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High Throughput PBTK: Open-Source Data and Tools for Dosimetry and Exposure Reconstruction

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PBK Modelling in Risk Assessment
Ispra, Italy

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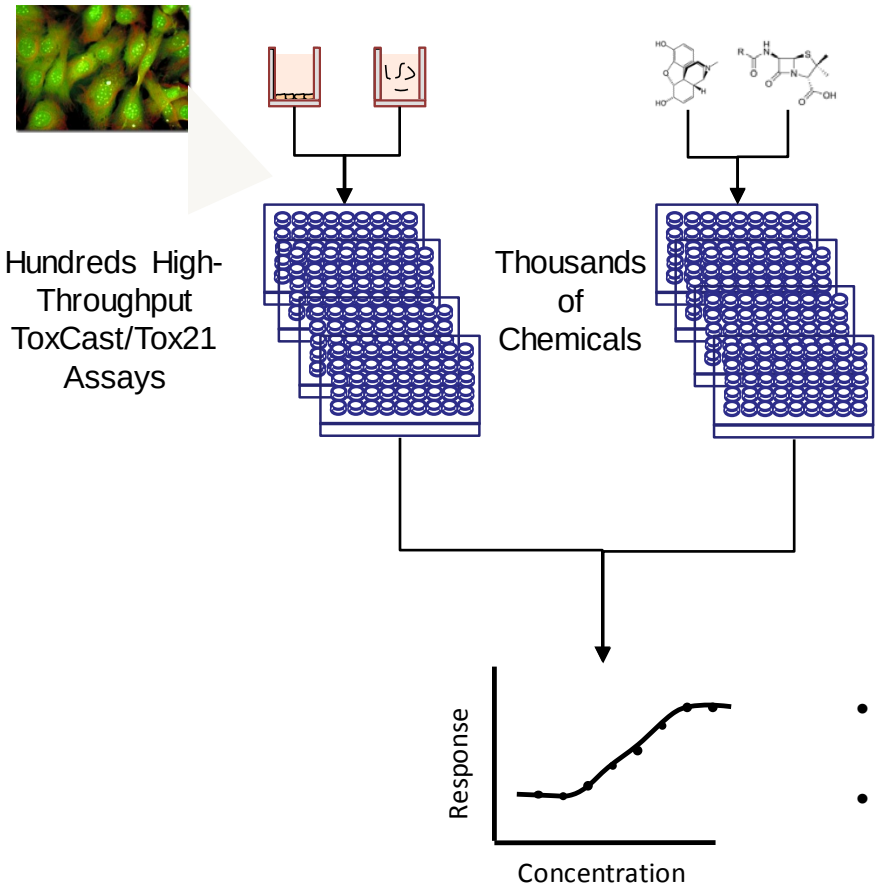
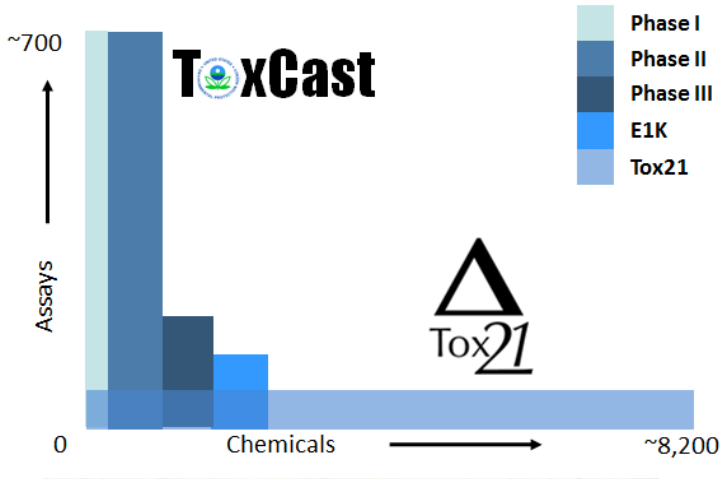
High-Throughput Assays Used to Screen Chemicals for Bioactivity

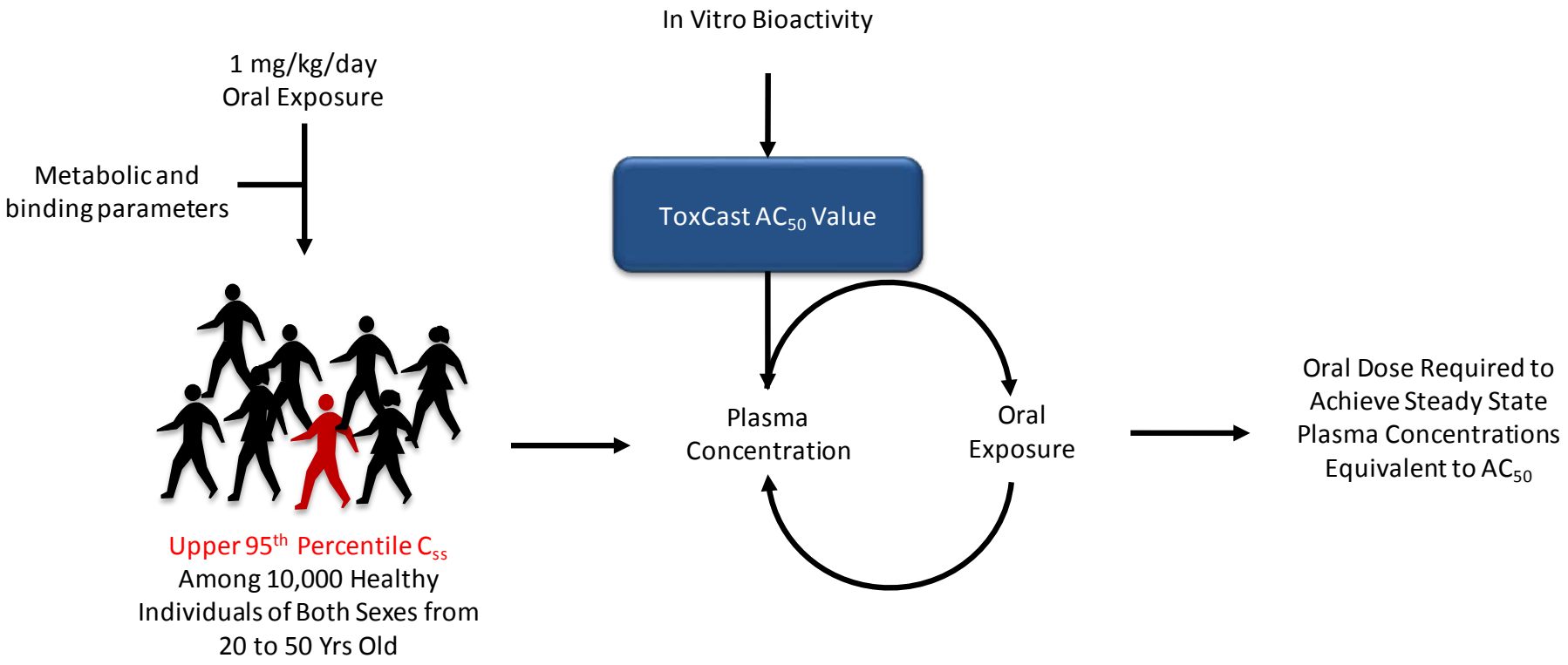




- Understanding of what cellular processes/pathways may be perturbed by a chemical
- Understanding of what amount of a chemical causes these perturbations

