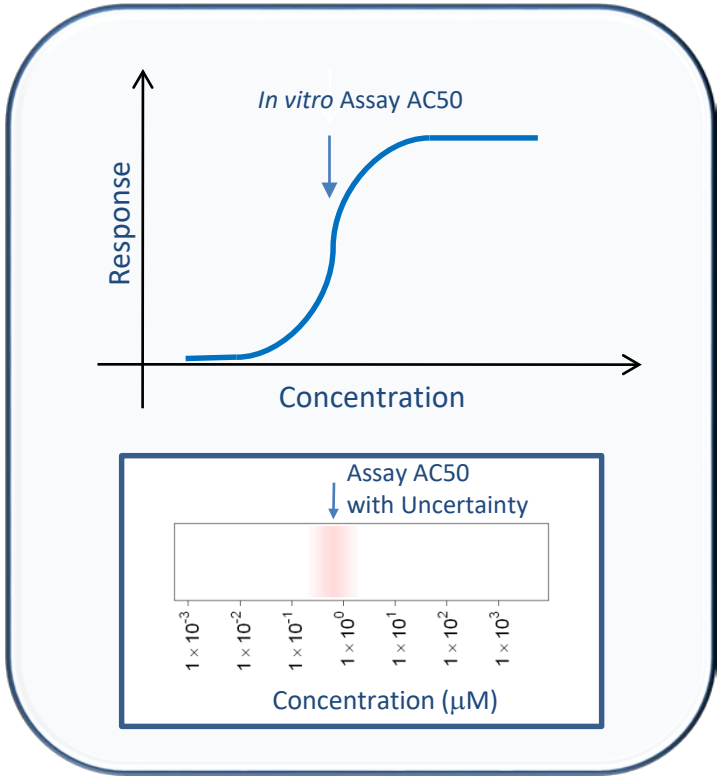
High Throughput Risk Prioritization

- **High throughput risk prioritization** relies on three components:
  1. high throughput **hazard** characterization
  2. high throughput **exposure** forecasts
  3. high throughput **toxicokinetics** (*i.e.*, dosimetry)
- While advances have been made in toxicity and exposure screening, TK methods applicable to 100s of chemicals are needed



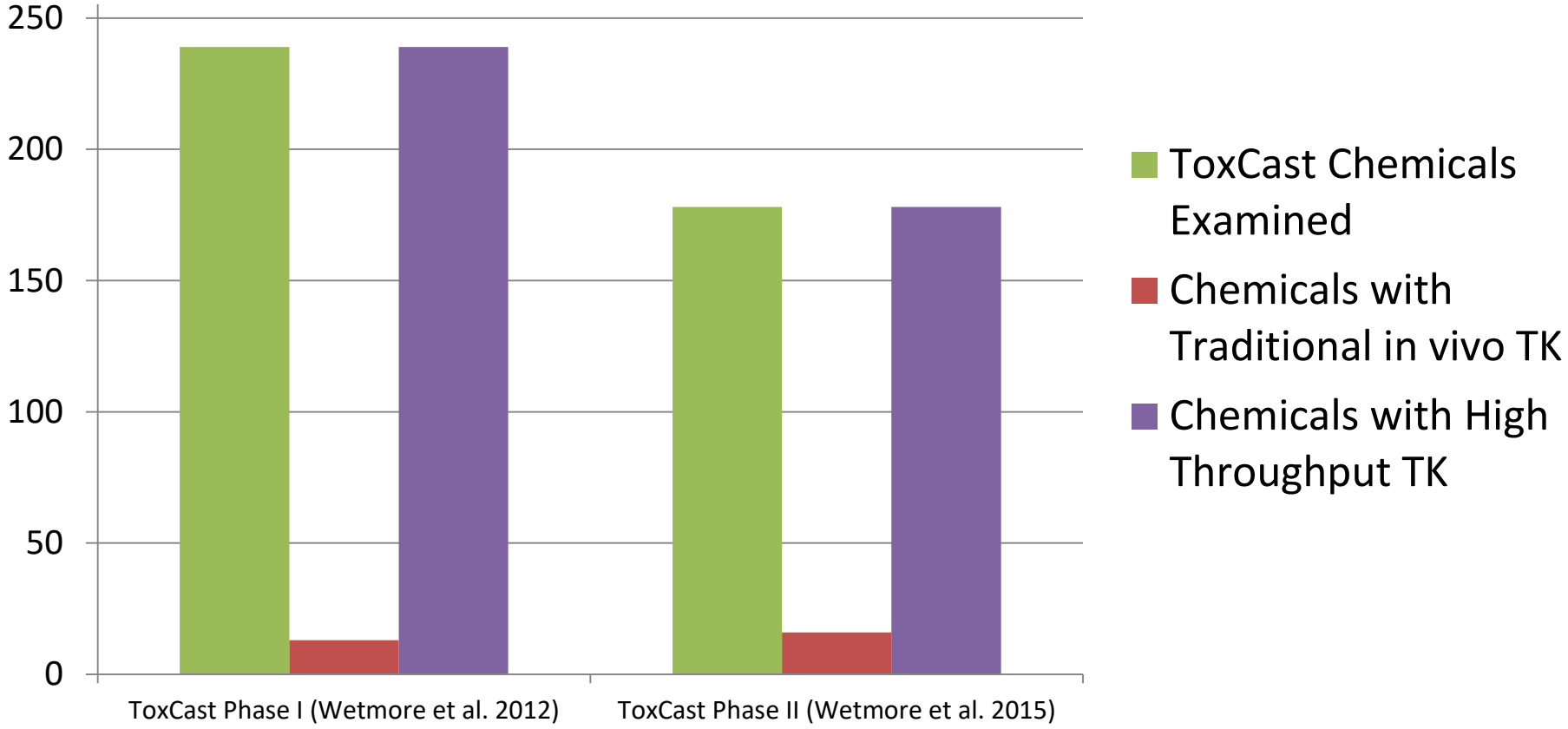
High-Throughput Bioactivity

- **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 >500 additional assays (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, Filer *et al.*, 2016)
- All data is public: http://actor.epa.gov/



The Need for *In Vitro* Toxicokinetics

- Studies like Wetmore et al. (2012,2015) used *in vitro* methods to provide TK for >500 chemicals to date



- Ongoing data collection by ToxCast contractor Cyprotex,
  - Upcoming publication of ~300 new compounds
  - Work by Derek Angus, Maria Bacolod, Jon Gilbert, Chris Strock

Reverse Dosimetry to Convert µM to mg/kg/day

New population simulator based on NHANES *biometrics*

Sex
Race/ethnicity
Age
Height
Weight
Serum creatinine

Regression equations from literature
(+ residual marginal variability)

Predict physiological quantities

Tissue masses
Tissue blood flows
GFR (kidney function)
Hepatocellularity

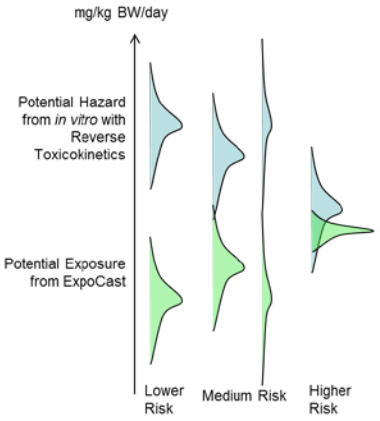
(Similar approach used in SimCYP [Jamei et al. 2009], GastroPlus, PopGen [McNally et al. 2014], P3M [Price et al. 2003], physB [Bosgra et al. 2012], etc.)

