High-Throughput Toxicokinetics for Rapid Risk Prioritization

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NARROWING THE IN VITRO TO IN VIVO TRANSLATION GAP

Predicting Drug Toxicity
June 21, 2018

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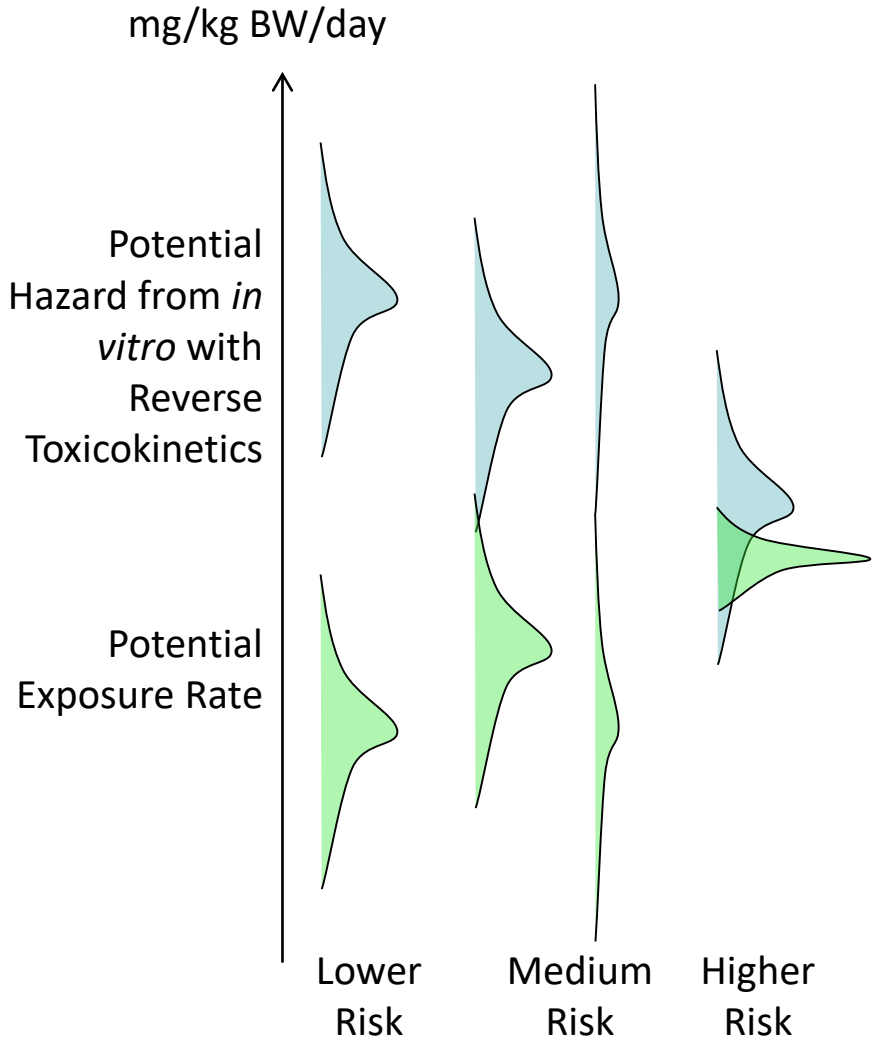
Chemical Regulation in the United States

- Park *et al.* (2012): At least 3221 chemicals in pooled human blood samples, many appear to be exogenous
- 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)
- Most other 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 updated in June, 2016 and new approach methodologies (NAMs) are being considered prioritize these existing and new chemicals for testing

November 29, 2014

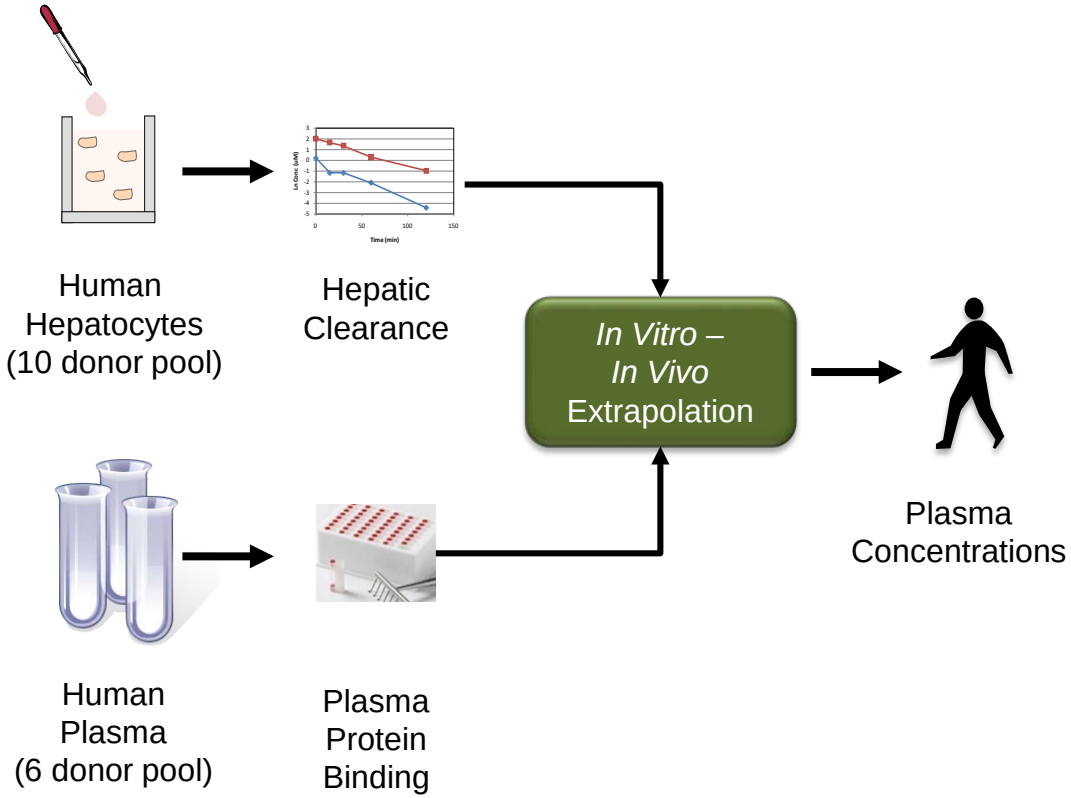
Chemical Risk = Hazard + Exposure

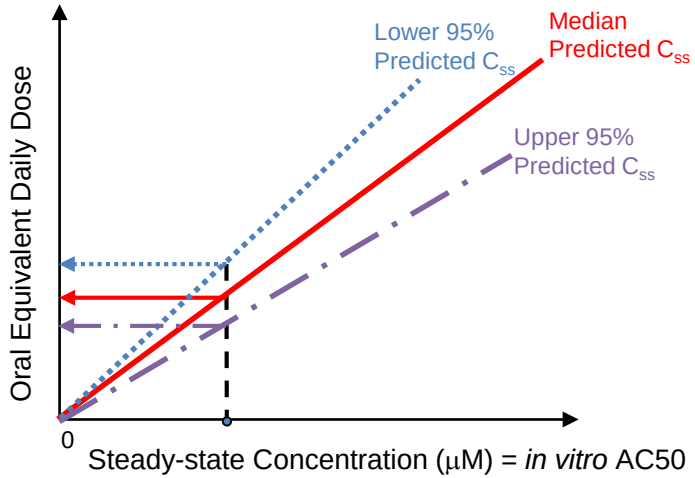
- National Research Council (1983) identified chemical risk as a function of both inherent hazard and exposure
- To address thousands of chemicals, we need to use NAMs to prioritize those chemicals most worthy of additional study
- **High throughput risk prioritization** needs:
  1. high throughput **hazard** 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



Rotroff et al. (2010)
Wetmore et al. (2012, 2014, 2015)