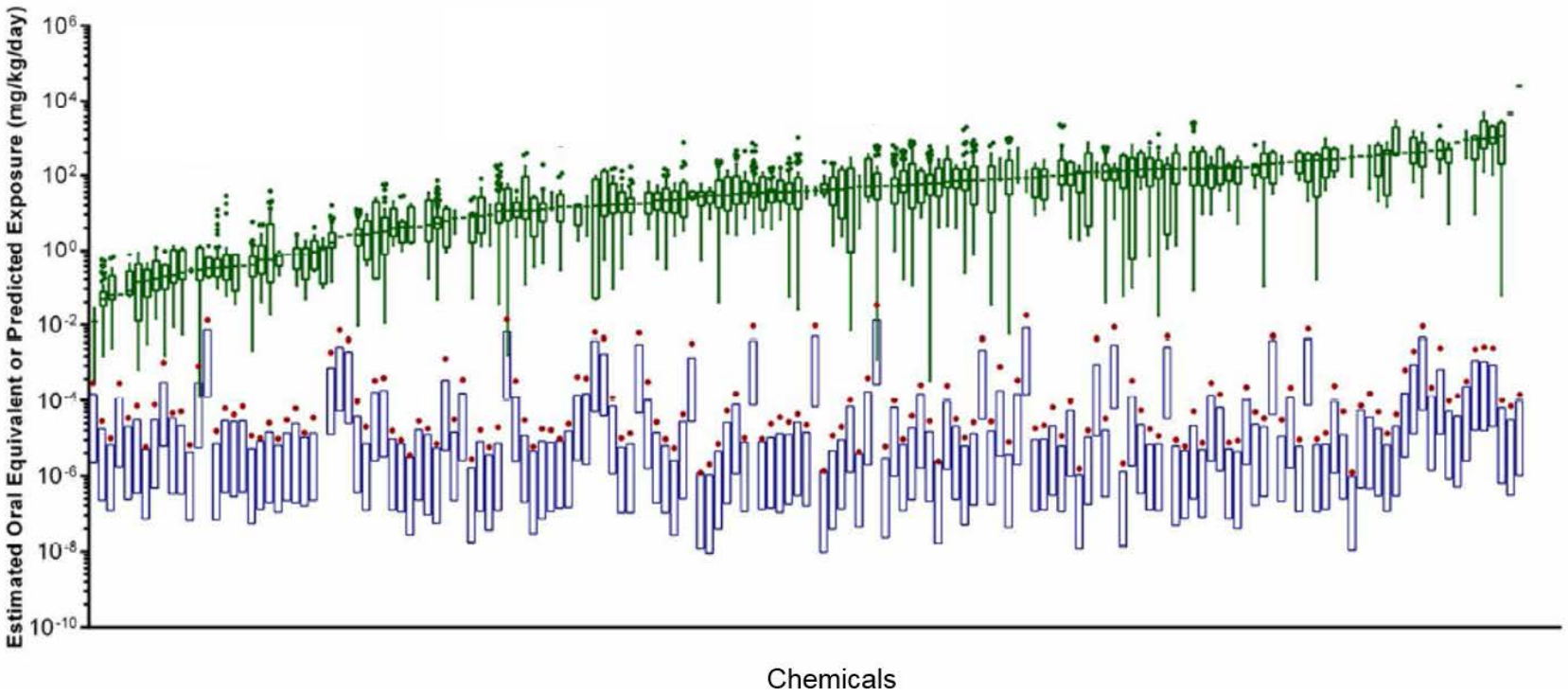
All data is public: http://actor.epa.gov/

Incorporating Toxicokinetics to Characterize Dose



Using Monte Carlo to capture variability:
Incorporates variability in blood flow rates, tissue size, binding, other physiologic parameters – during IVIVE simulations

HTTK to Derive Oral Equivalent Doses



Wetmore *et al.*, *Tox Sci.*, 2015

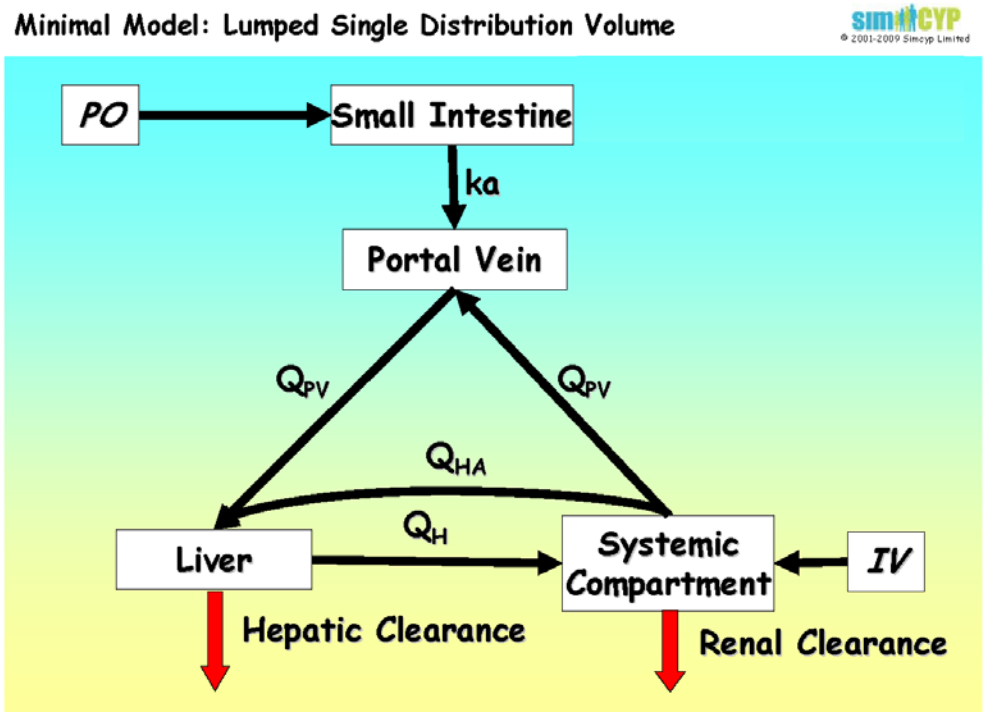
Translation from nominal *in vitro* concentrations to external dose equivalents --

- greater discrimination of chemical potencies
- direct comparator to exposure estimates to provide an activity:exposure metric

High Throughput Toxicokinetics (HTTK)

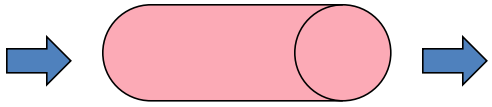
- *In vitro* plasma protein binding and metabolic clearance assays allow approximate hepatic and renal clearances to be calculated
- At steady state this allows conversion from concentration to administered dose
- 100% bioavailability assumed (very conservative assumption – may overestimate risk)

Jamei *et al*. (2009)



