# New Tricks with High Throughput Toxicokinetics

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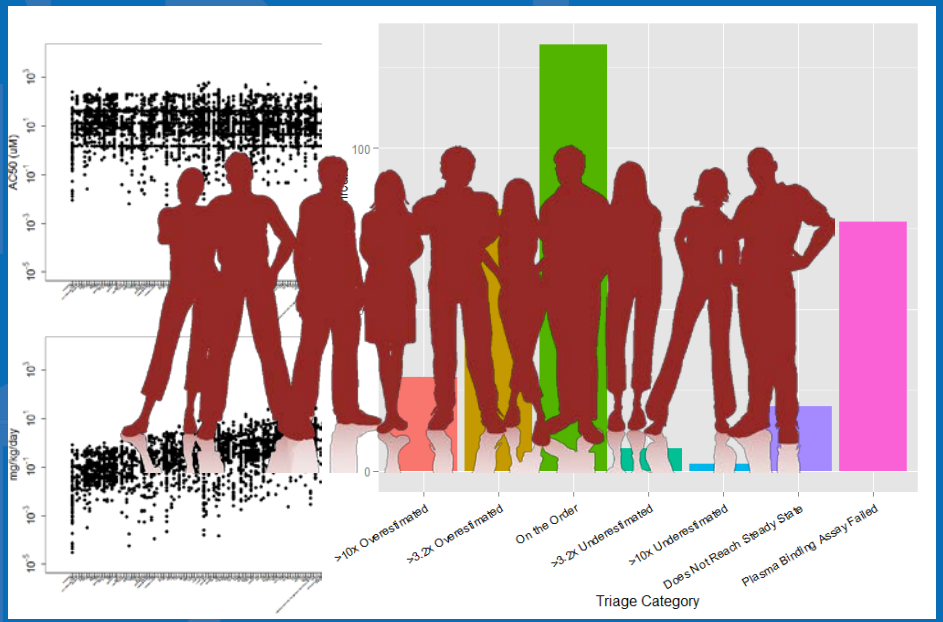


*Figure includes image from Thinkstock*

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# Introduction

- Toxicokinetics (TK) provides a bridge between HTS and HTE by predicting tissue concentrations due to exposure
  - Traditional TK methods are resource intensive

- Relatively high throughput TK (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)
  - A key application of HTTK has been "reverse dosimetry" (also called Reverse TK or RTK)
  - RTK can approximately convert *in vitro* HTS results to daily doses needed to produce similar levels in a human for comparison to exposure data (Wetmore, *et al.,* 2012)

- R is a free, open source programming language and software environment for statistical computing and graphics
  - R "packages" add special functionality and data for statistical analysis

- New R package "httk" freely available on CRAN allows RTK and other statistical analyses of 543 chemicals (more coming)

# Why Build Another PBTK Tool?

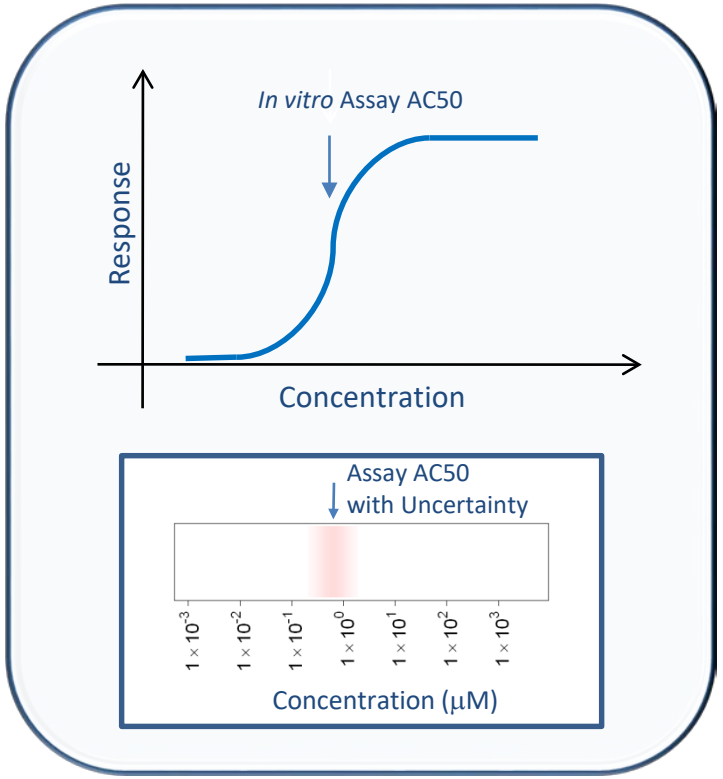
| | SimCYP | ADMET Predictor / GastroPlus | MEGen | httk |
|---|---|---|---|---|
| Maker | SimCYP Consortium / Certara | Simulations Plus | UK Health and Safety Laboratory (Loizou) | US EPA |
| Availability | License, but inexpensive for research | License, but inexpensive for research | Free: http://xnet.hsl.gov.uk/megen | Free: CRAN Repository |
| Population Variability Monte Carlo | Yes | No | No | Yes |
| Batch Mode | Yes | Yes | No | Yes |
| Physiological Data | Yes | Yes | Yes | Yes |
| Chemical-Specific Data Library | Clinical Drugs | No | No | Pharma and ToxCast Compounds: 443 PBTK, +100 steady-state only |
| 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 |



We want to do a statistical analysis (using R) for as many chemicals as possible

# 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)
- All data is public: http://actor.epa.gov/



# *In vitro* Bioactivity, HTTK, and *in Vivo* Toxic Doses

Comparison of HTTK predicted oral equivalent doses (box and whisker plots in mg/kg/day) with doses for no effect and low effect groups in animal studies

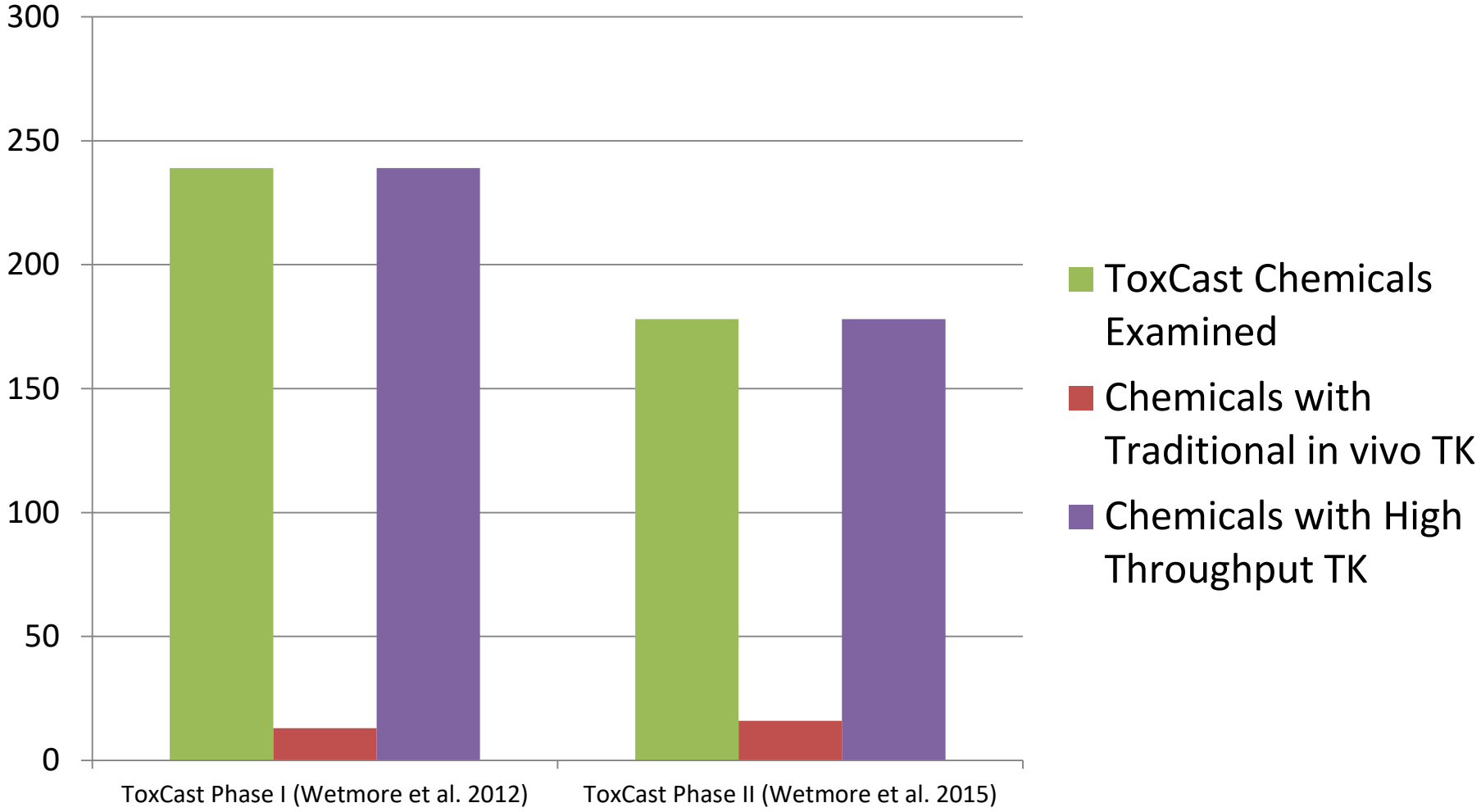
- **Lowest Observed Effect Level**
- **No Observed Effect Level (NEL)**
- **NEL/100**

Estimated chronic exposure levels from food residues are indicated by vertical red lines. All values are in mg/kg/day.

Judson *et al*. (2011)

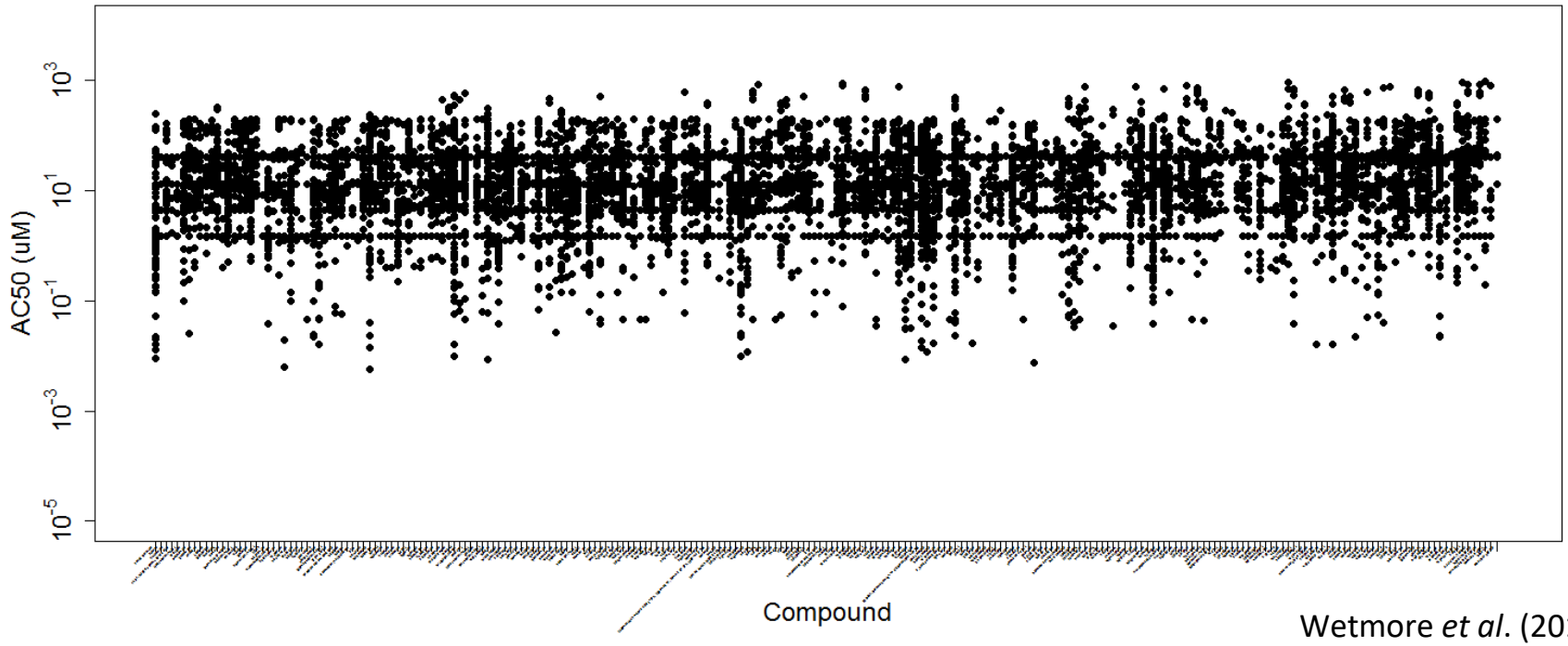
# The Need for *In Vitro* Toxicokinetics

United States Environmental Protection Agency




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

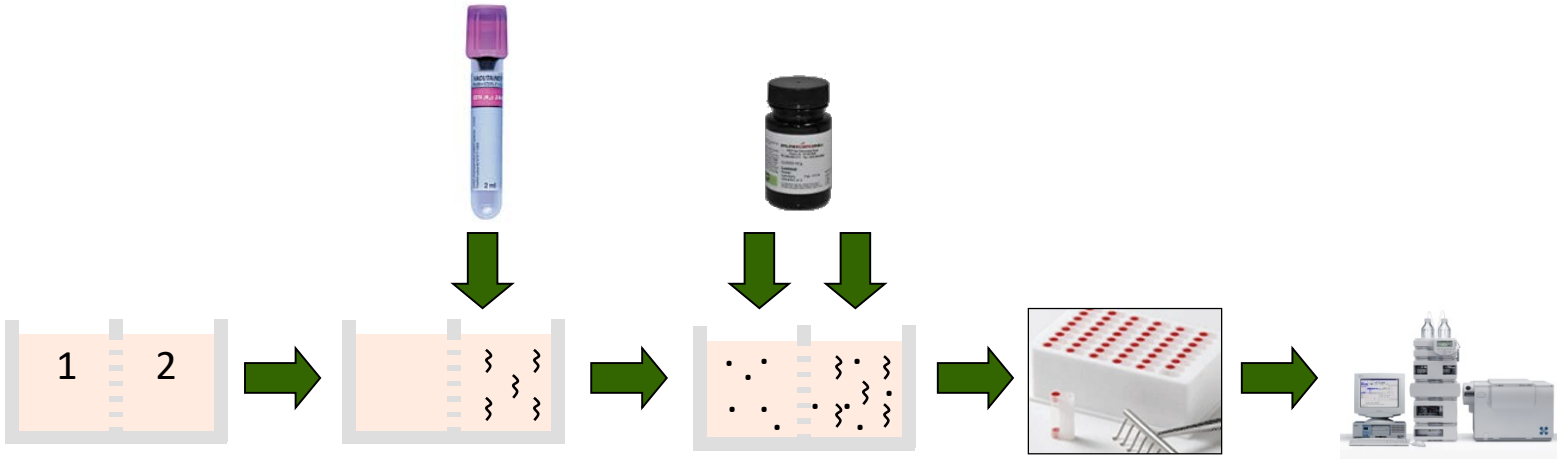
# ToxCast *in vitro* Bioactive Concentrations




Wetmore *et al*. (2012)

- One point for each chemical-*in vitro* assay combination with a systematic (Hill function) concentration response curve
- How can we use toxicokinetics to convert these to human doses?