Ring et al. (submitted)
httk v1.5

Life-stage and Demographic Specific Predictions

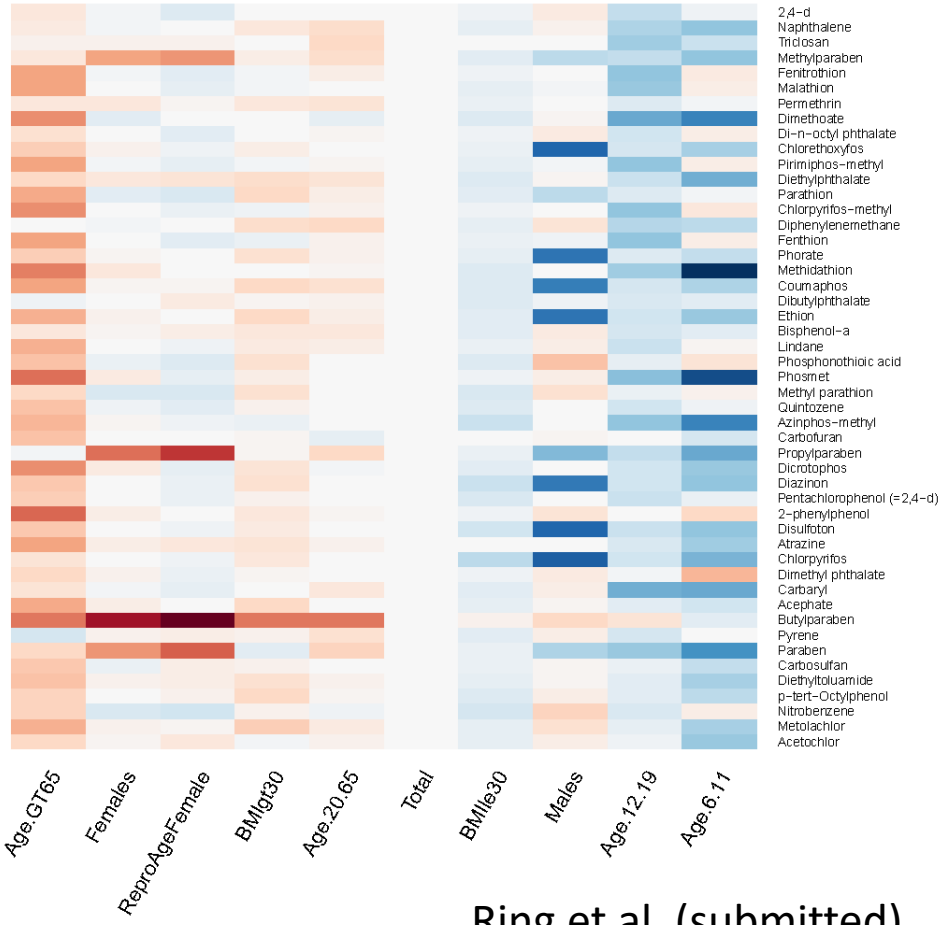
- Wambaugh *et al.* (2014) predictions of exposure rate (mg/kg/day) for various demographic groups

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



Change in Risk

Change in Activity:Exposure Ratio



Ring et al. (submitted)
httk v1.5

In vivo Predictive Ability and Domain of Applicability

- 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 environmental compounds, there will be no clinical trials

- Uncertainty must be well characterized ideally with rigorous statistical methodology
  - We will use direct comparison to *in vivo* data in order to get an empirical estimate of our uncertainty
  - Any approximations, omissions, or mistakes should work to increase the estimated uncertainty when evaluated systematically across chemicals

Statistical Analysis of High Throughput Toxicokinetics

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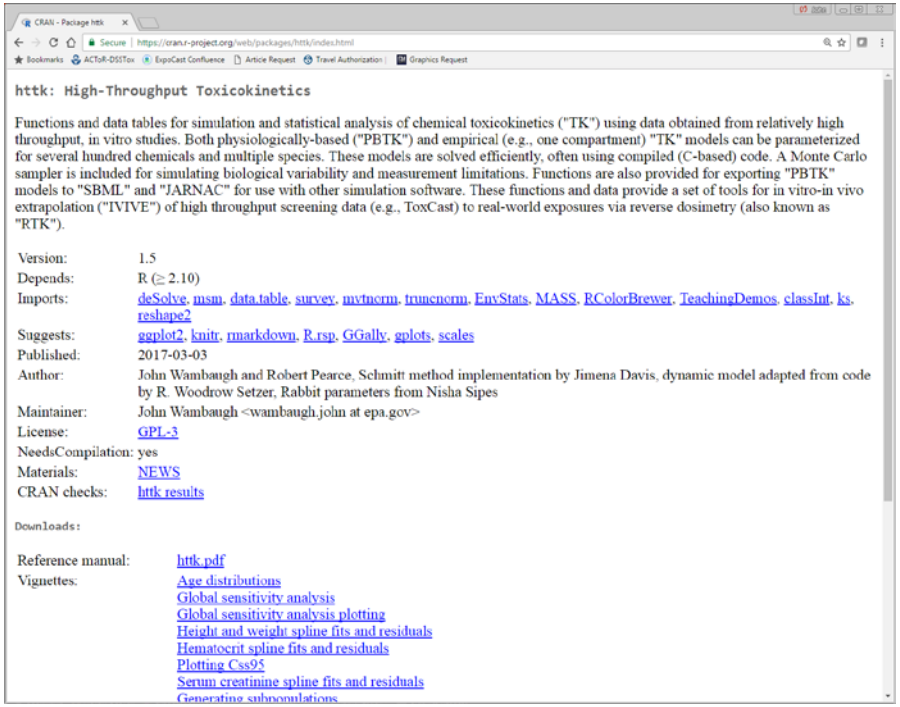


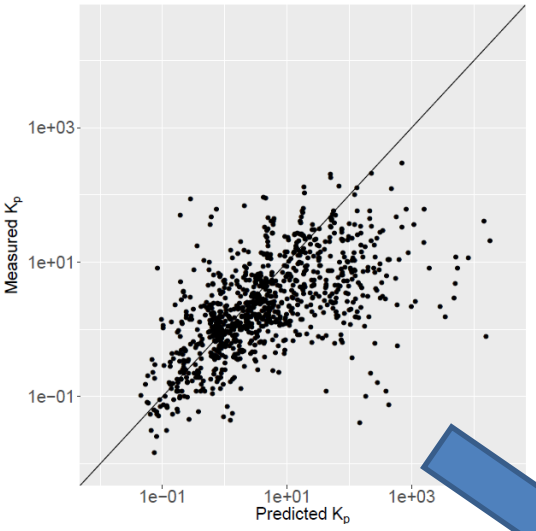
httk: High-Throughput Toxicokinetics

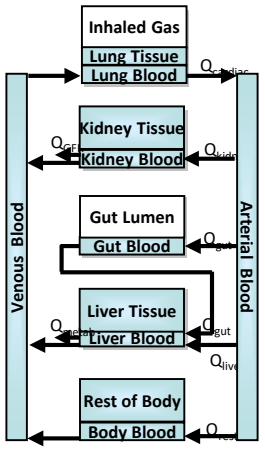
Functions and data tables for simulation and statistical analysis of chemical toxicokinetics ("TK") using data obtained from relatively high throughput, in vitro studies. Both physiologically-based ("PBTK") and empirical (e.g., one compartment) "TK" models can be parameterized for several hundred chemicals 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:	1.5
Depends:	R (≥ 2.10)
Imports:	deSolve, msm, data.table, survey, mvtnorm, truncnorm, EnvStats, MASS, RColorBrewer, TeachingDemos, classInt, ks, reshape2
Suggests:	ggplot2, knitr, rmarkdown, R.rsp, GGally, gplots, scales
Published:	2017-03-03
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
Materials:	NEWS
CRAN checks:	httk results

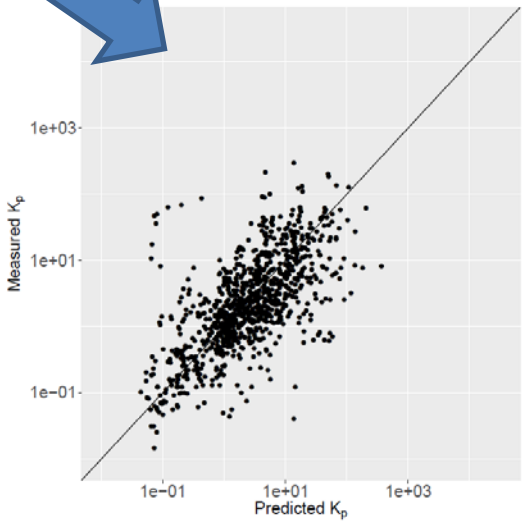
Downloads:

Reference manual:	httk.pdf
Vignettes:	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





Ongoing refinements:
High log P, ionization
(Pearce et al., in preparation)