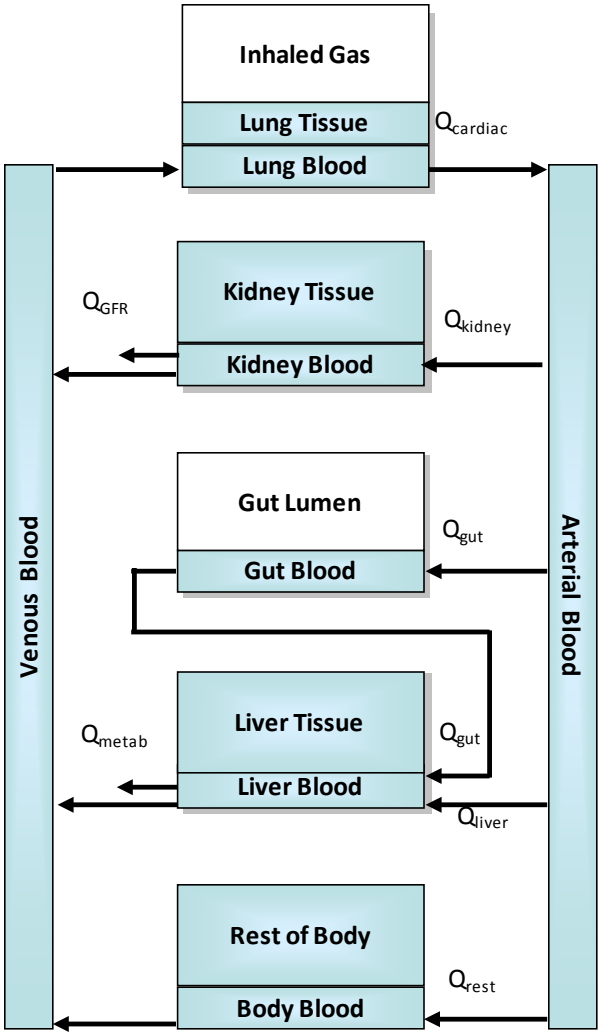
$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * \text{F}_{ub}) + \left(\text{Q}_{l} * \text{F}_{ub} * \frac{Cl_{int}}{\text{Q}_{l} + \text{F}_{ub} * Cl_{int}}\right)}$$

Oral dose in (mg/kg/day)



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

A General Physiologically-based Pharmacokinetic (PBPK) Model



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

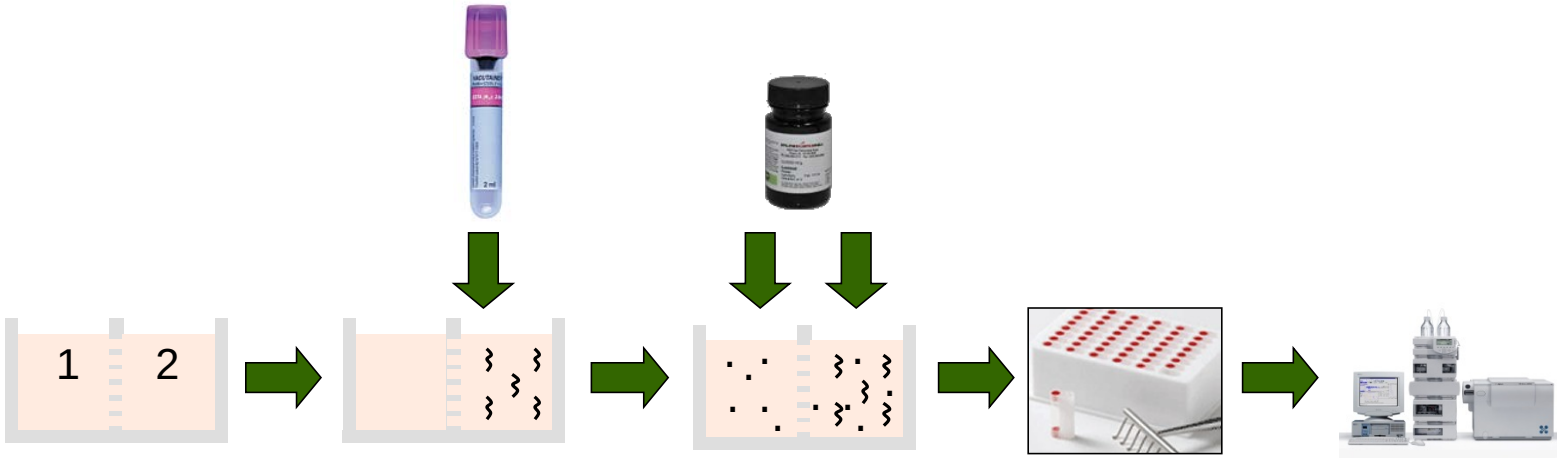
Exposures are absorbed from reservoirs (gut lumen)

Some specific tissues (lung, kidney, gut, and liver) are modeled explicitly, others (e.g. fat, brain, bones) are lumped into the "Rest of Body" compartment.

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

The only ways chemicals "leaves" the body are through metabolism (change into a metabolite) in the liver or excretion by glomerular filtration into the proximal tubules of the kidney (which filter into the lumen of the kidney).

Plasma Protein Binding (Fraction Unbound in Plasma)



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

RED Method: Waters *et al.* (2008)

Data on ToxCast chemicals initially collected at Hamner Institutes

- Rotroff *et al.* (2010) - Pilot study using 38 Phase I ToxCast Chemicals
- Wetmore *et al.* (2012) - Remainder of easily analyzed Phase I chemicals
- Wetmore *et al.* (2013) Rat PK for 50 ToxCast/ToxRefDB compounds

Intrinsic Hepatic Clearance (Cl_{int})

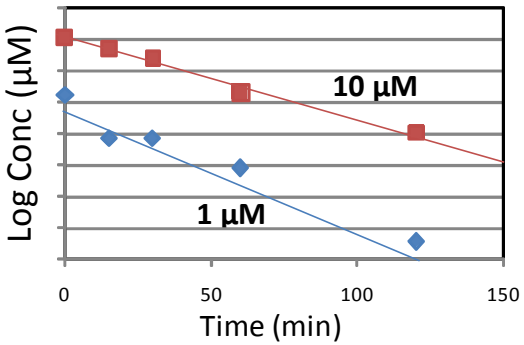
Cryopreserved Hepatocytes (10 donor pool for human)

Add Chemical (1 and 10 µM)

Remove Aliquots at 15, 30, 60, 120 min

Analytical Chemistry

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.