# Plasma Protein Binding (Fraction Unbound in Plasma)




**Double-wells connected by semi-permeable membrane on a Rapid Equilibrium Dialysis (RED) Plate**

**Add plasma (6 donor pool for human) to one well**

**Add chemical**

**Incubate plates to allow wells with and without protein to come to equilibrium**

**Determine concentration in both wells (analytical chemistry)**

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

- Data on ToxCast chemicals initially collected at Hamner Institutes

Published:

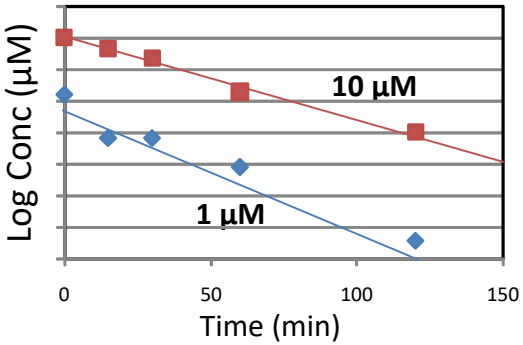
- Rotroff et al. (2010) - Pilot study using 38 Phase I ToxCast chemicals
- Wetmore et al. (2012) - Remainder of easily analyzed Phase I chemicals
- Wetmore et al. (2013) - Rat TK for 50 ToxCast/ToxRefDB compounds
- Wetmore et al. (2015) - ~200 ToxCast Phase II chemicals



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

# Intrinsic Hepatic Clearance


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

Published:

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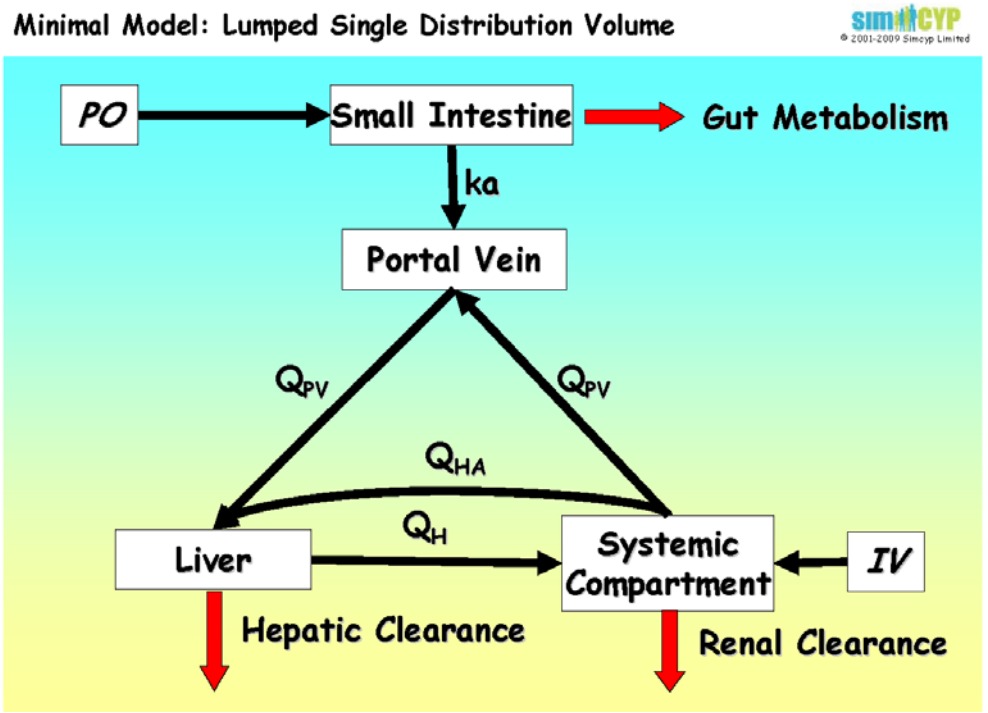
# High Throughput Toxicokinetics (HTTK)

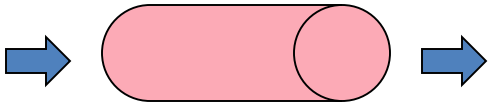
Jamei *et al*. (2009)

- *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



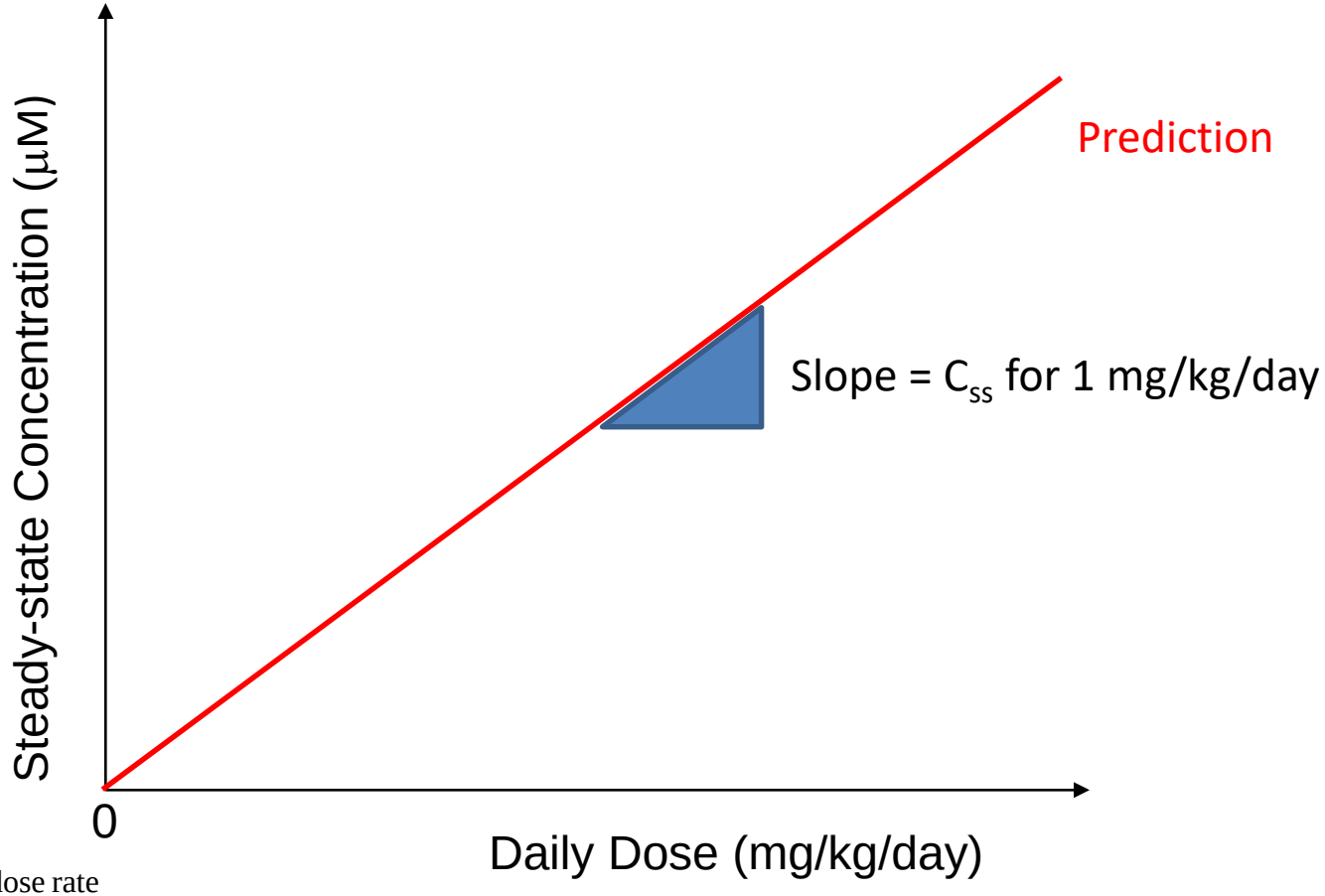

$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * \text{F}_{ub}) + \left(\text{Q}_l * \text{F}_{ub} * \frac{Cl_{int}}{\text{Q}_l + \text{F}_{ub} * Cl_{int}}\right)}$$

Oral dose in (mg/kg/day)



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

# Steady-State is Linear with Dose

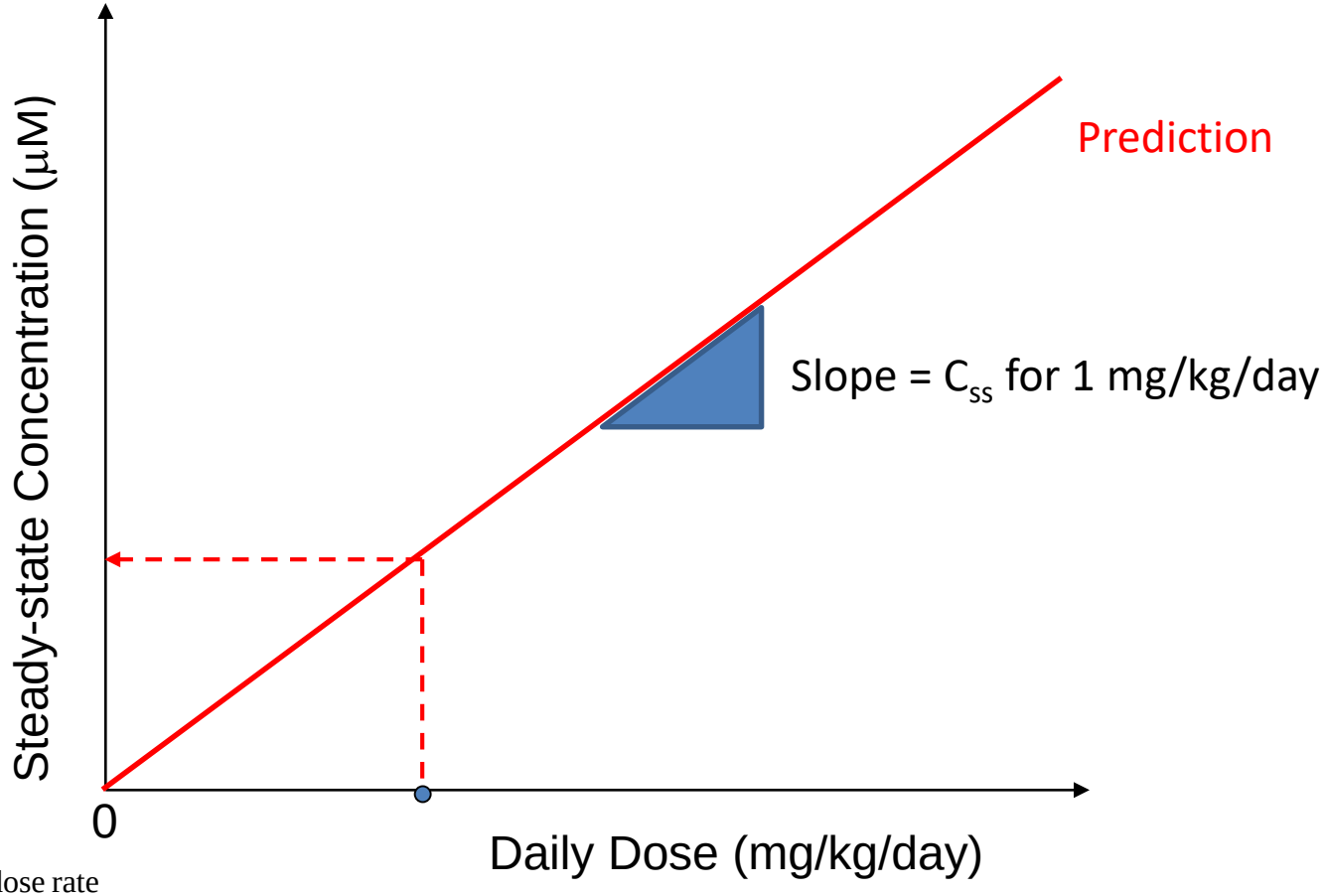



$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * \text{F}_{ub}) + \left(\text{Q}_l * \text{F}_{ub} * \frac{Cl_{int}}{\text{Q}_l + \text{F}_{ub} * Cl_{int}}\right)}$$

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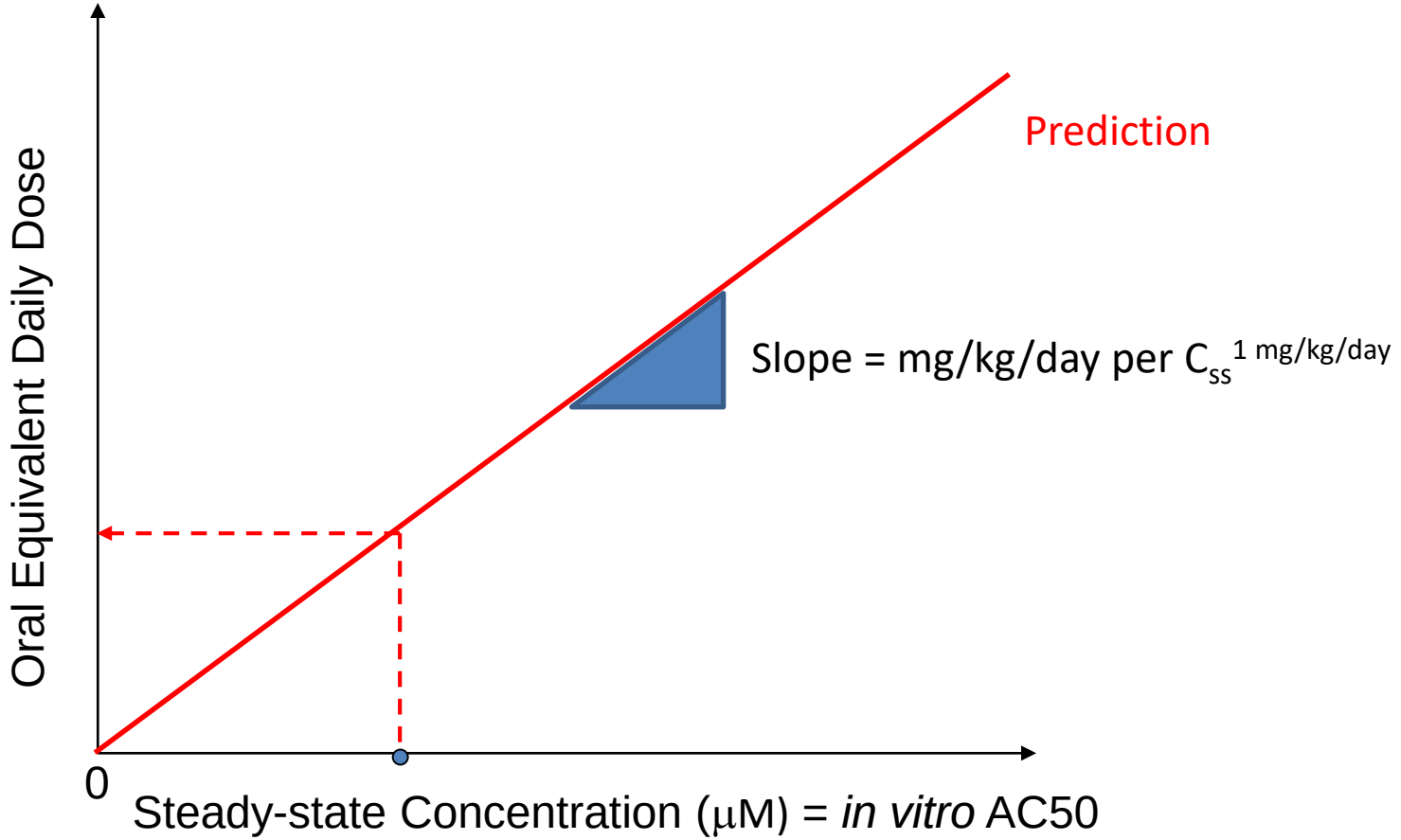
- Can calculate predicted steady-state concentration ($C_{ss}$) for a 1 mg/kg/day dose and multiply to get concentrations for other doses

Wetmore *et al*. (2012)