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



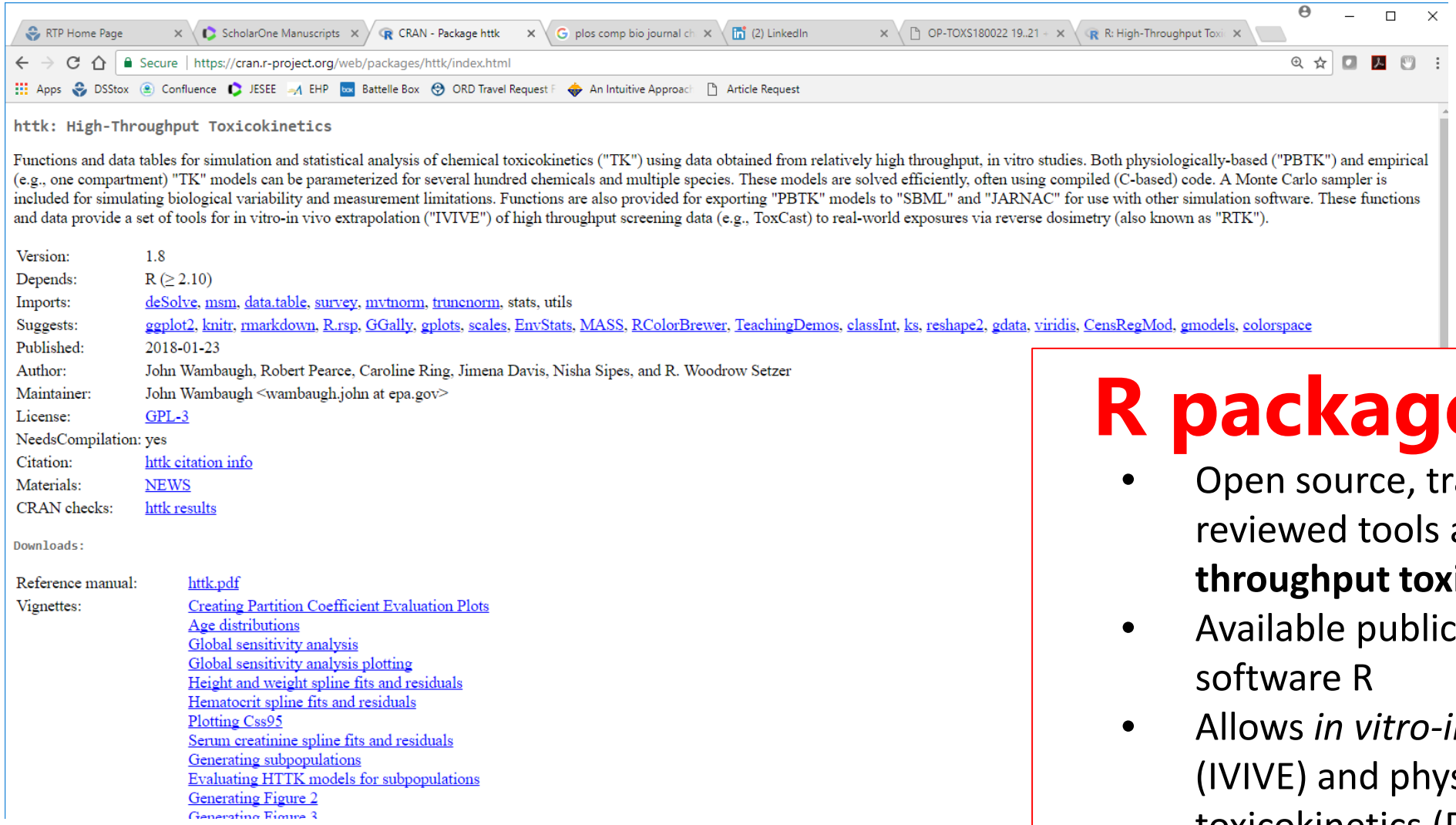


Rotroff et al. (2010) 35 chemicals
Wetmore et al. (2012) +204 chemicals
Wetmore et al. (2015) +163 chemicals
Wambaugh et al. (in prep.) + ~300 chemicals

- **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)

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

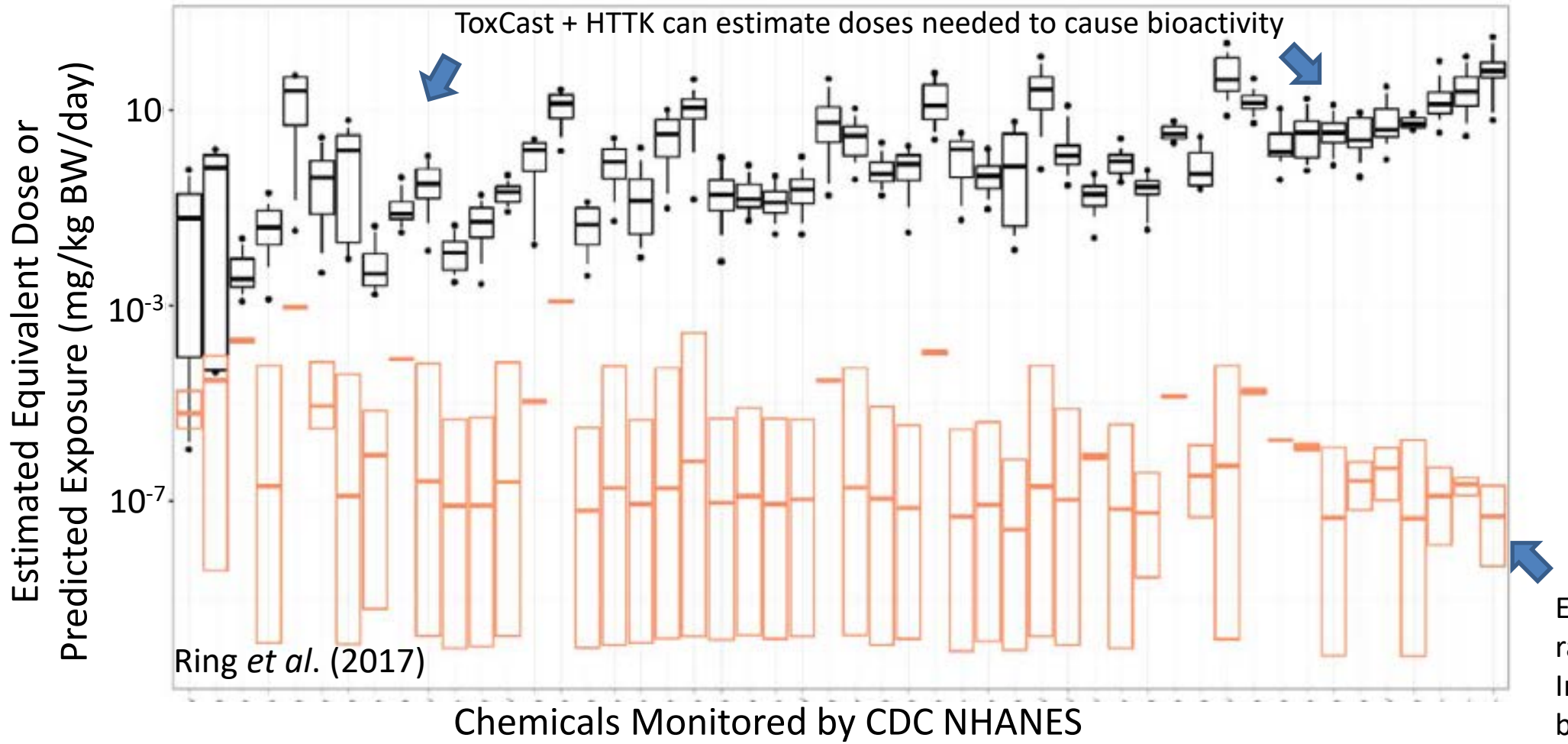
Downloads:

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-base toxicokinetics (PBTK)

High Throughput Risk Prioritization



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)

Variability

Different crayons have different colors, and none of them are the “average” color

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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

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

Predict physiological quantities

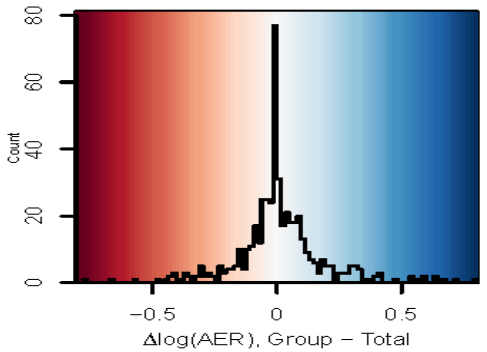
Tissue masses
Tissue blood flows
GFR (kidney function)
Hepatocellularity

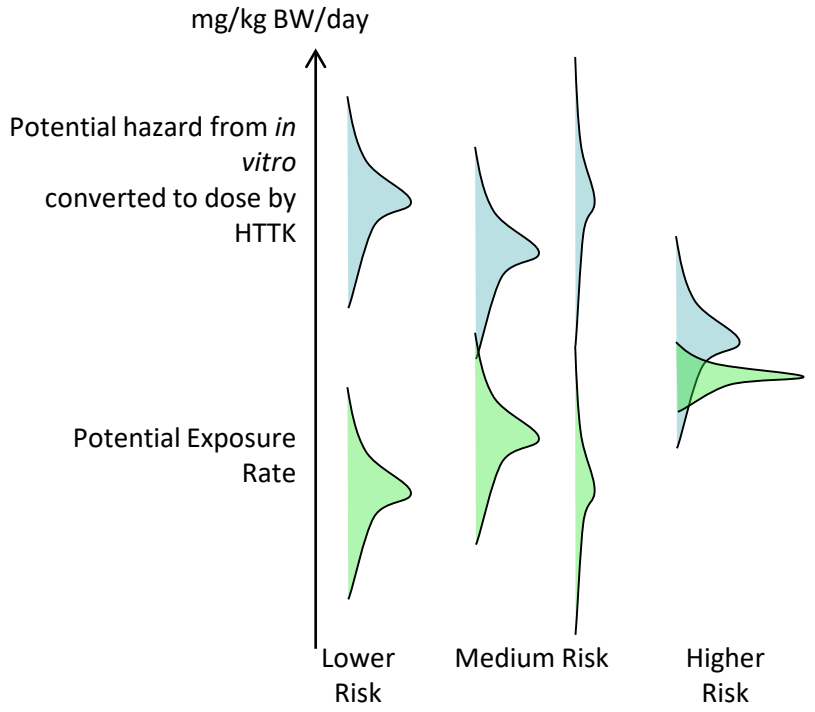
(Similar approach used in SimCYP [Jamei et al. 2009], GastroPlus, PopGen [McNally et al. 2014], P3M [Price et al. 2003], physB [Bosgra et al. 2012], etc.)