Cryopreserved hepatocyte Method: Shibata *et al.* (2002)

Data on ToxCast chemicals initially collected at Hamner Institutes

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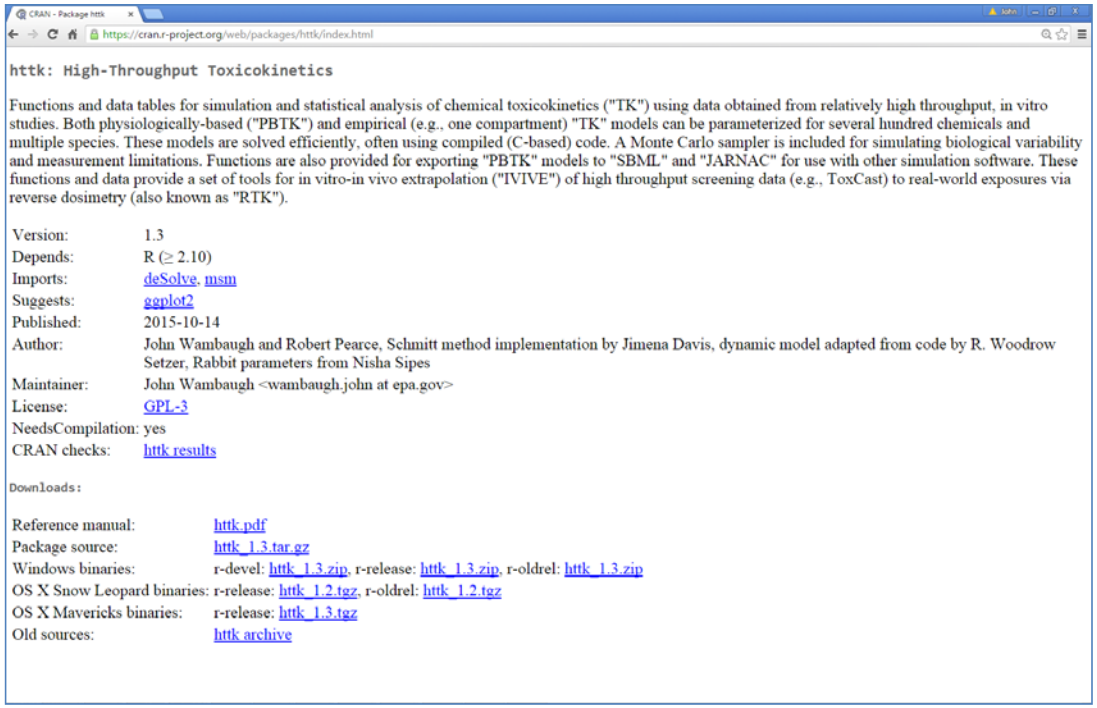
httk R Package



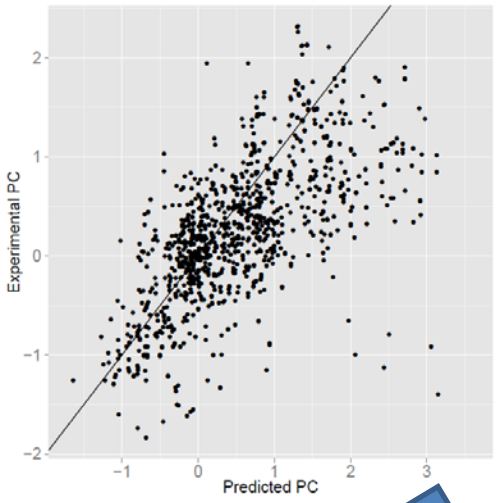
httk: High-Throughput Toxicokinetics

Functions and data tables for simulation and statistical analysis of chemical toxicokinetics ("TK") using data obtained from relatively high throughput, in vitro studies. Both physiologically-based ("PBTK") and empirical (e.g., one compartment) "TK" models can be parameterized for several hundred chemicals and multiple species. These models are solved efficiently, often using compiled (C-based) code. A Monte Carlo sampler is included for simulating biological variability and measurement limitations. Functions are also provided for exporting "PBTK" models to "SBML" and "JARNAC" for use with other simulation software. These functions and data provide a set of tools for in vitro-in vivo extrapolation ("IVIVE") of high throughput screening data (e.g., ToxCast) to real-world exposures via reverse dosimetry (also known as "RTK").

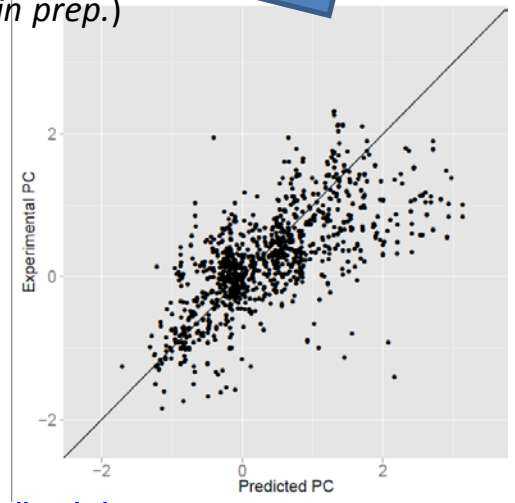
Version:	1.3
Depends:	R (≥ 2.10)
Imports:	deSolve, msm
Suggests:	ggplot2
Published:	2015-10-14
Author:	John Wambaugh and Robert Pearce, Schmitt method implementation by Jimena Davis, dynamic model adapted from code by R. Woodrow Setzer, Rabbit parameters from Nisha Sipes
Maintainer:	John Wambaugh <wambaugh.john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

Downloads:

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



Ongoing refinements:
High log P, better treatment of ionization
(Pearce et al., *in prep.*)

