Higher Throughput Toxicokinetics to Allow Extrapolation

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March 8, 2017

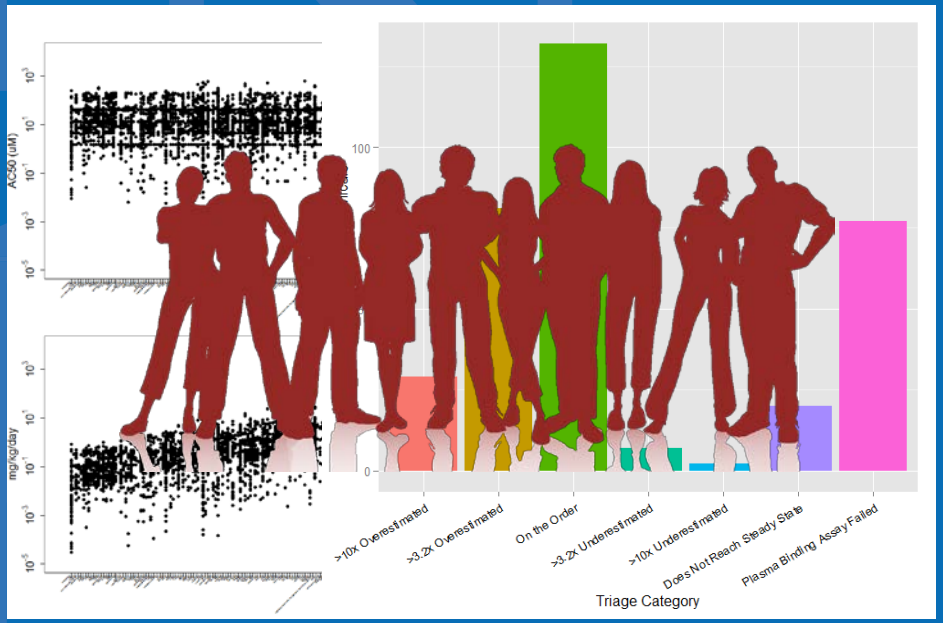

Figure includes image from Thinkstock

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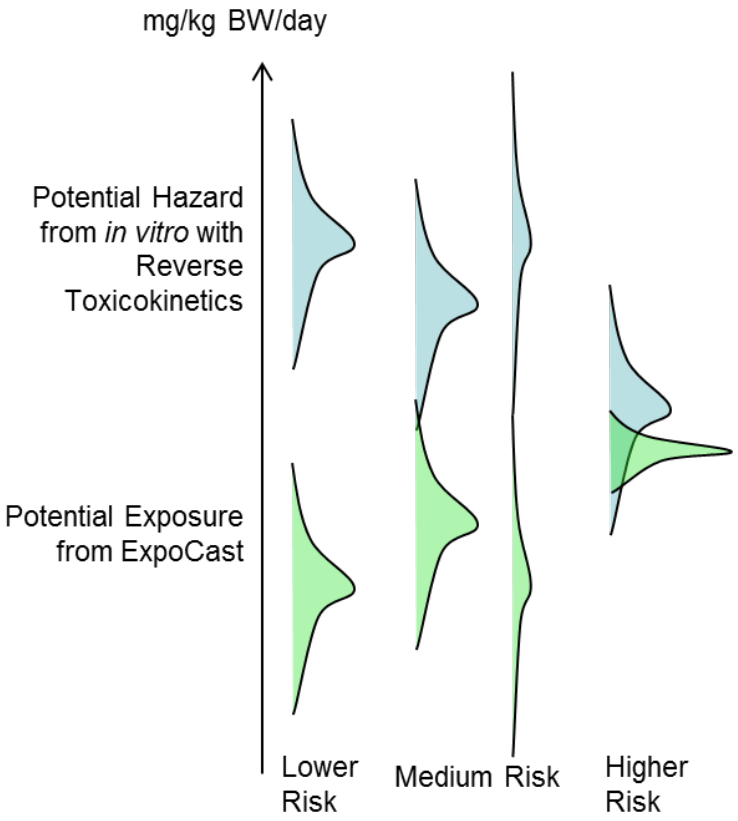
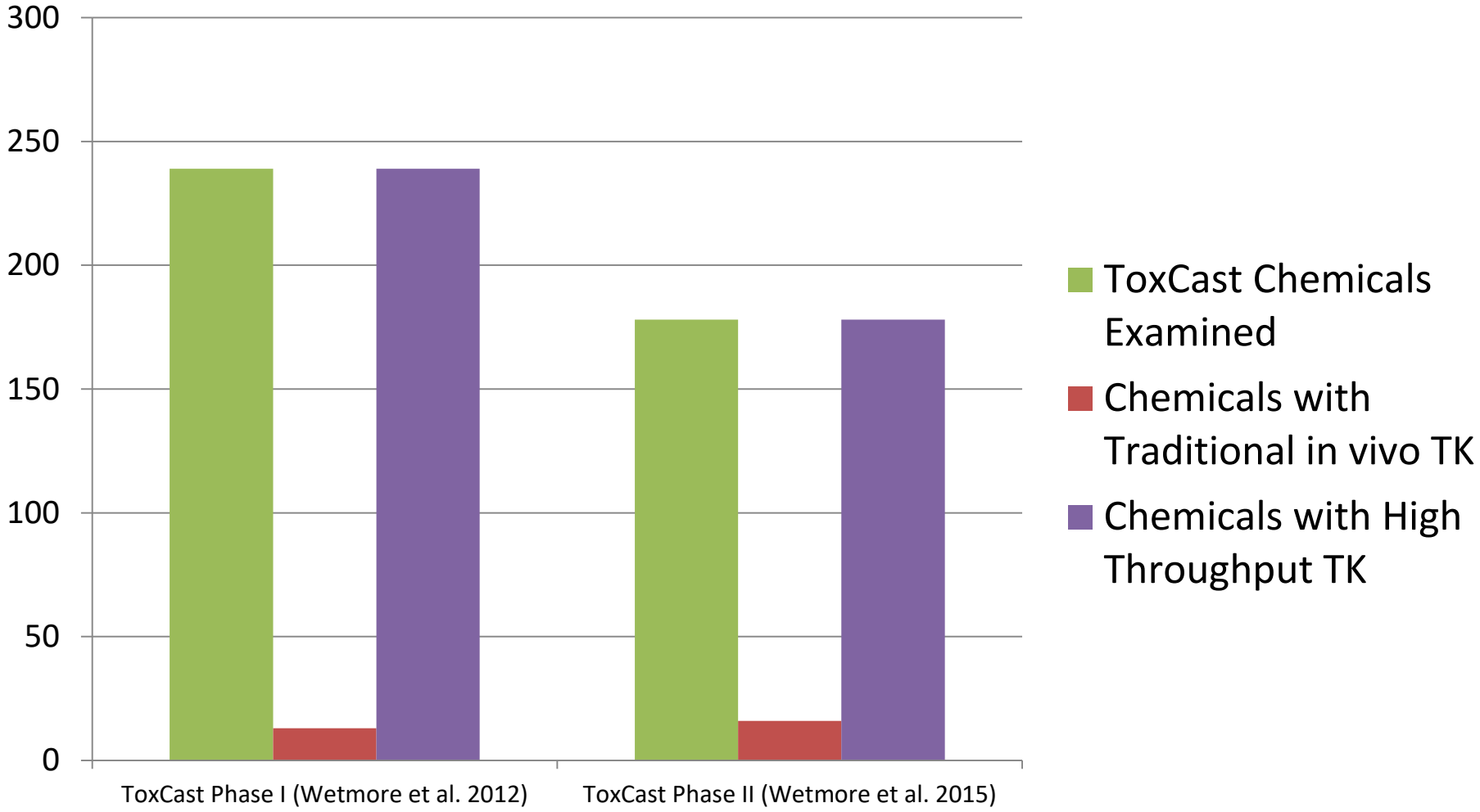
Introduction