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

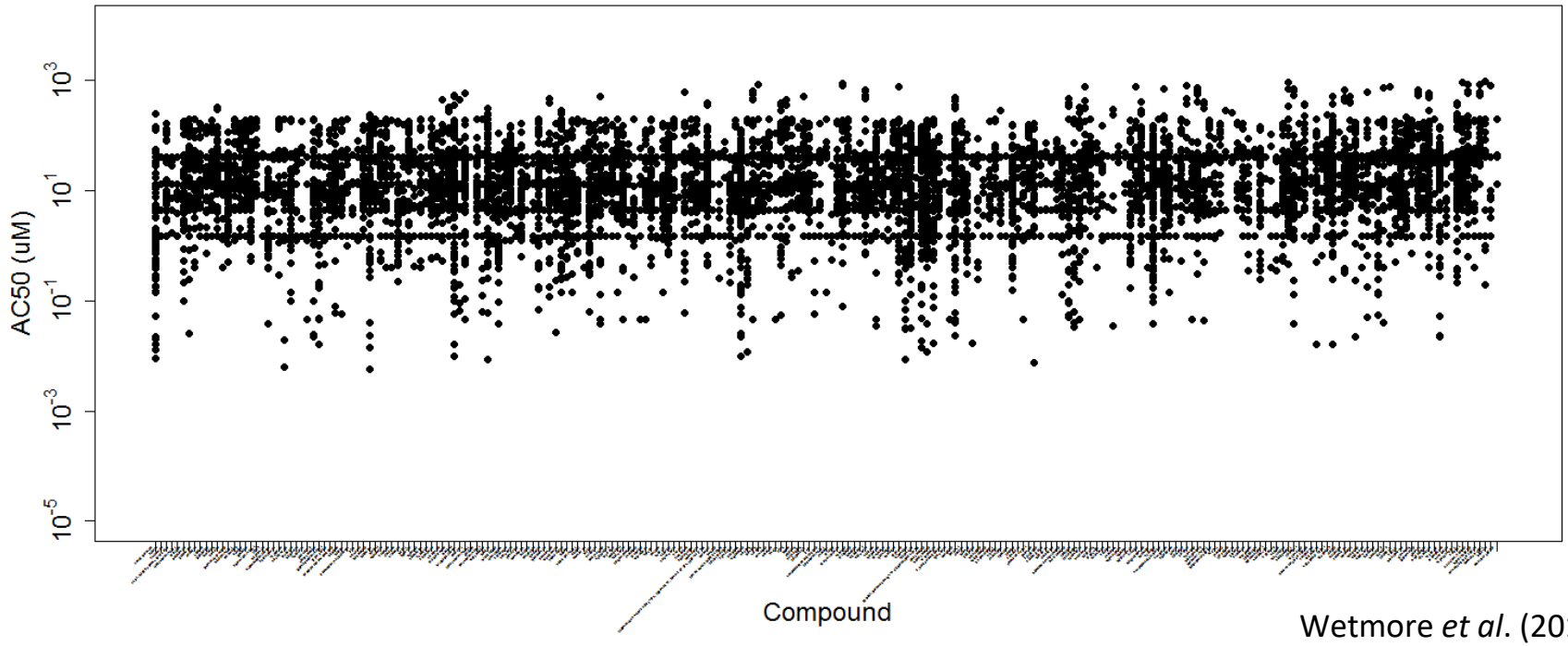



- Swap the axes (this is the "reverse" part of reverse dosimetry)
- Can divide bioactive concentration by $C_{ss}$ for for a 1 mg/kg/day dose to get oral equivalent dose



Wetmore *et al*. (2012)

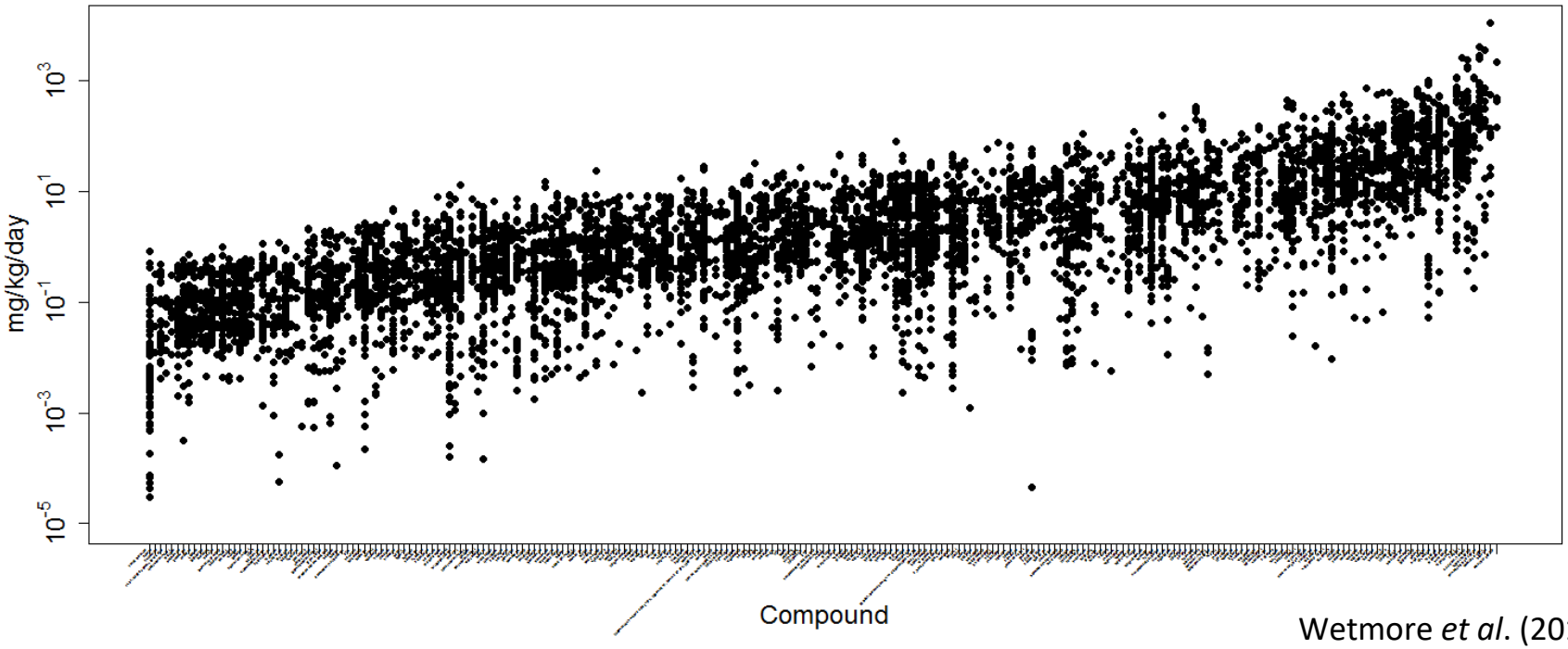
# ToxCast *in vitro* Bioactive Concentrations




Wetmore *et al*. (2012)

- It appears harder to prioritize on bioactive *in vitro* concentration without *in vivo* context

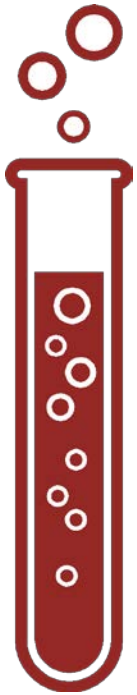
# HTTK Oral Equivalents




Wetmore *et al*. (2012)

- Translation from *in vitro* to steady-state oral equivalent doses allow greater discrimination between effective chemical potencies

# Reverse Dosimetry with HTTK



High Throughput *In Vitro* Bioactive Concentration

HTTK *in vitro* data

Simulated Human *In Vivo* Doses

Monte Carlo Simulation of Biological Variability

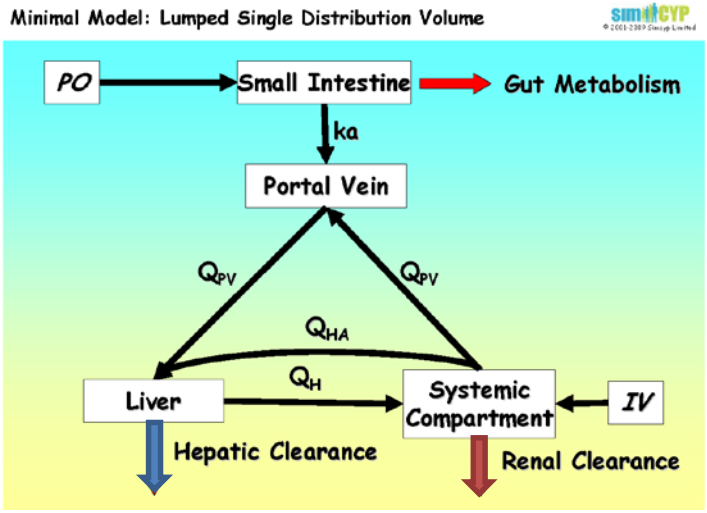
Combination of higher exposure and sensitivities

Populations that are More Sensitive

*Images from Thinkstock*

# Variability in this Steady-State TK Model

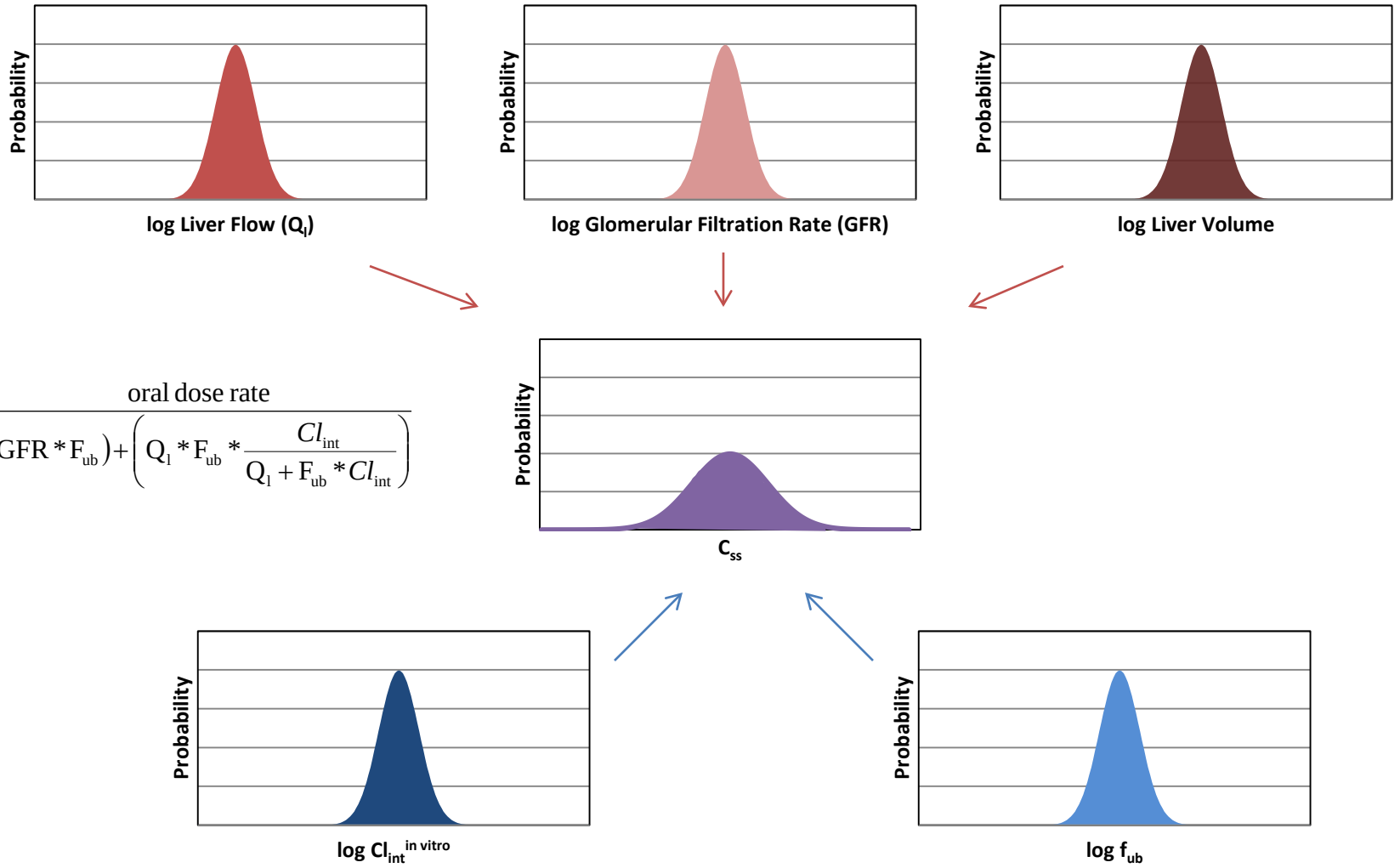
Jamei *et al*. (2009)




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

- *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

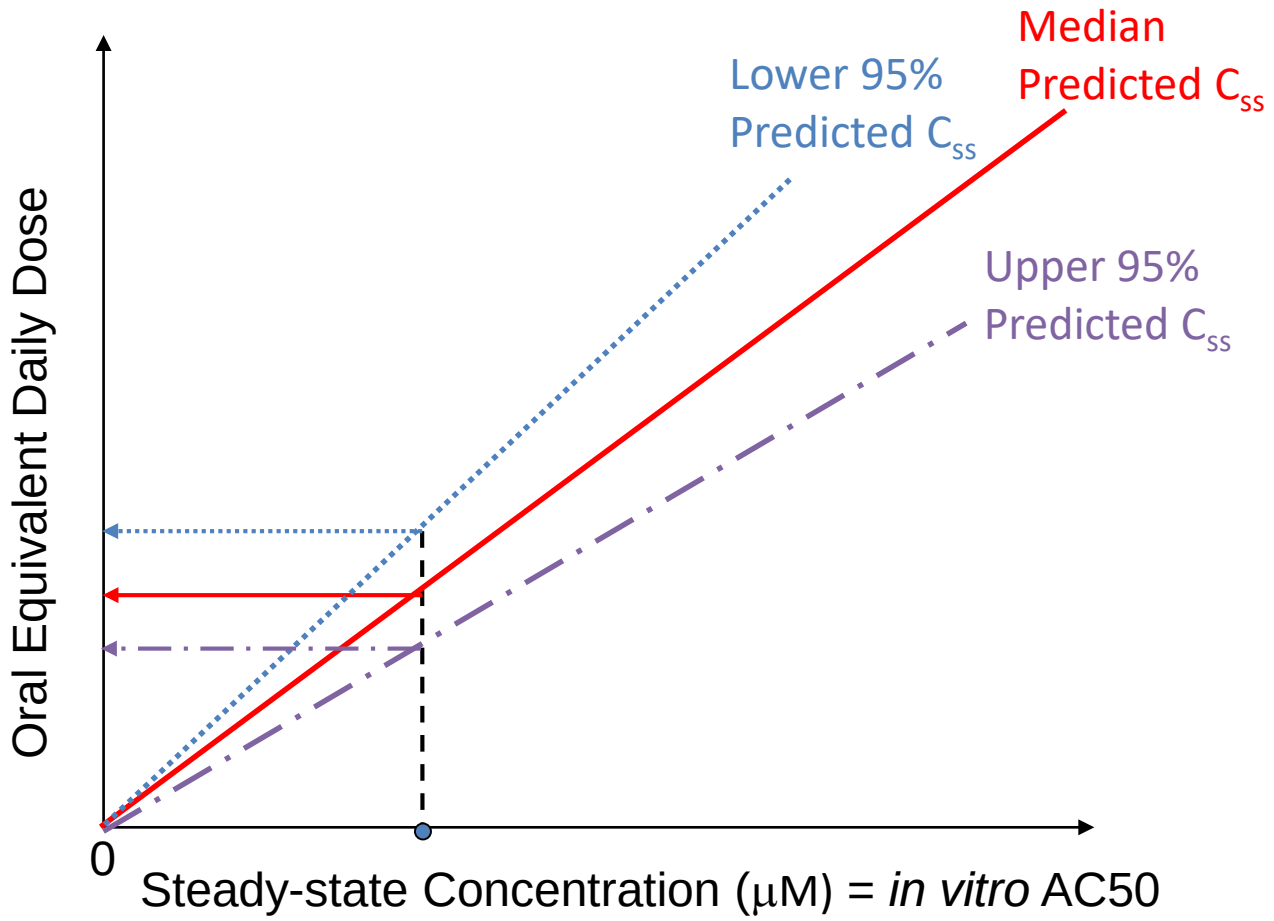
# Monte Carlo (MC) Approach to Variability: SimCYP (Pharma) Approach