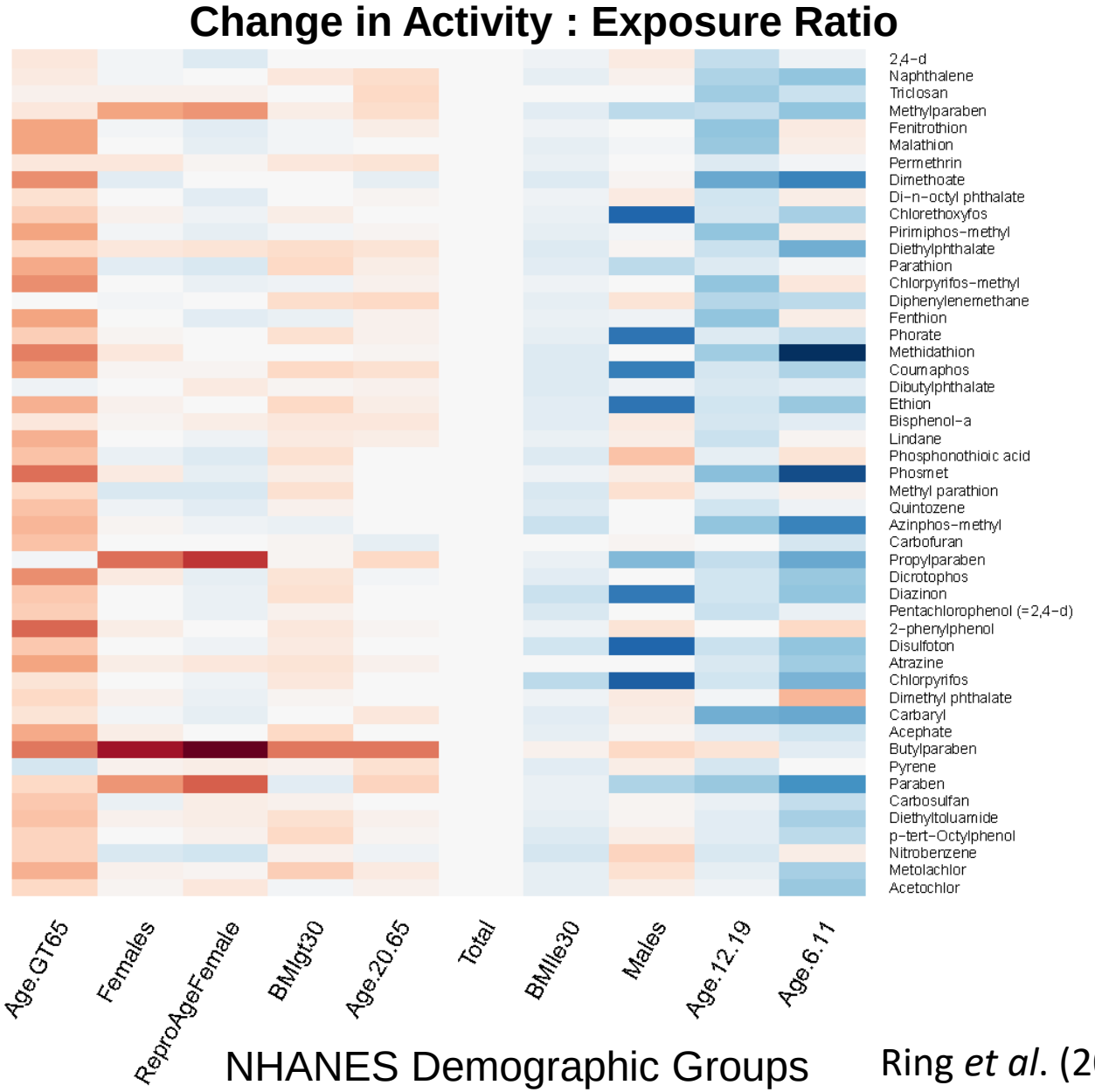
Ring *et al.* (2017)

Life-stage and Demographic Specific Predictions

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

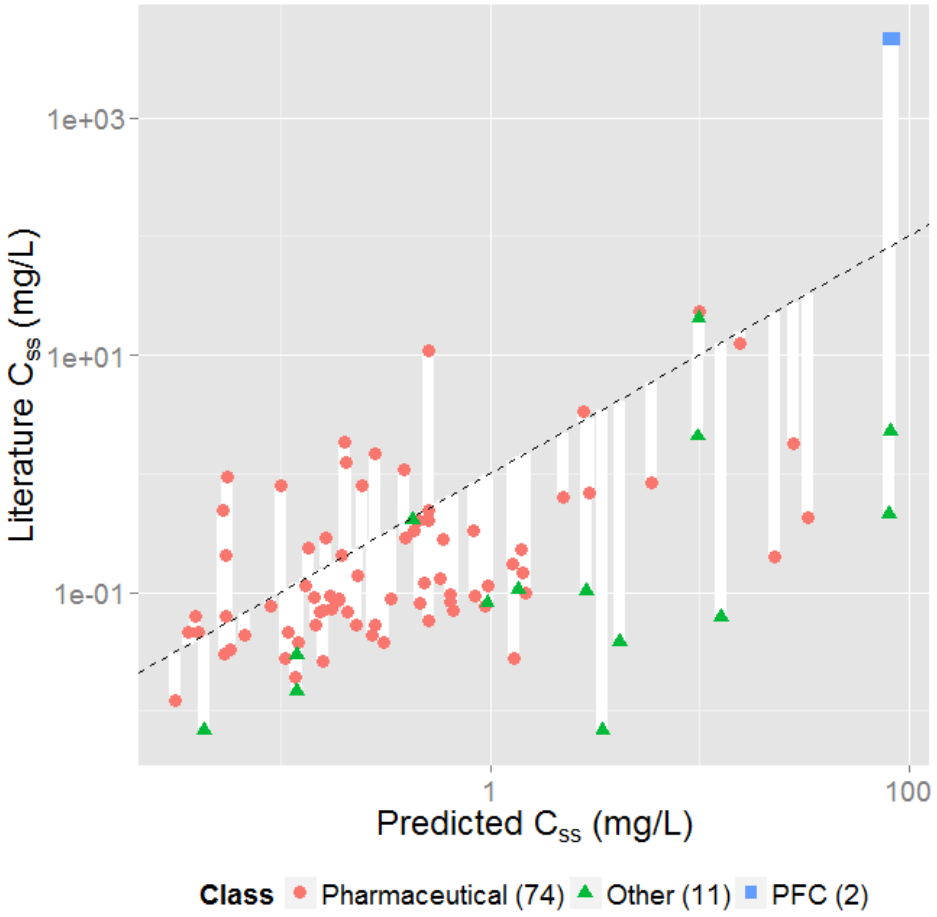




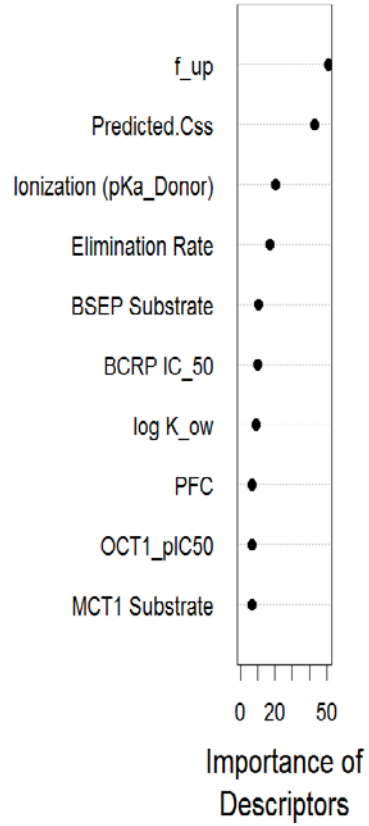


Ring *et al.* (2017)

Using *in vivo* Data to Evaluate RTK



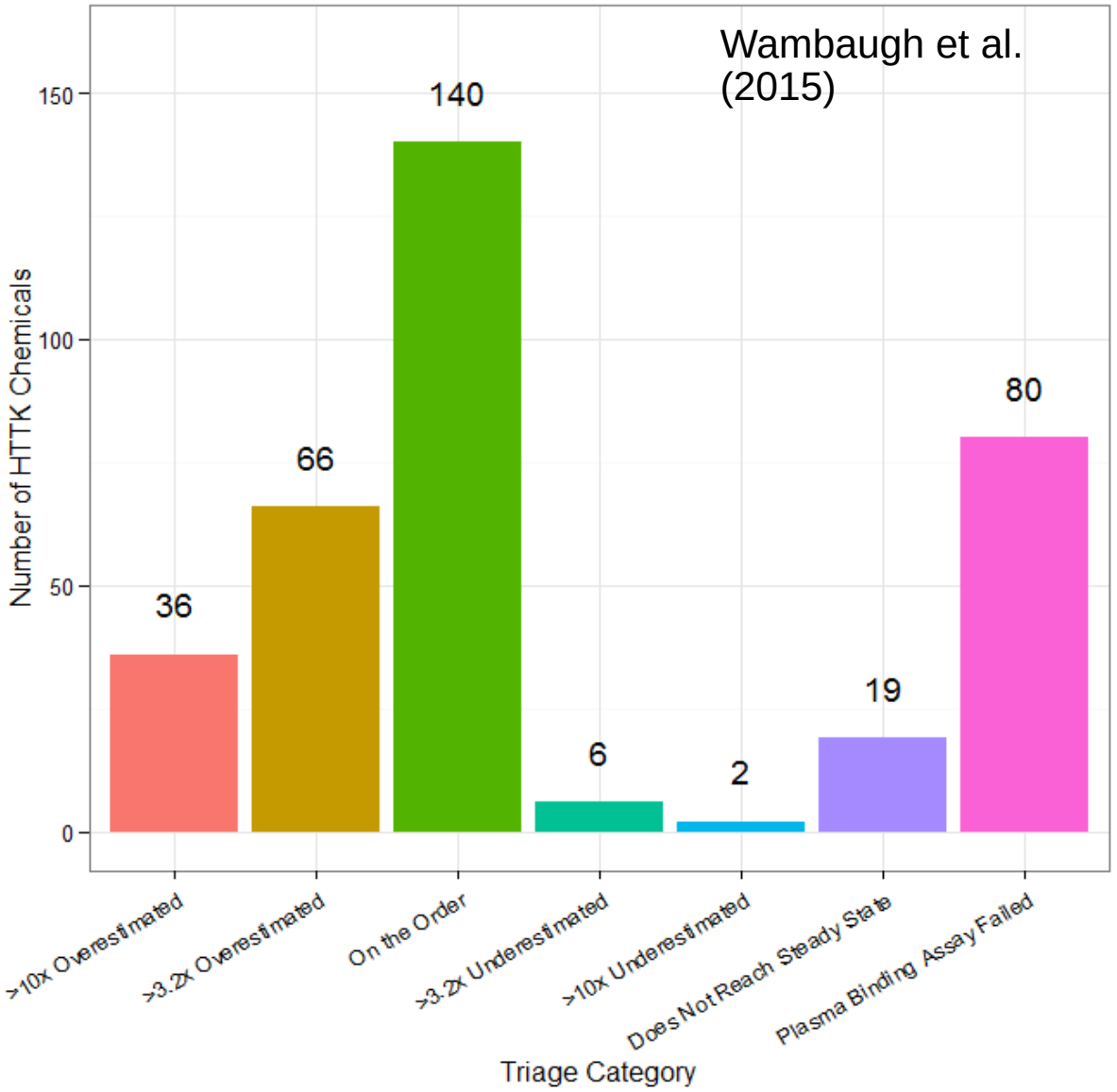
- When we compare the C_{ss} predicted from *in vitro* HTTK with *in vivo* C_{ss} values determined from the literature we find limited correlation (R^2 ~0.34)
- The dashed line indicates the identity (perfect predictor) line:
  - Over-predict for 65
  - Under-predict for 22
- The white lines indicate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