- 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?)



The Need for *In Vitro* Toxicokinetics

United States Environmental Protection Agency



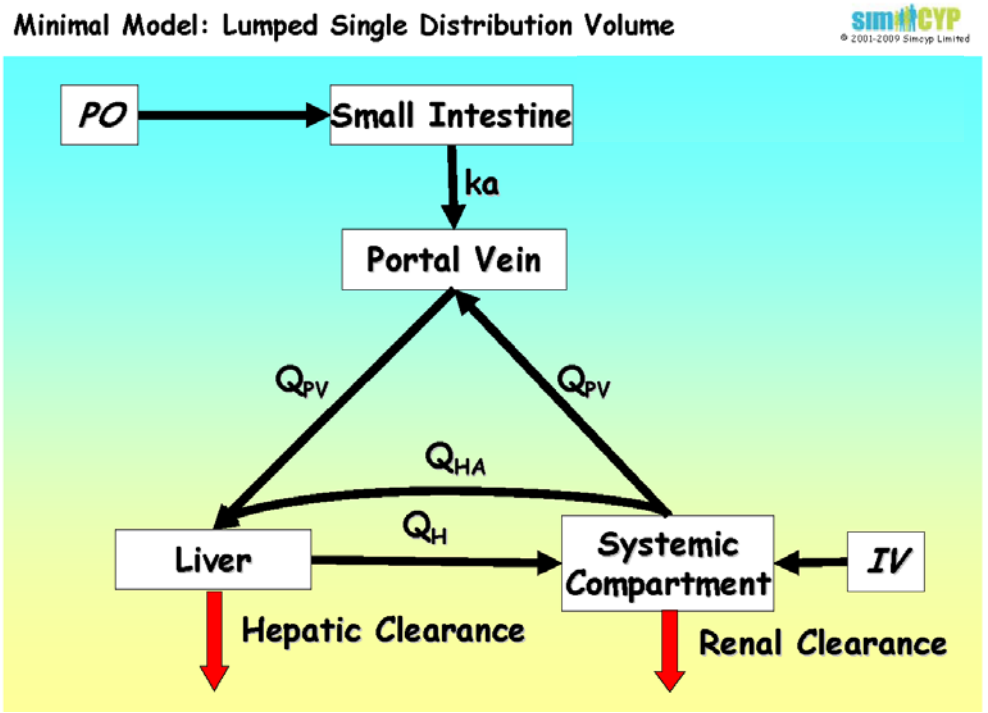
- Studies like Wetmore et al. (2012), address the need for TK data using *in vitro* methods

High Throughput Toxicokinetics (HTTK)

United States Environmental Protection Agency

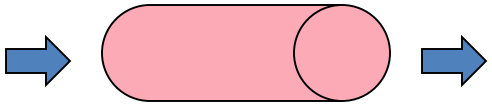
Jamei *et al*. (2009)

- *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



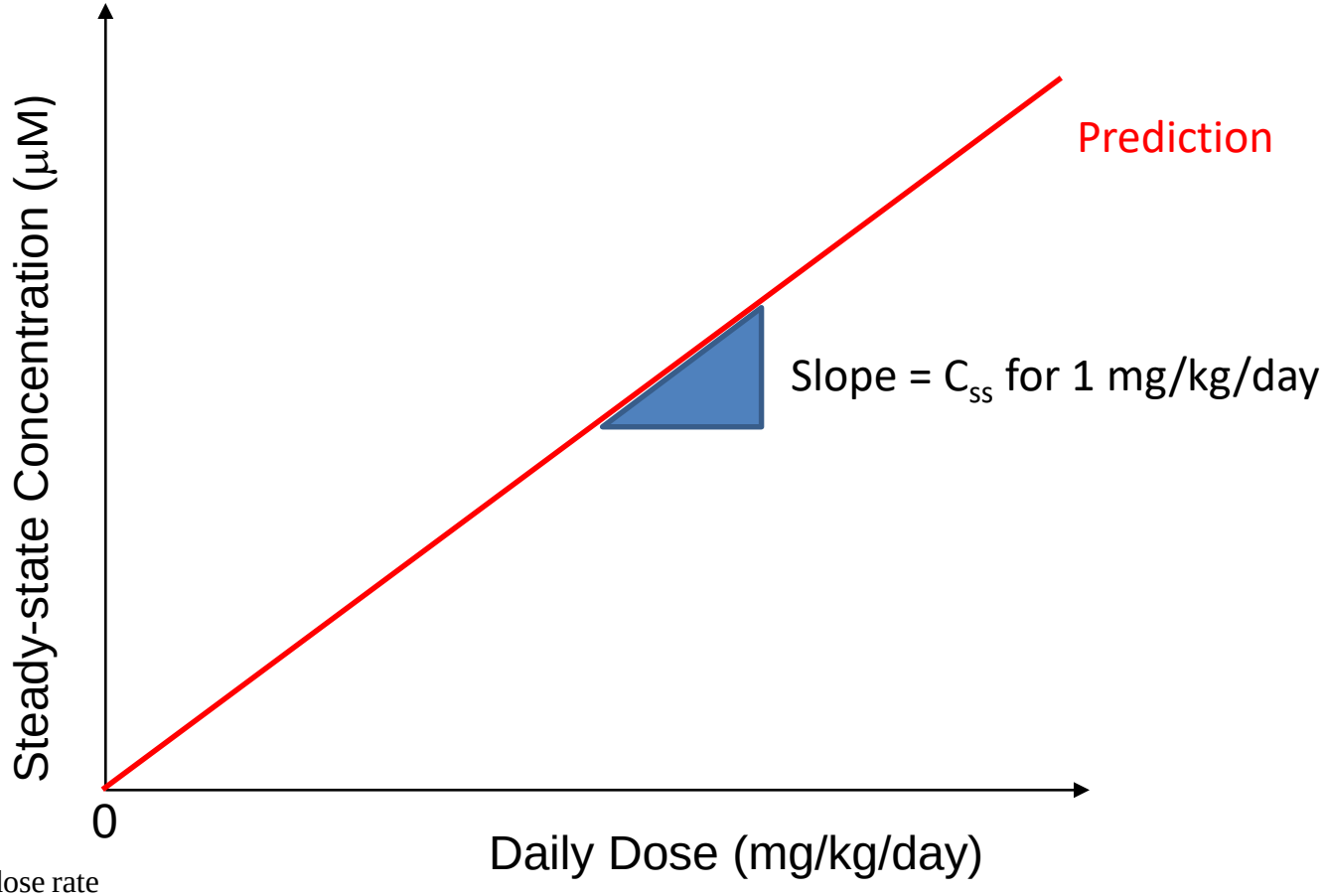
$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * \text{F}_{ub}) + \left(\text{Q}_l * \text{F}_{ub} * \frac{Cl_{int}}{\text{Q}_l + \text{F}_{ub} * Cl_{int}}\right)}$$