Wetmore *et al*. (2012)

# Steady-State In Vitro-In Vivo Extrapolation (IVIVE)




- The higher the predicted $C_{ss}$, the lower the oral equivalent dose, so the upper 95% predicted $C_{ss}$ from the MC has a lower oral equivalent dose

# Application to High Throughput Risk Prioritization

Prioritization as in Wetmore *et al*. (2012) Bioactivity, Dosimetry, and Exposure Paper

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

ExpoCast Exposure Predictions

**Near Field**

**Far Field**

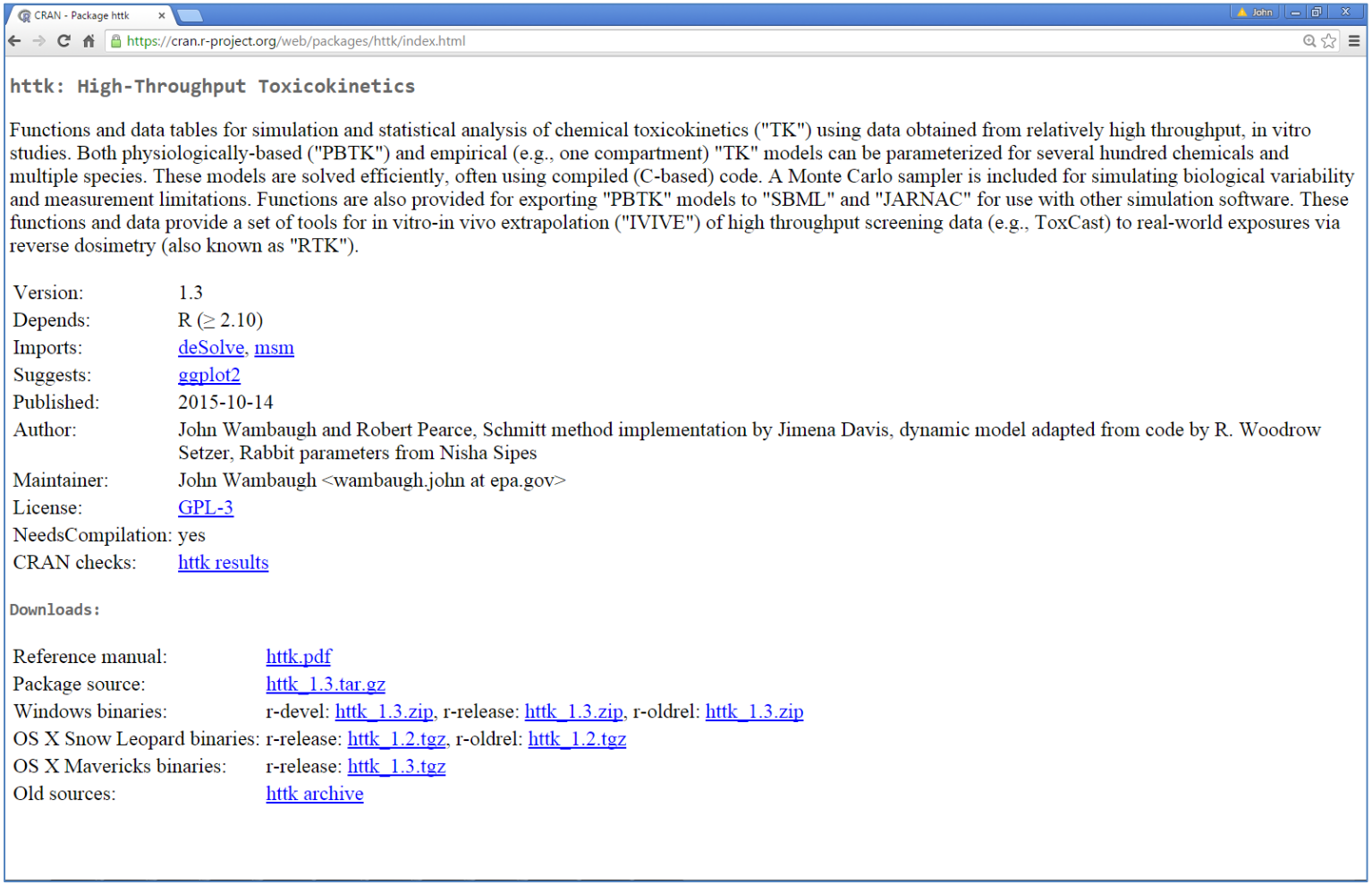
**ToxCast Chemicals**

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



**Also see poster "Computational Models to Correlate *In Vitro* to *In Vivo* Activity" by Nisha Sipes and Steve Ferguson, et al.**

# Steady State Concentrations with httk R Package


CRAN - Package httk

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

**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:**

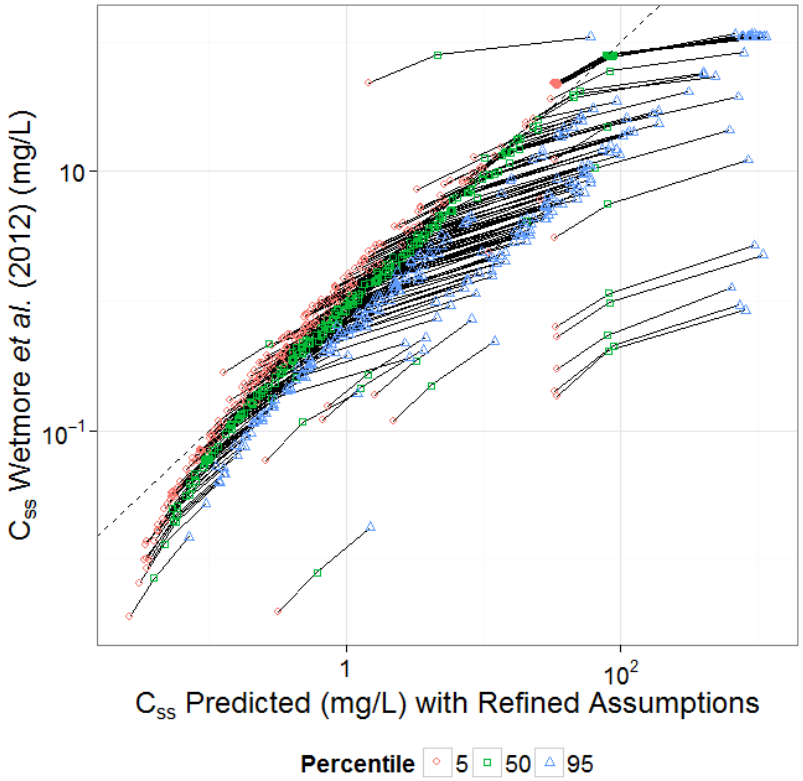
| | |
|---|---|
| Reference manual: | httk.pdf |
| Package source: | httk_1.3.tar.gz |
| Windows binaries: | r-devel: httk_1.3.zip, r-release: httk_1.3.zip, r-oldrel: httk_1.3.zip |
| OS X Snow Leopard binaries: | r-release: httk_1.2.tgz, r-oldrel: httk_1.2.tgz |
| OS X Mavericks binaries: | r-release: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



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

Can access from the R GUI: “Packages” then “Install Packages”

# Comparison Between httk and SimCYP



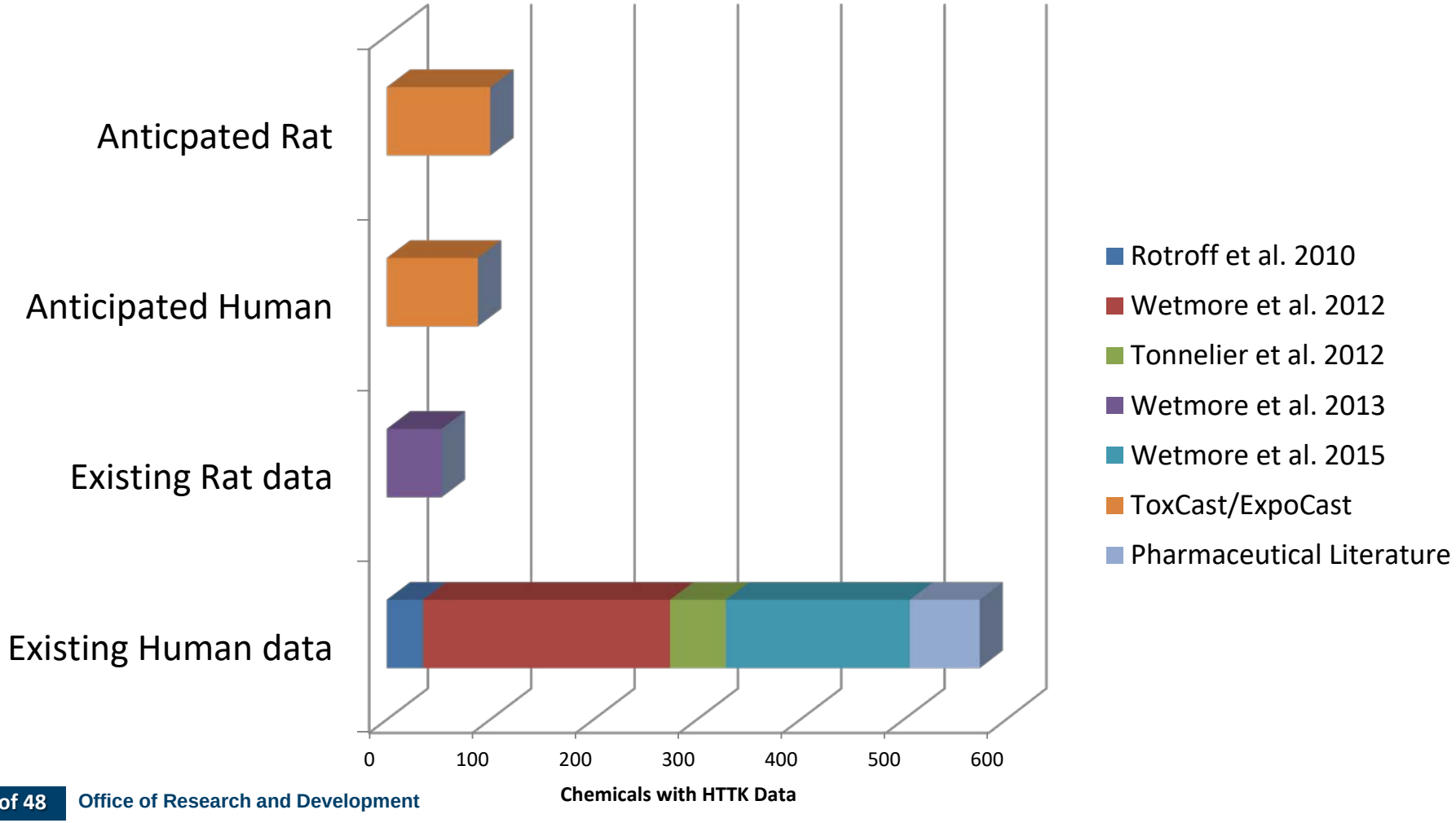

- In the Rotroff et al. (2010) and Wetmore et al. (2010) papers SimCYP was used to predict distributions of $C_{ss}$ from *in vitro* data
  - We show that our new we can reproduce the results from those publications for most chemicals using our implementation of Monte Carlo.
- Any one chemical's median and quantiles are connected by a dotted line.
- Hepatic clearance assays with p-values < 0.05 are considered "good".

The RED assay for measuring protein binding fails in some cases because the amount of free chemical is below the limit of detection. For those chemicals a default value of 0.5% free was used. We have replaced the default value with random draws from a uniform distribution from 0 to 1%.

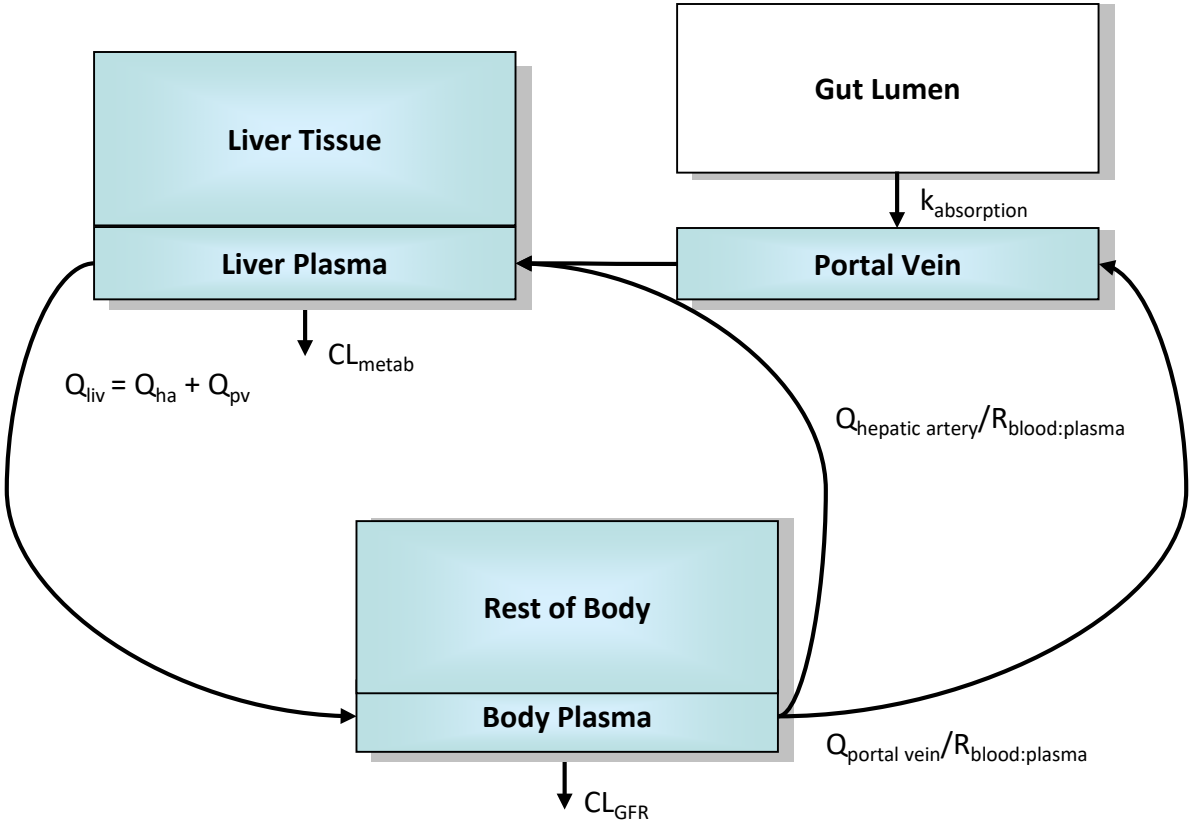


Wambaugh et al. (2015)