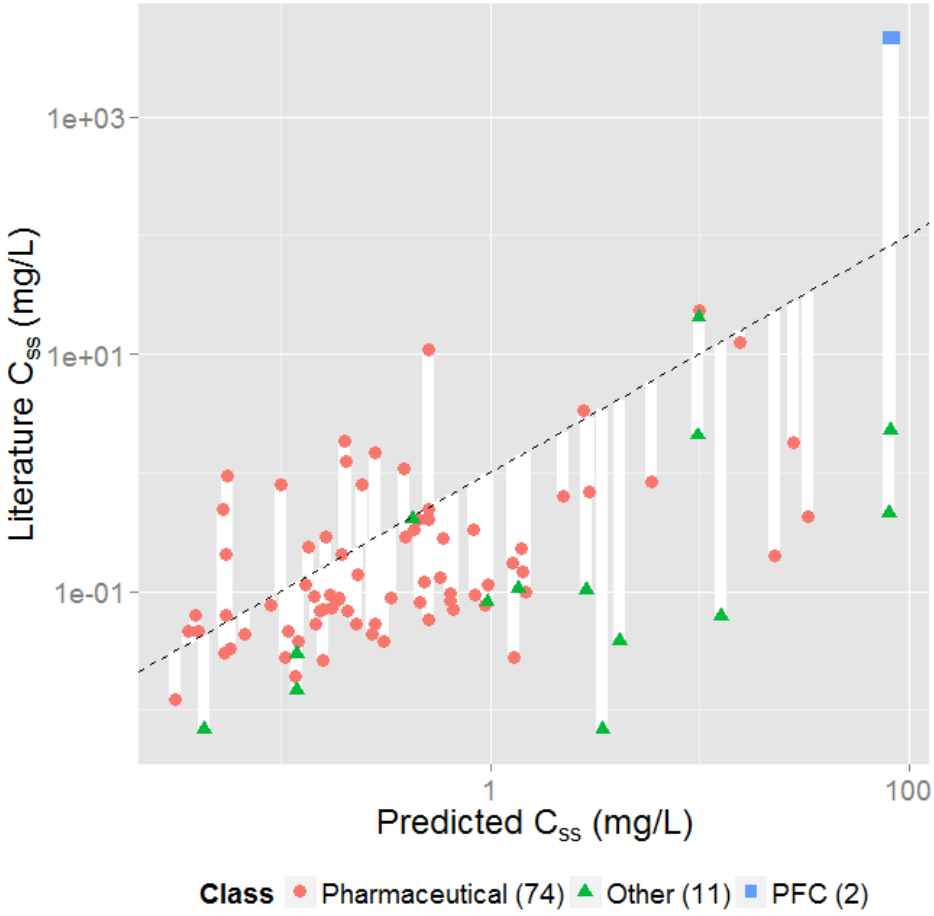


- "httk" R Package for reverse dosimetry and PBTK
- 543 Chemicals to date
- 100's of additional chemicals being studied
- Pearce *et al.* package documentation manuscript accepted at Journal of Statistical Software

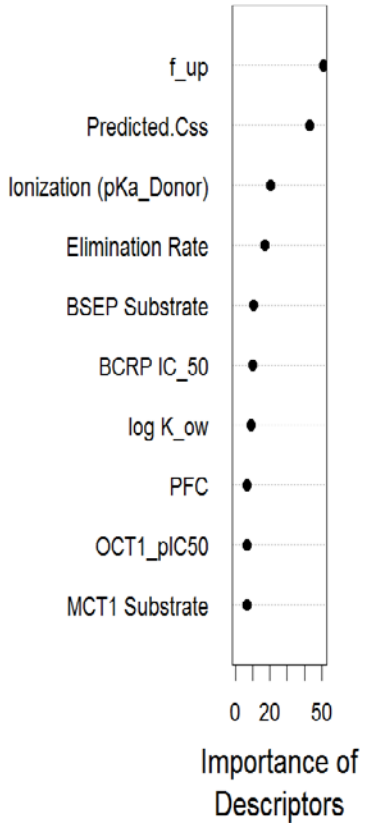
https://cran.r-project.org/web/packages/httk/

Can access this from the R GUI: "Packages" then "Install Packages"

Using *in vivo* Data to Evaluate HTTK



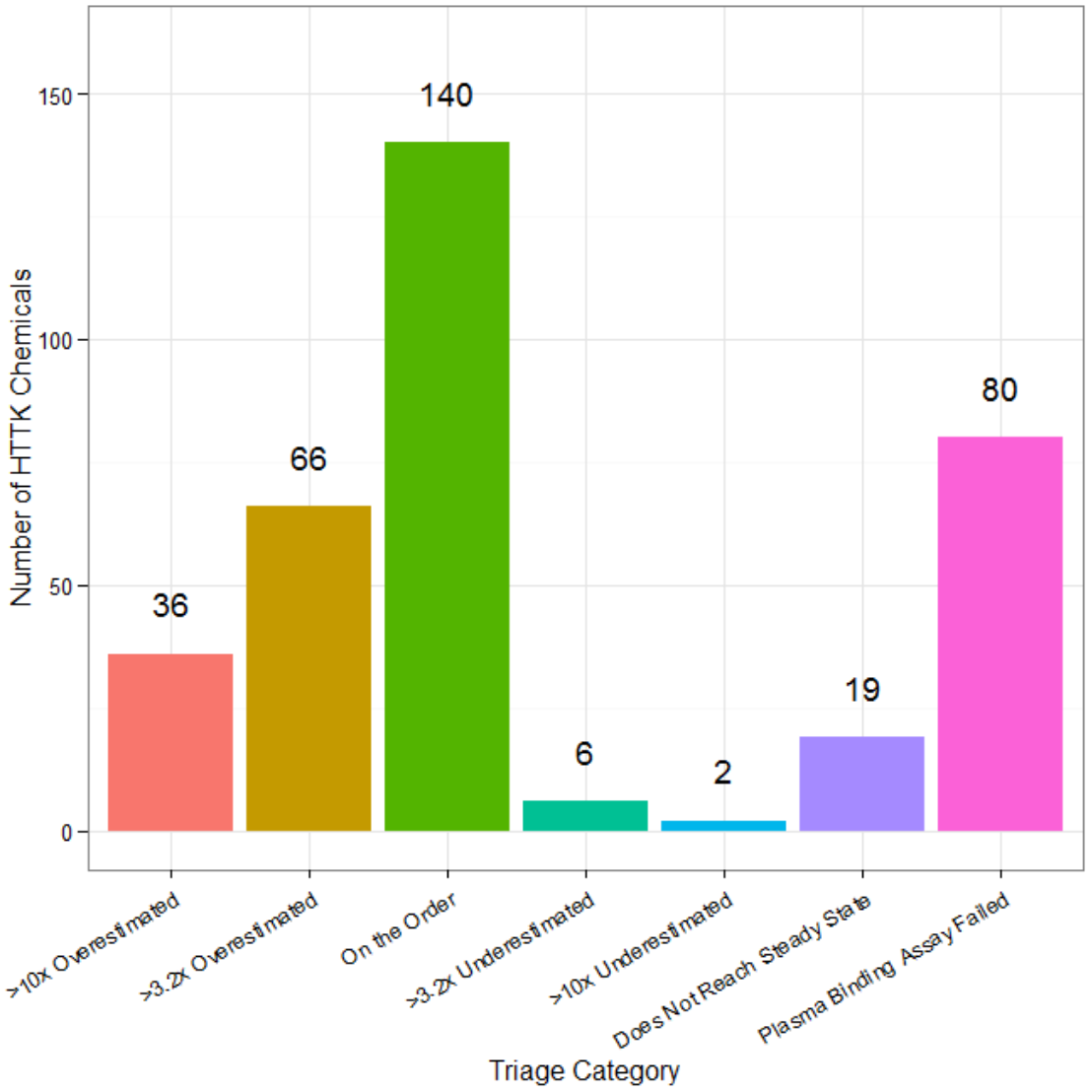
- When we compare the C_{ss} predicted from *in vitro* HTTK with *in vivo* C_{ss} values determined from the literature we find limited correlation (R^2 ~0.34)
- The dashed line indicates the identity (perfect predictor) line:
  - Over-predict for 65
  - Under-predict for 22
- The white lines indicate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



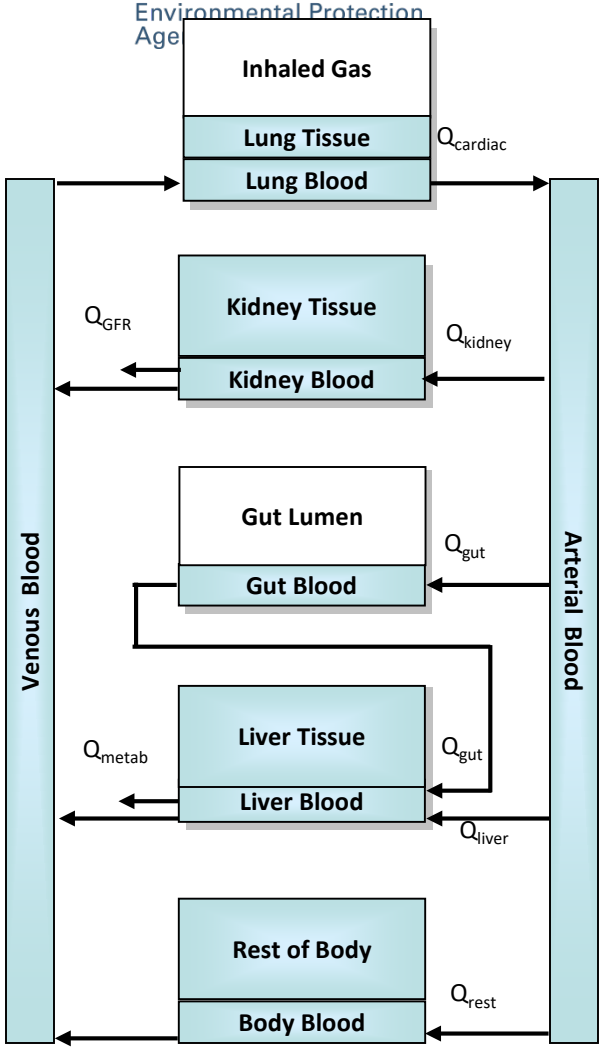
Wambaugh et al. (2015)