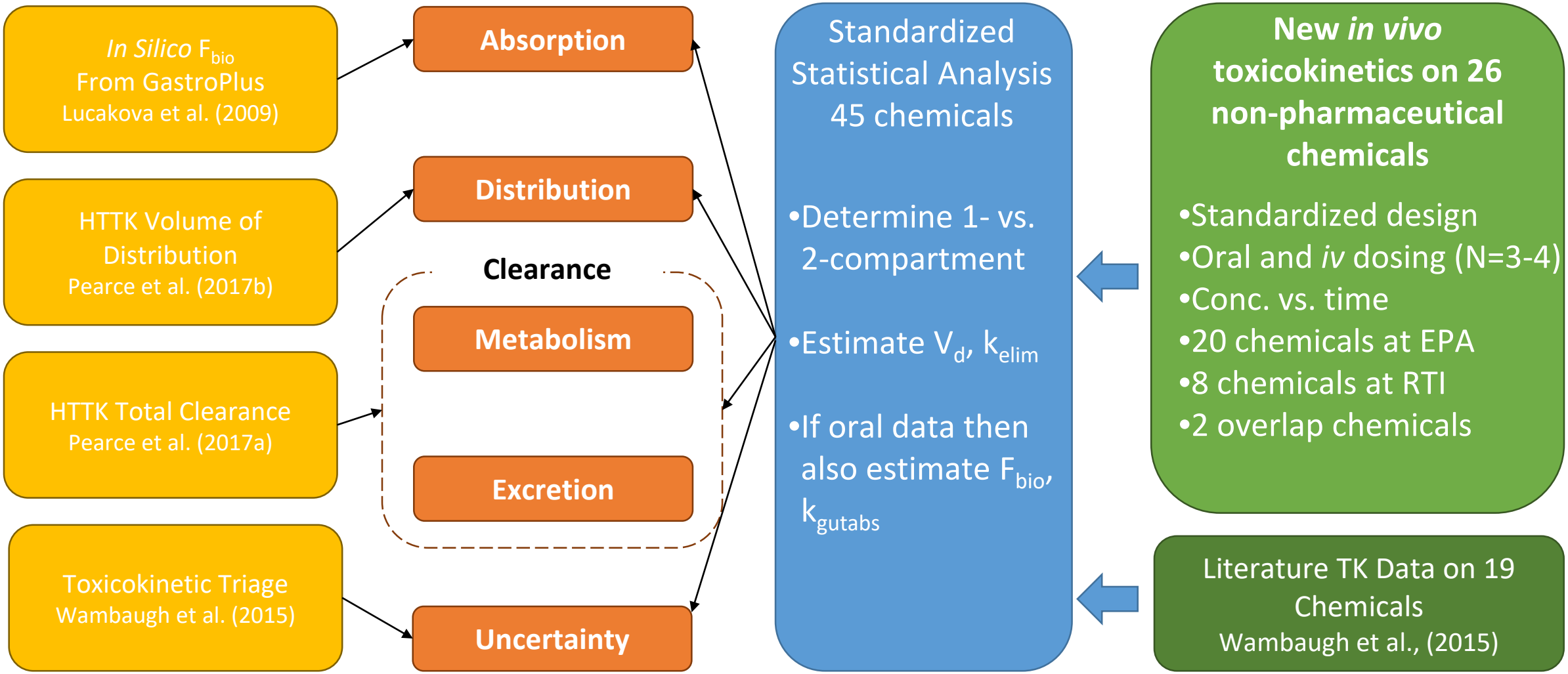
Wambaugh et al. (2015)

Toxicokinetic Triage

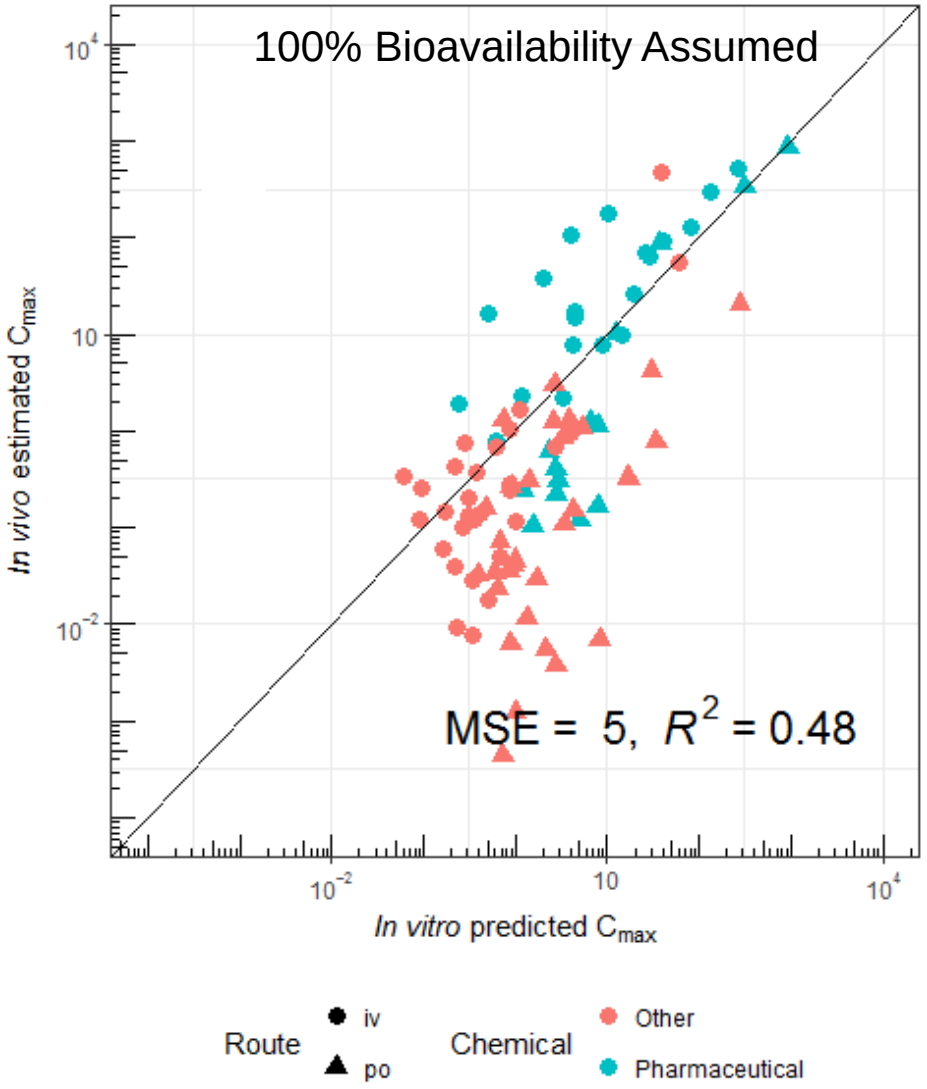
- Through comparison to *in vivo* data, a cross-validated (random forest) predictor of success or failure of HTTK has been constructed
- Add categories for chemicals that do not reach steady-state or for which plasma binding assay fails
- All chemicals can be placed into one of seven confidence categories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