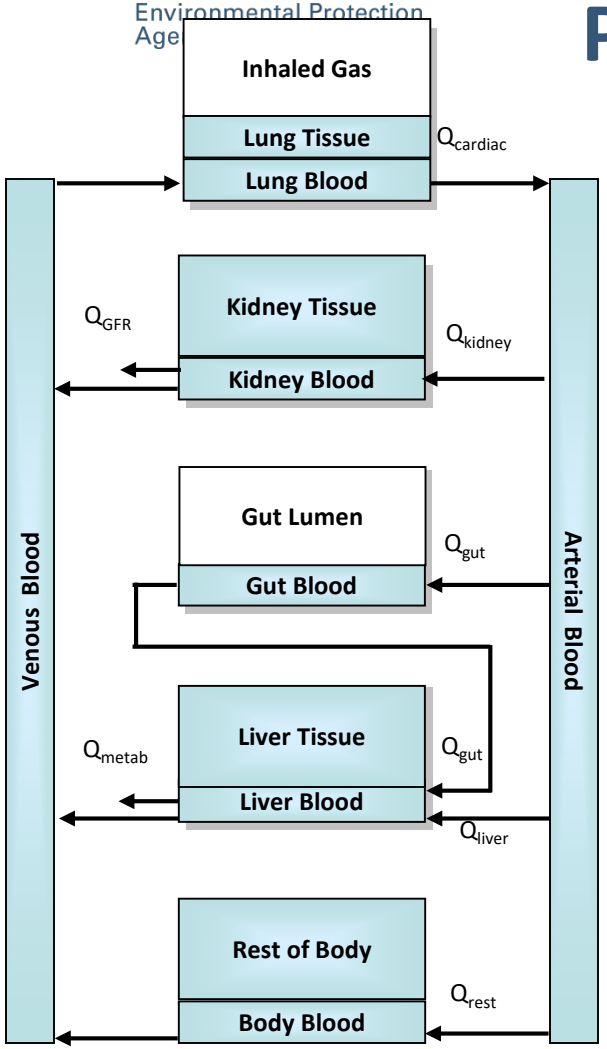
# Chemicals with HTTK Data



# Three Compartment (SimCYP) Model



# 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).

# Physiological Data

United States Environmental Protection Agency

| | Volume (L/kg) | | | | | Blood Flow (ml/min/kg) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Tissue** | **Mouse** | **Rat** | **Dog** | **Human** | **Rabbit** | **Mouse** | **Rat** | **Dog** | **Human** | **Rabbit** |
| **Adipose** | 0.07 | 0.07 | 0.05 | 0.21 | 0.05 | 10.80 | 1.60 | 3.50 | 3.71 | 12.80 |
| **Bone** | 0.05 | 0.04 | 0.04 | 0.07 | 0.04 | 23.31 | 36.11 | 1.30 | 3.36 | 36.11 |
| **Brain** | 0.02 | 0.01 | 0.01 | 0.02 | 0.01 | 13.20 | 5.20 | 4.50 | 10.00 | 5.20 |
| **Gut** | 0.04 | 0.03 | 0.04 | 0.02 | 0.05 | 72.50 | 39.20 | 23.00 | 16.43 | 44.40 |
| **Heart** | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 14.00 | 15.60 | 5.40 | 3.43 | 6.40 |
| **Kidneys** | 0.02 | 0.01 | 0.01 | 0.00 | 0.01 | 65.00 | 36.80 | 21.60 | 17.71 | 32.00 |
| **Liver** | 0.05 | 0.03 | 0.03 | 0.02 | 0.04 | 90.00 | 47.20 | 30.90 | 20.71 | 70.80 |
| **Lung** | 0.01 | 0.00 | 0.01 | 0.01 | 0.01 | 2.00 | 6.22 | 10.56 | 2.00 | 6.22 |
| **Muscle** | 0.37 | 0.39 | 0.44 | 0.38 | 0.54 | 45.50 | 30.00 | 25.00 | 10.71 | 62.00 |
| **Skin** | 0.15 | 0.17 | 0.17 | 0.03 | 0.04 | 20.50 | 23.20 | 10.00 | 4.29 | 23.20 |
| **Spleen** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 5.50 | 4.07 | 1.65 | 1.10 | 3.60 |
| **Rest** | 0.03 | 0.05 | 0.00 | 0.05 | 0.03 | 110.19 | 90.00 | 5.59 | 2.97 | 90.00 |

Volumes and flows from Schmitt (2008) + Nisha Sipes (Rabbit)

Other parameters from Davies and Morris (1993) + Nisha Sipes (Rabbit)

| | **Units** | **Mouse** | **Rat** | **Dog** | **Human** | **Rabbit** |
|---|---|---|---|---|---|---|
| **Total Body Water** | ml/kg | 725.00 | 668.00 | 603.60 | 600.00 | 716 |
| **Plasma Volume** | ml/kg | 50.00 | 31.20 | 51.50 | 42.86 | 44 |
| **Cardiac Output** | ml/min/kg | 400.00 | 296.00 | 120.00 | 80.00 | 212 |
| **Average BW** | kg | 0.02 | 0.25 | 10.00 | 70.00 | 2.5 |
| **Total Plasma Protein** | g/ml | 0.06 | 0.07 | 0.09 | 0.07 | 0.057 |
| **Plasma albumin** | g/ml | 0.03 | 0.03 | 0.03 | 0.04 | 0.0387 |
| **Plasma a-1-AGP** | g/ml | 0.01 | 0.02 | 0.00 | 0.00 | 0.0013 |
| **Hematocrit** | fraction | 0.45 | 0.46 | 0.42 | 0.44 | 0.36 |
| **Urine** | ml/min/kg | 0.035 | 0.139 | 0.021 | 0.014 | 0.0417 |
| **Bile** | ml/min/kg | 0.069 | 0.063 | 0.008 | 0.003 | 0.0833 |
| **GFR** | ml/min/kg | 14.0 | 5.2 | 6.1 | 1.8 | 3.12 |

# Schmitt (2008) Tissue Composition Data

| | Fraction of total volume[a] | | Fraction of cell volume[b] | | | Fraction of total lipid | | | |
|---|---|---|---|---|---|---|---|---|---|
| Tissue | Cells | Interstitium | Water | Lipid | Protein | Neutral Lipid[c] | Neutral Phospholipid[c] | Acidic Phospholipid[c] | pH[d] |
| Adipose | 0.86 | 0.14 | 0.03 | 0.92 | 0.06 | 1 | 0.0022 | 0.0006 | 7.10 |
| Bone | 0.9 | 0.1 | 0.26 | 0.02 | 0.21 | 0.85 | 0.11 | 0.04 | 7.00 |
| Brain | 1 | 0.004 | 0.79 | 0.11 | 0.08 | 0.39 | 0.48 | 0.13 | 7.10 |
| Gut | 0.9 | 0.096 | 0.78 | 0.07 | 0.15 | 0.69 | 0.26 | 0.05 | 7.00 |
| Heart | 0.86 | 0.14 | 0.7 | 0.11 | 0.19 | 0.48 | 0.43 | 0.09 | 7.10 |
| Kidneys | 0.78 | 0.22 | 0.73 | 0.06 | 0.21 | 0.26 | 0.61 | 0.13 | 7.22 |
| Liver | 0.82 | 0.18 | 0.68 | 0.08 | 0.21 | 0.29 | 0.59 | 0.11 | 7.23 |
| Lung | 0.5 | 0.5 | 0.74 | 0.04 | 0.11 | 0.51 | 0.38 | 0.11 | 6.60 |
| Muscle | 0.88 | 0.12 | 0.76 | 0.01 | 0.19 | 0.49 | 0.42 | 0.09 | 6.81 |
| Skin | 0.69 | 0.31 | 0.47 | 0.14 | 0.41 | 0.9 | 0.08 | 0.02 | 7.00 |
| Spleen | 0.79 | 0.21 | 0.75 | 0.02 | 0.23 | 0.3 | 0.54 | 0.15 | 7.00 |
| Red blood cells | 1 | – | 0.63 | 0.01 | 0.33 | 0.3 | 0.59 | 0.1 | 7.20 |

**a** Values taken from (Kawai et al., 1994). Original values given as fraction of total organ volume were rescaled to tissue volume by subtracting vascular volume

**b** Values taken from (ICRP, 1975). Original values given as fraction of total tissue mass were rescaled to cellular volume as follows: Water fraction of total tissue reduced by interstitial volume and subsequently all values normalized by cellular fraction.

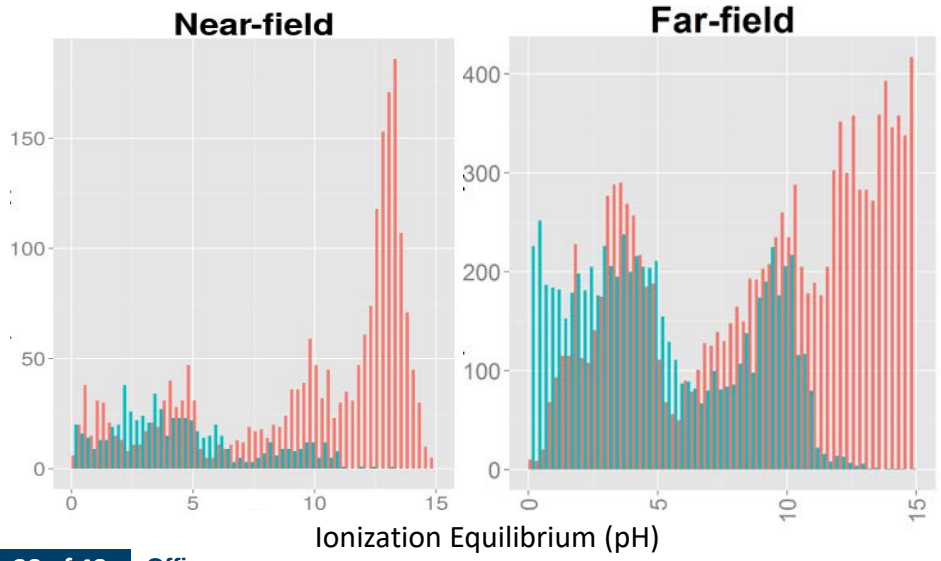
**c** Data taken from (Rodgers et al., 2005a).

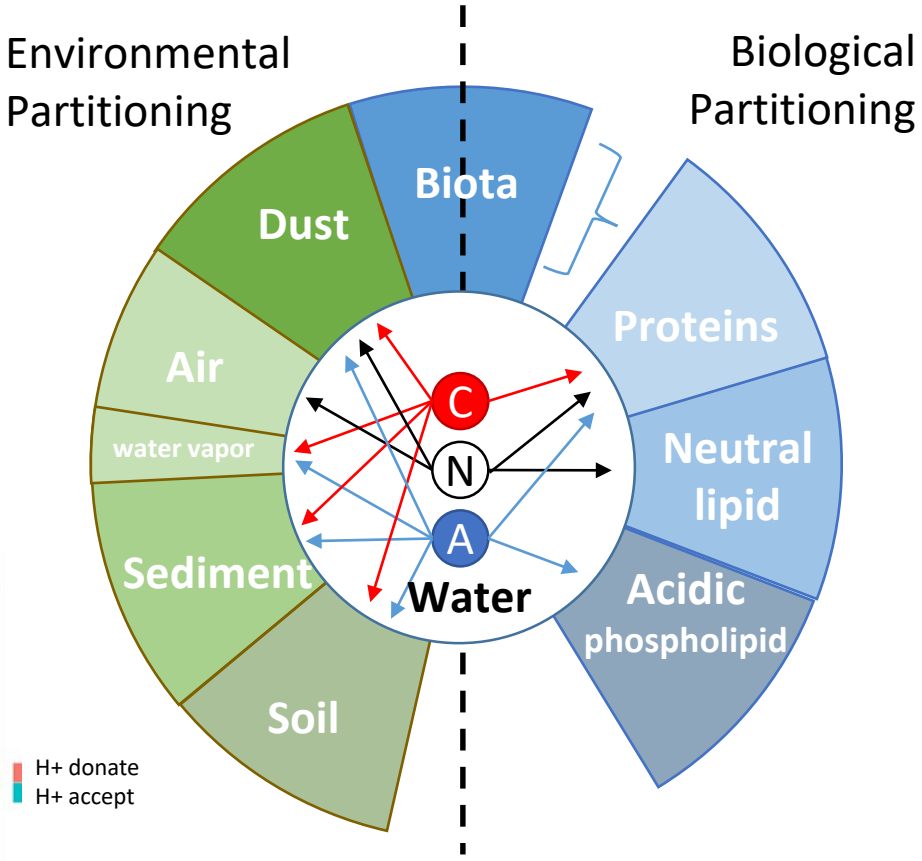
**d** Values taken from ([Waddell and Bates, 1969], [Malan et al., 1985], [Wood and Schaefer, 1978], [Schanker and Less, 1977], [Harrison and Walker, 1979] and [Civelek et al., 1996]). Mean values were calculated when more than one value was found for the same tissue.

**e** Data taken from (Gomez et al., 2002).

# Prediction of Ionization

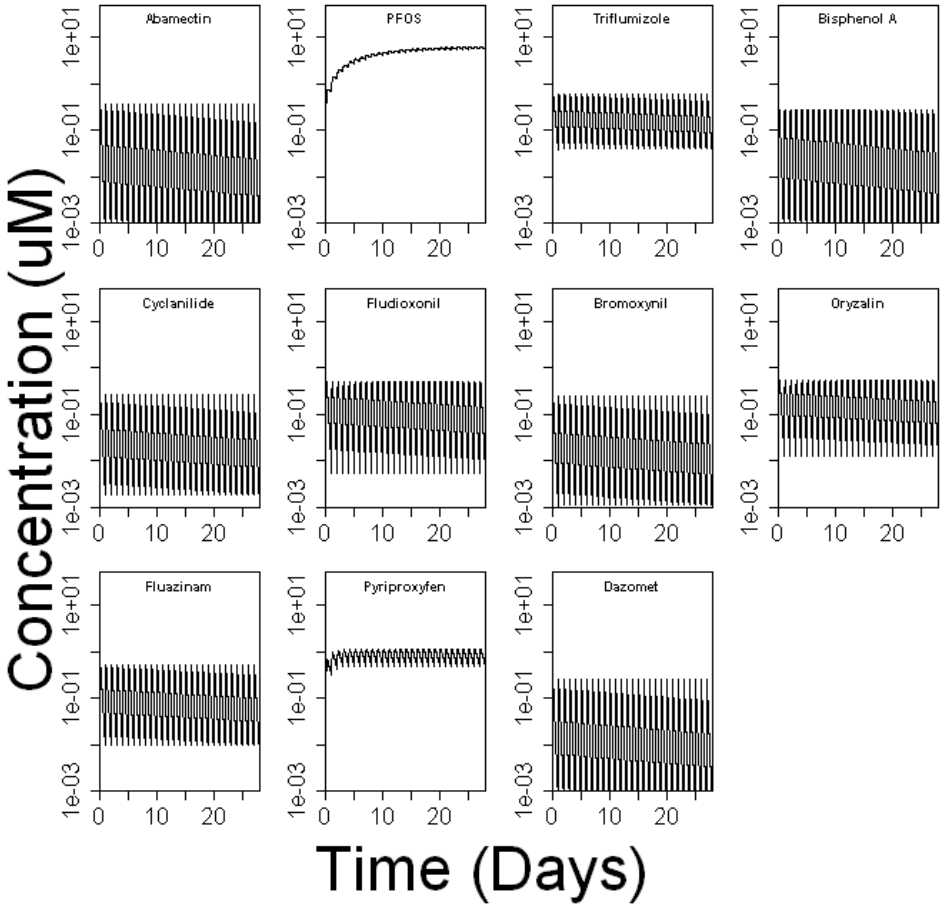
- Neutral and ionized species of the same molecule will partition differently into environmental and biological media
- Better models are needed for predicting pKa at different pH for chemicals




Project lead Cory Strope (Hamner)