Reasons for C_{ss} Over-prediction - Opportunities for Refinement

- Not all routes of metabolic clearance are captured
  - Extrahepatic (intestinal, renal, etc.) metabolism
  - Non-hepatocyte-mediated clearance
- Hepatocyte suspensions unable to detect clearance of low turnover compounds
- Absorption / Bioavailability assumed 100%
- Restrictive vs. Nonrestrictive clearance
- Conservative assumptions drive poor predictive ability for chemicals known to be rapidly cleared *in vivo*

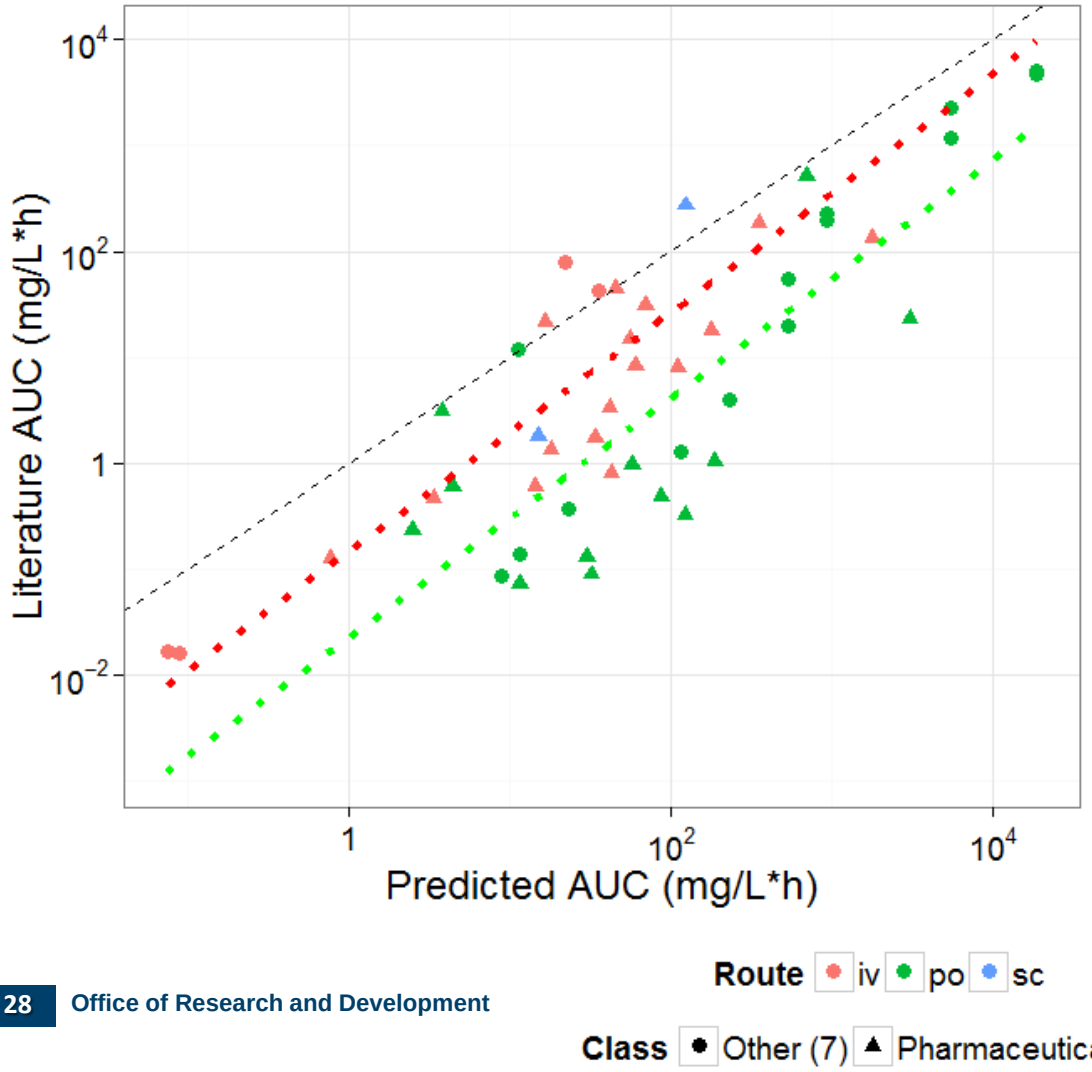
Slide from Barbara Wetmore

A General Physiologically-based Toxicokinetic (PBTK) Model



- "httk" also 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.
- 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).

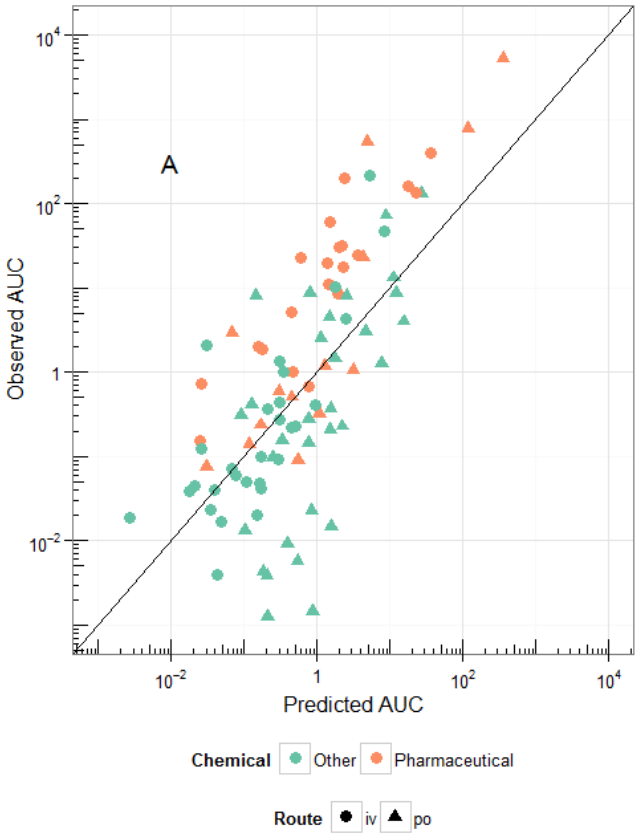
Evaluating HT-PBTK Predictions with *In Vivo* Data



- PBTK 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

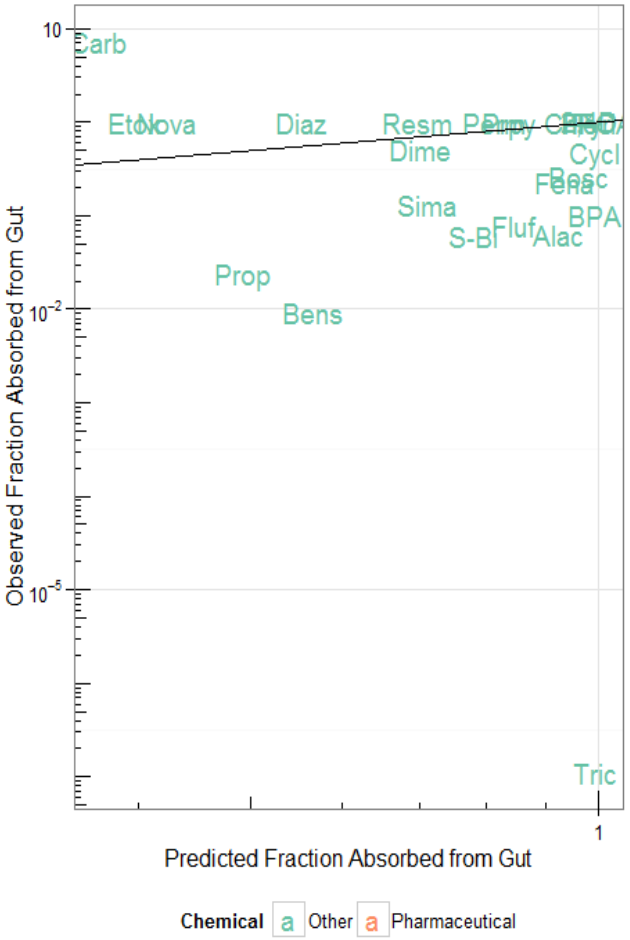
Wambaugh et al. (2015)