Oral dose in (mg/kg/day)



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

Steady-State is Linear with Dose



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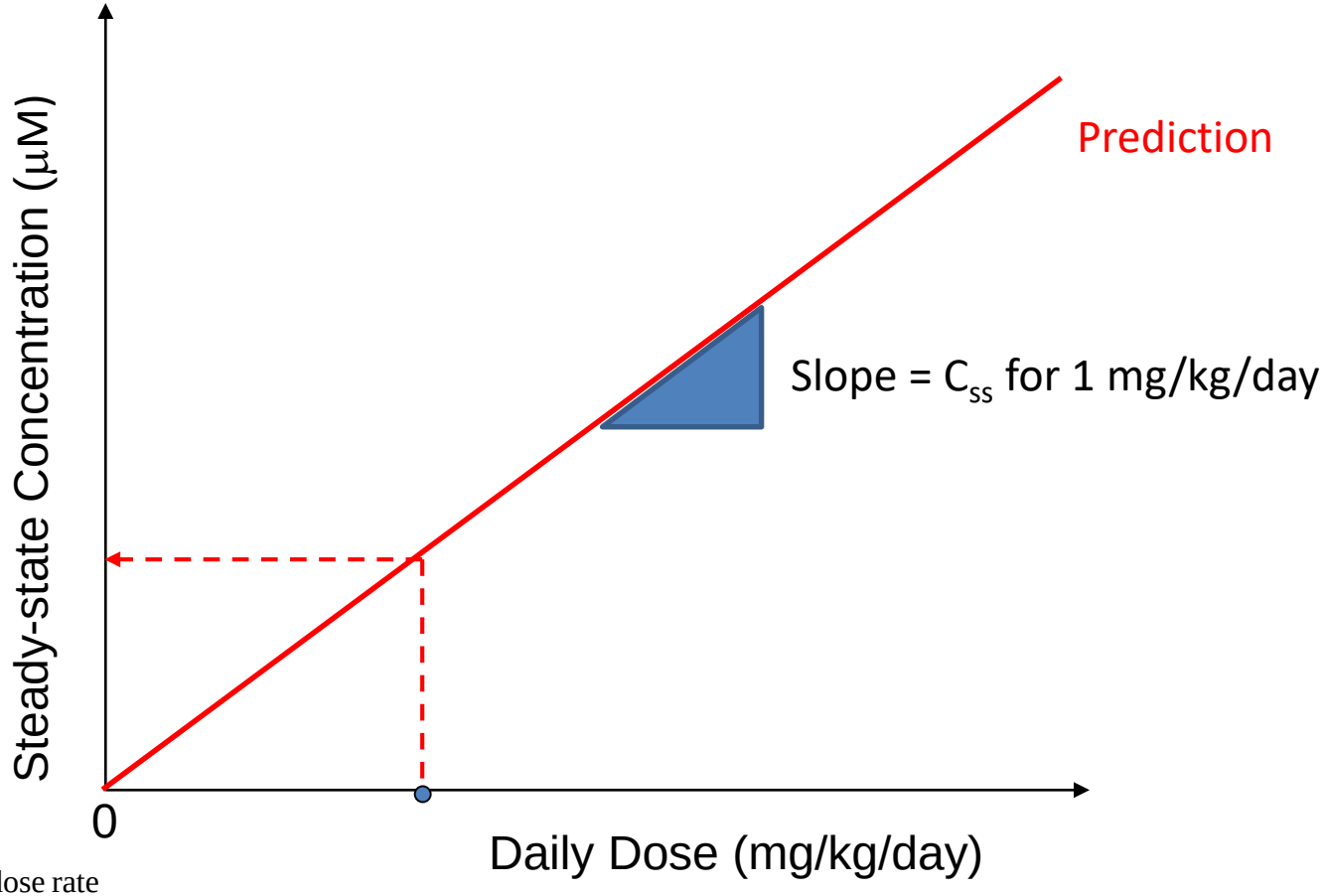
- Can calculate predicted steady-state concentration (C_{ss}) for a 1 mg/kg/day dose and multiply to get concentrations for other doses

Wetmore *et al*. (2012)