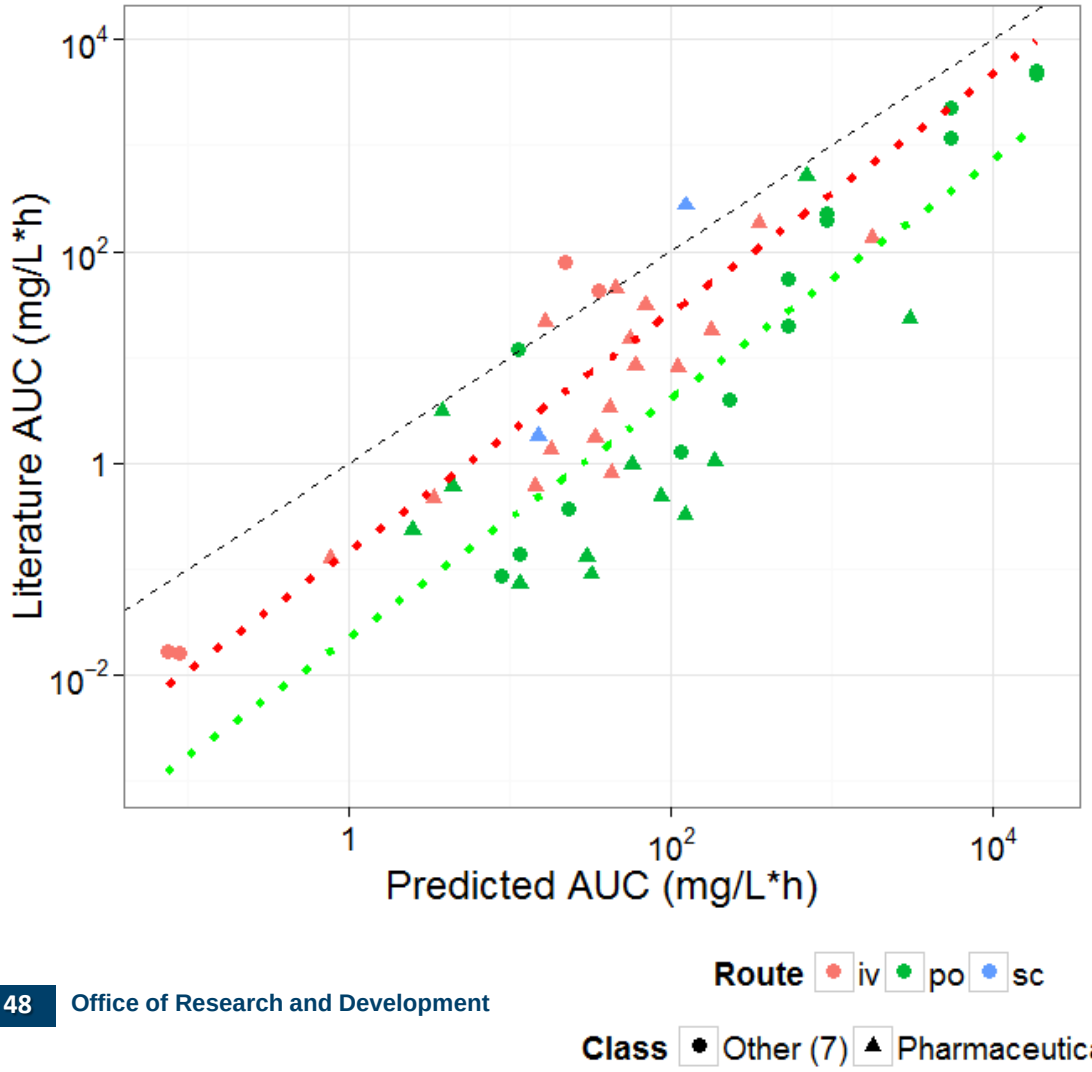
# Predicted PK Metrics




Example at left: Human hepatic concentration of various chemicals as a function of 28 daily doses (10 mg/kg/day)

Can predict mean and peak concentration and time integrated area under the curve (AUC) for various tissues

# Evaluating HTPBPK Predictions from *In Vitro* Data



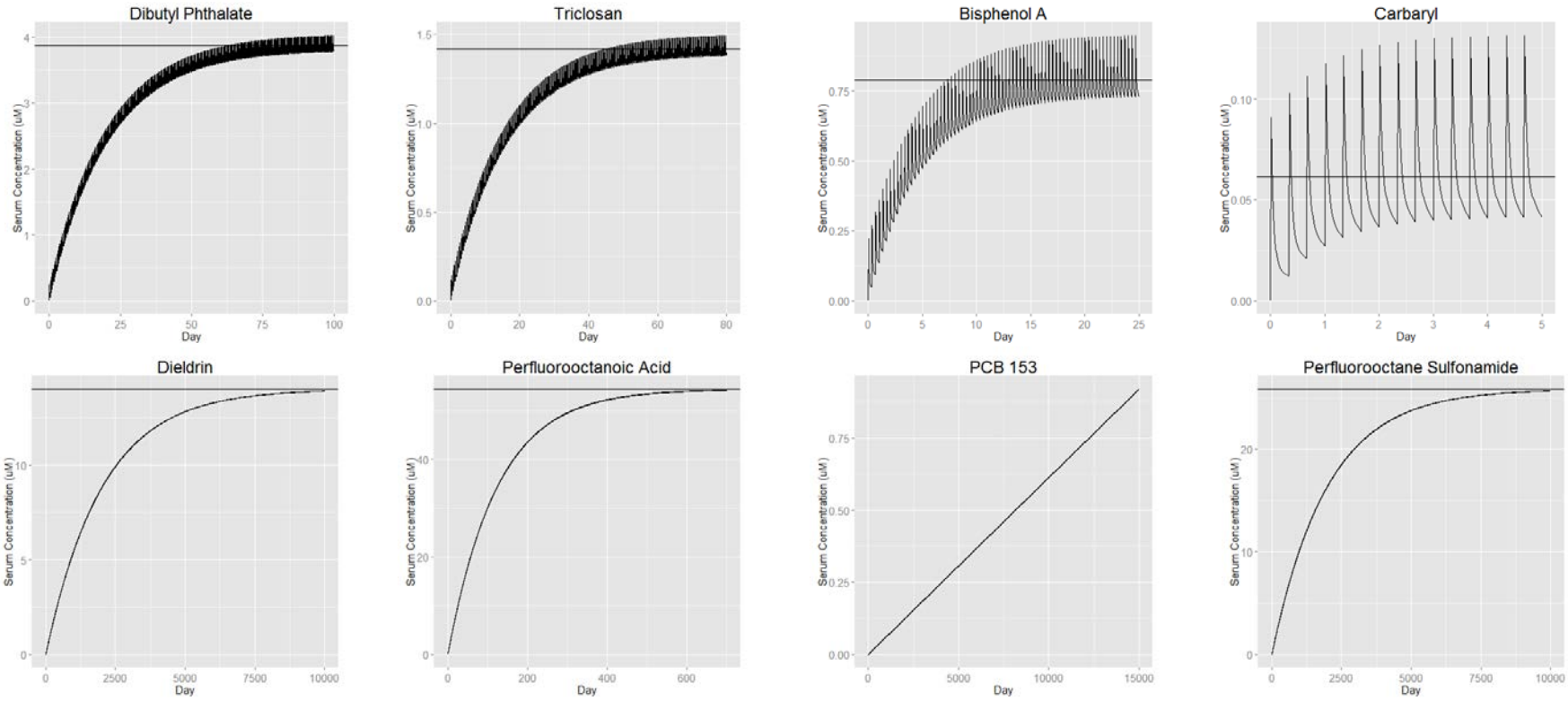

- HTPBPK predictions for the AUC (time integrated plasma concentration or Area Under the Curve)
- *in vivo* measurements from the literature for various treatments (dose and route) of rat.
- Predictions are generally conservative – *i.e.*, predicted AUC higher than measured
- Oral dose AUC ~6.4x higher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

Wambaugh et al. (2015)

# calc_stats Examples

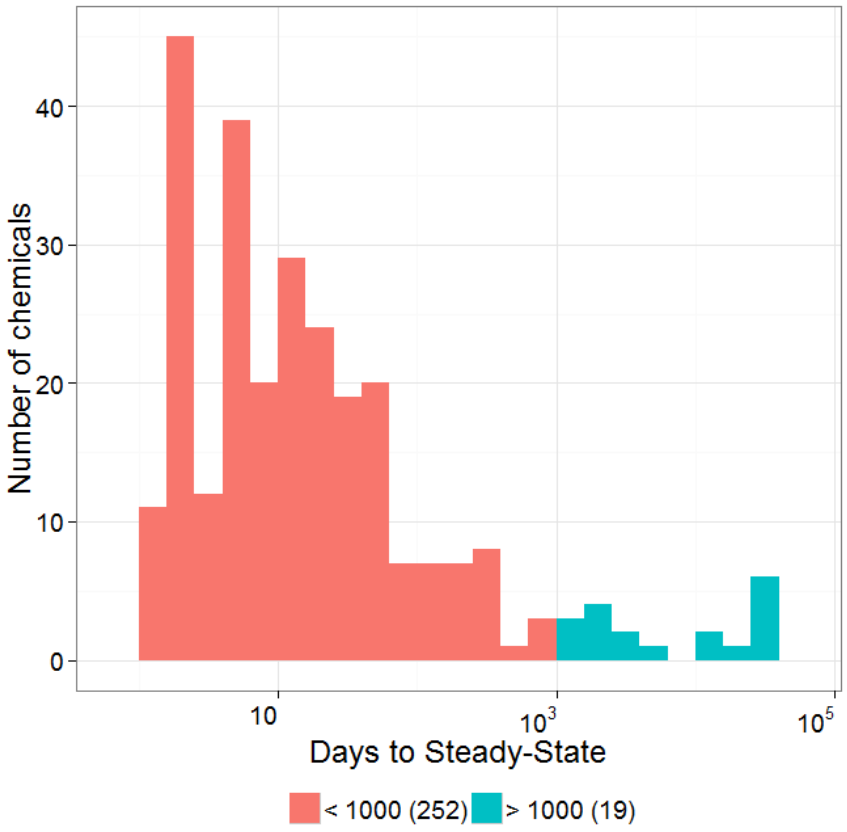
```
library(httk)

#A Function to get PK summary statistics from the PBPK model:
help(calc_stats)
# 28 day human study (20 mg/kg/day) for Abamectin:
calc_stats(days=28,chem.name="bisphenol a", dose=20)
# Units default to µM but can use mg/L:
calc_stats(days=28,chem.name="bisphenol a", dose=20,output.units="mg/L")
# Same study in a mouse:
calc_stats(days=28,chem.name="bisphenol a", dose=20,species="mouse")
```

# PBPK Simulated Approach to Steady-State




Figures from Robert Pearce

# Evaluation of Steady-State Predictions

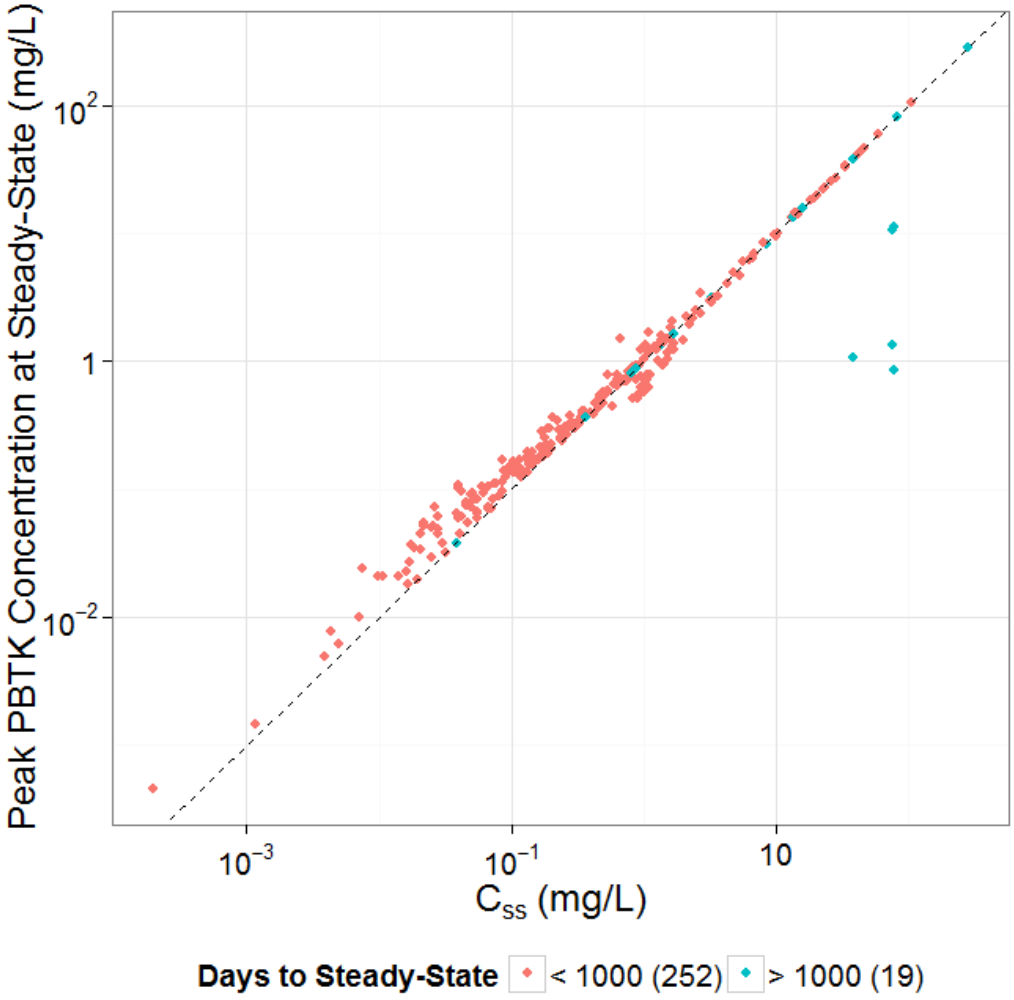



- Using HTPBTK model and assuming three daily doses (every eight hours)
- This allows us to evaluate the plausibility of the steady-state dosing assumption.
- We find that the majority of chemicals reach steady state in a few weeks
- A second population of chemicals never reach steady state.