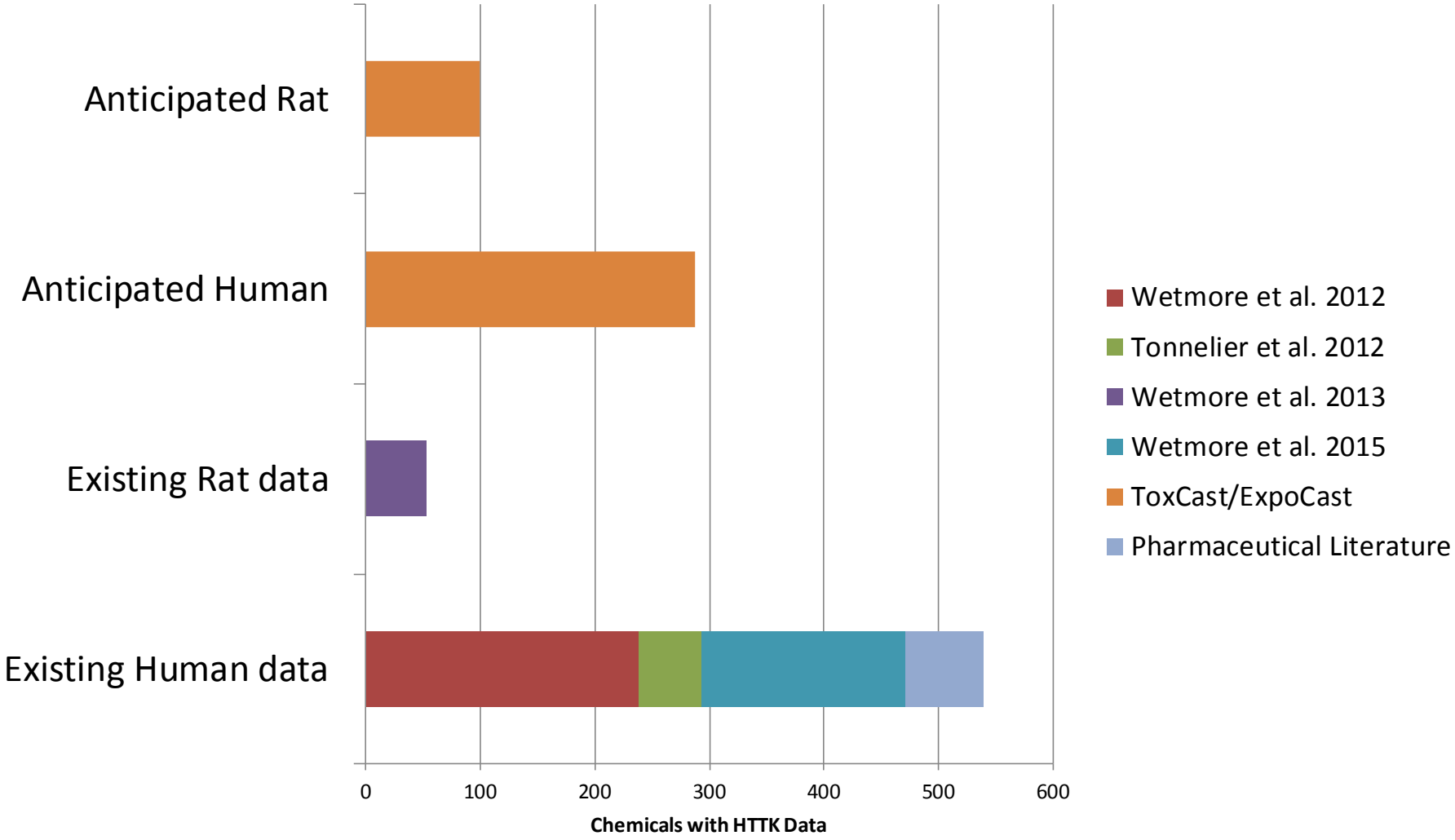


"httk" R Package
543 Chemicals to date
Lead programmer Robert Pearce
Wambaugh *et al.* (2015), Pearce *et al. in press*

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

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

Chemicals with HTTK Data



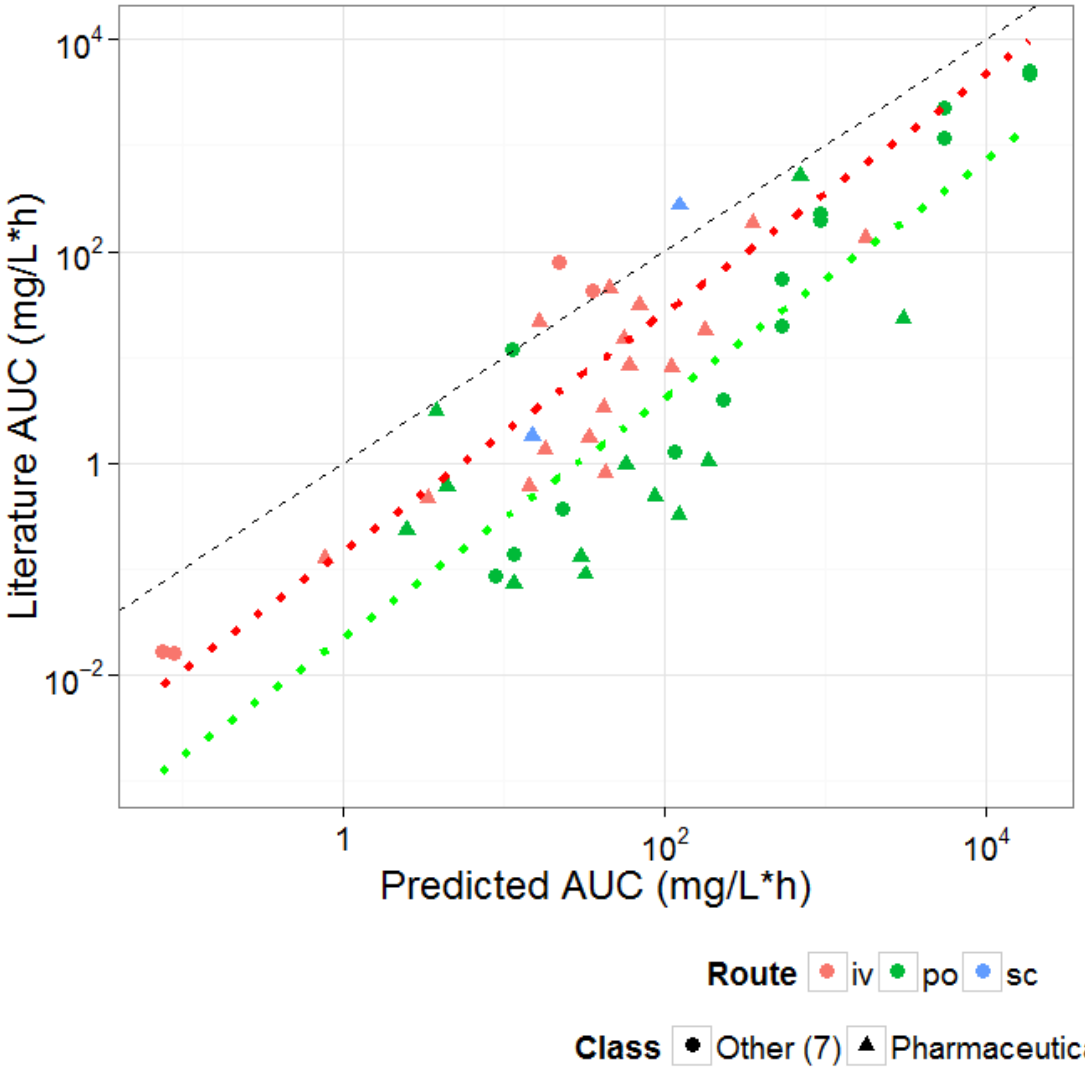
In vivo Predictive Ability and Domain of Applicability

- 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

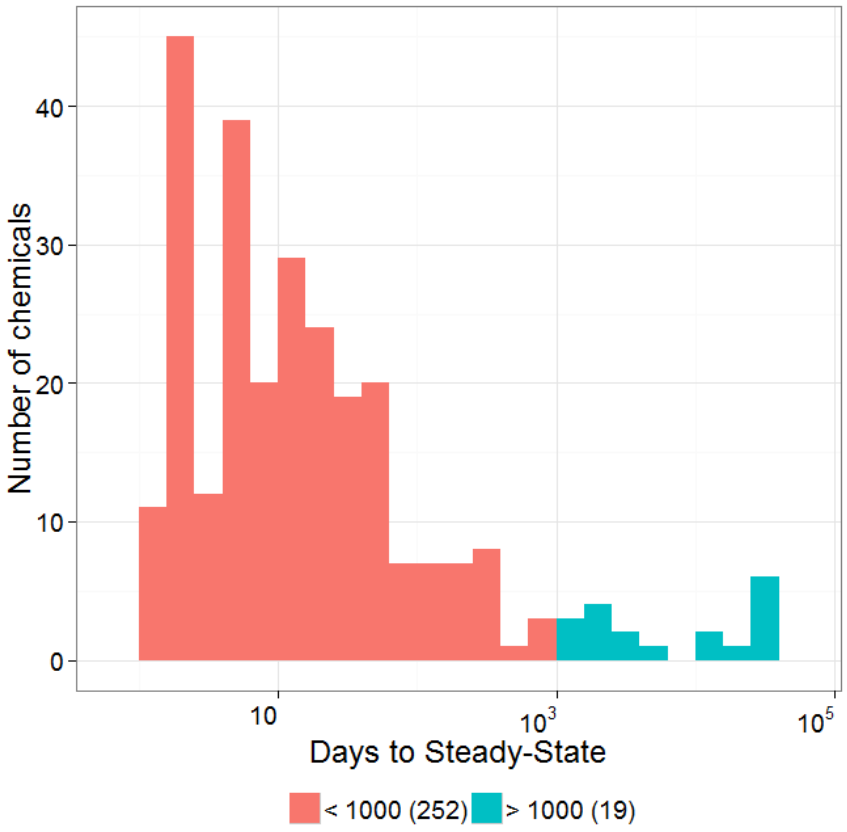
Evaluating HTPBPK Predictions from *In Vitro* Data