Steady-State is Linear with Dose



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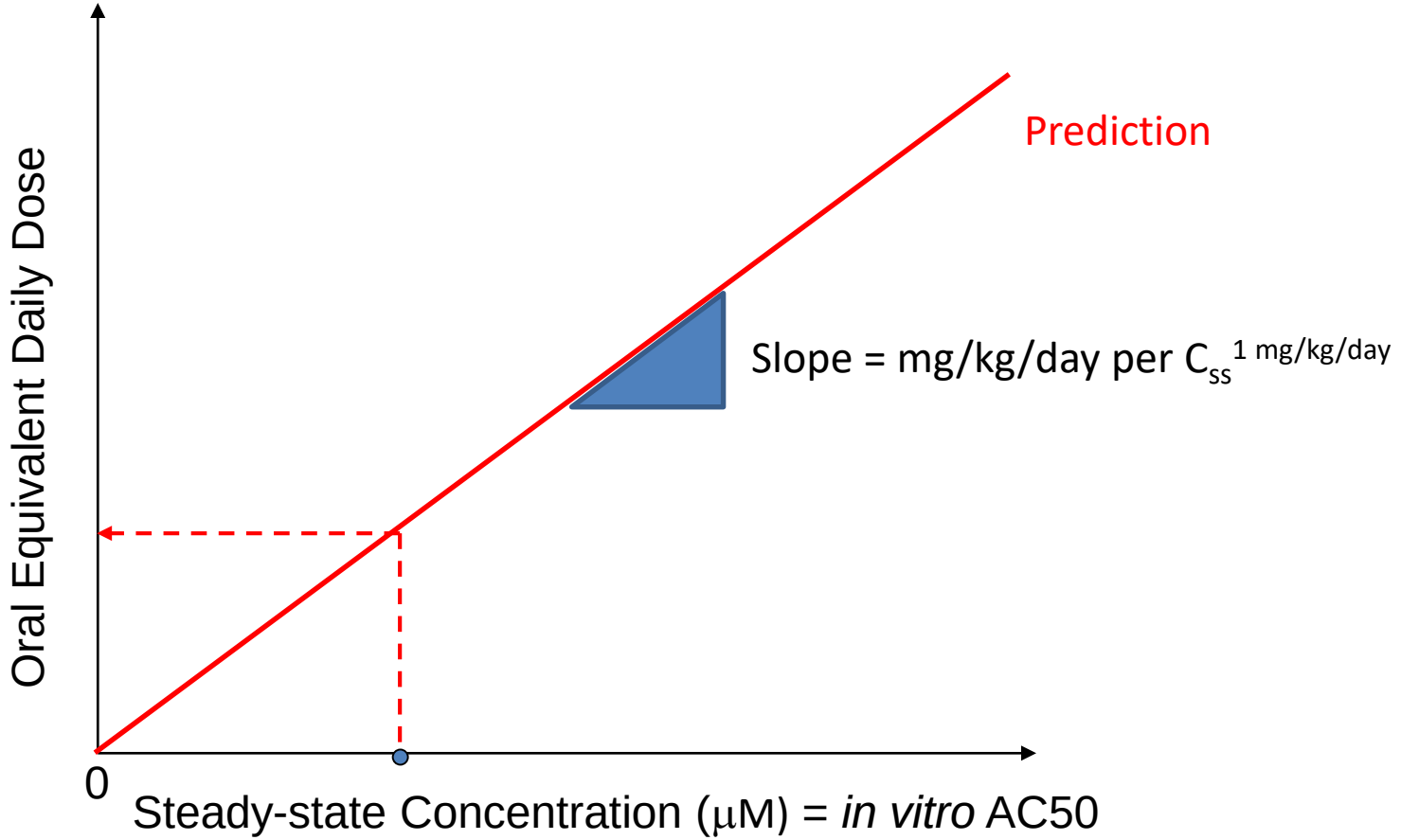
Wetmore *et al*. (2012)

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)

Integrated Bioactivity:Exposure Ratio

(Wetmore et al. 2012, 2014, 2015)

$$\text{IBER} = \frac{\text{Bioactive Dose}}{\text{Estimated exposure}}$$

IBER <=1 : Exposure potentially high enough to cause bioactivity

IBER >> 1: Exposure less likely to be high enough to cause bioactivity

(figure adapted from Wetmore *et al.* 2012)