Wambaugh et al. (2015)

# 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)

# *In vivo* Predictive Ability and Domain of Applicability

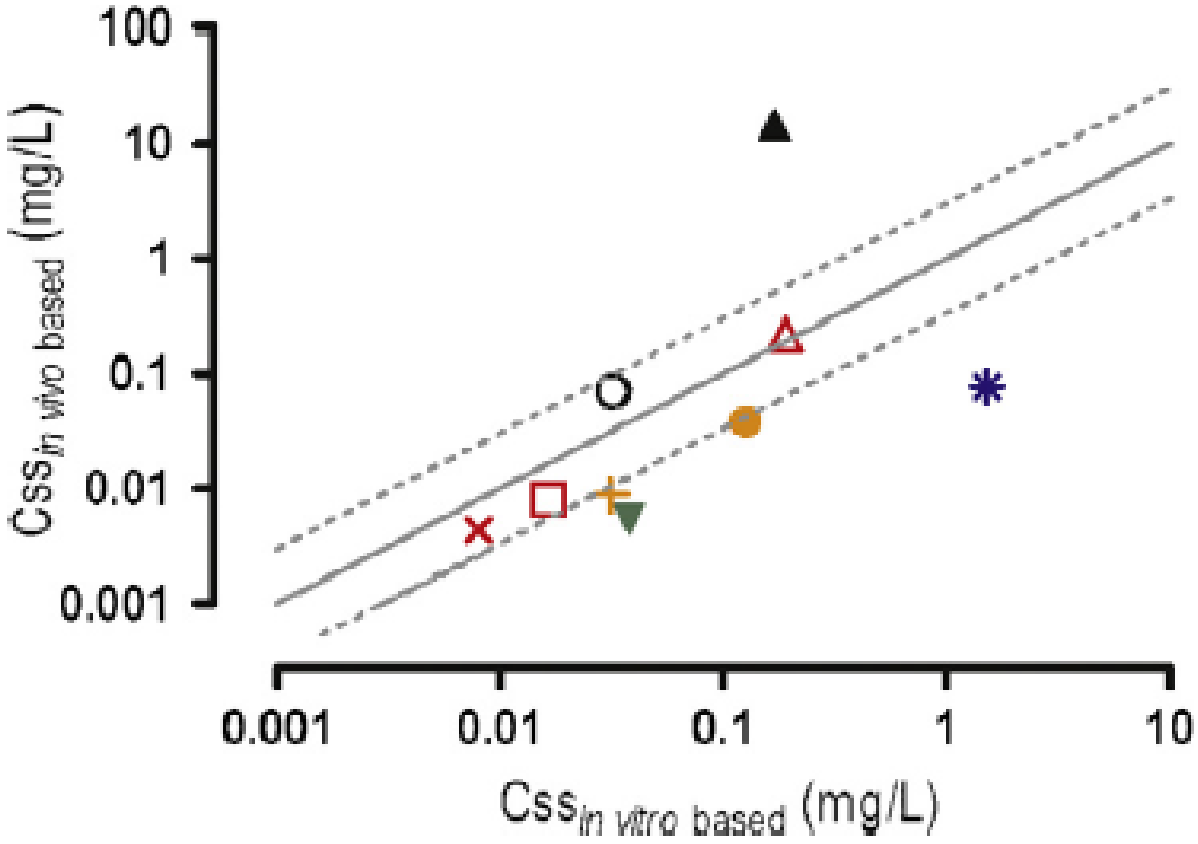
- In drug development, HTTK methods estimate therapeutic doses for clinical studies – predicted concentrations are typically on the order of values measured in clinical trials (Wang, 2010)

- 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

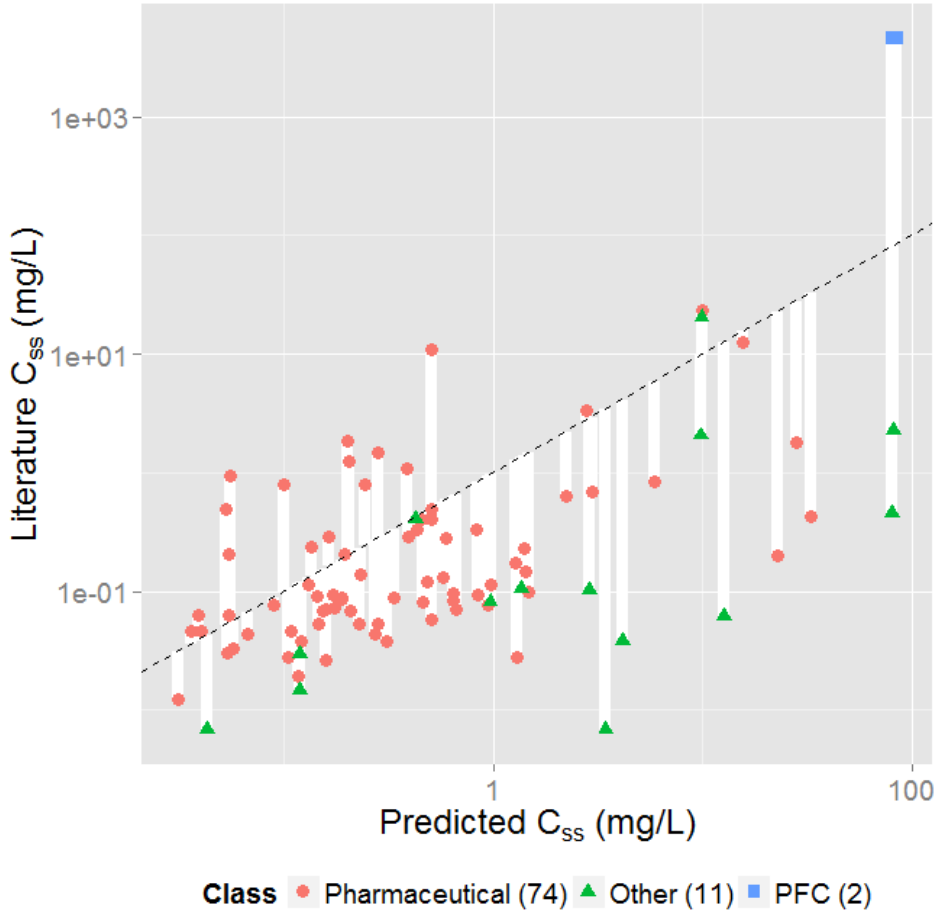
# Characterizing Uncertainty in HTTK

Yoon *et al.* (2014): Manual curation of chemical specific PK models allowed direct evaluation of HTTK IVIVE predictions

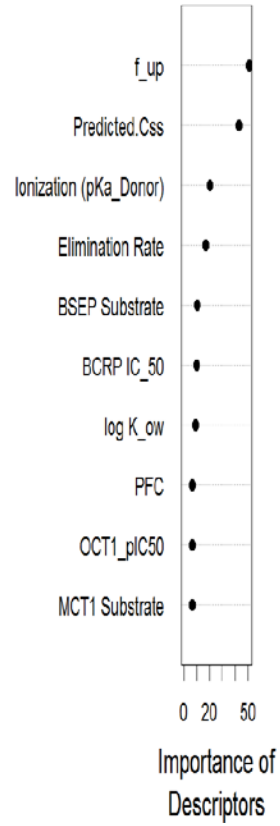



Wang (2010): In vitro predictions typically within a factor of three for pharmaceuticals

# 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)

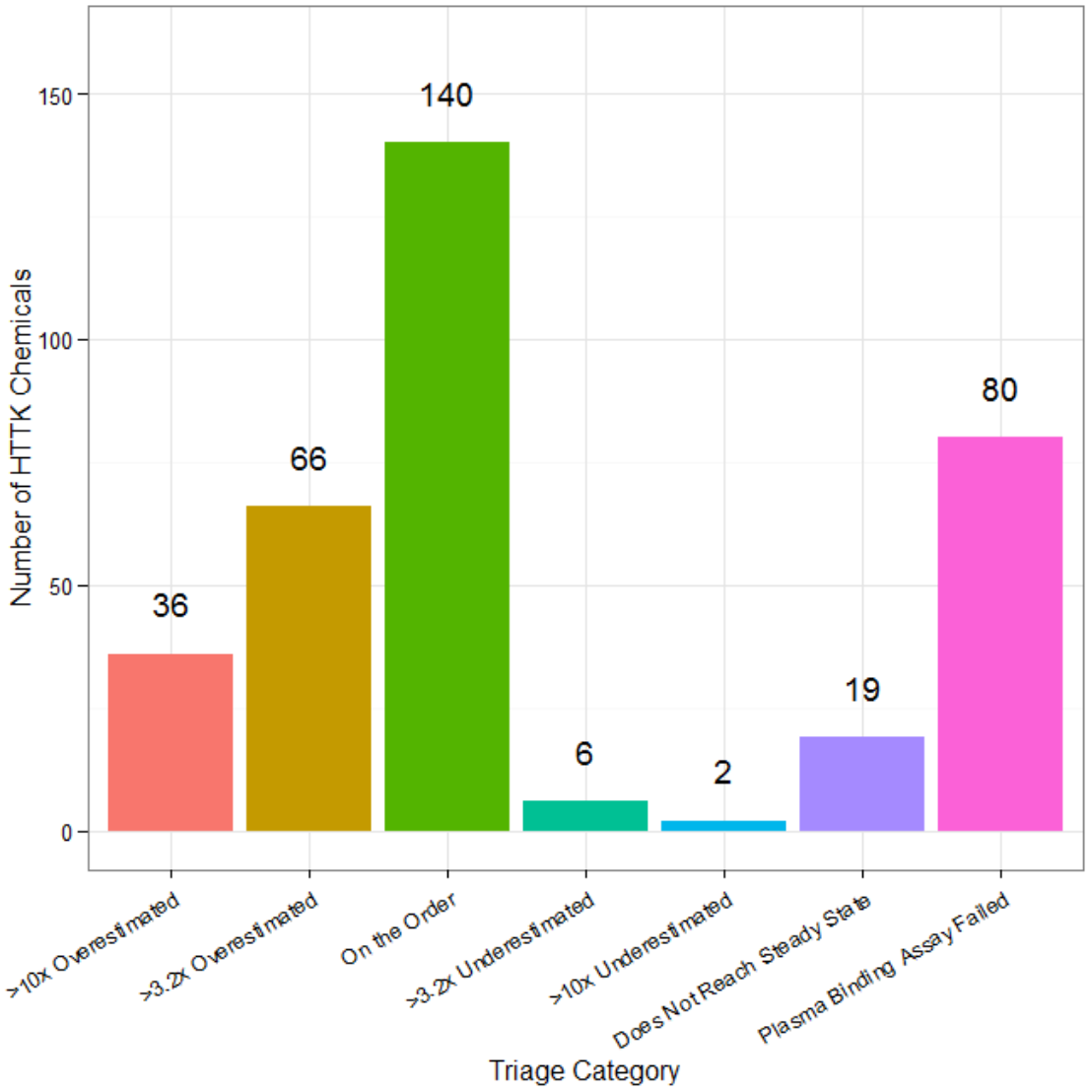





Wambaugh et al. (2015)

# 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)

# Calibrated Exposure Predictions for 7968 Chemicals

$R^2 \approx 0.5$ indicates that we can predict 50% of the chemical to chemical variability in **geometric mean NHANES exposure rates** (this does not cover highly exposed individuals)

Same five predictors work for all NHANES demographic groups analyzed – stratified by age, sex, and body-mass index:

- Industrial and Consumer use
- Pesticide Inert
- Pesticide Active
- Industrial but no Consumer use
- Production Volume

Wambaugh et al. (2014)

# Application to High Throughput Risk Prioritization

Prioritization as in Wetmore *et al*. (2012) Bioactivity, Dosimetry, and Exposure Paper

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

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"



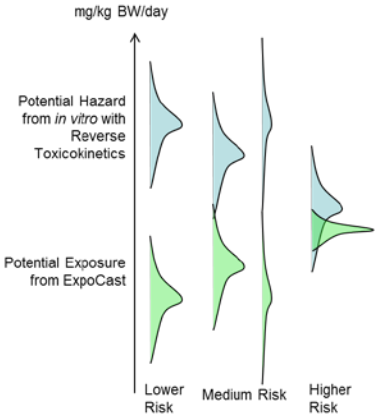
**Also see poster "Computational Models to Correlate *In Vitro* to *In Vivo* Activity" by Nisha Sipes and Steve Ferguson, et al.**

# Life-stage and Demographic Specific Predictions

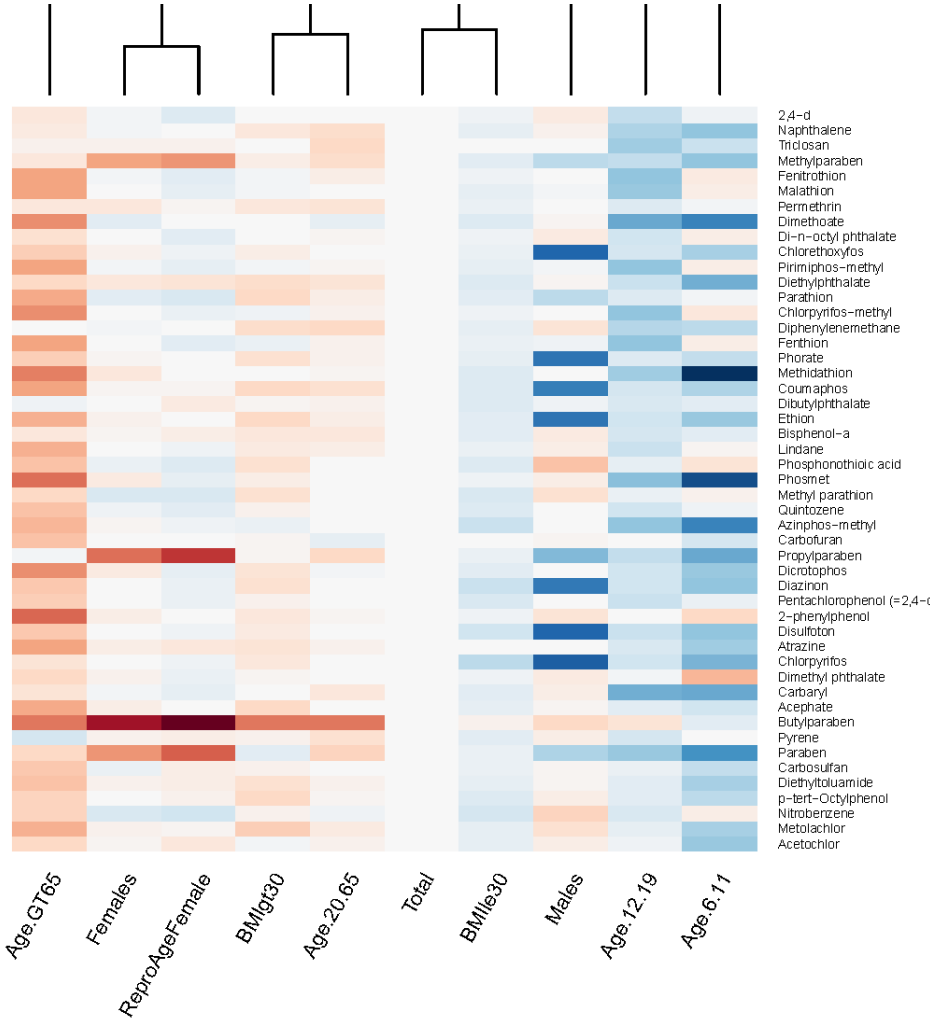
United States Environmental Protection Agency

- 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

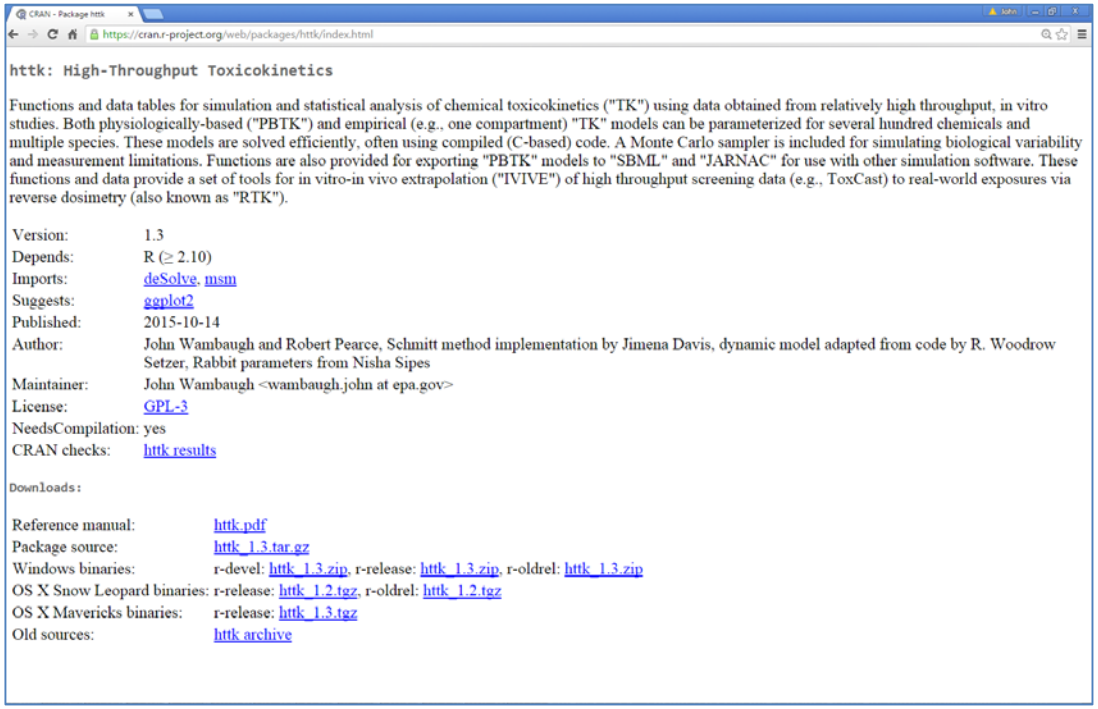



Change in Risk




Work by Caroline Ring (NCCT)