New Data for Evaluation

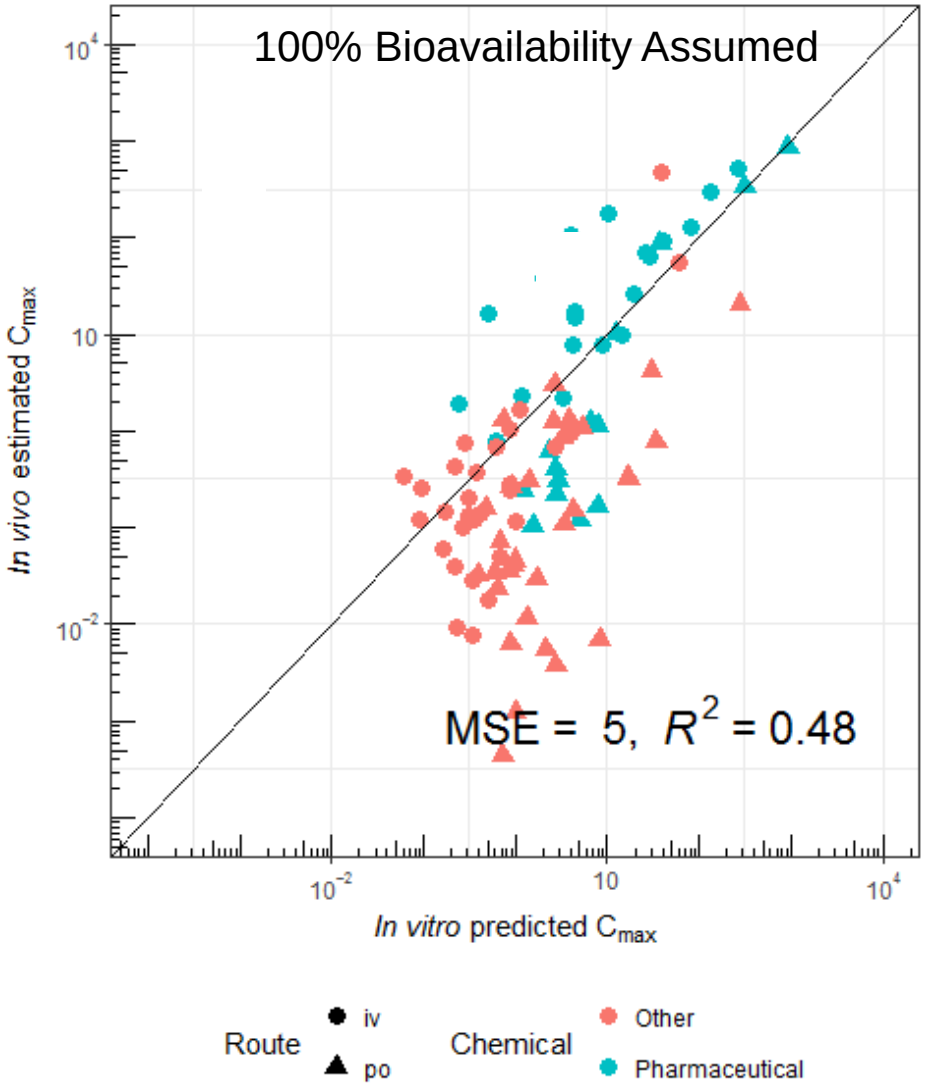


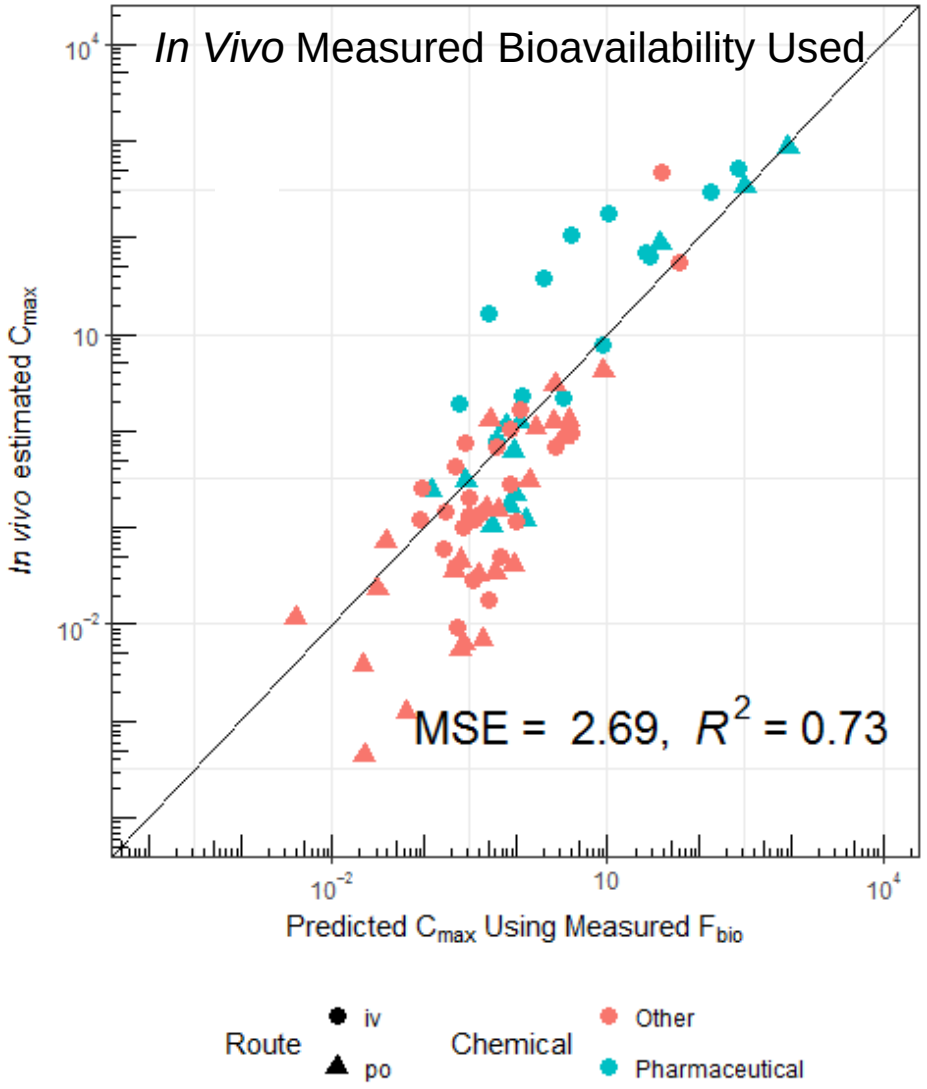
Impact of Oral Bioavailability



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

Impact of Oral Bioavailability



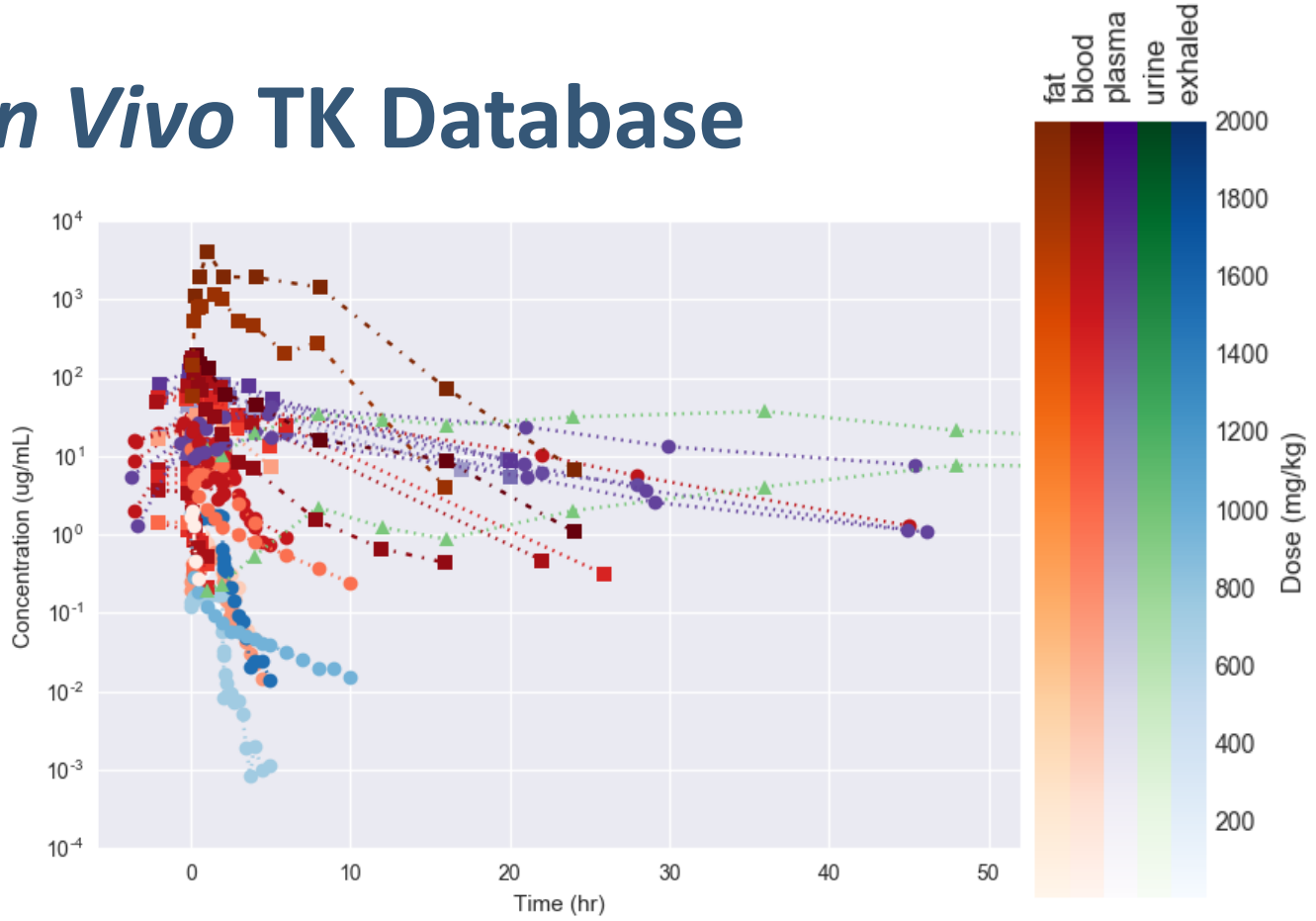


Greg Honda (NCCT) made a SOT2018 presentation on using Caco2 *in vitro* data to predict absorption for ~300 ToxCast chemicals

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:
  - 175 analytes (EPA, National Toxicology Program, literature)
  - Routes: Intravenous, dermal, oral, sub-cutaneous, and inhalation exposure
  - Species: dog, frog, human, monkey, mouse, rabbit, rat
  - Media: plasma, as well as adipose, bile, blood, brain, digestive tract, exhaled air, heart, kidney, liver, lung, muscle, pancreas, serum, skin, spleen, testes, thymus, urine
  - Multiple studies per chemical



- 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

Risa Sayre and Chris Grulke

Uncertainty

Until I open the box, I don't know what colors I have...