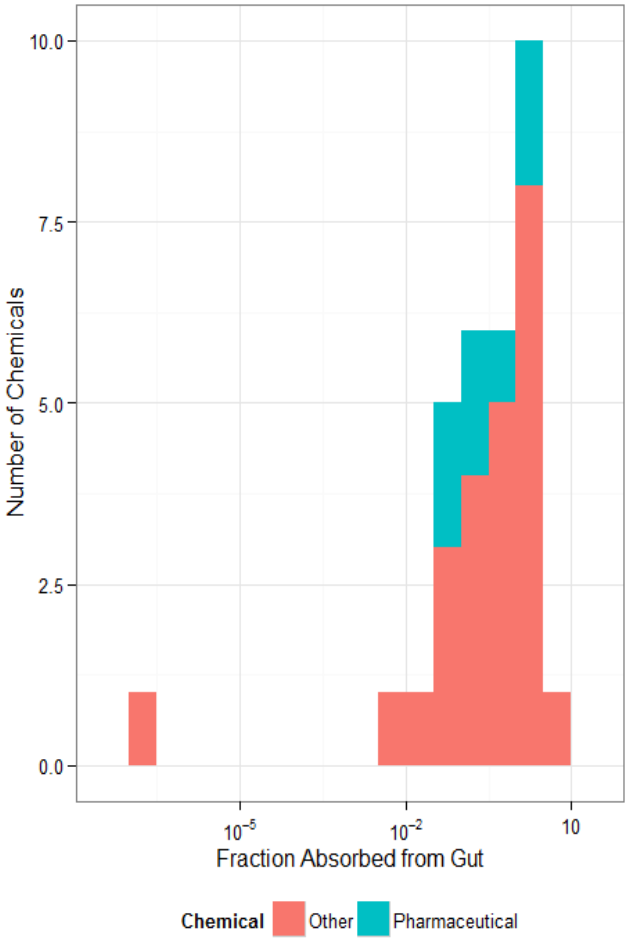
Analyzing New *In Vivo* Data (Rat)



- Oral and *iv* studies for 26 ToxCast compounds
  - Collaboration with NHEERL (Mike Hughes and Jane Ellen Simmons)
  - Additional work by Research Triangle Institute (Tim Fennell)
- Can estimate
  - Fraction absorbed
  - Absorption Rate
  - Elimination Rate
  - Volume of Distribution

Analyzing New *In Vivo* Data (Rat): Oral Absorption





- *In silico* methods do not correctly predict absorption
- Oral and *iv* studies for 26 ToxCast compounds
  - Collaboration with NHEERL (Mike Hughes)
  - Additional work by Research Triangle Institute
- Can estimate:
  - Fraction absorbed
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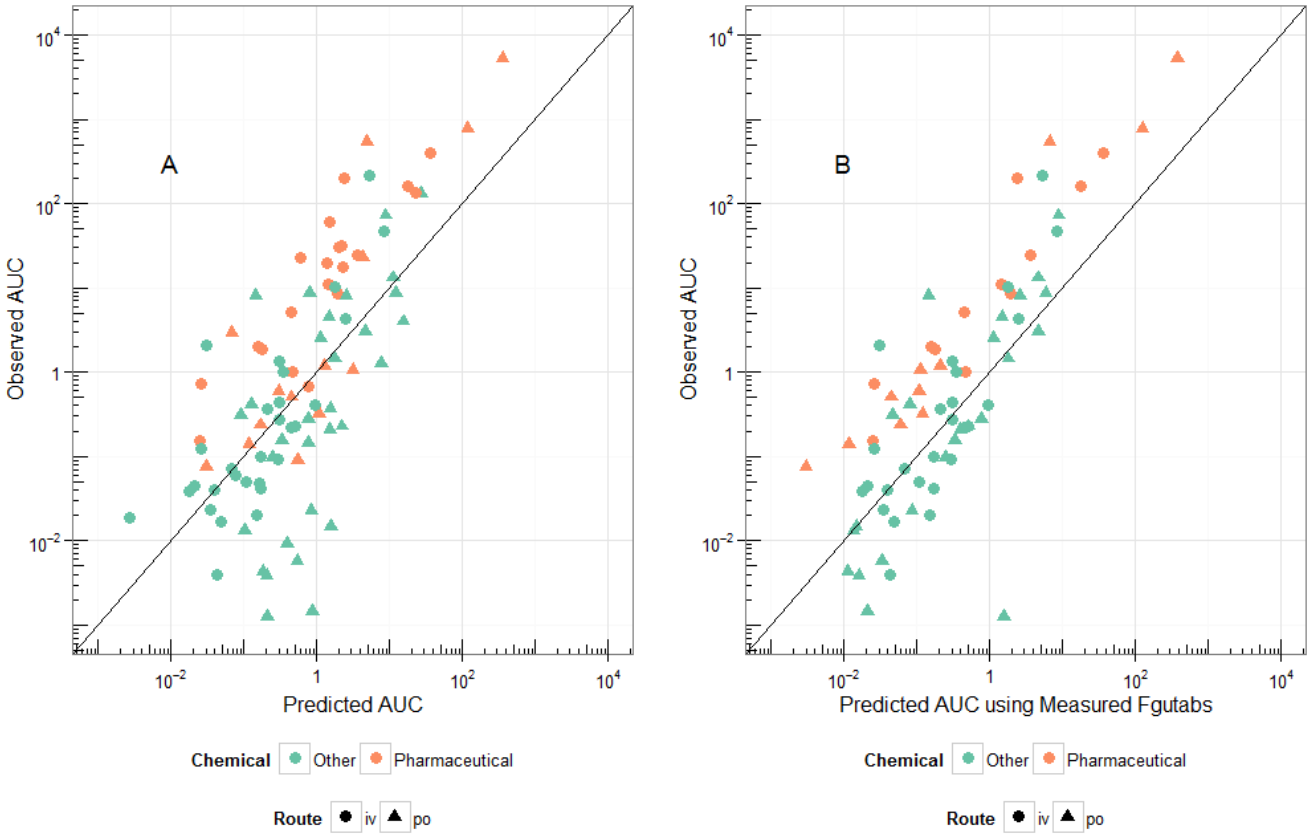
  - Elimination Rate
  - Volume of Distribution

Bioavailability prediction from Nisha Sipes, Steve Ferguson, John DiBella

Analyzing New *In Vivo* Data (Rat)



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- Can estimate
  - Fraction absorbed
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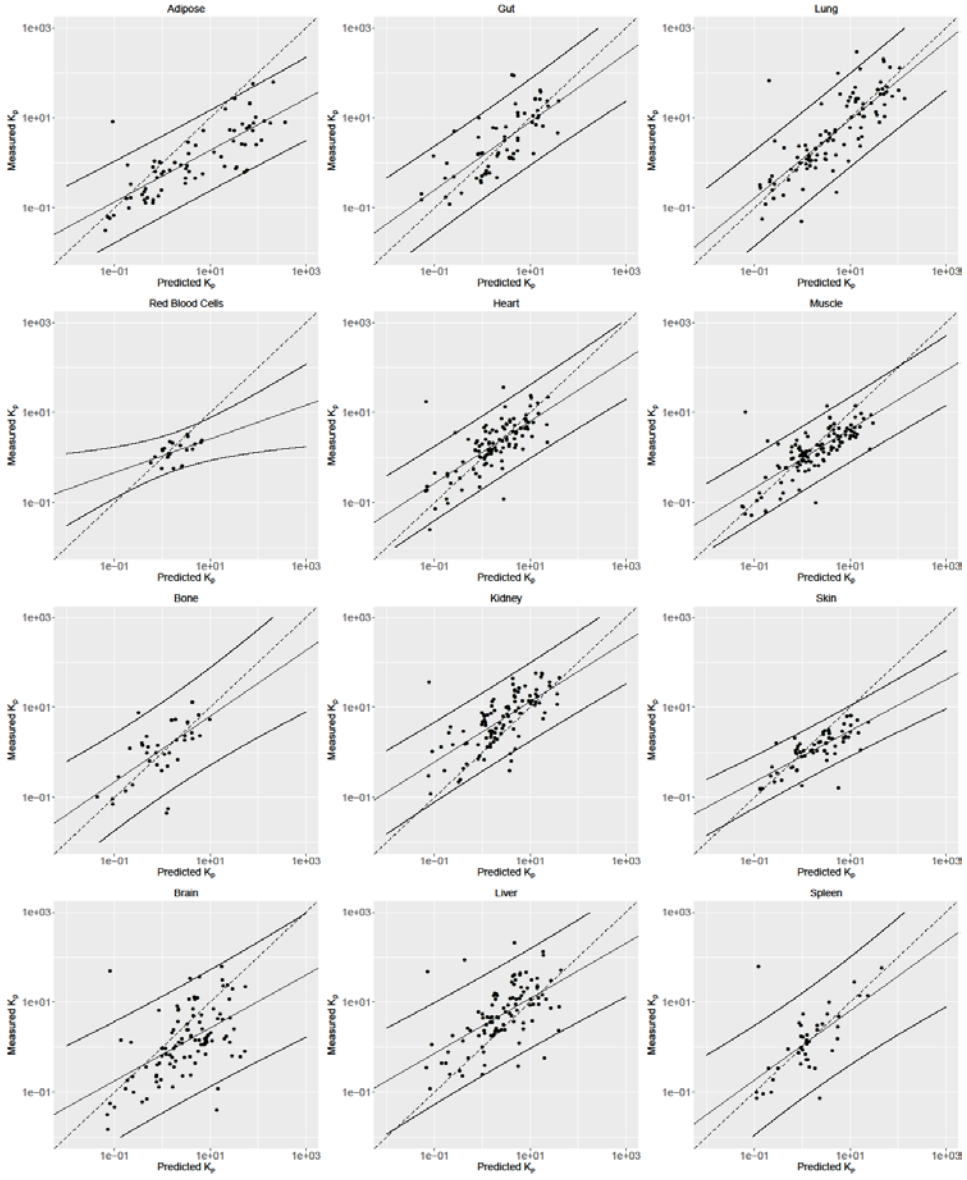
  - Elimination Rate
  - Volume of Distribution