# httk R Package



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

**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 (eventual Pearce et al. manuscript)

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

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

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

# Version history for the "httk" R Package

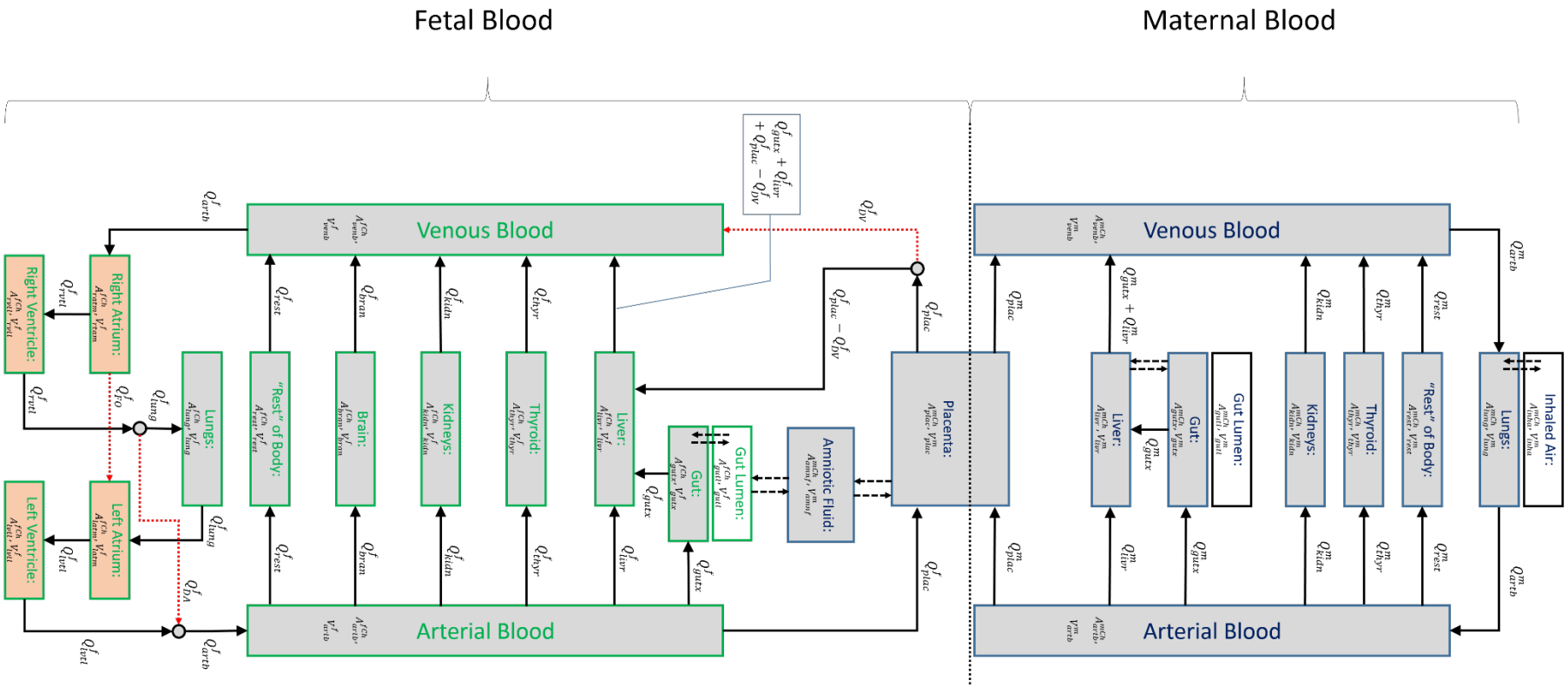
The publicly available R package contains code and data that has been part of peer-reviewed publications

- Version 1.1 accompanied "Toxicokinetic Triage for Environmental Chemicals" Wambaugh et al. (2015) Tox. Sci.
- Version 1.2 accompanied "httk: R Package for High-Throughput Toxicokinetics" Pearce et al., submitted to Journal of Statistical Software
- Version 1.3 accompanied "Incorporating High-Throughput Exposure Predictions with Dosimetry-Adjusted *In Vitro* Bioactivity to Inform Chemical Toxicity Testing" Wetmore et al., (2015) Tox. Sci.
- Version 1.4 is in development to accompany Ring et al., in preparation

We maintain internal versions containing data and code that has yet to be peer reviewed.

Lead programmer Robert Pearce

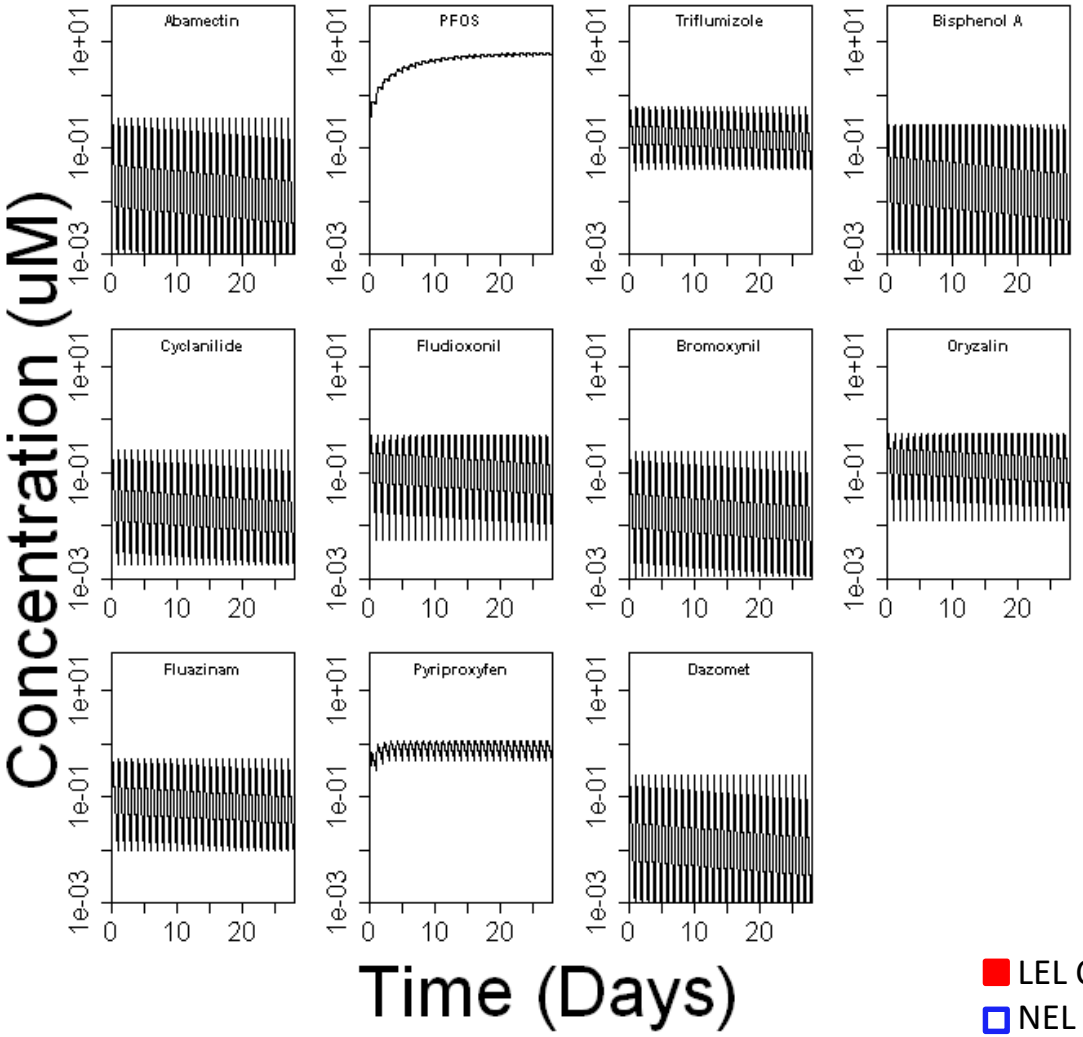
# Gestational Version of PBTK model Under Development




Dustin Kapraun, Eric Watt, and Robert Pearce of NCCT are developing a model for the httk package that allows fetal tissue concentration predictions for 443 chemicals

# HTPBTK Predicted Metrics

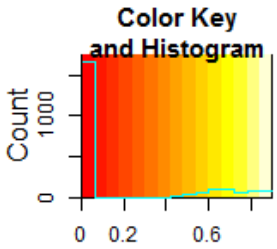
- With HTPBTK we can predict the concentration time course for various tissues
- For now, we identify mean serum concentration for no effect level (NEL) and low effect level (LEL) treatments
- Look for coincident gene expression changes



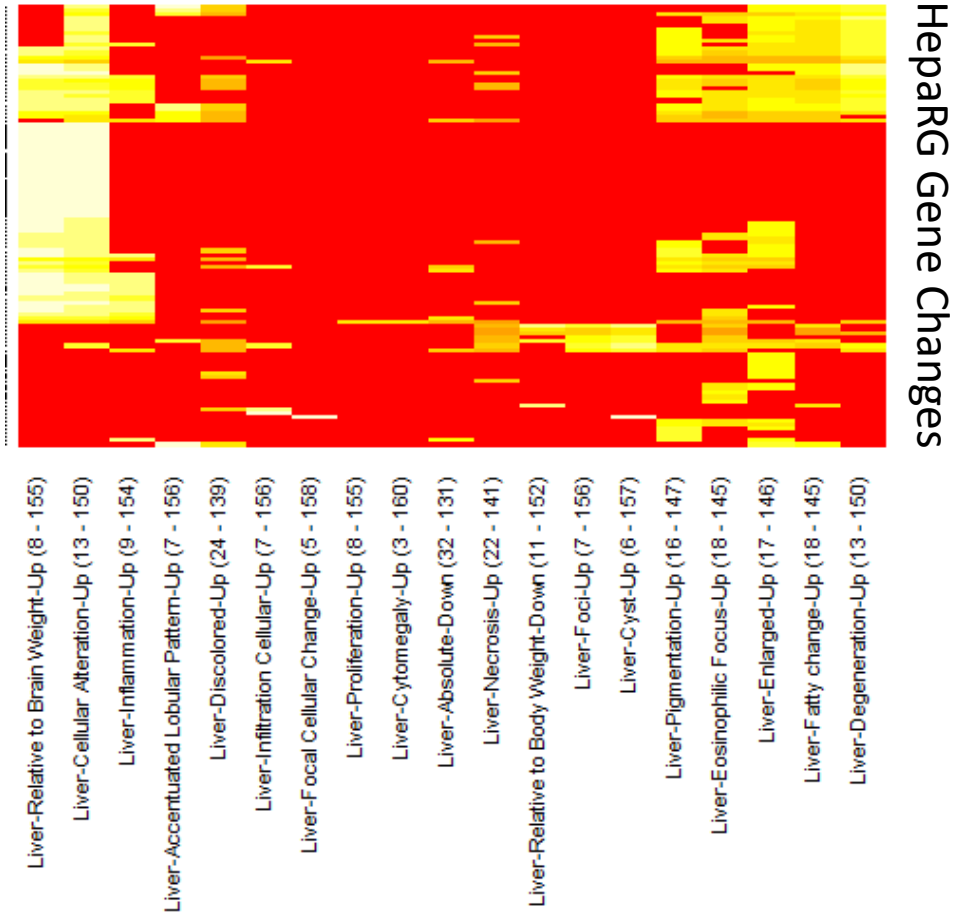
# Correlating HepaRG with Toxicity

United States Environmental Protection Agency

- We get higher balanced accuracies than ToxCast Phase I using the results of the HepaRG assay and non-steady-state PK (from the "httk" R package




Balanced Accuracy




ToxRef Liver Phenotypes (# Pos - # Neg Chems)

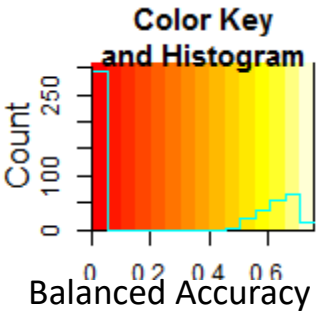
# Correlating HepaRG with Toxicity

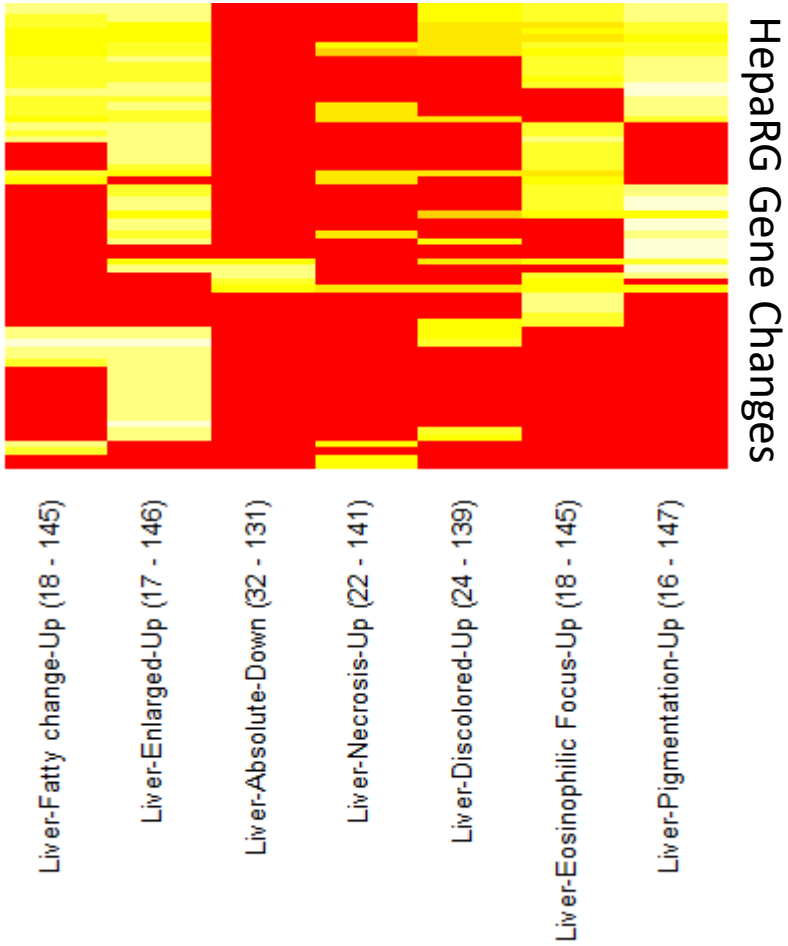
United States Environmental Protection Agency

- We get higher balanced accuracies than ToxCast Phase I using the results of the HepaRG assay and non-steady-state PK (from the "httk" R package

- Here we subset for just those effects with more than 15 positive chemicals

- Highest balanced accuracy is 0.76



# 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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Caroline Ring*
Woody Setzer
Rusty Thomas
**John Wambaugh**
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**NRMRL**
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