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

Wambaugh et al. (2015)

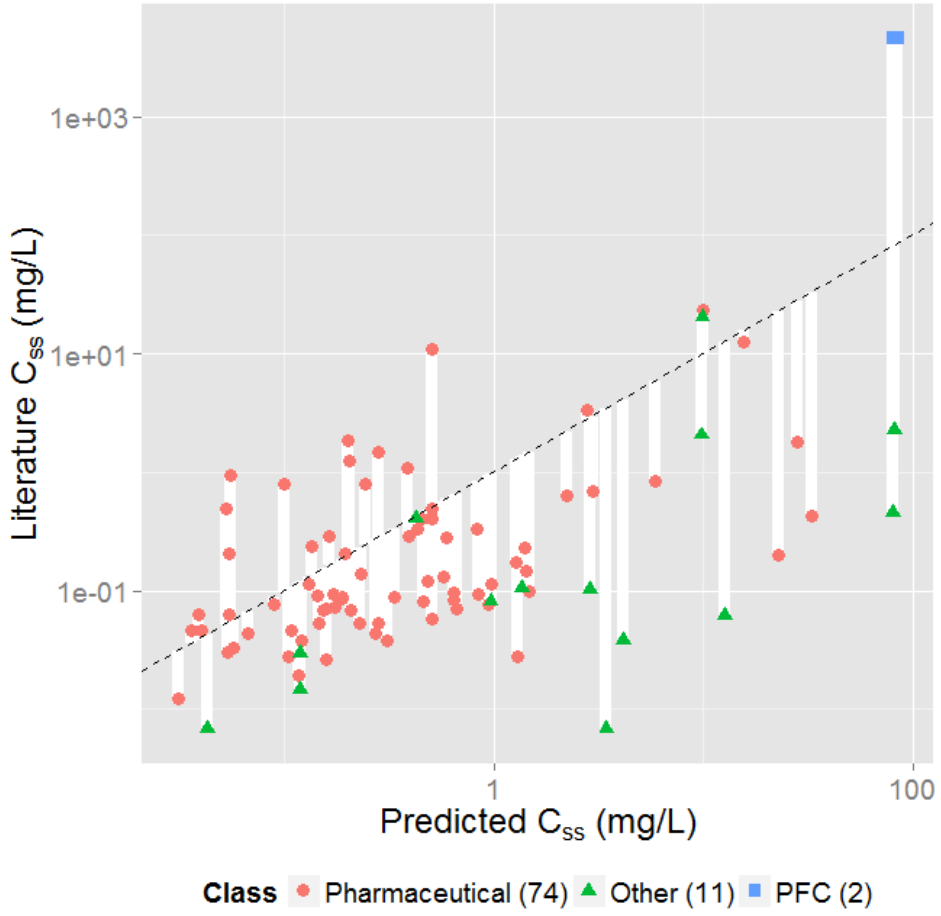
Evaluation of Steady-State Predictions



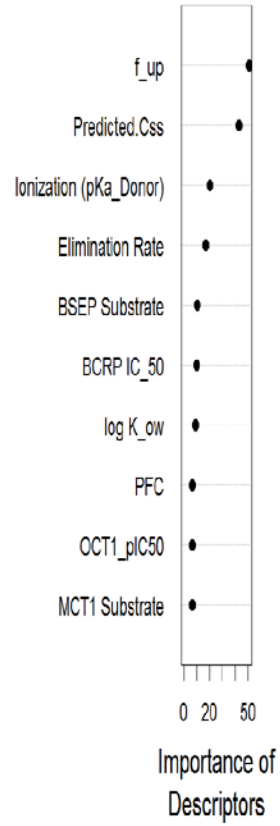
- Using HTPBTK model and assuming three daily doses (every eight hours)
- This allows us to evaluate the plausibility of the steady-state dosing assumption.
- We find that the majority of chemicals reach steady state in a few weeks
- A second population of chemicals never reach steady state.

Wambaugh et al. (2015)

Using *in vivo* Data to Evaluate RTK



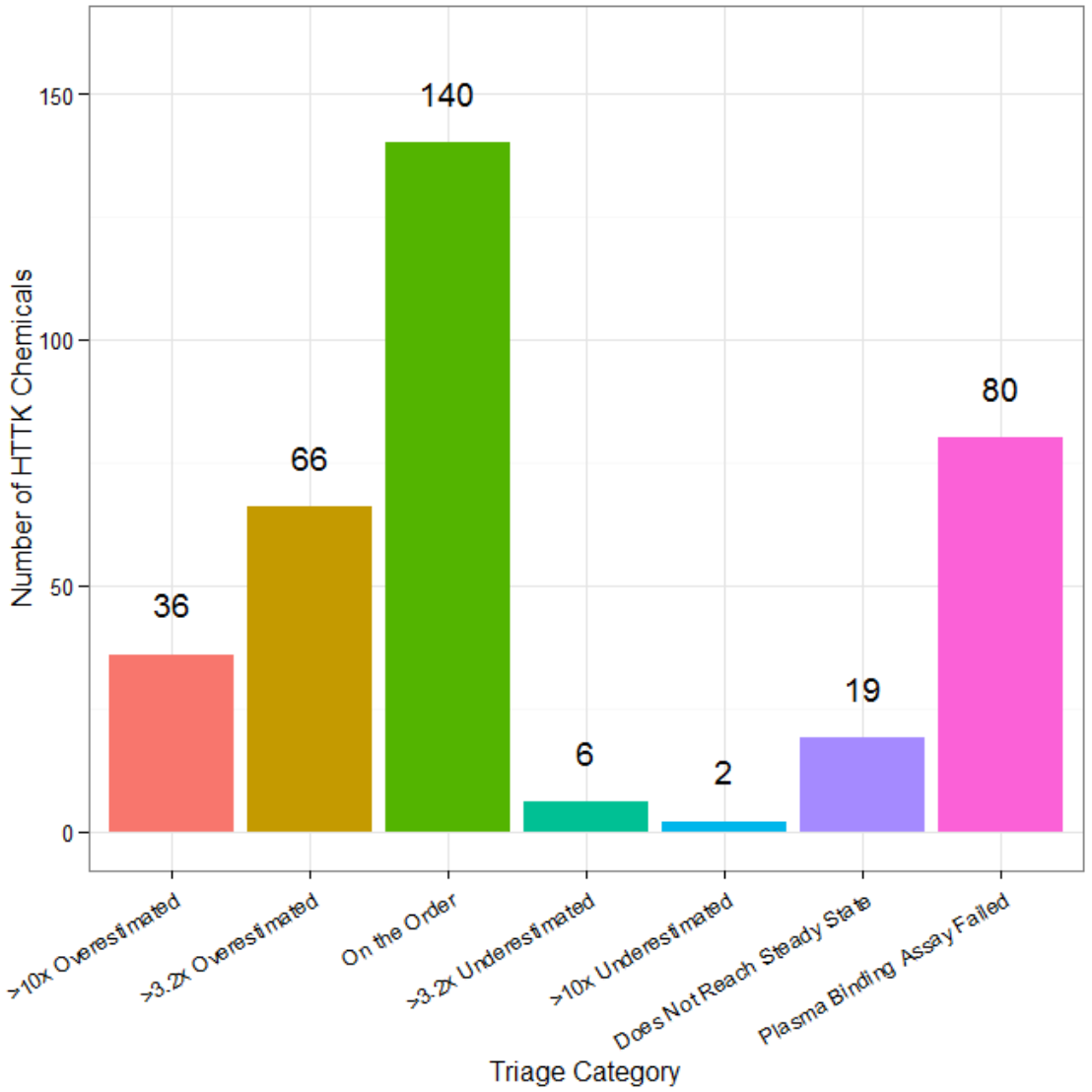
- When we compare the C_{ss} predicted from *in vitro* HTTK with *in vivo* C_{ss} values determined from the literature we find limited correlation (R^2 ~0.34)
- The dashed line indicates the identity (perfect predictor) line:
  - Over-predict for 65
  - Under-predict for 22
- The white lines indicate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