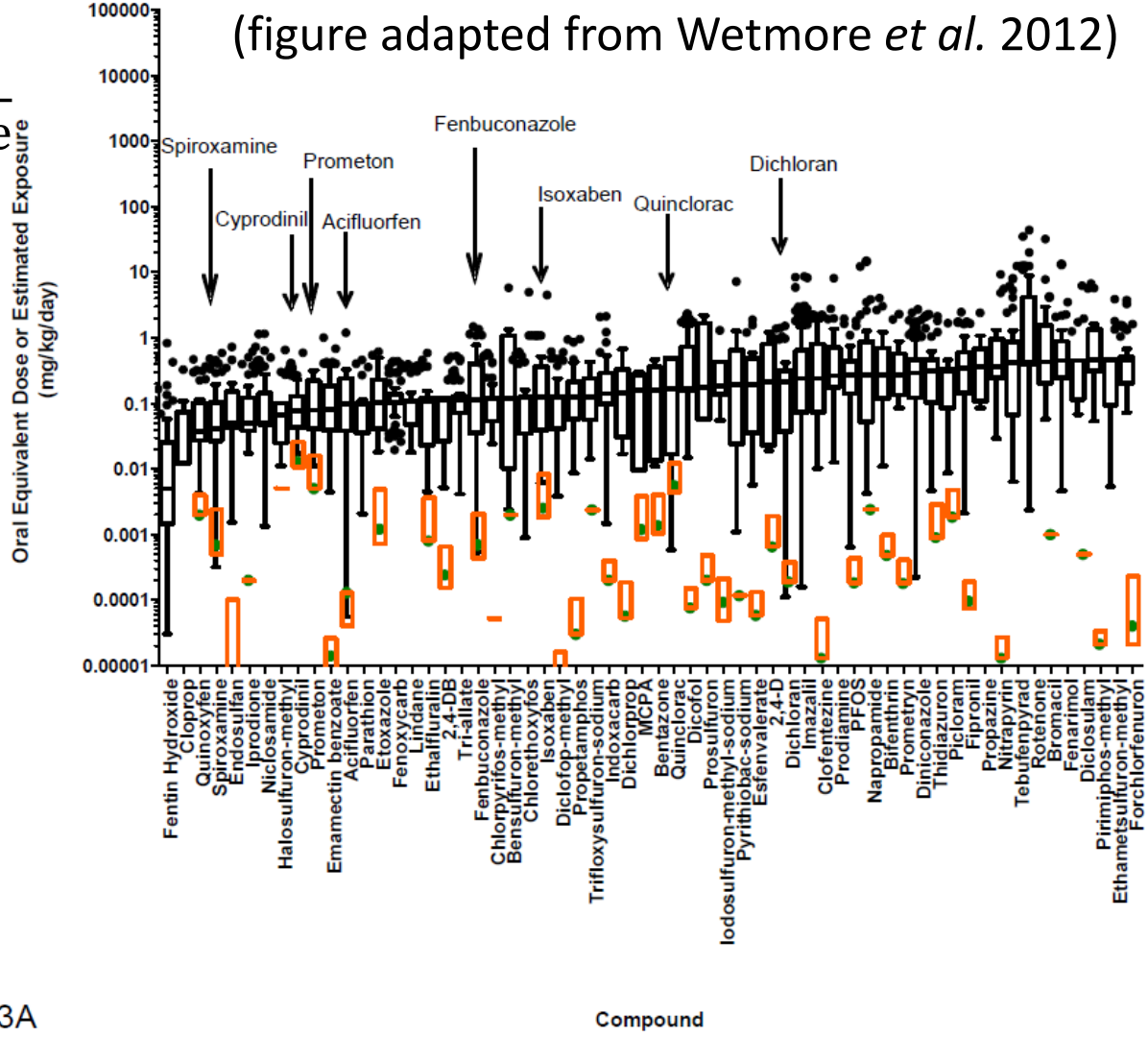


Fig. 3A

Slide from Caroline Ring

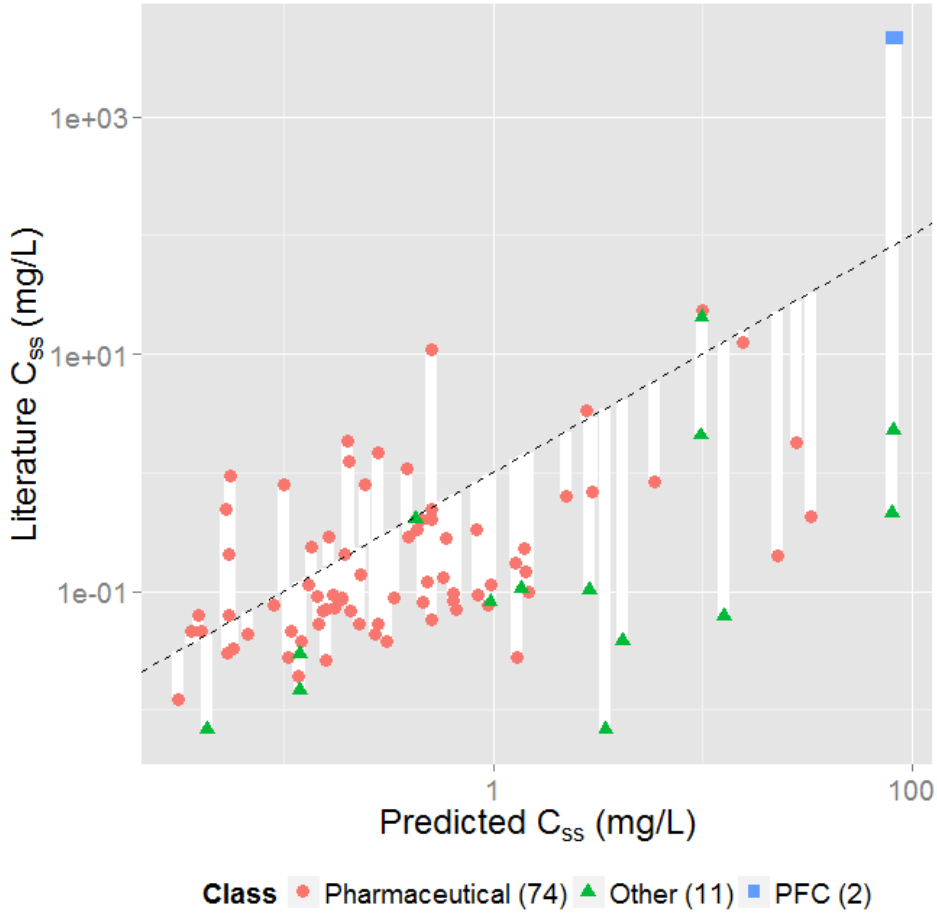
In vivo Predictive Ability and Domain of Applicability

- 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)

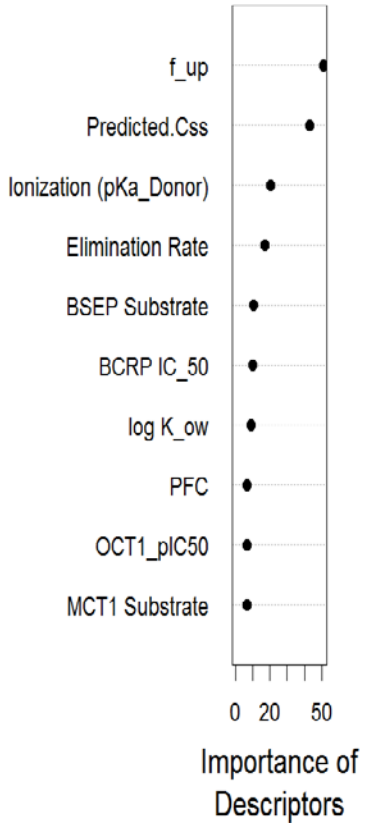
- For environmental compounds, there will be no clinical trials