- **Cyprotex is now measuring bioavailability (CACO2) for all HTTK chemicals**
  - Work by Derek Angus and Chris Strock

Analyzing Old *In Vivo* Data (Rat)



- Curating literature for measurements of chemical-specific partition coefficients (PC) in rat
  - 945 tissue-specific PC
  - 137 unique chemicals
- Calibrating *in silico* predictors (Schmitt, 2008) to actual performance
  - Tissue-specific estimates of predictor bias and uncertainty
- Research initiated by Woody Setzer and Jimena Davis, ongoing analysis by Robert Pearce

Figure from Robert Pearce

Evaluation of Calibrations to Rat *In Vivo* Data

- Partition coefficient calibrations were evaluated with human measured volumes of distribution for 498 chemicals from Obach (2008)
  - Volume of distribution calculated as sum of tissues weighted by partition coefficients
- Calibration to *in vivo* rat data improved 106 chemicals by at least a factor of 3
- Additional model refinements improved 61 by more than a factor of 10

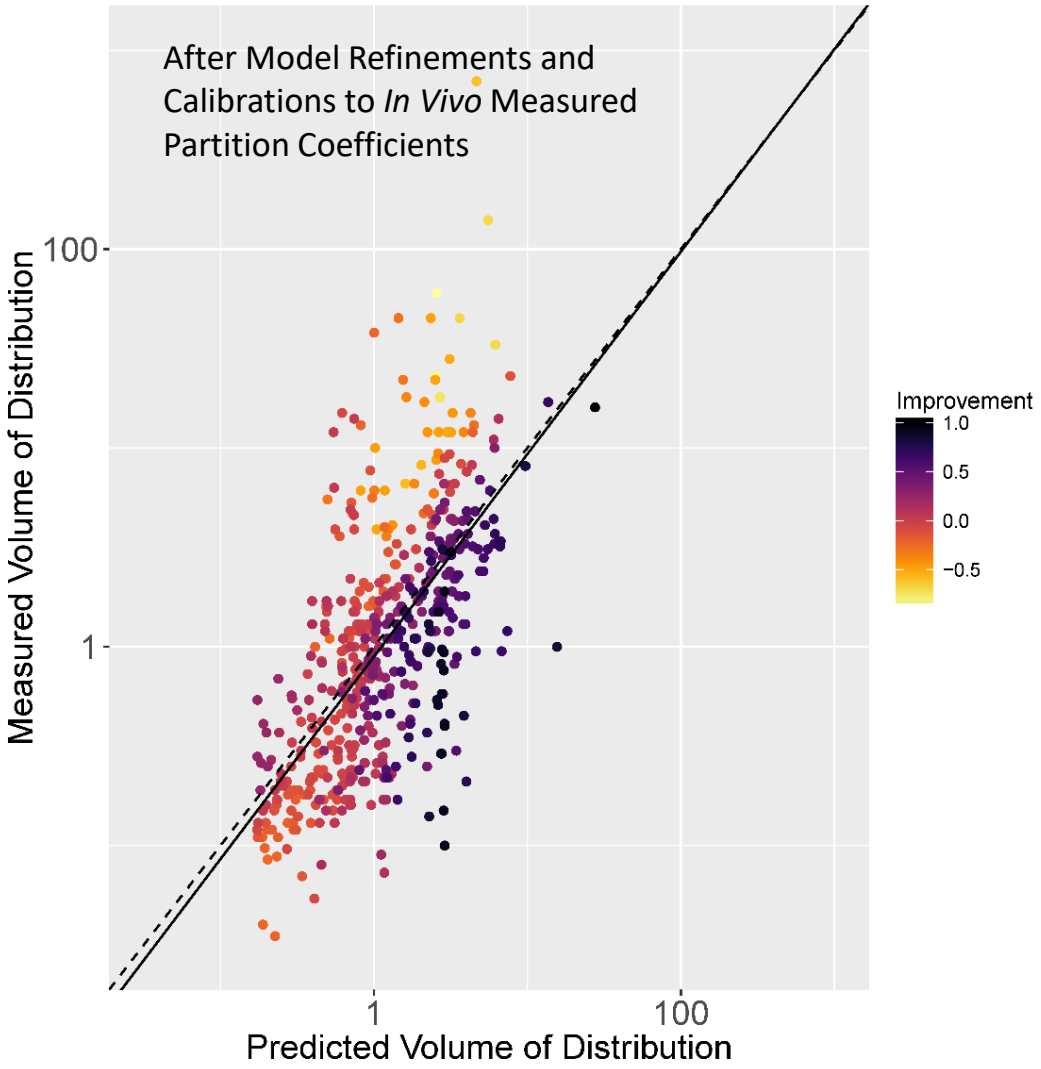


Figure from Robert Pearce

Further Evaluation with New *in vivo* Data

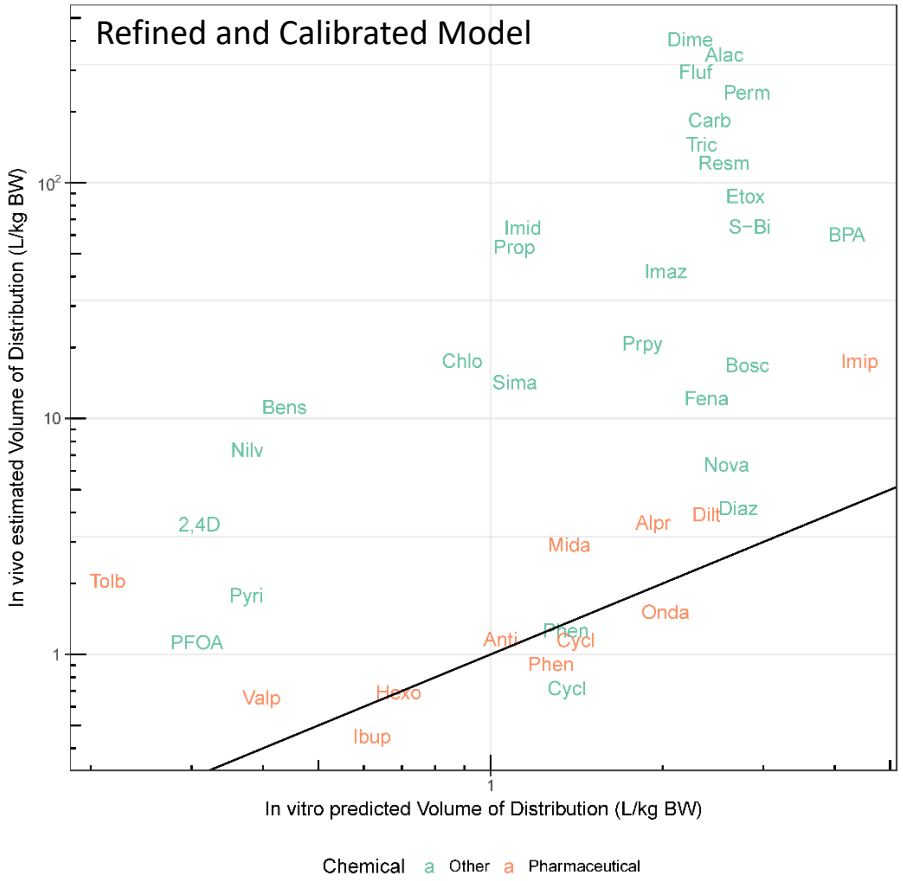


Figure from Robert Pearce

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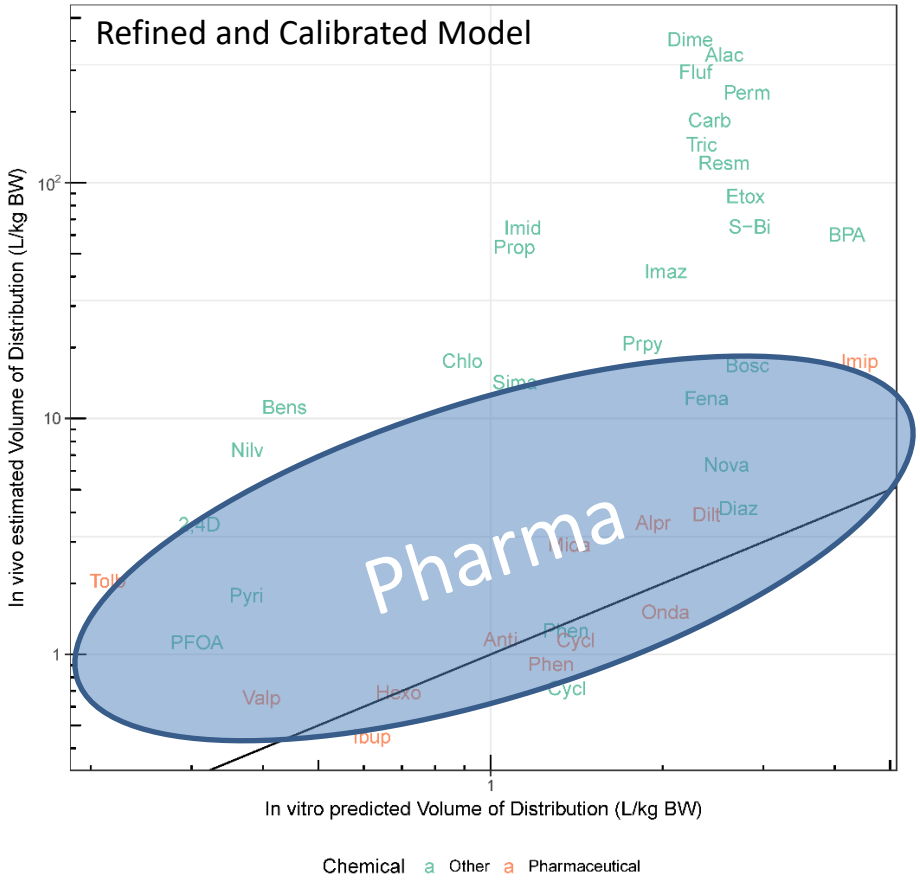

Figure from Robert Pearce

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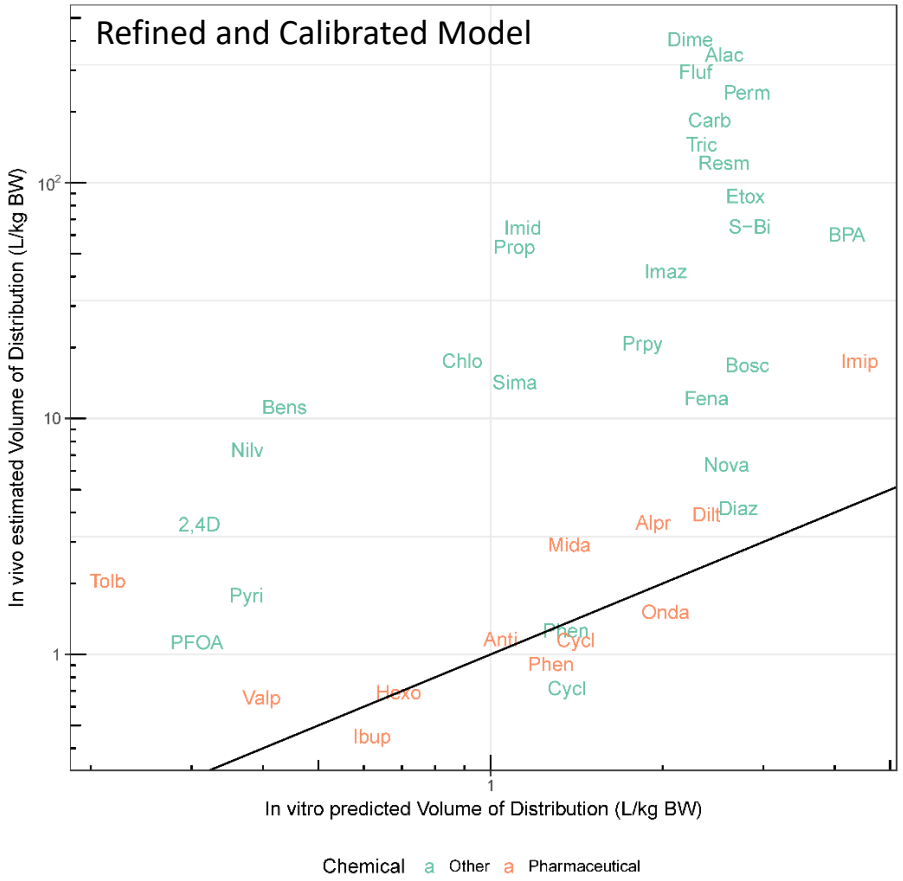
Further Evaluation with New *in vivo* Data