Wambaugh et al. (2015)

Toxicokinetic Triage

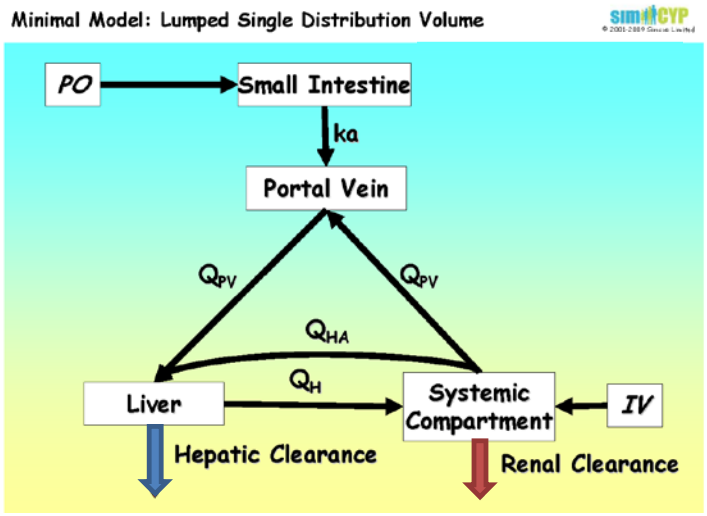
- Through comparison to *in vivo* data, a cross-validated (random forest) predictor of success or failure of HTTK has been constructed
- Add categories for chemicals that do not reach steady-state or for which plasma binding assay fails
- All chemicals can be placed into one of seven confidence categories



Wambaugh et al. (2015)

Variability in this Steady-State TK Model

Jamei *et al*. (2009)



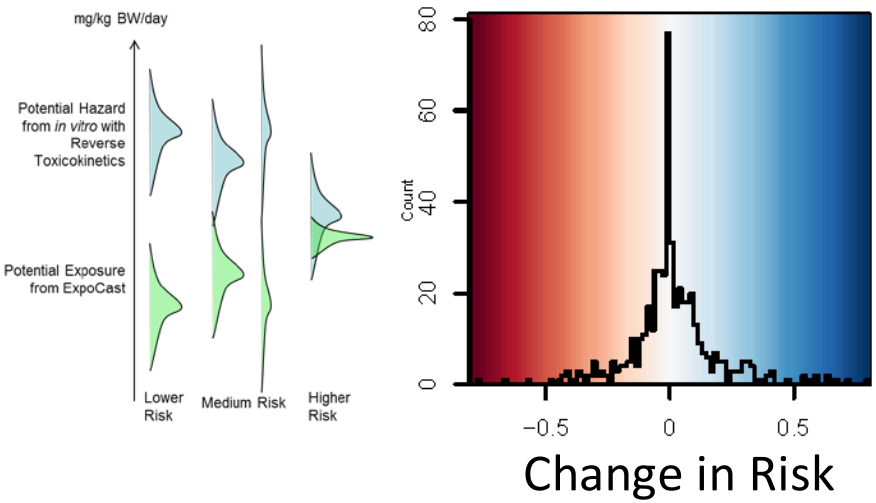
$$C_{ss} = \frac{\text{oral dose rate}}{\underbrace{(GFR * F_{ub})}_{\text{(Passive) Renal Clearance}} + \underbrace{\left(Q_l * F_{ub} * \frac{Cl_{int}}{Q_l + F_{ub} * Cl_{int}}\right)}_{\text{Hepatic Clearance (Metabolism)}}}$$

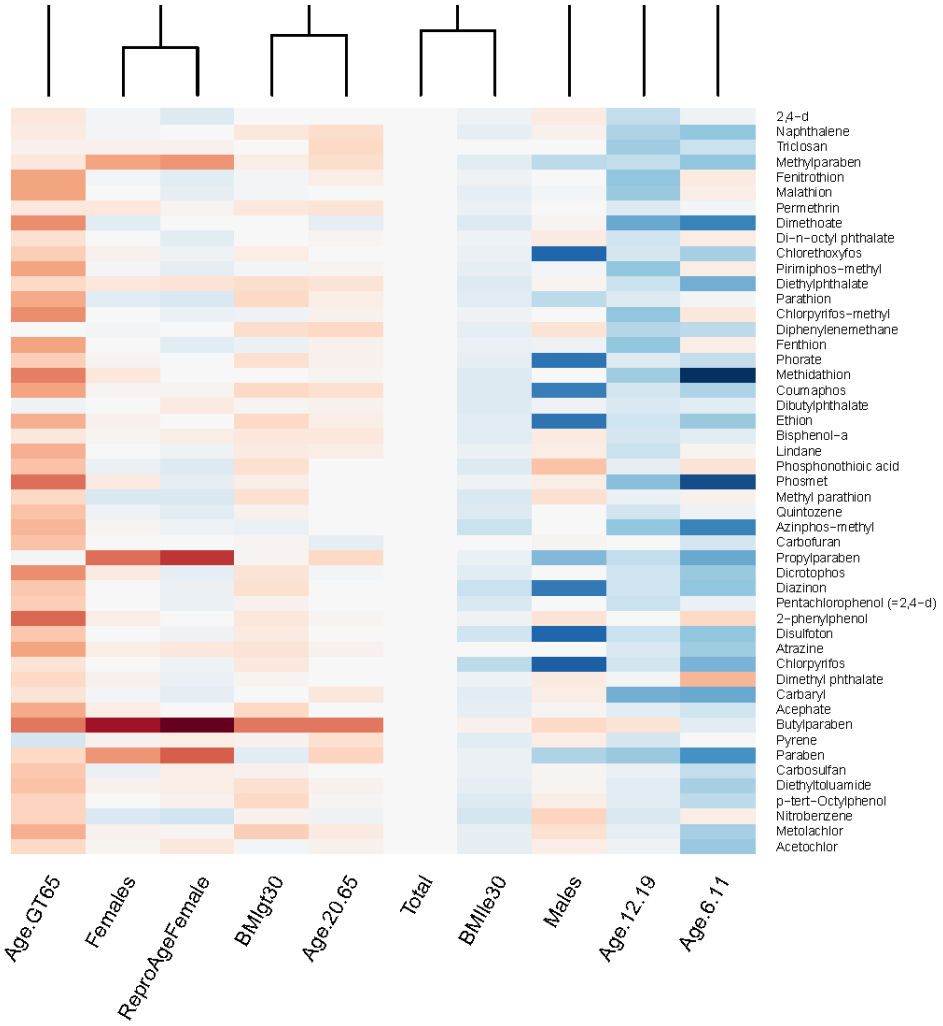
- *In vitro* clearance (µL/min/10^6 hepatocytes) is scaled to a whole organ clearance using the density of hepatocytes per gram of liver and the volume of the liver (which varies between individuals)
- Glomerular filtration rate (GFR) and blood flow to the liver (Q_l) both vary from individual to individual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