- 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

Using *in vivo* Data to Evaluate HTTK



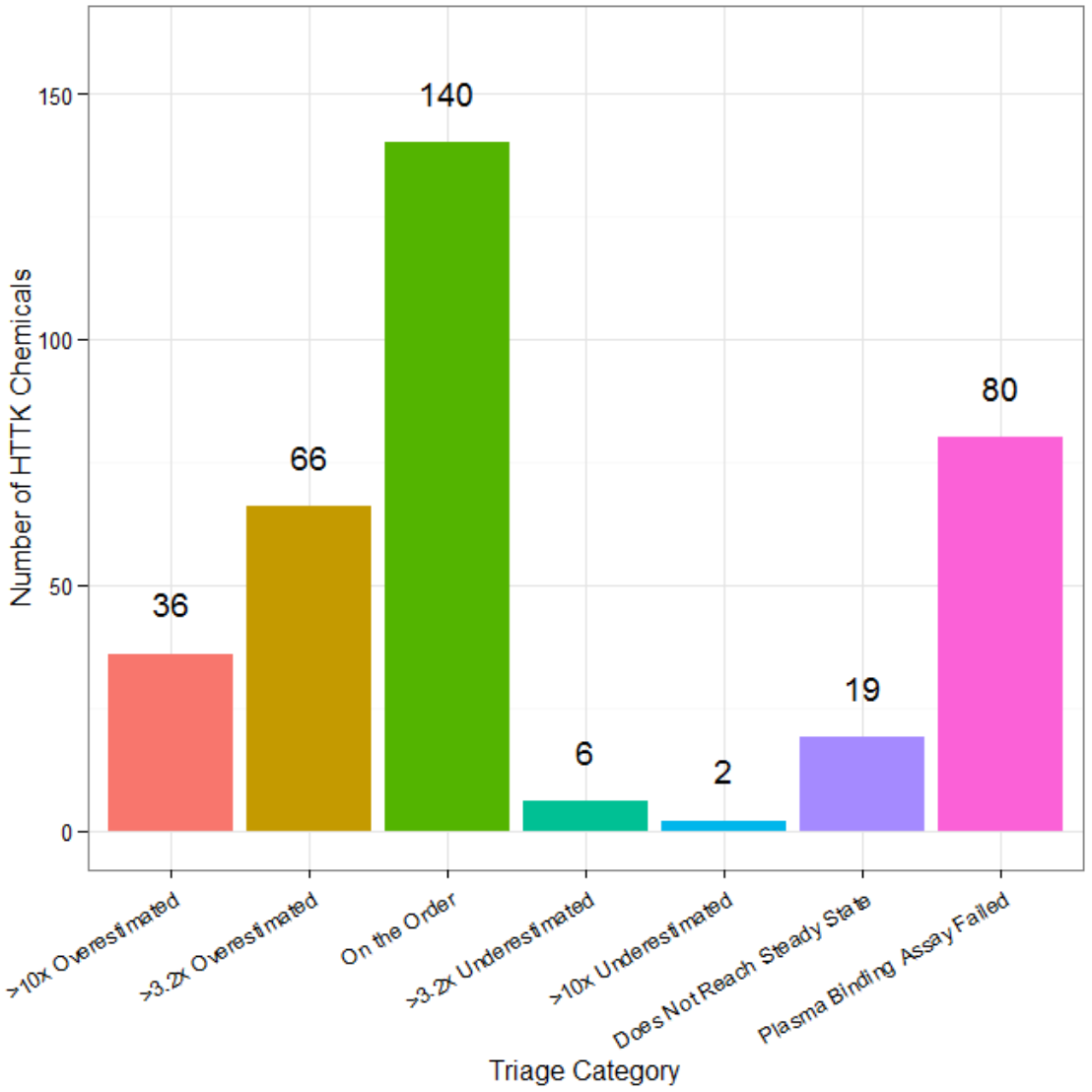
- When we compare the C_{ss} predicted from *in vitro* HTTK with *in vivo* C_{ss} values determined from the literature we find limited correlation (R^2 ~0.34)
- The dashed line indicates the identity (perfect predictor) line:
  - Over-predict for 65
  - Under-predict for 22
- The white lines indicate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



Wambaugh et al. (2015)

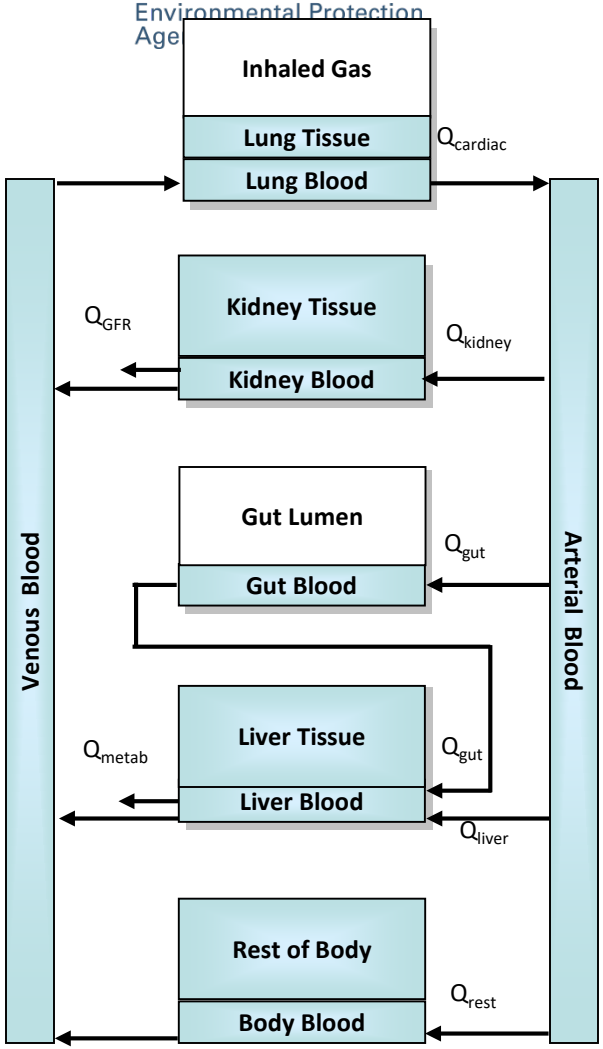
Toxicokinetic Triage

- 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



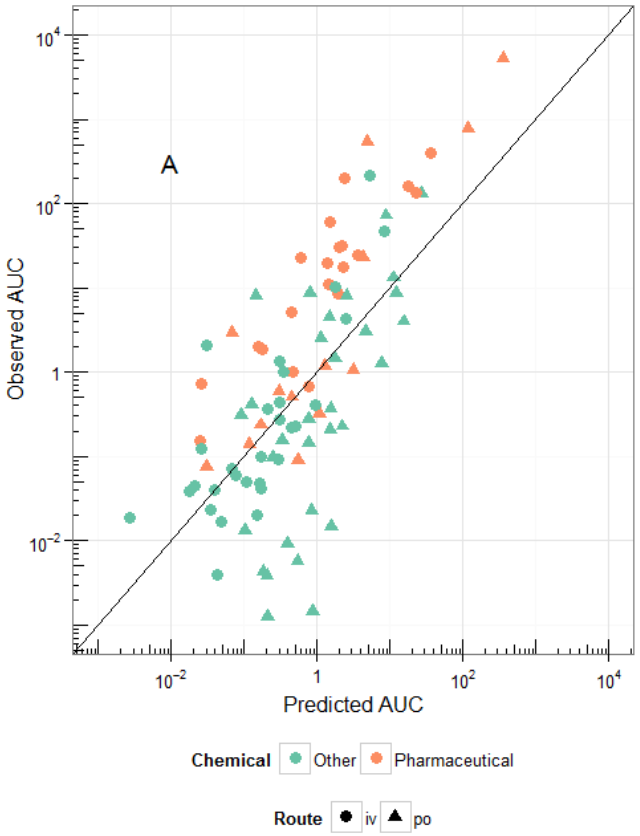
Wambaugh et al. (2015)