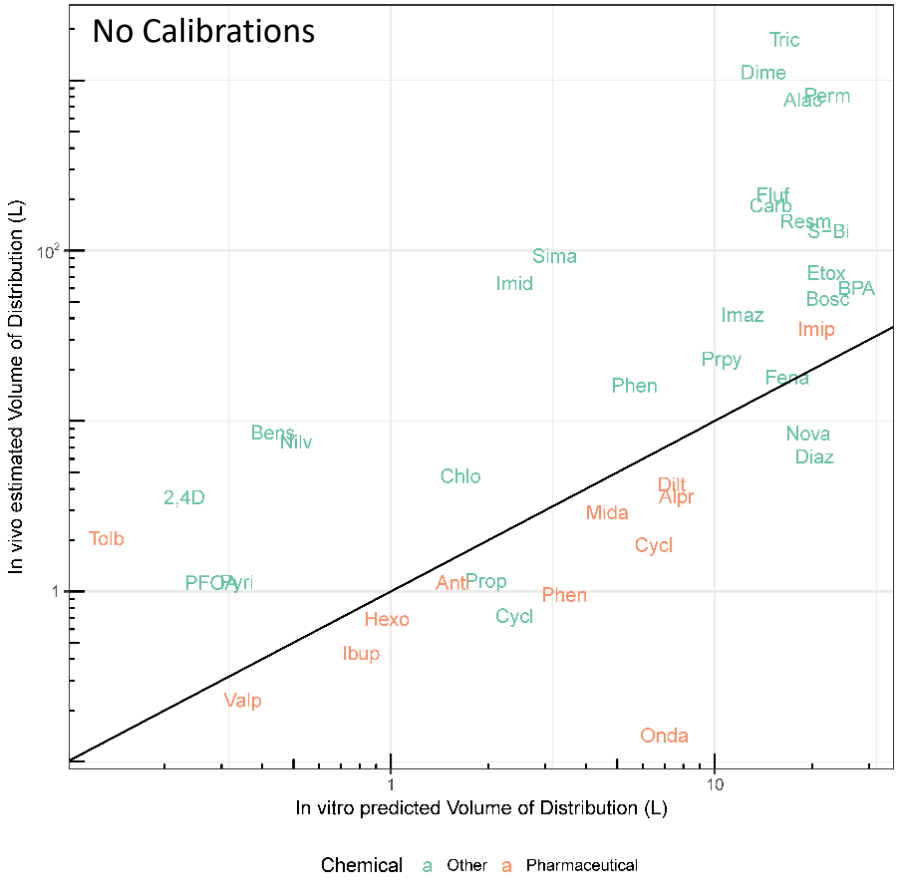


Figure from Robert Pearce

Application to High Throughput Risk Prioritization

More Plausible Biologically Active Exposures

mg/kg BW/day

ToxCast-derived Receptor Bioactivity Converted to mg/kg/day with HTT

ExpoCast Exposure Predictions

Near Field

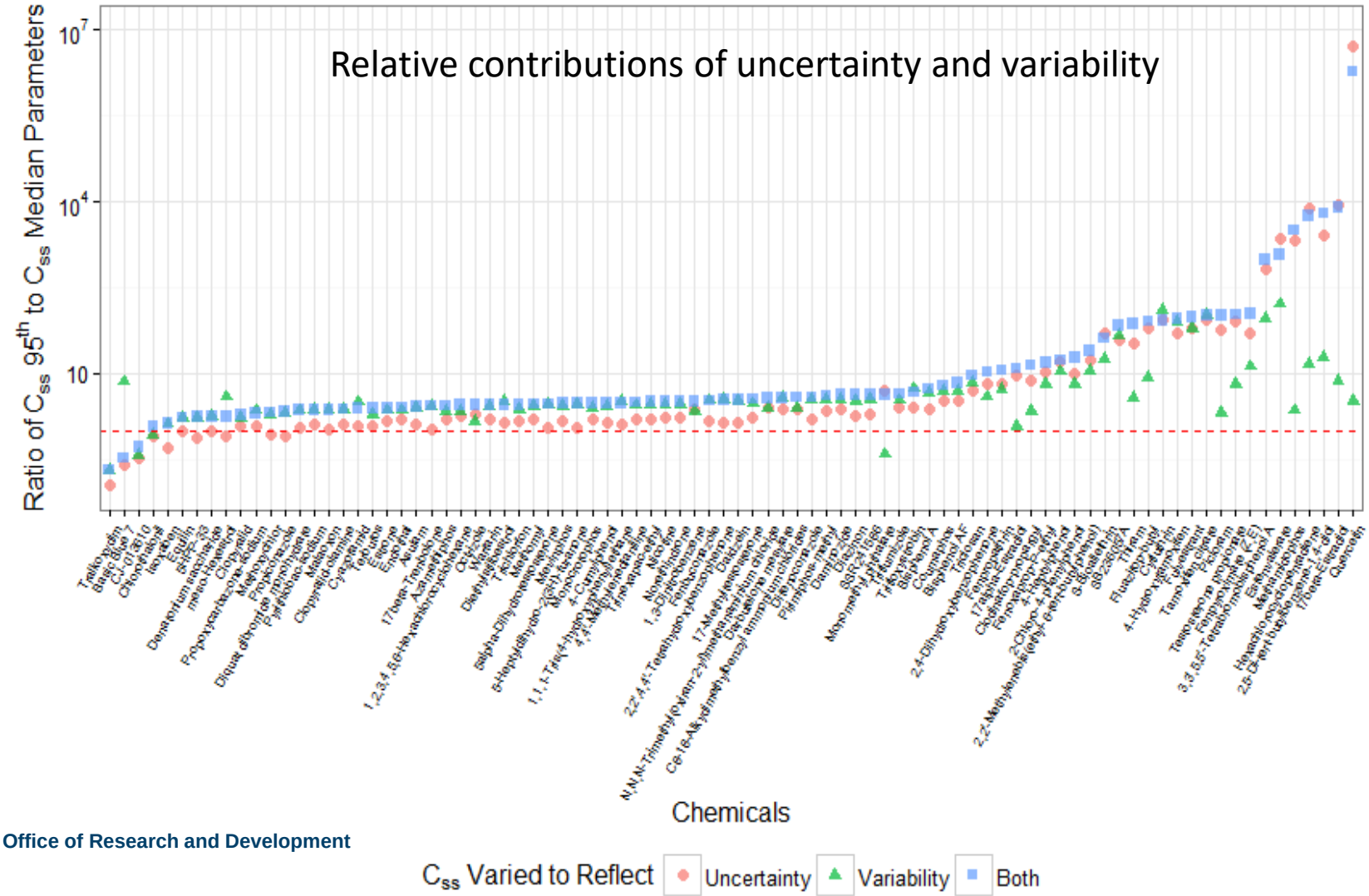
Far Field

ToxCast Chemicals

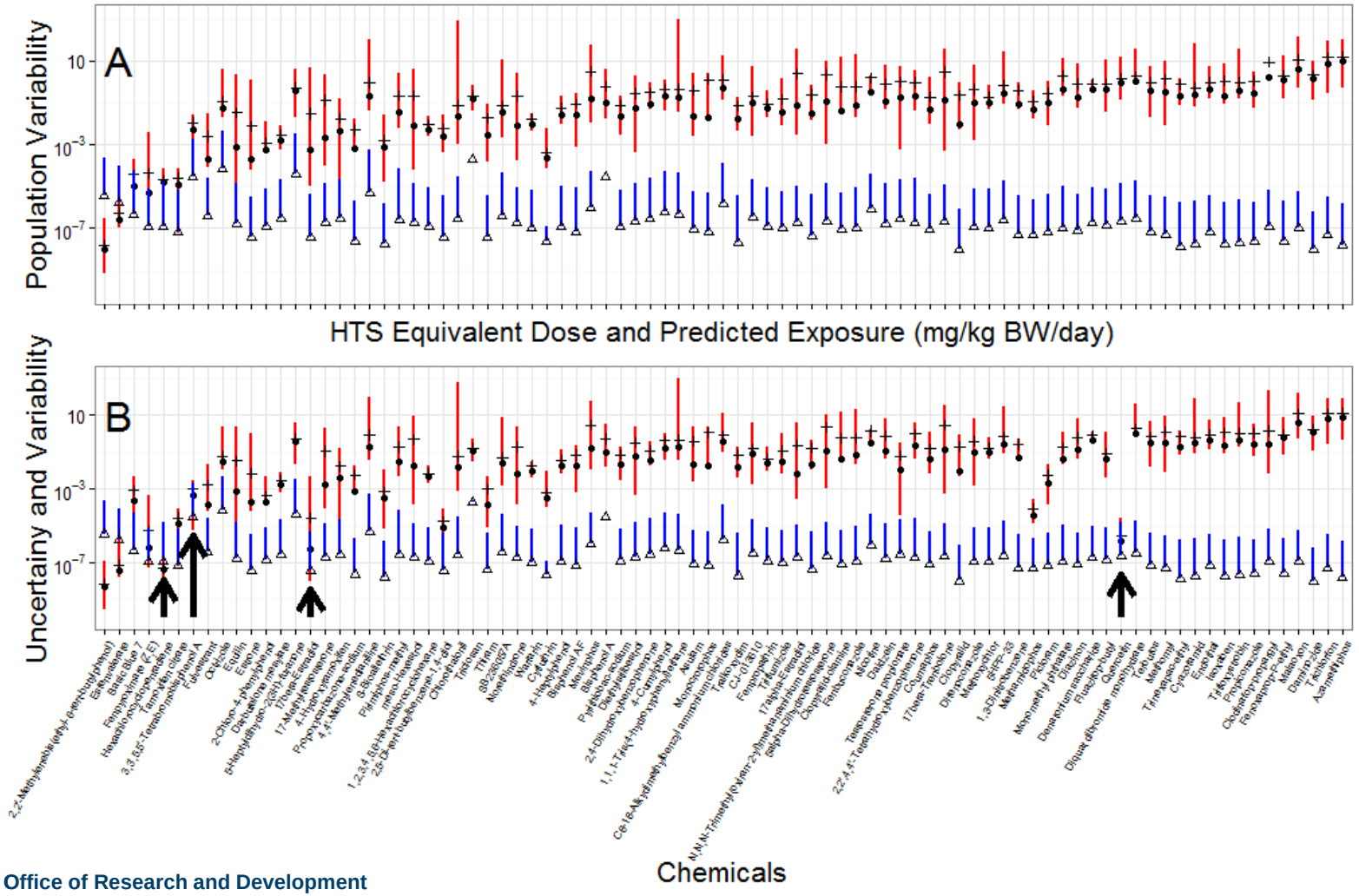
December, 2014 Panel:
"Scientific Issues Associated with Integrated Endocrine Bioactivity and Exposure-Based Prioritization and Screening"

DOCKET NUMBER:
EPA–HQ–OPP–2014–0614

Propagating Measurement Uncertainty



Propagating Measurement Uncertainty



Summary

- Toxicokinetics (TK) provides a bridge between HTS and HTE by predicting tissue concentrations due to exposure
- HTTK methods developed for pharmaceuticals have been adapted to environmental testing
- A primary application of HTTK is “Reverse Dosimetry” or RTK
  - Can infer daily doses that produce plasma concentrations equivalent to the bioactive concentrations and reconstruct exposure from biomarkers, **but:**
- We must consider “domain of applicability”. One way is to evaluate against *in vivo* data for large numbers of chemicals
  - Collected new PK data from *in vivo* studies (EPA/NHEERL and Research Triangle Institute)
  - Organizing data from literature studies into computable format
- 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
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Risa Sayre*
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