Life-stage and Demographic Specific Predictions

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

- New version of httk R package (Ring *et al.*, in preparation) allows prediction of parameters based on actual NHANES biometrics





Work by Caroline Ring (formerly of NCCT)

Summary

- Use of HTTK with HTS data provides an *in vivo* dose context that can be directly compared to exposure predictions, useful in risk-based prioritization.
- HTTK R package freely available on CRAN; models of varying complexity (1 compartment to multi-compartment PBTK) available for chemical analyses
- Upcoming refinements to the package:
  - Additional human and rat data
  - NHANES-derived population variability
  - Revised partition coefficient prediction
  - Human gestational PBTK
  - Inhalation exposure route
- Ongoing Efforts Consider Domain of Applicability
  - Analyses of 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

Acknowledgments

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[a] former members, NCCT

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Mark Sochaski

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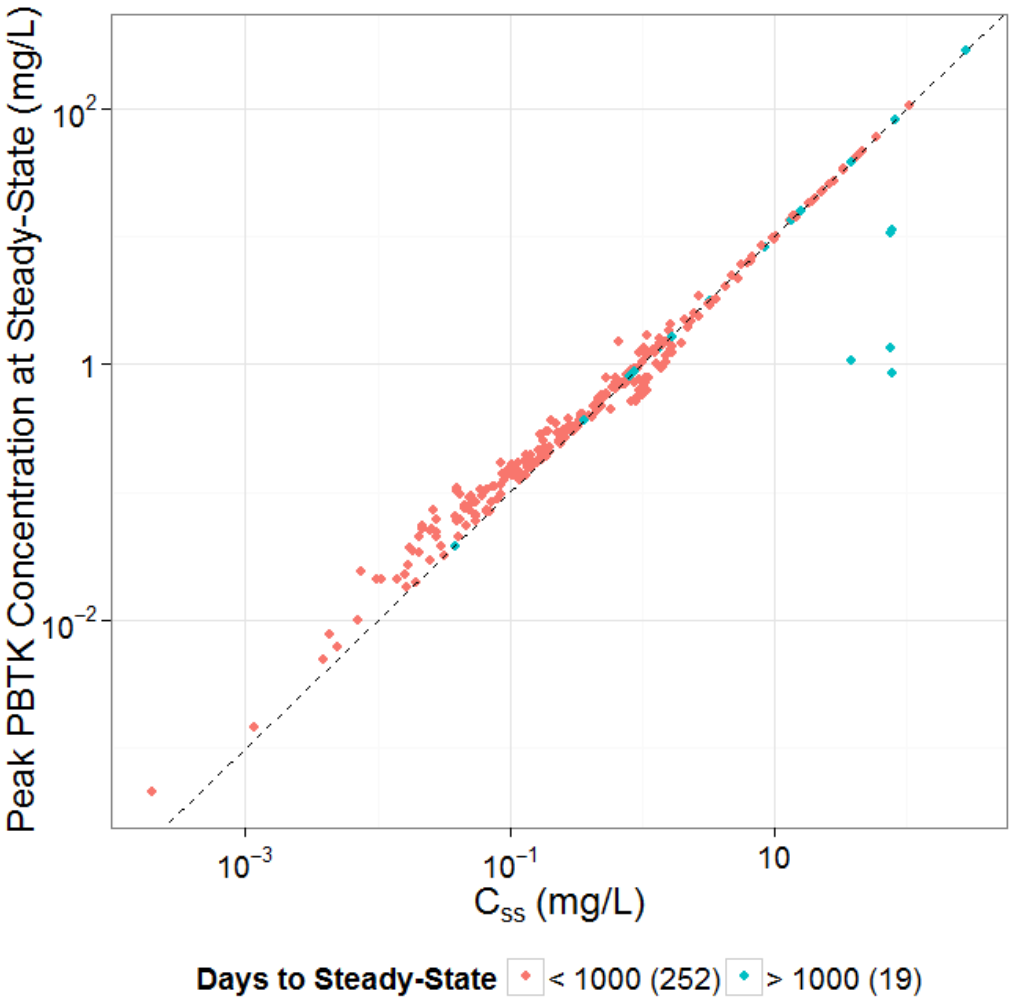
Extra Slides

Why Build Another PBTK Tool?

	SimCYP	ADMET Predictor / GastroPlus	MEGen	httk
Creator/Owner:	SimCYP Consortium / Certara	Simulations Plus	UK Health and Safety Laboratory (Loizou)	US EPA
Availability	License; but inexpensive for basic research	License, but inexpensive for basic research	Free: http://xnet.hsl.gov.uk/megen	Free: CRAN Repository
Monte Carlo Simulation	Yes	No	No	Yes
Batch Mode	Yes	Yes	No	Yes
Physiologic Data	Yes	Yes	Yes	Yes
Chemical-Specific Library	Pharma	No	No	Pharma and ToxCast (>500 and growing)
Export Function	No	No	Matlab and AcslX	SBML and Jarnac
R Integration	No	No	No	Yes
Easy Reverse Dosimetry	Yes	Yes	No	Yes
Future Proof XML	No	No	Yes	No

Goal: An open-source (and transparent) platform for rapid chemical analysis

Peak Concentration vs. C_{ss}



Peak serum concentrations from the HTPBPK model are compared against the steady-state concentration predicted by the three compartment model for a constant infusion exposure (as in Wetmore et al. 2012)

The dashed, identity (1:1) line indicates that for most compounds the peak concentrations are very similar to C_{ss}.

Wambaugh et al. (2015)