A General Physiologically-based Toxicokinetic (PBTK) Model



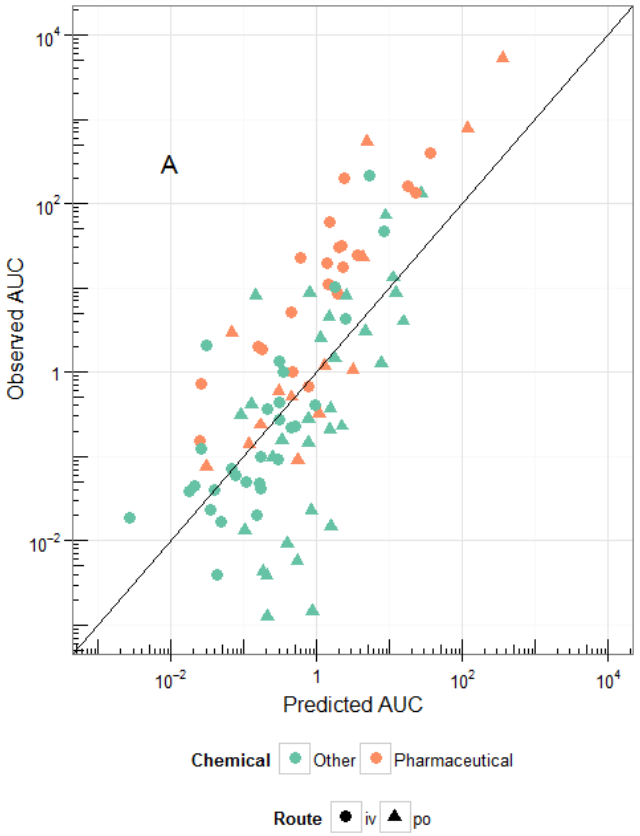
- Some tissues (e.g. arterial blood) are simple compartments, while others (e.g. kidney) are compound compartments consisting of separate blood and tissue sections with constant partitioning (i.e., tissue specific partition coefficients)
- Exposures are absorbed from reservoirs (gut lumen)
- Some specific 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).

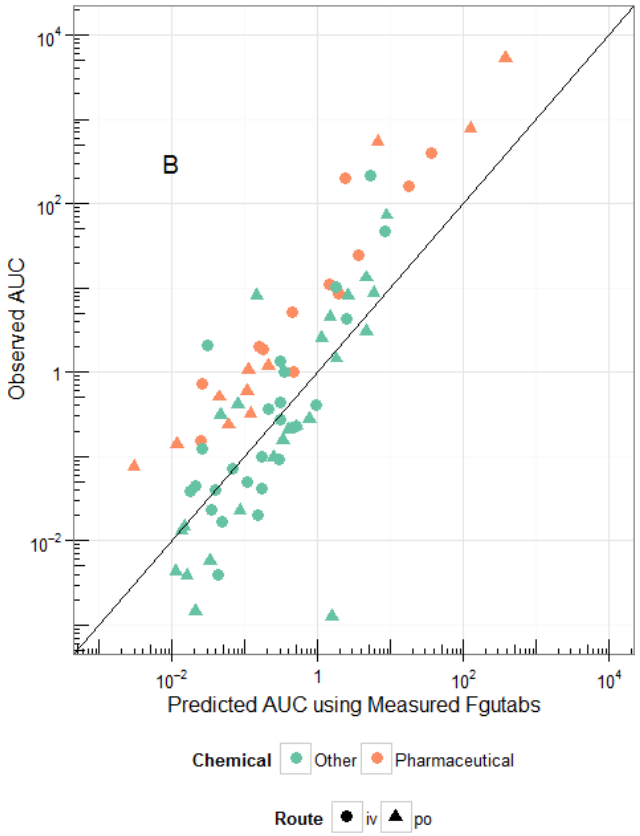
Evaluating *In Vitro* PBTK Predictions with *In Vivo* Data



- PBTK predictions for the AUC (time integrated plasma concentration or Area Under the Curve)
- 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

Evaluating *In Vitro* PBTK Predictions with *In Vivo* Data





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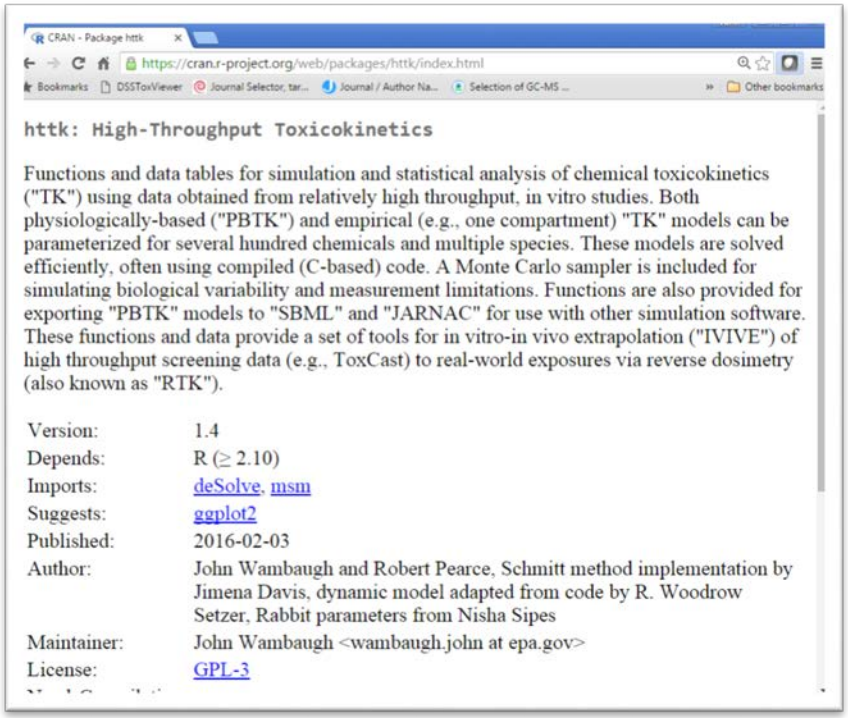
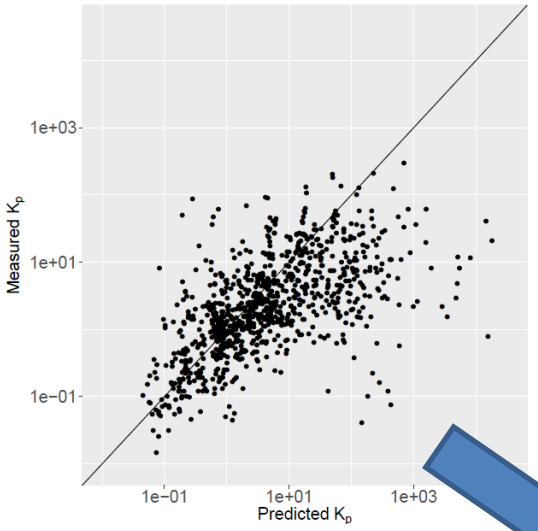
Cyprotex is now measuring bioavailability (CACO2) for all HTTK chemicals