...especially if my five-year-old has been around.

Analytical Chemistry is an HTTK Bottleneck

- Need to develop a chemical-specific method for quantitating amount of chemical *in vitro*
  - This is very different from HTS where same readout (e.g., bioluminescence) can be used for most chemicals

- In Wetmore et al. (2012), the rapid equilibrium dialysis (RED) assay (Waters et al. 2008) failed for fraction unbound in plasma (f_{up}) 38% of the chemicals.

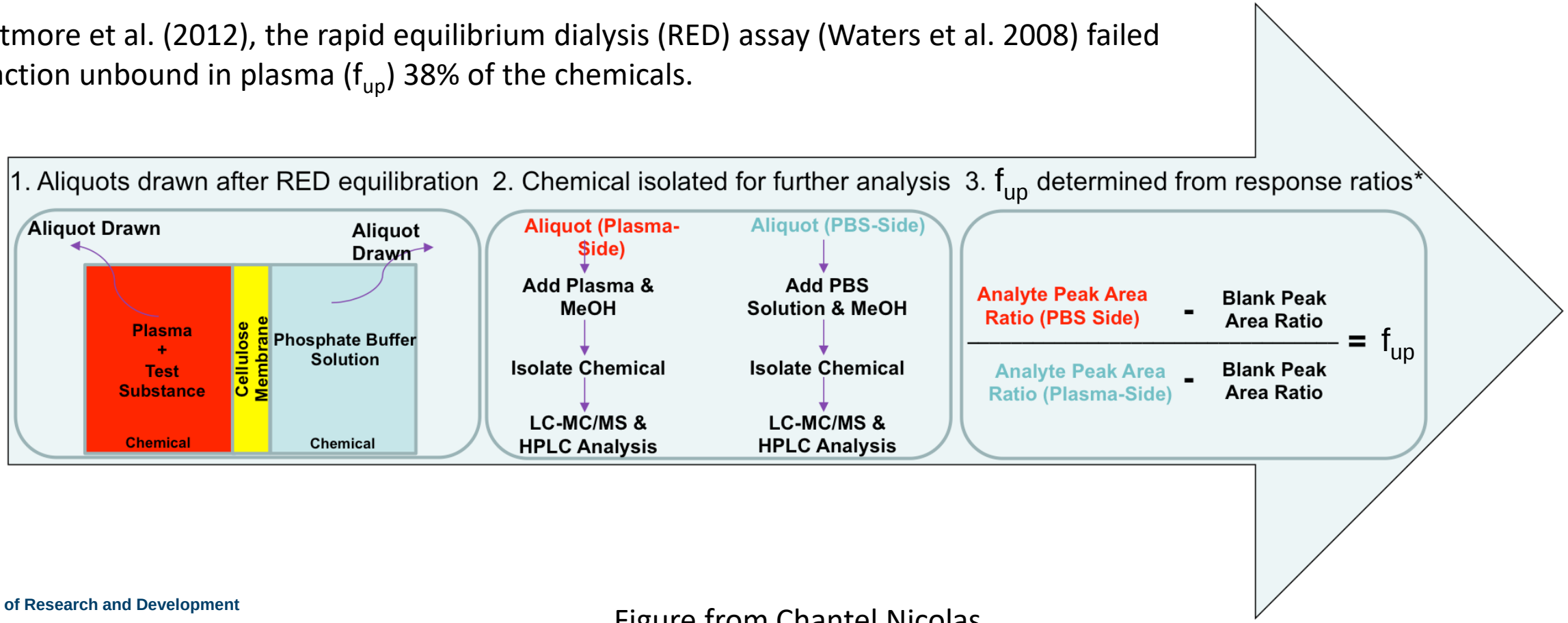
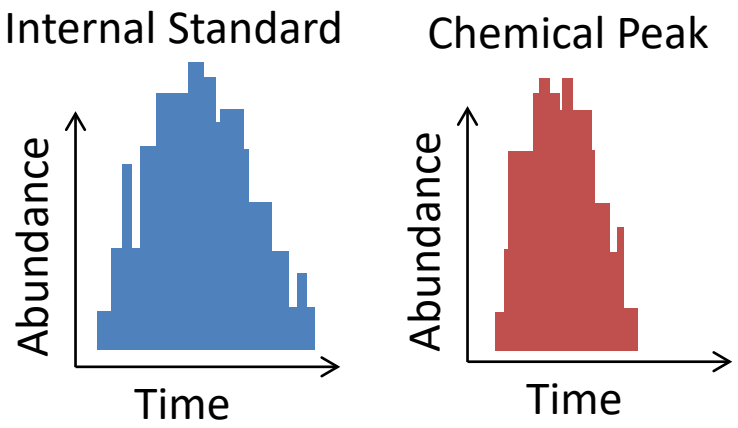


Figure from Chantel Nicolas

New HTTK Measurements and Uncertainty Analysis

The HTTK *in vitro* assays need to measure differences in chemical concentration



- Area of the internal standard (ITSD) at a known, fixed concentration fluctuates with time
- Find a peak that corresponds to chemical of interest, and then follow the ratio R of the chemical peak to the ITSD

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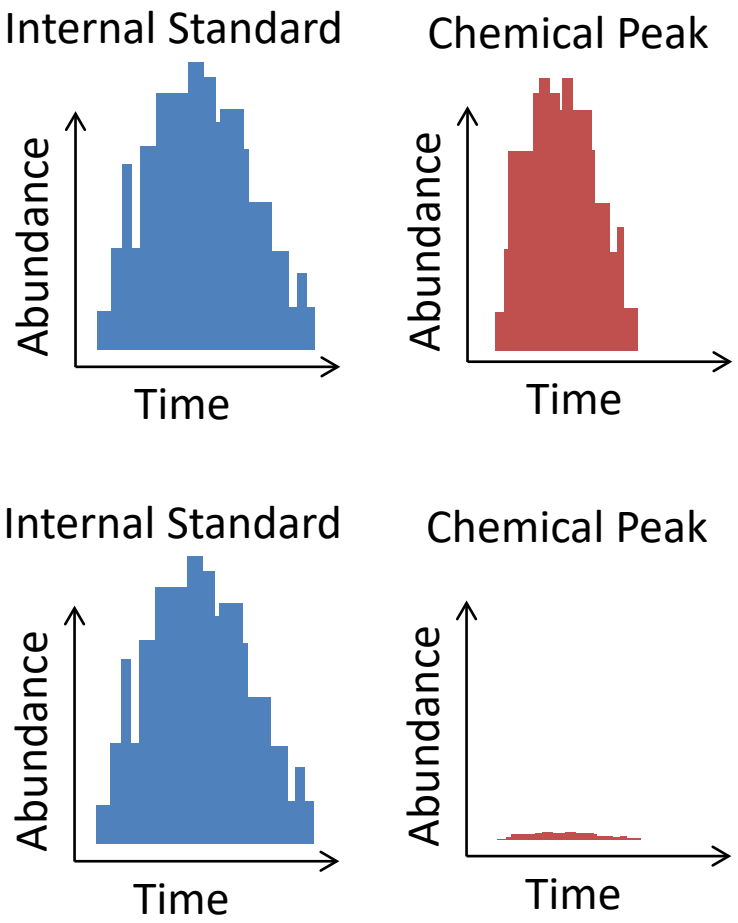
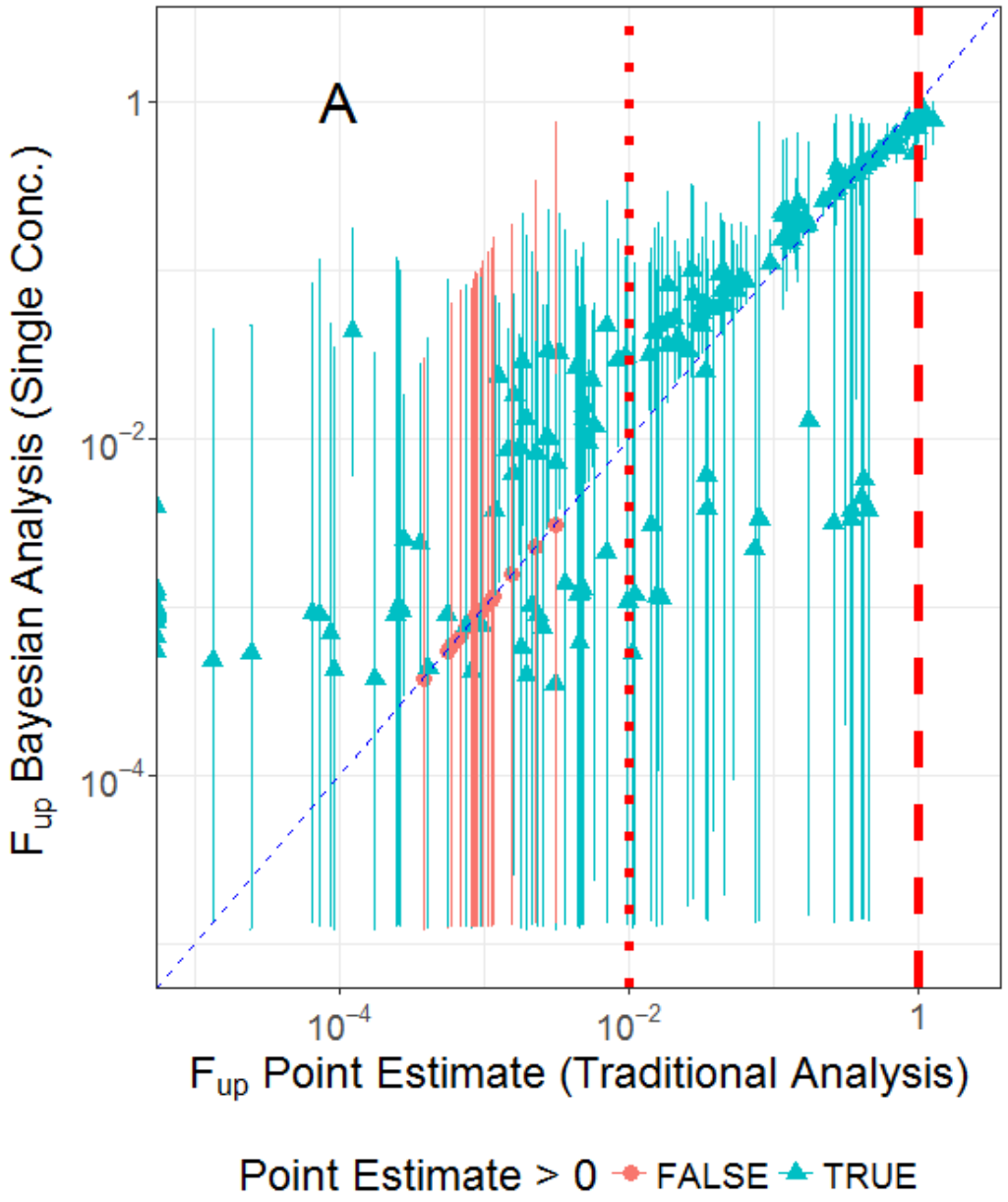