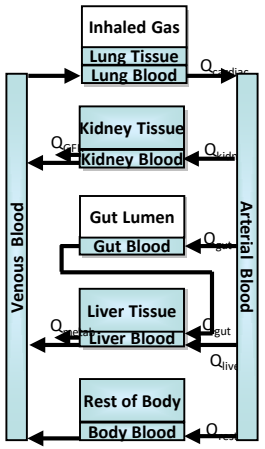
httk: An Up-to-Date Tool

United States Environmental Protection Agency

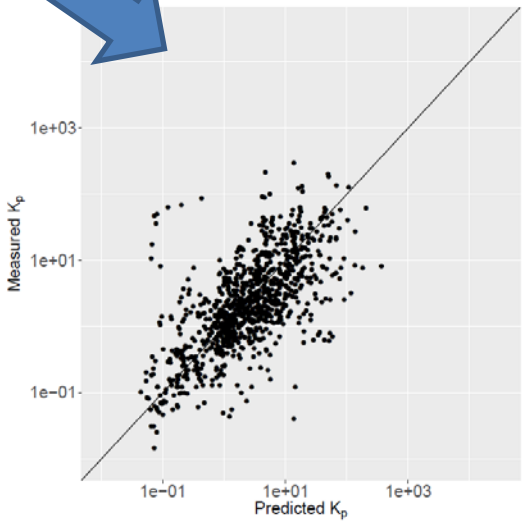
Old versions are archived







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

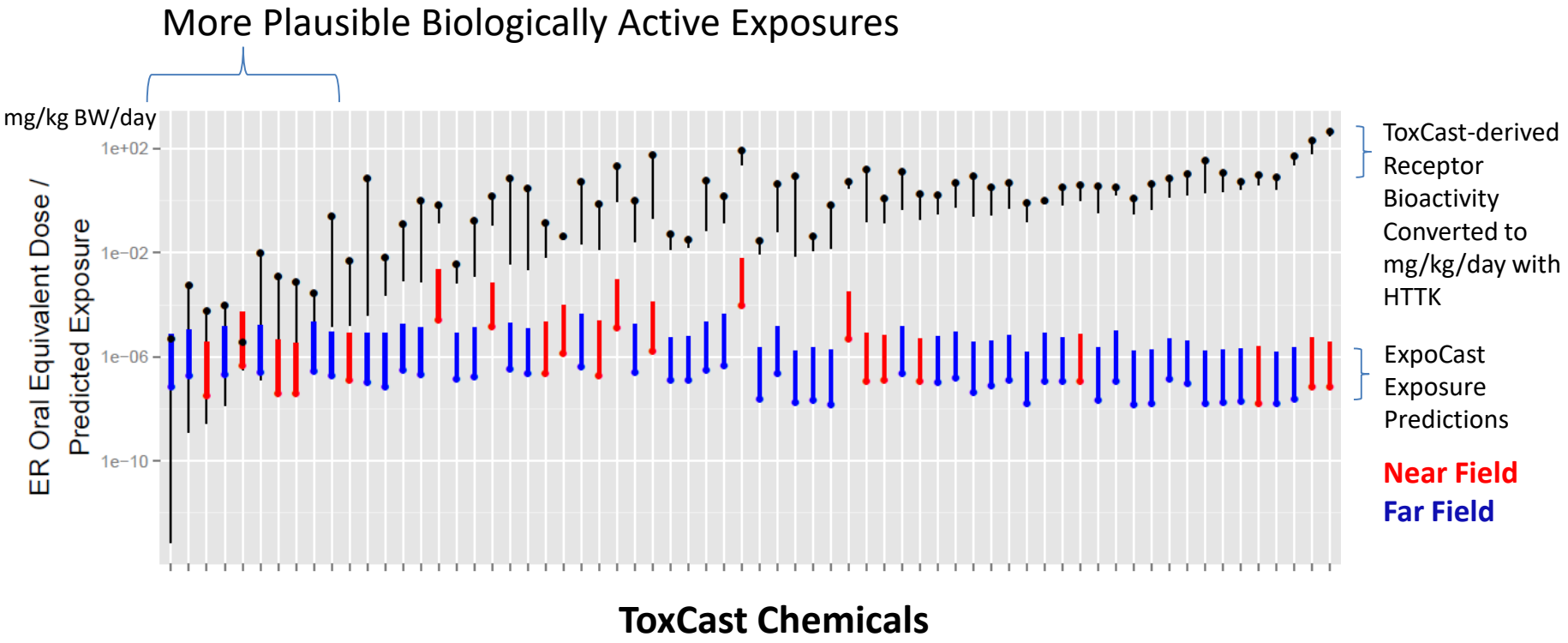


- "httk" R Package for reverse dosimetry and PBTK
- 543 Chemicals to date
- 100's of additional chemicals being studied
- Pearce *et al.* package documentation manuscript accepted at Journal of Statistical Software

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

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

Application to High Throughput Risk Prioritization



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

DOCKET NUMBER:
EPA–HQ–OPP–2014–0614

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, **but:**
- We must consider domain of applicability
  - Collected new PK data from *in vivo* studies (EPA/NHEERL and Research Triangle Institute)
  - Organizing data from larger, systematic studies (e.g., National Toxicology Program) into computable format
- New R package “httk” freely available on CRAN allows statistical analyses
  - Analysis has been submitted

EPA United States Environmental Protection Agency

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

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