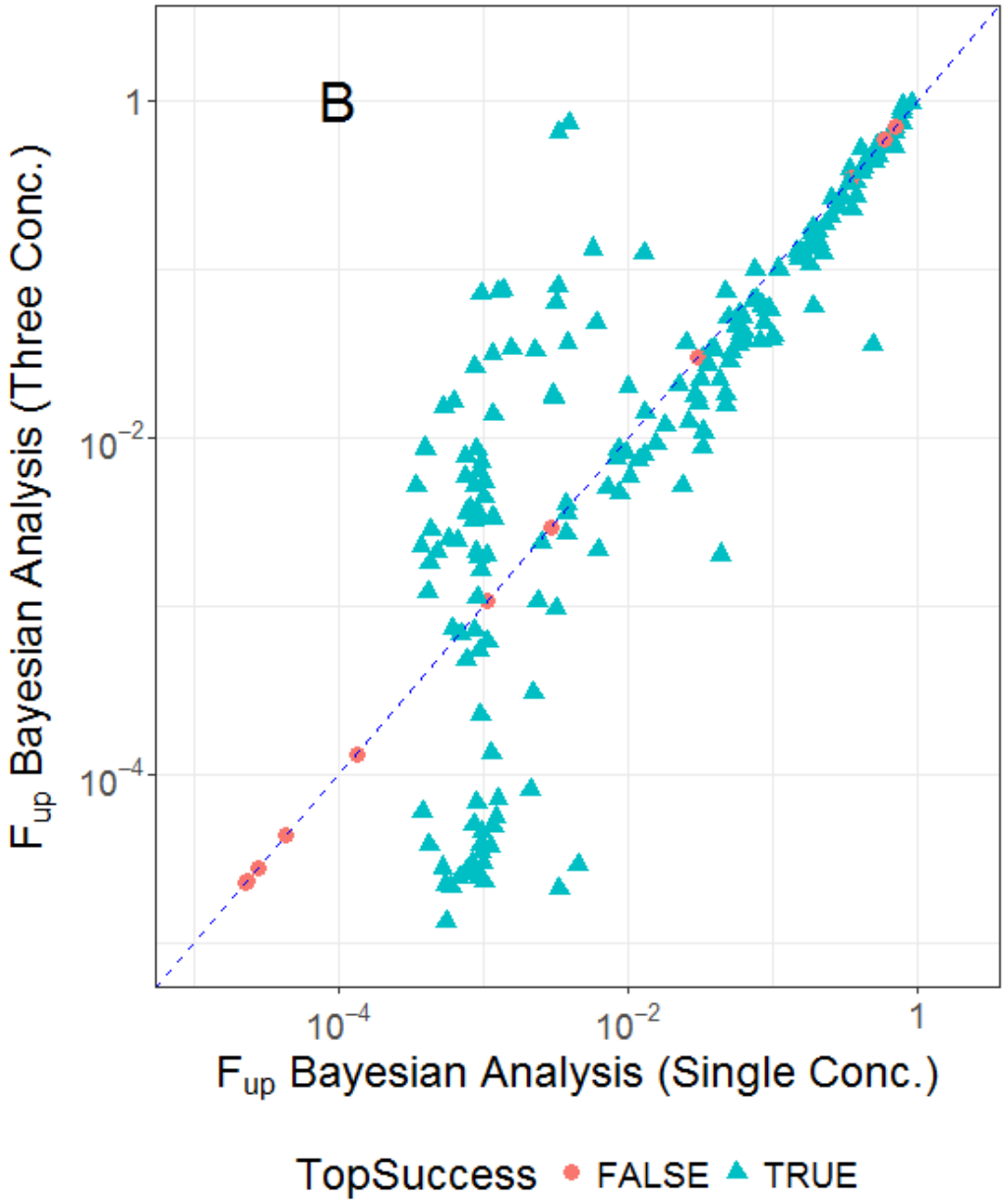
- Area of the internal standard (ITSD) at a known, fixed concentration fluctuates with time
- Find a peak that corresponds to chemical of interest, and then follow the ratio R of the chemical peak to the ITSD
- For new measurements HTTK (>200 compounds to data) performed by Cyprotex, we have modified RED protocol to use a titration of plasma protein (10%, 30%, 100%) of physiological concentration
  - Keeps chemical concentration in the same range
- Analyzed data in Bayesian framework that included a model for analytical chemistry
  - Bayesian approach gives a credible interval (range of values that would be consistent with the data) – quantitative uncertainty

Results of Bayesian Analysis for PPB



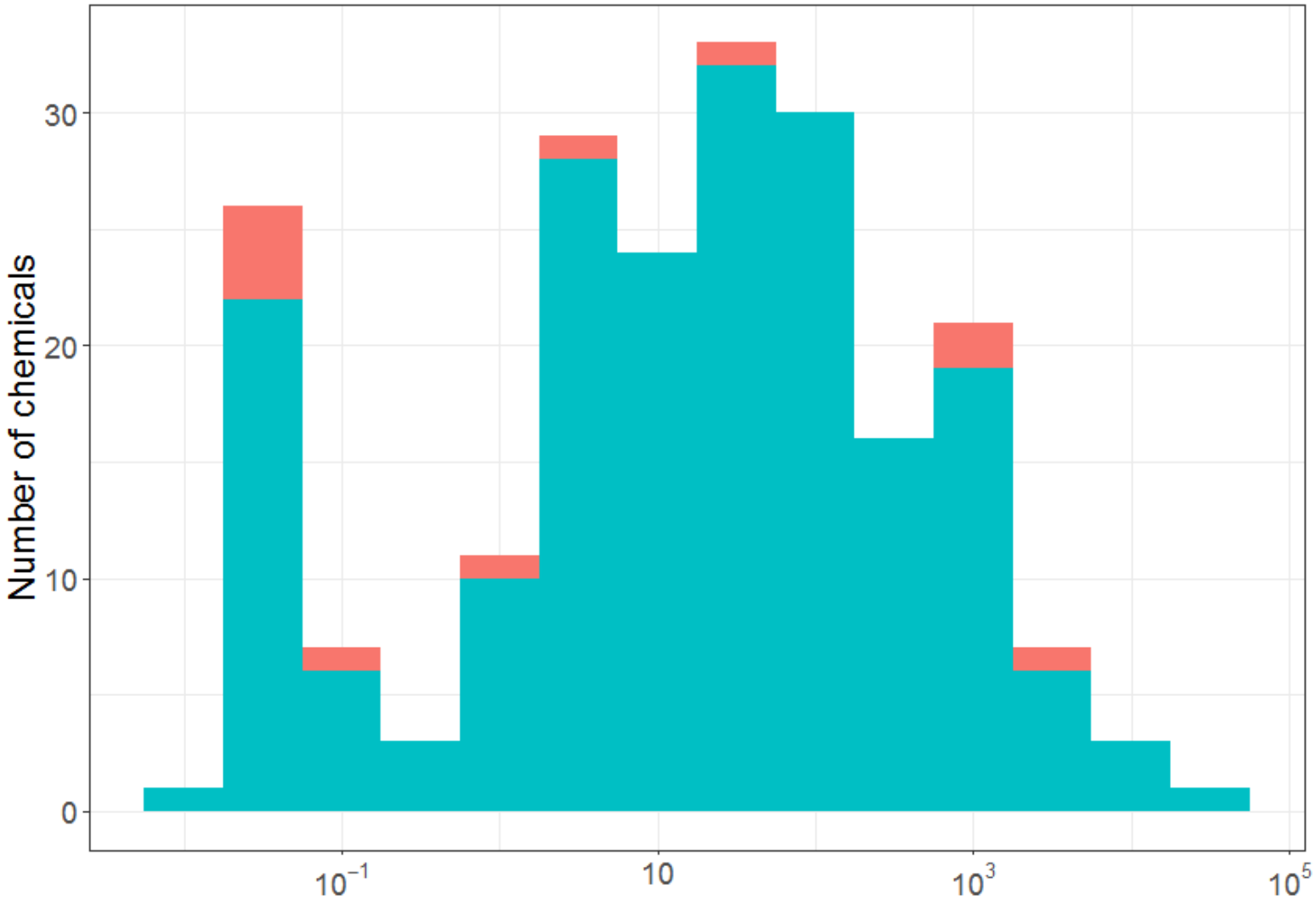
- Previous method allowed values above and below zero, those observations now increase measurement error estimate
- Medians from Bayesian analysis correlate with point estimates from previous method
- Larger values track with each other better (Wetmore *et al.. 2*012) average LOD was ~1%)

Results for Plasma Protein Titration



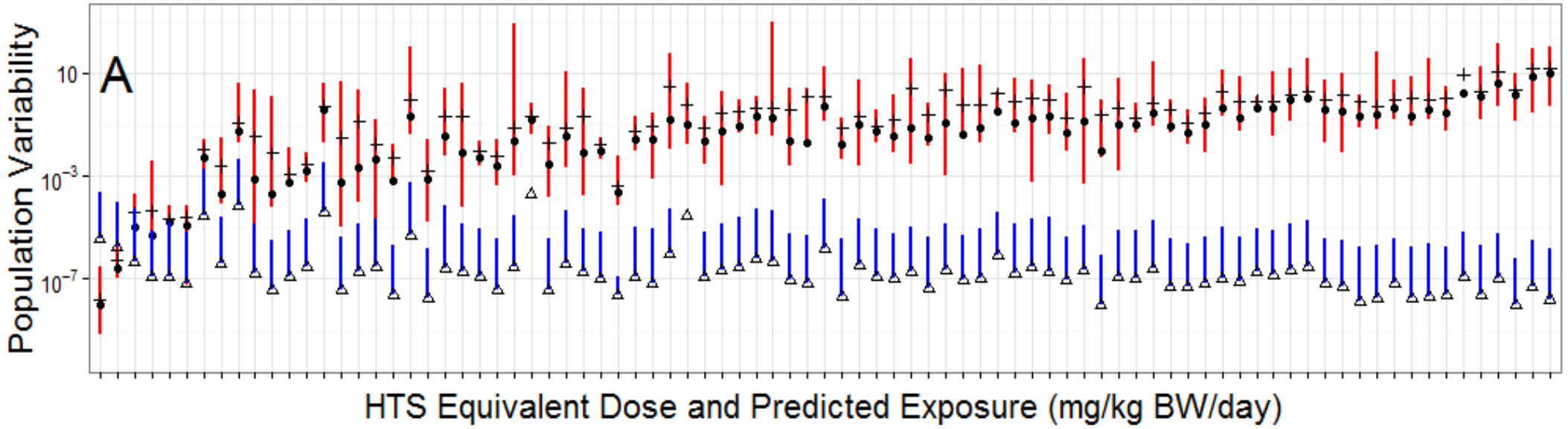
- Analysis of rapid equilibrium dialysis performed at 100%, 30%, and 10% of physiologic protein concentration
- Seven chemicals that had no measurement at 100% concentration now have a value
- Generally correlate, especially for higher F_{up}'s

Estimate of Protein Binding Affinity



A major benefit of three protein titration protocol is that you get an estimate of binding affinity for each chemical (some are very uncertain)

Relative Contribution of Uncertainty and Variability



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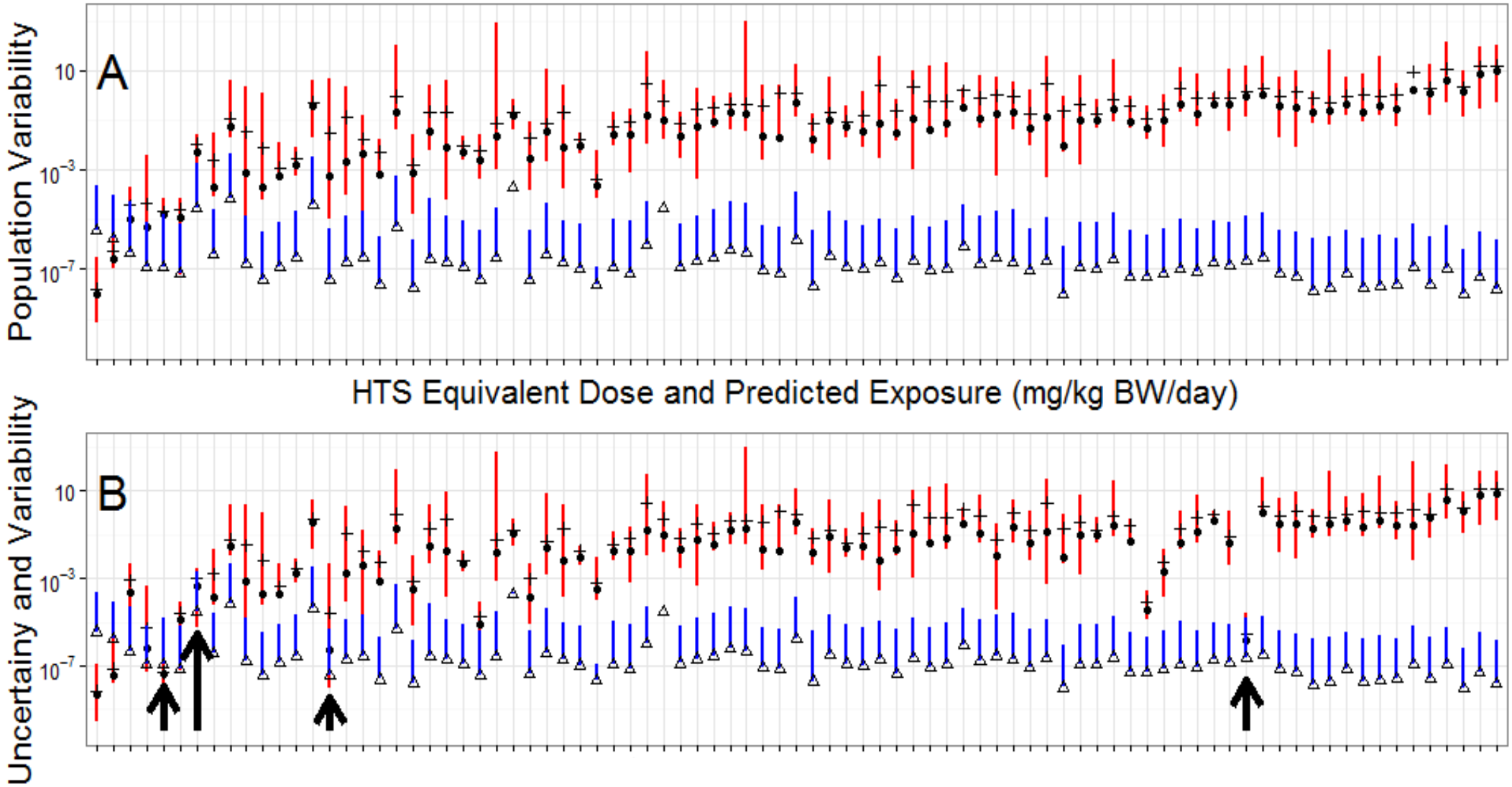

Four chemicals where uncertainty in HTTK data changes ratio of activity to exposure enough to cause overlap

Conclusions

- HTTK allows dosimetric adjustment of high-throughput screening (HTS) data across thousands of chemicals.
- Assessments of the impact of uncertainty and variability on these TK values and subsequent predictions are needed to guide data interpretation and provide overall confidence in the new approach methodologies
- New, chemical-specific *in vitro* experiments have been conducted by Cyprotex, using a revised protocol for measuring protein binding
- Bayesian methods were developed to provide chemical-specific uncertainty estimates for two *in vitro* TK parameters: plasma protein binding (f_{up}) and intrinsic hepatic clearance (Cl_{int}),
- Overall, variability contributed more significantly to C_{ss} estimations of the 95th percentile
- All EPA HTTK data and models are made publically available upon publication through the R “httk” package (Pearce et al., 2017)

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Netherlands Organisation for Applied Scientific Research (TNO)
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ScitoVation
Harvey Clewell
Kamel Mansouri
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Silent Spring Institute
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Southwest Research Institute
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Kristin Favela
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Lesa Aylward
Tox Strategies
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University of Michigan
Olivier Jolliet
University of North Carolina, Chapel Hill
Alex Tropsha

EPA United States Environmental Protection Agency

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